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CHEMICAL PATHOLOGY

BEING A DISCUSSION OF GENERAL PATHOLOGY FROM THE STANDPOINT OF THE CHEMICAL PROCESSES INVOLVED

BY

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FIFTH EDITION, REVISED AND RESET

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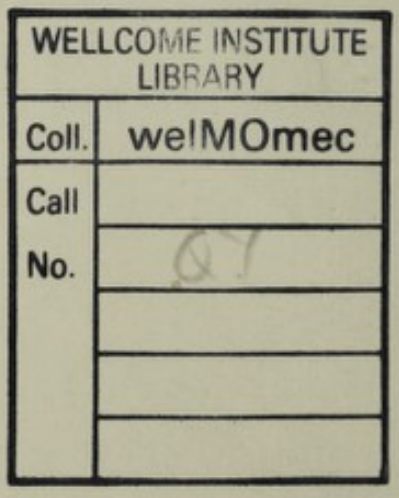

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TO

Ludvig Hektoen

THIS BOOK IS RESPECTFULLY DEDICATED, AS A

SLIGHT TOKEN OF THE GRATITUDE AND

ESTEEM OF HIS PUPIL

PREFACE TO THE FIFTH EDITION

In the five years that have passed since the fourth edition of this book was prepared, the development of interest and investigation in the chemical problems of biology and medicine has been greater, at least as evidenced by volume of publication, than in any previous five year period. To consider exhaustively all these contributions would require a work of several volumes, and would be a comparatively easy task. Believing that the chief claim for consideration this book has had in the past has depended on its containing a reasonably complete résumé of our knowledge of the topics covered within a single volume of moderate size, the same policy of compression has again been followed. In order to include as far as possible what has seemed most essential without unduly increasing the bulk of the material, much of the older work has been eliminated, and to a very large extent the entire book has been rewritten. Few pages of the previous edition remain unchanged, and numerous sections are entirely new. Much of the preliminary discussion of the principles of biological and physical chemistry of the introductory chapter of former editions has been omitted, in the belief that these principles are now generally known by the modern physician and medical student, which was far from the case when the first edition was prepared eighteen years ago. The chapters on immunity have been rearranged in a more logical way, and the number of chapters increased by two without a corresponding increase in volume. In general, however, the scope and character of the book remains unchanged. The author recognizes fully that it would be easily possible to report the existing state of knowledge on the topics covered in "Chemical Pathology" in a much briefer space, if only completely established evidence were included. With the object of serving as a guide to investigators, and with the hope of stimulating further investigations, much more than this minimal amount of existing evidence is included.

As with all previous editions, my indebtedness must be acknowledged to numerous colleagues who have kindly read over the sections of this book which most closely concern their own fields, and especially to the members of my Department and of the Otho S. A. Sprague Memorial Institute, who have made many useful suggestions. The chapter on Diabetes is largely contributed by Dr. R. T. Woodyatt, Director of the Laboratory of Clinical Research of the Sprague Institute.

H. G. W.

Chicago, Ill.,
November, 1925.

PREFACE TO THE FIRST EDITION

During the past score of years the subject of biological chemistry has attracted the attention and labors of a constantly increasing number of investigators, many of whom have, for one reason or another, been interested in pathological conditions. Sometimes the physiologist has sought for light on his problems in the evidence afforded by related pathological conditions. Frequently clinicians have studied the metabolic changes and the composition of the products of disease processes. Relatively seldom, unfortunately, has the pathologist attacked his problems by chemical methods. From the above and other sources have come scattered fragments of information concerning the chemical changes that occur in pathological phenomena. Only when bearing upon conditions such as gout and diabetes, which concern alike the physiologist, the clinician, and the pathologist, have the fragments been moulded together into a homogeneous whole. For the most part they still remain isolated, uncorrelated, frequently unconfirmed items of information, scattered through medical, chemical, physiological, and physical literature.

It has been the aim of the writer to collect these scattered fragments as completely as possible, and to use them as a basis for a consideration of General Pathology from the standpoint of the chemical processes which occur in pathological conditions. Owing to the diffusely scattered conditions of the literature on which this work is based, it cannot be claimed that all of the many contributions from which useful information might be obtained have been noticed; but it is hoped that a sufficiently thorough collection of material has been made to afford a fair basis for a consideration of "Chemical Pathology." The time seems ripe for an effort of this nature. Within the past few years great and encouraging advances have been made in biological chemistry, which in many instances seem to throw light upon pathological processes. In medicine, the use of chemical methods in the study of clinical manifestations has become more general, and has yielded valuable information. Pathologists have come to feel that the opportunities for the acquirement of knowledge by means of morphological studies have become reduced to a minimum, while the fields of pathological physiology and chemistry lie still almost unexplored. The development of research upon the subject of natural and acquired immunity has presented innumerable problems, all of which are essentially chemical. And perhaps most important of all is the general awakening of an appreciation of the importance of physiological chemistry to medical science, which has led to the introduction of laboratory courses on this subject in every medical school worthy of the name.

A book on Chemical Pathology should, therefore, seek to supply information to a varied group of readers. It should furnish collateral reading to the student who for the first time goes over the subject of General Pathology, which his text-books usually consider chiefly from the morphological standpoint. It should exploit to the graduate in medicine the advances that are being made along lines that are of fundamental importance to clinical medicine. It should serve for the investigator in biological chemistry or in pathology as a source of information concerning the ground upon which the two subjects overlap—the "Grenzgebiete" of Pathology and Physiological Chemistry. And, above all, it should afford a guide to the sources of our knowledge of these subjects, since nothing but direct familarity with the original reports of the investigators themselves can give the student an impersonal view of the actual status of the questions under consideration. On account of this multiplicity of the objects in view, it has often been necessary to consider certain topics from more than one standpoint; which explains, perhaps, certain apparent irregularities in the style and manner of treatment.

It has been assumed that the reader has at least an elementary knowledge of organic and physiological chemistry. For the benefit of those whose studies in these subjects date back some years, it has seemed advisable to include in an introductory chapter an epitome of the more modern views concerning the chemistry of the protein molecule, the composition of the animal cell, and the principles of physical chemistry, in as far as they apply to biological problems. The general consideration of "Enzymes" in Chapter II is written with a similar object. In discussing these fundamental topics it has seemed advisable to omit detailed references to the numerous original sources,—these may be found quoted in the special text-books cited in the foot-notes; but in presenting the more distinctly pathological topics the attempt has been made to render all the important literature available to the reader and investigator. To economize space, a complete bibliography has not been inserted when this exists already collected in some readily accessible review or original article; hence the references cited in the foot-notes will generally be found to include only the more recent publications. These references have been so selected, however, that they will be found to furnish bibliographical matter sufficient to lead the investigator to all the important literature on the topics covered in this book. As to those subjects (such as gout, diabetes, and gastro-intestinal putrefaction) which, because of their great practical clinical interest, have already been discussed in available monographs at greater length than the scope of this work would permit, it has seemed appropriate merely to summarize the most recent views and advances, referring the reader to the special treatises for the general and historical discussions.

It is with the greatest pleasure that I acknowledge my indebtedness to many colleagues in the University of Chicago, who have kindly read the sections of my manuscript that touch upon their own special fields, and whose criticism and advice have been of the greatest assistance;

their number alone prevents my thanking them by name. Most particularly, however, must I express my debt to my former instructor, Professor Lafayette B. Mendel, of Yale University, whose kindly criticism and suggestions have been of inestimable value. For constant assistance in the preparation of the manuscript, and for the revision of the bibliography, I am indebted to my wife.

H. G. W.

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CHEMICAL PATHOLOGY

CHAPTER I

INTRODUCTION

THE CHEMISTRY AND PHYSICS OF THE CELL

Since Virchow founded modern pathology, the unit of all anatomical considerations of disease has been the cell, and in physiology the same unit has been found equally useful. Although we know that within each cell are many different chemical substances, and that numerous different enzymes and other agencies are exerting their influences upon them, yet we find that the reactions are all profoundly affected by the environment in which they occur, and it is the structure of the cell that determines the environment of its chemical constituents. All chemical reactions are modified by physical influences, and an enzyme may have quite a different effect upon a substance when it acts in a test-tube from what it will have when in a living cell, whose structure permits the diffusion of one substance while preventing that of another, and where countless other substances and enzymes may participate in the changes. The cell is the structural unit of the living organism, and as by its physical properties it modifies chemical processes, so it becomes practically the unit in physiological and pathological chemistry. All consideration of the chemistry of disease must thus refer back to the chemistry and physics of the normal cell, and on this account a brief résumé of these subjects may serve as a fitting introduction to the strictly pathological matter to follow.[1]

As applied to the animal tissues, the term "cell" is entirely a misnomer, for it describes accurately only such forms of "cells" as are found in plants, in which the prominent feature is the limiting wall, forming a cell to enclose a fluid content. In most instances the animal "cell" answers better to the definition, "a mass of protoplasm;" but usage makes language, and no possible confusion can arise from the prevailing universal use of the original term, except, perhaps, that

[1] Of necessity, only so much of the very extensive literature on cell structure and cell chemistry can be considered as will have direct bearing upon the subject matter to follow, referring the reader for more detailed information to such works as Wilson's "The Cell in Development and Inheritance;" Mathews' "Physiological Chemistry;" Hammarsten's "Physiological Chemistry;" Gurwitsch's "Morphologie und Biologie der Zelle;" Höber's "Physikalische Chemie der Zelle und der Gewebe;" Hamburger's "Osmotischer Druck und Ionenlehre;" Loeb's "Dynamics of Living Matter;" Oppenheimer's "Handbuch der Biochemie;" and "General Cytology," edited by E. V. Cowdry, University of Chicago Press, 1924.

the term is prone to carry with it the thought of the walls of the cell being much more prominent than they really are. This is not so unfortunate a result, perhaps, for, as we shall see later, the limiting surfaces of the cell, even when too thin to be readily demonstrable, may play a much more important part in cell chemistry than their appearance indicates.

The morphological division of the cell into cell wall, cytoplasm, nucleus, and nucleolus can hardly be followed out chemically, for if we surmount to some extent the difficulties in the way of studying the different portions separately, we find that the differences between them are rather quantitative than qualitative. And, furthermore, however different the cells of one organ or tissue may appear from those of another organ or tissue under the microscope, when analyzed by the chemical methods at present at our disposal we find the differences very slight indeed. Certain substances are found in every living cell, and in about the same proportions; hence they are assumed to be the most important constituents of protoplasm, and are sometimes called the *primary* constituents of the cell. Many other *secondary* constituents may also be present, some of which are so nearly universal that we are not sure but that they really are primary cell components; such are fat and glycogen. Others are characteristic of certain cells, such as melanin and keratin, or specific products of cell metabolism, such as mucin and the specific enzymes. The great histological and chemical differences existing between different tissues depend often on these secondary products, as in fat tissue and squamous epithelium; or upon the intercellular substance, as with connective tissue, cartilage, bone, etc., which may be looked upon as products of cell activity.

Protoplasm, as the term is generally used, includes the various primary constituents with the fluids permeating or dissolving them, but does not include the more conspicuous secondary constituents, such as fat droplets, pigment granules, etc., nor the cell membrane when such exists. Evidently it is a very indefinite term, to be avoided as much as possible, particularly because of the confusion as to whether it includes the nucleus or not, different authors differing in this respect in their usage of the word. The anatomical distinction of nucleoplasm and cytoplasm avoids this difficulty.

CHEMISTRY OF THE ESSENTIAL CELL CONSTITUENTS

To enumerate exactly the primary or essential constituents of the cell is not possible, but practically they may be grouped under the headings of proteins, lipins, carbohydrates, salts, and water.

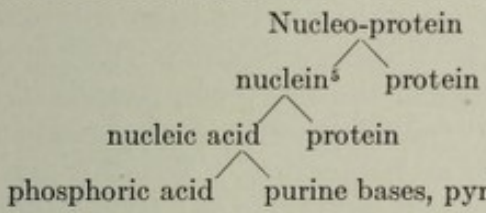
The Proteins of the Cell.—Proteins are classified into *simple proteins*, of which egg and serum albumin are types; and *compound* or *conjugated proteins*, which are characterized by having some special non-protein group which can be split off, leaving behind a characteristic protein residue, *e. g.*, nucleo-proteins, glyco-proteins. As primary cell constituents the following varieties of proteins may be mentioned; albumin, globulin, nucleo-protein, glucoprotein, phospho-protein, and insoluble proteins. At one time it was thought that cytoplasm consisted chiefly

of albumin, like white of egg, but we now know that this forms but a small part of the cell proteins, often occurring only as traces. It is held by some that true albumin occurs only as a building or intermediate cleavage product of the more complicated forms of cellular proteins, and is itself of relatively slight importance in cell life, not participating in chemical changes except as a food-stuff.

Globulins occur in all cells, but in small amounts in most animal cells except the muscles, whose chief proteins belong to this or a closely related group. An important feature of the globulins is the low temperature at which they coagulate—some so low that Halliburton[2] believes it possible that they may be coagulated within the cells during high fevers.

Hammarsten has long maintained that simple proteins form a relatively insignificant part of the cytoplasm, in opposition to the once-prevalent view that the nucleo-proteins were limited to the nucleus, and that the cytoplasm was chiefly albumin and globulin. The general trend of opinion as influenced by the results of researches has been favorable to his contentions, and we shall probably not be far wrong in accepting his statement that—"The chief mass of the protein substances of the cells does not consist of proteins in the ordinary sense, but consists of more complex phosphorized bodies, and that the globulins and albumins are to be considered as nutritive materials for the cells or as destructive products in the chemical transformation of the protoplasm."[3]

Nucleo-proteins[4] are considered to be the most important constituents of the cell, both in quantity and in relation to cell activity. In structure the nucleo-proteins are very complex, as indicated by the different products yielded on hydrolytic cleavage of the molecule. Furthermore, there are many varieties, depending both upon the nature and proportions of the component parts. They may be described as consisting of two primary constituents—(1) nucleic acid and (2) a protein body, in chemical combination with each other like a salt. The term *nucleic acid* covers a large group of substances, which are characterized, on the one hand, by their frequent occurrence bound with proteins, and, on the other hand, by their yielding phosphoric acid and purine bases, pyrimidines and pentoses or hexoses on cleavage. Diagrammatically the manner of cleavage of the nucleo-proteins may be indicated as follows:



In the cell the nucleo-proteins probably exist partly as solid structures, *e. g.*, the chromatin framework of the nucleus, and partly dissolved in the plasma. An interesting phenomenon is the alteration in the chromatin nucleo-proteins during cell division, when they seem to lose part of the combined protein and approach more nearly pure nucleic acid—just as inorganic salts occur with the acids and bases saturating each other more or less incompletely, *e. g.*, mono-, di-, and tri-basic phosphates. In this we have a chemical explanation of the intensity of the staining of dividing nuclei by basic dyes.[6]

Phosphoproteins resemble nucleo-proteins to the extent that they also yield phosphoric acid, and are somewhat similar in solubility and digestibility. They are essentially different, however, in that they *do not yield nucleic acid* or purine bases on cleavage. Probably members of this group are also constant components of cells.

[2] Halliburton and Mott, Archives of Neurology, 1903 (2), 727; also see Halliburton's "Chemistry of Muscle and Nerve."

[3] A different view is taken by Whiteside, Anat. Record, 1923 (26), 91.

[4] See review by Steudel, Klin. Woch., 1923 (2), 1789.

[5] Probably nuclein should be considered as merely one variety of nucleoprotein, with less protein than the other varieties.

[6] The chemistry of the nucleo-proteins is discussed in the chapter on Uric Acid Metabolism and Gout, Chap. XXV.

Gluco proteins (or *glyco-proteins*) and *phospho-glucoproteins* are also believed to occur frequently or constantly in protoplasm. They are compounds of proteins with a sugar or sugar-like group, which probably usually contains nitrogen, thus differing from the ordinary hexoses and pentoses.

Insoluble proteins, or bodies resembling the coagulated proteins in their lack of solubility in various fluids, are left behind after the other proteins have been extracted from the cells. Their significance is not known; whether to a large extent artificially produced or whether a normal structural element of the cell.

Fats and Lipoids (Lipins)[7]

Lipoids is a term in common use but of indefinite significance; most usually it comprehends the intracellular substances which are soluble in ordinary fat solvents, but which are not simple fats or fatty acids, lecithin and cholesterol being the most important of the lipoids.

For the entire group of fats and lipoids the term *lipins* has been proposed by Gies and Rosenbloom.[8] Lipoids and ordinary fats, that is, lipins, occur in all cells, but their demonstration is not always readily possible. The microscopic appearance of a cell, even when special stains for fat are used, gives no correct idea of the amount of lipins actually present. Thus normal kidneys contain 15 to 18 per cent. in their dry substance, but none of this can be detected readily with the microscope. A kidney which seems microscopically the site of marked fatty degeneration may show no more fat when examined chemically than a normal kidney, which in section appears to be quite free from fat. This is because some of the intracellular fat is so bound, chemically or physically, with the proteins that it cannot be seen, nor can it be stained by the dyes ordinarily used for that purpose; only when degenerative changes of certain kinds have liberated it from combination does it become visible and stainable by ordinary methods (Rosenfeld). By the special fixation method of Ciaccio the fatty compounds of even normal cells may be made stainable (Bell),[9] showing that the so-called masked fat is really in a not altogether invisible form. Whether the intracellular fat has any function other than that of serving as a food-stuff is not known, but there can be no question of the importance of the phosphorized fats, or phospholipins.

Phospholipins are primary cell-constituents and are probably important both in metabolism and physically. Hammarsten regards them as concerned in the building up of the nucleus. As will be shown later, many of the most essential physical properties of the living cell depend upon the presence in it of lipoids, of which phosphatids are apparently the chief. Of the ether-soluble substances in the heart, for example, 69 to 70 per cent. are phosphatids, representing 8 per cent. of the dry weight of the myocardium. Many different substances have been described as phosphatids, but the chemical identity of but few is sufficiently established. Of these the most important are *lecithin* and *cephalin*, which are most intimately asso-

[7] Full discussion in "Lecithin and Allied Substances (The Lipins)," by Hugh MacLean, Monographs on Biochemistry, London, 1918.

[8] MacLean[7] uses "lipin" to include "substances of a fat-like nature yielding on hydrolysis fatty acids or derivatives of fatty acids and containing in their molecule either N, or N and P." As there is need for a term covering the fats, phosphatids, cholesterols and related bodies, the suggestion of Gies and Rosenbloom is followed for the present in this book, and the word lipin used with the broader significance.

[9] Jour. Med. Res., 1911 (19), 539.

ciated. It has been held by some that there are many phospholipins, which may be specific for different cells, tissues, and species, but it seems more probable that these supposed specific lipoidal substances are merely mixtures of lecithin, cephalin and their derivatives in varying proportions (Levene).[10]

Cholesterol is nearly as universally present as lecithin. It exists both free and in combination with fatty acids, for cholesterol is an alcohol and not at all similar to the fats chemically, although very similar physically. The empirical formula is $C_{27}H_{43}OH$ or $C_{27}H_{43}OH$, and it is related to the terpenes. It seems to be relatively inert chemically, and therefore is probably important chiefly because of its effect on the physical properties of the cells.[11] However, recent studies on vitamins have shown that pure cholesterol after treatment with ultraviolet light may assume the properties of the antirachitic vitamin, which fact suggests that cholesterol may have more active functions than has hitherto been appreciated.

Doubly Refractive Lipoids and Myelins.[12]—In practically all normal tissues there are present droplets of l poid nature which are characterized by showing prominent crosses when examined with crossed Nicol prisms (anisotropic), the adrenal and corpus luteum containing them most abundantly. Chemically they seem to be mixtures of various lipoids in inconstant proportions, but probably the anisotropic character is most usually dependent upon the presence of cholesterol esters. The term myelin was first applied by Virchow to peculiar fatty substances found in various normal and pathological tissues, because they showed physical characters similar to those of the myelin substance of nerves, but as many of these substances are doubly refractive, or can be easily made so, some authors use the term myelin as if it were synonymous with doubly refractive lipoids. There are, however, myelins which are not always doubly refractive, and also doubly refractive lipoids which do not swell up in water to form myelin figures, etc., as is characteristic of true myelins. Chemically, however, the myelins and doubly refractive substances are probably related, consisting of mixtures of cholesterol, cholesterol esters, phospholipins and perhaps soaps, in varying proportion. They will be considered further in discussing Fatty Metamorphosis, Chap. XVIII.

Carbohydrates

The third great class of food-stuffs, the carbohydrates, is represented in the cell by *pentoses* and *hexoses* combined with proteins and with lipoids, and also by *glycogen*, which exists free. Glycogen is difficult to isolate in minute quantities and, therefore, although it is not found in all cells by our present methods, yet it may well be that it is a constant constituent of the protoplasm. There is no evidence, however, that it is anything more than a source of heat and energy to the cell. Its properties and occurrence will be considered more fully in the discussion of glycogenic infiltration. Since glycogen is formed from dextrose and is constantly breaking down into dextrose, it is probable that the latter is also contantly present in the cells.

Inorganic Substances

Up to this point the substances of the cytoplasm that have been discussed have all been organic compounds which do not naturally exist independent from living or once living cells, yet the inorganic substances of the protoplasm are also of vital importance. As Mann says, "so-called pure ash-free proteins are chemically inert, and, in the true sense of the word, dead bodies. What puts life into them is the presence of electrolytes." The various salts of K, Na, Ca, Mg, and Fe, which all cells contain, exist not merely dissolved in the water of the cell, but in part they are combined with the organic constituents of the protoplasm as *ions*, both anions and cations, united in chemical combination to the large protein molecule (ion-proteins).[13] Possibly the proteins participate in vital chemical processes only as ion compounds with inorganic elements. It is extremely difficult, indeed almost impossible, to secure proteins entirely free from inorganic substances (ash-free proteins). The fact that

[10] A full review on the phosphatids is given by Levene and Rolf, Physiol. Reviews, 1921 (1), 327.

[11] See Degkwitz, Klin. Woch., 1924 (3), 1572.

[12] See Adami, Jour. Amer. Med. Assoc., 1907 (48), 463; Karwicka, Ziegler's Beitr., 1911 (50), 437; Schultze, Ergebnisse Pathol., 1909 (13, pt. 2), 253; Bang, Ergebnisse Physiol., 1907 (6), 131; 1909 (8), 463.

[13] See J. Loeb, Science, 1919 (50), 439.

inorganic substances are held in the cells chemically rather than by simple diffusion into them from the surrounding fluids is suggested by the great difference in the proportions of various salts in the cells and in the extra-cellular fluids.[14] Thus potassium is nearly always much more abundant in the cells than in the tissue fluids, while sodium is more abundant in the fluids. Phosphoric acid is also more abundant in the cells, and chlorin in the plasma. In cells iron seems to exist chiefly in combination with the nucleo-proteins.[15]

THE PHYSICAL CHEMISTRY OF THE CELL AND ITS CONSTITUENTS[16]

From the standpoint of physical chemistry the cell consists of a collection of colloids and crystalloids, electrolytes and non-electrolytes, dissolved in water, in lipoids, and in each other, surrounded by a semi-permeable membrane, and perhaps subdivided by similar membranes or surfaces. Physical chemical processes, as we shall see later, play an all-important part in the life phenomena of the cell. Thus, if there were no electrolytes in the cell, electric charges could not be carried about in it, and hence chemical reactions could not occur. It is this fact that makes the inorganic salts of such vital importance to the cell life. To repeat Mann's words, it is the electrolytes that put life into the proteins. Water is almost absolutely non-dissociated, and proteins so little that for some time it was doubted if they really did ionize. Probably all soluble substances do dissociate to a certain minimal degree, but dissociation is so slight for most of the constituents of the cell, that without the salts there would be little chemical activity possible, and hence life would be absent or at a very low ebb indeed. As before mentioned, the inorganic salts probably exist in the cell not only as salts, but also, and perhaps chiefly, as ions and ionic compounds with the cell proteins.[17] Many applications of the facts and theories of ionization have been made in physiology and a few applications have also been made in pathology, especially the relation of ions to edema, to diuresis and glycosuria, and also to problems of immunity. No attempt will be made here to go further into the observations and theories concerning ionization or its rôle in physiology, but for more extensive information as well as for bibliography the works mentioned below may be referred to.[18]

[14] This disparity may depend partly on purely physical properties through the Donnan equilibrium (q.v.) effect of colloidal solutions (See J. Loeb, "Proteins and the Theory of Colloidal Behavior," 1924).

[15] See Macallum on Microchemistry, Ergebnisse Physiol., 1908 (7), 552.

[16] See Bayliss, "Principles of General Physiology," London, and Höber, "Physikalische Chemie der Zelle und der Gewebe," for a more extensive discussion of these topics.

[17] A good brief review of the electrochemical properties of proteins is given by Handowsky, Klin. Woch., 1923 (2), 267.

[18] "Physical Chemistry in the Service of Medicine," Wolfgang Pauli, translation by M. H. Fischer, New York. "Physikalische Chemie der Zelle und der Gewebe," Höber, Leipzig. "Osmotische Druck und Ionenlehre in den medicinischen Wissenschaften," Hamburger, Wiesbaden. "Studies in General Physiology," Loeb, University of Chicago Press. "Dynamics of Living Matter," Loeb, Columbia University Press, New York. "Physical Chemistry of Vital Phenomena," McClendon, Princeton Univ. Press. "Protoplasmic Action and Nervous Action," R. S. Lillie, Univ. Chicago Press.

Diffusion and Osmosis.—Although the non-electrolytes do not ionize to any considerable extent, and therefore are relatively inactive chemically, the crystalloidal non-electrolytes, of which sugar and urea are the two chief examples among the cell constituents, possess in common with the electrolytes the important property of diffusion. By this process the exchange of chemical substances between the blood and the cell is largely brought about, by it the chemical composition of the different parts of the cell and between different cells is equalized, and without it chemical change would be practically impossible. The processes of diffusion and osmosis are studied especially readily in plant cells, for, unlike animal cells, the typical cell of plant tissue possesses a distinct wall, usually cellulose, lined internally by a layer of protoplasm which incloses a mass of aqueous solution, the cell sap, containing sugar and various other solutes. The outer wall is readily permeable by water and by most solutes, whereas the protoplasmic layer inside it behaves like a semipermeable membrane, which permits water to pass through readily but hinders greatly the passage of most solutes; that it is somewhat permeable is attested by the fact that the cell sap contains solutes derived from the external fluids. As a result of this arrangement there is a constant tendency for the cavity of the cell to be distended by water and for the solutes within it to exert their considerable pressure upon the cell wall. Because of the strength of the cellulose layer the cell can withstand great pressures that would tear apart the tender protoplasmic layer that really determines the osmotic pressure that causes the rigidity or *turgor* of plant cells, and explains the ability of a tender green shoot to hold itself upright or horizontal in the air; and it is the force that enables growing roots to lift great stones or tear apart rocks in whose clefts they grow. If certain plant cells are placed in distilled water, the pressure may rise to such an extent that the cells burst, and it was through studying this phenomenon that Pfeffer worked out the basis of our present knowledge of osmotic pressure. If the cell is placed in a solution of greater concentration than its cell sap, the pressure outside will be greater than that inside and the protoplasmic membrane will be forced away from the cellulose wall, while its central cavity shrinks and perhaps disappears entirely, the protoplasm forming a ball in the center. This is practically what occurs when a plant stem is cut and it "wilts"—the water is removed by evaporation, the osmotic pressure outside the cells becomes greater than that inside, and the water passes out. Likewise when a plant cell dies the turgor is lost because the membrane becomes permeable, and so pressure soon becomes the same on both sides of the cell wall.

In animal cells the wall is not so highly developed as in plants, nor is it backed up by a rigid material like cellulose; indeed for many animal cells there is no well-defined wall and the protoplasm appears to be naked. Nevertheless the behavior of the animal cells indicates that they do possess what resembles a cell wall, in that they behave when in solutions as if they were surrounded by a semipermeable diffusion membrane. The degree to which phenomena of this nature are shown varies with different cells; with red corpuscles, for example, the osmotic pressure influences are very marked, as shown by the wrinkling or crenation of the corpuscles when they are placed in fluids of higher concentration than the blood plasma, and by their swelling and disintegration with escape of the hemoglobin (*hemolysis*) when they are put into distilled water or solutions of less concentration than the plasma. Other tissue cells seem to undergo more or less alteration from changes in the osmotic pressure in the fluids surrounding them. The diffusion membrane that surrounds the cell is generally not well defined, and for most cells seems to be but a surface condensation of the protoplasm, perhaps formed through the effects of surface tension. It seems probable that this surface diffusion membrane contains a large proportion of cell lipoids, *i. e.*, cholesterol and phospholipins (for the red corpuscles this is practically certain); hence substances soluble in lipoids penetrate the cell readily, while to many substances insoluble in lipoids the cell is nearly or quite impermeable (Overton). Probably the wall of the animal cell is not so nearly semipermeable as is that of the plant cell, for nowhere in the animal body do we get such turgor in the cells as we see in plant tissues. Lacking a cellulose wall, animal cells could not develop such an internal pressure without rupturing and such a process of rupturing (*plasmorrhexis, plasmoptysis*) does not seem to be a normal occurrence in animal tissues. However, in animal tissue the internal osmotic pressure of the cell is largely counterbalanced by the osmotic pressure of the fluids outside the cell. We shall be most nearly correct, probably, if we look upon the animal cell as possessing a delicate diffusion membrane at its surface, through which water passes more readily than do most crystalloids, and through which colloids pass almost not at all, but the exclusion of each of these types of substances is merely relative and not absolute. Within the cell, also, the colloids possibly exist as a more or less well-developed emulsion, with a practically

limitless amount of surface formation all through the protoplasm; such a structure could permit the endless number of reactions of a living cell to go on side by side in the same cell.

Since osmotic pressure, exactly like gas pressure, is presumably produced by the bombarding of the walls of the container by particles in the solution, the amount of pressure will vary in proportion to the number of particles present. With non-electrolytes, such as sugar and urea, the moving particles seem to be molecules, and so a solution of sugar or urea will produce an osmotic pressure directly proportional to the number of molecules it contains. In the case of the electrolytes, however, the ions produce pressure as well as the molecules, and hence an electrolyte in solution will produce a relatively high osmotic pressure as compared with an equivalent solution of a non-electrolyte, since each molecule may yield two or more ions. Colloids, however, exert so slight an osmotic pressure that it is difficult of detection; this probably depends on the great size and slight motility of their molecules. In the many and important osmotic processes of the animal organism, therefore, the colloids take no part except in helping to form the diffusion membrane, and in preventing the diffusion of one another.[19]

Under ordinary conditions colloids do not greatly modify the diffusion of crystalloids through a solution containing both classes of matter, hence the fact that a cell is full of dissolved colloids does not seriously affect the osmotic properties of the intracellular crystalloids, provided the colloids are not condensed in such a way as to form diffusion membranes. But as all the cleavage products of proteins after they have passed the peptone stage are crystalloids, by decomposition of the intracellular proteins the osmotic pressure may be greatly raised. As long as the cell is living there can be no constancy in composition, for metabolic processes, by producing from colloids that have almost no osmotic pressure crystalloidal substances that have much more osmotic pressure, cause intracellular osmotic conditions to be continually varying. As a result, streams of diffusing particles are moving about in every direction, setting up new chemical reactions and consequent new osmotic currents. The greater the difference in osmotic pressure between a cell and its environment, and between the different parts of the same cell, the more powerful the osmotic effects, and as a result the greater the capacity for accomplishing work.

Indeed, *we may look upon cell life as a constant attempt at the establishment of equilibrium, both chemical and osmotic, which is never achieved* because the move towards one sort of equilibrium is always against the other. All the food-stuffs—fats, carbohydrates, and proteins—are characterized by being colloids when intact and crystalloids when disintegrated, thus:

colloidal proteins ⇄ crystalloidal amino acids
colloidal glycogen ⇄ crystalloidal sugar
nondiffusible fats ⇄ diffusible soaps and glycerol.

In consequence of this, if the crystalloids diffuse from the blood into a cell there is at once an excess of this end of the equation, and, hastened by the intracellular enzymes, partial synthesis to the colloid soon occurs

[19] Under experimental conditions it is found that the nature of the membrane greatly modifies the osmotic pressure; for if a given colloid is soluble in a certain membrane and a certain crystalloid is not, the colloid will diffuse through the membrane while the crystalloid is held back. (Kahlenberg, Jour. Physical Chem., 1906 (10), 141.)

to establish chemical equilibrium. Chemical changes in the crystalloids, by oxidation, reduction, or hydrolysis, upset this chemical equilibrium, and hence further diffusion, synthesis, and hydrolysis continue, one upsetting the other continuously. If equilibrium were established we should have no further reactions, and the cells would be inactive. *The constant upsetting of the equilibrium is what constitutes cell life.*

It is apparent that osmotic pressure and osmosis must be of essential importance in absorption from the alimentary canal, in absorption and excretion between the cells and the blood stream, and in secretion by glandular organs; but it is also certain that they are no less important in all the less obvious chemical and physical processes of the cell.[20] In pathological processes osmotic pressure may play an equally important rôle, and the facts discussed in the preceding paragraphs will be alluded to frequently in subsequent chapters.

Colloidal Properties of Protoplasm.[21]—The chief constituents of the cells, in fact nearly all the primary constituents except the inorganic salts, are organic colloids, and therefore *the properties of the cells are largely dependent upon the properties of the colloids.* The effect of colloids upon chemical processes going on within their solutions or gels is surprisingly small. Salts in solution in a thick gel of agar or gelatin will diffuse almost as rapidly as in water; they will also ionize as rapidly as in watery solutions, and chemical reactions occur with nearly the same speed and completeness as if the colloids were absent. Furthermore it makes little difference whether these processes are measured in a colloidal solution that is liquid, or after it has set in the gel form. These facts merely indicate that the colloids do not greatly impede the movements of molecules or ions in solutions. On the other hand, as before mentioned, colloids diffuse very slowly into each other. Hence, in the cell the colloids are quite fixed in their positions, whereas the crystalloids may wander about freely, and this arrangement is certainly of great importance in biologic processes. Pauli suggests the probability that the fixation of the colloid causes the cell to have different properties in different parts, and so various reactions may occur independently in different areas of the cytoplasm. The possibility of the correctness of this view is increased when we consider that the enzymes are colloids, for there is much evidence to show that they are distributed in just such an uneven manner within the cells.

Although colloids permit the passage of dissolved crystalloids through them, they greatly interfere with the movement of larger particles. This property accounts for the ability of colloids to hold many insoluble sub-

[20] For further consideration of the subject of osmotic pressure in these relations see: Livingston, "The Rôle of Diffusion and Osmotic Pressure in Plants," University of Chicago Press, Chicago; Czapek, "Biochemie der Pflanzen," Jena. Also, Spiro, Pauli and Höber, all previously cited.

[21] For full discussions of the nature of colloids see: Höber, "Physikalische Chemie der Zelle," Leipzig; Bechhold, "Colloids in Biology and Medicine," translated by J. G. M. Bullowa, 1919; "The Elements of Colloidal Chemistry," Freundlich, translated by George Barger; Wo. Ostwald, "Grundriss der Kolloidchemie," and "Theoretical and Applied Colloid Chemistry," both translated by M. H. Fischer.

stances in such extremely fine suspensions that they seem superficially to be in true solution. If, for example, sodium phosphate is added to a solution of casein in lime-water, the calcium phosphate formed does not precipitate. It is not in solution, however, but rather exists as a suspension of very finely divided particles of the salt which the colloid keeps from aggregating into particles large enough to be visible or to overcome the viscosity of the fluid and sink to the bottom. Probably in this way many substances, including calcium salts, are carried in the blood, held in permanent suspension by the proteins. Substances thus finely divided will have extremely large surface area for reactions, and, therefore, will undoubtedly undergo changes with considerable rapidity and facility, although not in solution. Colloids also modify the distribution of crystalloids in solutions, as exhibited by

The Donnan Equilibrium.[22]—A Donnan equilibrium exists when two salt solutions are separated from one another by a membrane which is impermeable for one of the sorts of ions present on one side, although freely permeable for the remaining ions in the solutions. The non-diffusible ions exert an effect because they cause the freely permeable ions to be unequally distributed on both sides of the membrane. This may be illustrated by the following example. If we fill a collodion sac with a sodium gelatinate solution and suspend it in a sodium chloride solution, the following situation will arise: The collodion being freely permeable for all the ions except the gelatinate ion there will be a diffusion to establish an equilibrium, but this equilibrium is modified because of the presence of the non-diffusible gelatinate ions which causes the partition of sodium and chlorine ions to be unequal. The outer solution will be a pure sodium chloride solution while in the inner solution there will be, besides the original sodium gelatinate, also a certain quantity of NaCl. Consequently in the outer fluid the quantity of Cl ions will be greater and the quantity of the Na ions less than in the inner fluid. In the body, the capillary wall, being impermeable to protein, gives rise to a Donnan equilibrium and it is known that the tissue fluids contain more chloride than the blood. Also in support of this theory is the finding by R. Loeb, Atchley and Palmer that the blood is richer in sodium than are transudates, and the observation that when ascitic fluid and the serum of the patient are separated by a membrane there is no alteration in the original composition, showing that in the living body the capillary walls have accomplished the same equilibrium as the collodion membranes produce *in vitro*.[23]

The Structure of Colloids and of Protoplasm.[24]—Two very different sorts of substances are usually included under the term colloid, because they show the essential features of colloids in most respects; but as in many other respects they are quite unlike each other, it may be well to distinguish between them in some way. As a type of one class we may take gelatin; of the other, such a substance as colloidal arsenious sulfide. Gelatin solutions form gels upon cooling or evaporation, and redissolve when heated or when more solvent is added. Arsenious sulfide does not form gels upon cooling, and when solidified in any way, does not redissolve. In addition, the gelatin type is very viscous, and is not coagulated by the presence of salts unless these are added in large

[22] See review by Hitchcock, Physiol. Reviews, 1924 (4), 505.

[23] An extensive study of the electrolyte and water distribution in the blood by Van Slyke, Wu and McLean (Jour. Biol. Chem., 1923 (56), 765) indicates the validity of the application of pure physicochemical concepts to the problems of equilibria in the blood and tissues, and the significance of the Donnan equilibrium as a biological principle.

[24] Review by Harper, Amer. Jour. Botany, 1919 (6), 273.

amounts; while the other type does not render the fluid in which it is dissolved appreciably more viscid, and it forms a precipitate immediately if minute amounts of electrolytes are introduced. As the former type resembles in many details the true solutions, while the latter approaches more closely to the suspensions, it has been proposed to distinguish them by the terms "colloidal solution" and "colloidal suspension,"[25] which terms correspond to the more generally used terms "emulsoids" and "suspensoids." Of the two types, the colloidal solutions are by far the more important in biological considerations, since the colloidal suspensions are usually prepared artifically and seldom occur in nature, *e. g.*, Bredig's colloidal suspensions of the noble metals.

The colloidal solutions of proteins are of two types—one, such as albumin, forms a coagulum when heated, which under ordinary conditions is not reversible; that is, it does not again go into solution. Gelatin, however, becomes more fluid when heated, and when cooled it forms a gel which is readily reversible to the soluble form under the influence of heat. Within the cell, as far as we know, occur only the first type, the proteins that form non-reversible coagula.

An extensive study of the physical structure of the colloids was made by Hardy,[26] who found that the figures obtained in the solidification of protein solutions by fixing agents, such as bichloride of mercury or formalin, *bear a striking resemblance to the finer structures of protoplasm* as described by cytologists. There is produced an open network structure with spherical masses at the nodal points, or minute vesicles hollowed out in a solid mass, or a honeycomb appearance, or, when the concentration of the colloid is very slight, perhaps there is only a precipitation of fine granules of protein such as we often see in histological preparations of edematous cells and tissues. All these forms seem to depend chiefly upon the concentration of the colloid. The important fact is that when the chemicals ordinarily used as fixatives of cells for histological purposes act upon solutions of colloids that are perfectly homogeneous, they produce very constant and characteristic formations which recall at once the structures found in the protoplasm of hardened cells. Moreover, the use of different fixing agents, such as osmic acid, formalin, and bichloride of mercury, produces just the same differences in the structure of colloidal solutions that they produce in the protoplasm of cells hardened by them. Neither are the appearances seen in unfixed specimens reliable indications of the true structure of the living protoplasm. Granules of secretion may disappear after or during the death of the cell (*e. g.*, glycogen) or they may swell up (*e. g.*, mucin granules), thus giving the appearance of a network or honeycomb which is then incorrectly ascribed to the protoplasm itself. Death of the cells, even when not produced by external influences, seems to be accompanied by coagulation of some parts of the cell constituents, and hence a cell examined in anythng but its normal living condition, an extremely difficult

[25] Noyes, American Chemical Journal, 1905 (27), 85.
[26] Journal of Physiology, 1899 (24), 158.

matter, will not present a true idea of how it appears and is composed while in that condition. By microdissection with the Barber pipette method it is possible to study the properties of unaltered living cytoplasm, and Seifriz[27] concludes from his studies that protoplasm is a homogeneous structureless solution, probably an emulsion hydrosol, *i. e.*, a colloid in which both phases are liquid, one of them, the dispersion medium, being water. Normal cytoplasm is at all times nonmiscible in water, but readily degenerates into a condition in which it is miscible. Its viscosity varies from that of a somewhat rigid gel to nearly that of water and the structural appearance will presumably vary with changes in the proportion of water in the protoplasmic emulsion.[28]

If, with these facts in mind, we consider the theories of morphologists as to the finer structure of the cell protoplasm, which are based upon studies of cells fixed in various hardening agents, it becomes evident that the possibility is very real that the "foam structure" advocated by Bütschli, or the "thread," "reticular," and "pseudo-alveolar" structures of Fromann, Arnold, Reinke, and others, are all simply the effect of fixatives upon colloid solutions. The objection always advanced to these theories of protoplasmic structure, namely, that the structures described were at least in part artificial productions, not present in the normal living cell, and variously described and interpreted by different investigators, because each worked with a different hardening fluid or different technic, is strongly supported by these observations upon colloids.[29] This matter will receive further consideration in the next section.

THE STRUCTURE OF THE CELL IN RELATION TO ITS CHEMICAL AND PHYSICAL PROPERTIES[30]

It is obviously impossible to separate nuclei, nucleoli, cytoplasm, and cell membranes from each other (except with sperm heads) and to isolate them in quantities sufficient for analysis, and therefore we are still quite uncertain as to just the chemical differences that exist between them. That there are differences is certain, and by means of micro-chemical reactions, by comparing analyses of cells in which nucleus or cytoplasm predominate, and by studying their physico-chemical relations to one another, we have arrived at more or less tangible ideas on the question of the relation of the structural elements of the cell to its composition.

The Nucleus[31]

Although the nucleus presents, morphologically, a sharp isolation from the cytoplasm, and displays equally sharp tinctorial differences,

[27] Biol. Bull., 1918 (34), 307.

[28] See Giersberg, Arch. Entwickl. Organ., 1922 (51), 150.

[29] See de Moulin, Nederland. Tijdschr. Geneesk., 1923 (67), 1734.

[30] Reviews of the significance of cell structure for pathology are given by Benda and Ernst in Zentrlbl. allg. Path., 1914, Bd. 25, Ergänzungsheft.

[31] Earlier literature by Albrecht, "Pathologie der Zelle," Lubarsch-Ostertag, Ergeb. der. allg. Pathol., 1899 (6), 1900; see also Kossel, Münch. med. Woch., 1911 (58), 65.

it is probable that the chemical differences between nucleus and cytoplasm are quantitative rather than qualitative. Physically the nucleus, when not in mitosis, seems to be a fluid, possessing no visible structure except the nucleoli and the delicate peripheral membrane (Chambers).[32] The characteristic affinity of certain elements of the nucleus for basic stains depends upon the presence, in the nucleus, of nucleoproteins in large proportion, and to a limited degree nucleoproteins are characteristic of nuclei. Their affinity for basic dyes depends upon the nucleic acid radical.[33] For example, the heads of spermatozoa contain nucleic acid bound to simple proteins in such a way that it readily forms a salt or salt-like combination with basic dyes, and so the sperm heads appear intensely stained by alum-hematoxylin, etc. Ordinary chromatin threads of nuclei appear to contain protein somewhat more firmly bound in their nucleoprotein molecules, and hence stain less intensely than do the spermatozoa heads, except when in karyokinesis, when the chromatin nucleoprotein seems to approach that of the spermatozoa in avidity for basic dyes. We also have nucleoproteins with the nucleic acid so thoroughly saturated by protein that they do not stain at all with basic dyes, and these seem to exist principally in the cytoplasm, and also to form the ground-substance of the nuclei, occupying the spaces between the chromatin particles (this achromatic substance of the nuclei is called *linin* or *plastin* by some cytologists). Besides the chromatin and the nucleoli, there is a peculiar chromatophile substance, suspended in the finer part of the nuclear structure in the same manner as the chromatin itself is in the coarser portions; this was called *lanthanin* by Heidenhain,[34] and is probably similar to the substances also described as *parachromatin* and *paralinin*. Undoubtedly the other forms of proteins found in the cell, such as globulin, albumin, and nucleoalbumin, exist both in the nucleoplasm and in the cytoplasm, the essential difference being that the proportion of nucleoprotein is much greater in the nucleus. As nucleoproteins are little affected by peptic digestion, it is possible to isolate nuclear elements, especially the chromatin, for analytic purposes, and it has been demonstrated by this means also that nuclein is the chief constituent of the staining elements. The distribution in the nucleus of the other primary constituents of the cytoplasm, such as lecithin, cholesterol, and inorganic salts has not yet been worked out, except that Macallum[35] found that nuclei contain no chloride, as indicated by their not staining with silver nitrate, and also no potassium,[36] but the chromatin contains firmly bound iron.[37]

Nucleoli, which are not present in all nuclei, differ from the other nuclear structures in having an affinity for acid rather than for basic dyes,[38] at least in fixed tissues.

[32] "General Cytology," 1924, p. 265.
[33] See Stuedel and Osato, Zeit. physiol. Chem., 1923 (124), 227.
[34] Festschr. f. Kölliker, 1892, p. 128.
[35] Proceedings of the Royal Society, 1905 (76), 217.
[36] Jour. of Physiol., 1905 (32), 95.
[37] Apparently the nucleus contains calcium (Loew, Pharm. Monatsh., 1924 (5), 141).
[38] Nucleoli of nerve-cells are an exception, being basophilic.

Their chemical composition has not been ascertained. Zacharias considers the nucleoli as composed of nuclein well saturated with protein, because of its staining reactions and its relative insolubility in alkalies, and classes it with plastin or linin, which forms the achromatic part of the nucleus and is also present in the cytoplasm. Macallum[39] found that they reacted for organic phosphorus microchemically, but less strongly than did chromatin fibers.

The **nuclear membrane** is an uncertain structure, at times dense and staining as if formed of a layer of chromatin, in other cells staining like the cytoplasm with which it seems to be continuous, in most cells disappearing during karyokinesis, and in some protozoa being entirely absent. Naturally the composition of the nuclear membrane is unknown, but it is probable that it acts as a diffusion membrane of partially semipermeable character, maintaining different conditions in nucleus and cytoplasm.

Functionally, the nucleus is the essential element of the cell; an isolated nucleus with but a minimum of cytoplasm may be able to develop new cytoplasm, but isolated cytoplasm soon disintegrates, although it may manifest life for some time by movement and chemical activities. It has been frequently suggested that the nucleus controls oxidative processes, and there is some microchemical evidence for this.[40] Lynch[41] calls attention to the improbability that the part of the cell most removed from the oxygen should be the organ of oxidation, and finds evidence that the function of the nucleus is that of organic synthesis. An enucleated cell may move, respire, digest, respond to stimuli and exhibit any activity which is dependent solely upon catabolic or destructive processes of protoplasm. The group of phenomena which it never shows are those of growth, regeneration, and division, *i. e.*, those depending on synthetic activities.

It should be mentioned that certain cells, such as bacteria and algæ, seem to have no true nuclei, but Macallum[42] found that the forms he examined gave reactions for phosphorus and iron in a way similar to the nucleoproteins of a nucleus, suggesting that in such cells the nuclear elements are diffused through the cell rather than differentiated. To quote Wilson:[1] "The term 'nucleus' and 'cell body' should probably be regarded as only topographical expressions, denoting two differentiated areas in a common structural basis."

Because of the relative acidity of the nuclei they are electrically negative to the cytoplasm, particularly when in karyokinesis, and the chromatic elements of the nucleus can be shown to carry a negative electric charge.[43] Sperm-heads in isotonic cane-sugar solution move rapidly—2,000 microns a minute—toward the anode, when a current is passed through the solution; and leucocytes also go toward the anode under the same conditions, the rate depending upon the proportion of nucleoplasm and cytoplasm, large leucocytes sometimes even going slowly toward the cathode[44] while lymphocytes move towards the anode.[45]

[39] Proc. of the Royal Society, 1898 (63), 467.
[40] See Osterhout, Science, 1917 (46), 367.
[41] Amer. Jour. Physiol., 1919 (48), 258.
[42] "Studies from the University of Toronto," 1900.
[43] Pentamalli, Arch. Entwickl. u. Org., 1912 (34), 444; McClendon, Proc. Soc. Exp. Biol. and Med., 1910 (7), 111; Hardy, Jour. Physiol., 1913 (47), 108.
[44] R. S. Lillie, Amer. Jour. Physiol., 1903 (8), 273.
[45] See Abramson, Amer. Jour. Med. Sci., 1925 (41), 445.

The Sertoli cells of the testicle, which have a round mass of cytoplasm with a number of miniature spermatozoa heads at one side, orient themselves in the current so that the side or end containing the spermatozoa drags the mass of cytoplasm toward the positive pole.

The Cytoplasm

The cytoplasm, as before mentioned, contains all the primary cellular constituents, and also such secondary constituents as the particular cell possesses. Nucleoproteins are undoubtedly present in unknown proportions, but with the nucleic acid well saturated by proteins, and perhaps also to a large extent combined with carbohydrates to form the gluconucleoproteins. Sometimes the nucleoproteins of the cytoplasm may be partly of the unsaturated class, and show an affinity for basic stains, as in the case of the Nissl bodies of the nerve-cells, the basophilic granules of mast cells,[46] and perhaps also the cytoplasm of plasma cells. The great question concerning the cytoplasm is its structure—whether homogeneous, alveolar, areolar, fibrillar, foam-like, or granular. On a previous page have been mentioned the experiments of Hardy, which show that homogeneous solutions of protein, when fixed by the same reagents as are used in the customary fixation of histological materials, may show quite the same microscopical structures as are shown by the cytoplasm of cells. Network, foam, and alveolar structures are produced in albumin and gelatin solutions when they are hardened by bichloride of mercury, osmic acid, formalin, etc., and the same characteristic differences that are produced in cells by these different reagents are likewise produced in the hardened protein solution. Protein structures hardened under strain form radiating structures resembling centrosomes and the radiating threads seen in cells. If elder pith is saturated with protein solutions and then hardened, sectioned, and stained by the usual methods, appearances resembling closely the structure of a hardened cell may be found in the spaces of the pith—even a central, nucleus-like mass may be suspended in a network of anastomosing threads. These and many other experiments indicate that much of the work done on cell structure by studying hardened cells, cannot be considered of value in deciding the structure of living cells; nevertheless, the fact remains that many cells that can be observed while alive and uninjured under the microscope are seen to have a definite structure in the cytoplasm, *e. g.*, sea-urchin eggs, which show a characteristic alveolar structure.

A compromise view of the structure of protoplasm (and cytoplasm in particular) which takes account of what appear to be facts brought out on both sides of the question, is that while in some cells definite structural arrangements of the cytoplasm exist, in most cells the proteins are chiefly in a homogeneous solution.[27] The structures seen in fixed cells, except the mitochondria, chromatin threads, nuclear

[46] Herwerden (Arch. Zellforsch., 1913 (19), 431) found that the basophilic granules are disintegrated specifically by nuclease, supporting the view that they are nucleic acid compounds.

membrane, nucleoli, and centrosomes, are probably produced by the coagulation of the proteins, and are not present during life. When a framework does exist, it is a fair inference, by analogy with the cell membrane and the stroma of the red corpuscles, that the cell lipoids are largely responsible for its formation; and perhaps they are also responsible for the fact that fragments of protoplasm are immiscible in water.[47] This question of the presence or absence of structure in the cytoplasm is of more importance than as a mere morphological problem, for if the cytoplasm is subdivided into innumerable little chambers, each surrounded by a membrane, it is probable that processes of diffusion and conditions of osmotic pressure will be very different from what they would be if the cytoplasm were a simple homogeneous colloid solution, like a lump of semisolid gelatin or agar. In such colloidal masses diffusion and osmosis go on almost as if there were no colloids in the solvent at all, whereas most membrane structures that are found in living tissues seem to have a decidedly semipermeable character.

From what we know at the present time of intracellular physics and chemistry there is no necessity for assuming that semipermeable septa exist within the cell. All the intracellular processes with which we are familiar *could* go on without such structures. It is not necessary to assume a compartment structure to explain the possibility of different chemical reactions going on in different parts of the cell at the same time, for most of the cell reactions seem to depend on enzymes, which we know are not readily diffusible in solutions of colloids, and, therefore, might remain fixed without requiring any enclosing walls or retaining framework. Certainly, many cells are free from structural cytoplasm, for we see particles of solid matter moving about within them quite freely. In some cells the nuclei migrate about in the cell, as also do digestive and excretory vacuoles, which motion would seem to be rather destructive if the protoplasm had a structure at all permanent.

When a portion of the cytoplasm is cut free from the body of certain cells it at once forms a round drop, just as any insoluble fluid would do in another of different surface tension, and not at all as if it were bound into a fixed structure by a framework. Other cells, however, retain their form under the same conditions. The structure of even so evidently complicated a cytoplasm as that of striated muscle fibers is in doubt; a classical observation on this point is the passage of a minute worm through the substance of a muscle-cell, its progress being as unimpeded as if there were no such things as disks, bands, rods, and striæ in the cell. Many features of ameboid movement also seem to indicate that the cytoplasm follows much the same laws as a drop of fluid in a heterogeneous medium, for we can make a drop of mercury or of chloroform in water, or of oil in weak alcohol, react to various stimuli in much the same way that an ameba would. If we look upon the cytoplasm as a drop of emulsion colloid,[48] the surfaces of the particles in the emulsion furnish of themselves ade-

[47] See Seifriz, Ann. Botany, 1921 (35), 269.
[48] See Clowes, Jour. Physical Chem., 1916 (20), 407.

quate explanation of many of the phenomena of isolation of chemical reactions, etc., without lacking in harmony with the evidences of structural homogeneity. This hypothesis fits all sides of the problem and has many supporters at the present time.[49]

The question of structure in the nucleus is quite a different matter, so far as the chromatin threads and the nucleolus are concerned. In ameboid movement the nucleus seems to play a passive rôle and to be dragged about by the cytoplasm, indicating quite a high degree of rigidity. It is probable, however, that the achromatic portion between the chromatin threads and granules has much the same structure or lack of structure as the cytoplasm.

The various secretory granules, fat-droplets, pigment-granules, glycogen granules, keratin, etc., that may lie in the cytoplasm, are inconstant constituents, varying with different cells, and under varying conditions in the same cells, and lie beyond the scope of our discussion of the general composition of the cell. According to Ruzicka[50] there is contained in all cells, both in nucleus and cytoplasm, an insoluble substance which corresponds structurally to the "plastin" of the cytologists, and chemically is related to the reticulins and other albuminoids; this he looks upon as the ground substance of the cells, corresponding to the albuminoid ground substance or stroma of organized tissues.

Certain of the granulations observed in the cytoplasm of cells seem to be definite, constant structures of the living protoplasm, and these are now called *mitochondria*, which term includes many forms of granules described under various names.[51] Their solubility and staining reactions suggest that they contain phospholipins, perhaps associated with proteins. Their functional importance is indicated by the fact that usually their number varies directly with the metabolic activity of the cells, and they may be related to histogenesis.

Other histological cellular structures also permit of more or less satisfactory identification by microchemical methods, and Unna[52] especially has contributed to this field. By staining sections with dyes of varying reaction, after extracting the sections with various solvents, he has obtained evidence of the chemical nature of some of the cell structures, although it is by no means certain that the conclusions drawn will all be verified. In the nucleolus he finds a substance resembling globulin, the granuloplasm of the cell body he regards as an albumose, the spongioplasm as histone, mast cell granules as mucin or mucoid substances. Nissl bodies he holds to be albumose, although others have believed them to be nucleins.[53]

The Cell Wall[54]

The cell membrane in most animal cells is inconspicuous structurally, but in discussing osmosis it was shown that it is of the greatest biological importance. There is no direct chemical or microscopical evidence at hand showing the composition of the animal cell membrane, but by observations on its behavior when the cells are in solutions of different sorts, facts have been collected indicating that phospholipins and cholesterol, and probably allied fat-like bodies, are prominent constituents. The

[49] See Alsberg, Science, 1911 (34), 97.

[50] Arch. f. Zellforsch., 1908 (1), 587.

[51] See review by Cowdry, Amer. Jour. Anat., 1916 (19), 423; Carnegie Inst. Publ., No. 25, 1918; "General Cytology," 1924, p. 311.

[53] See Unna, Berl. klin. Woch., 1914 (51), 444; Mühlmann, Arch. mikr. Anat., 1914 (85), 361; Marui, Mitt. allg. Path. Univ. Sendai, 1922 (1), 413.

[54] See Zangger, "Ueber Membranen und Membranenfunktionen," Ergebnisse d. Physiol., 1908 (7), 99; also R. S. Lillie, "The Rôle of Membranes in Cell Processes," Popular Science Monthly, Feb., 1913.

[52] See review by Gans, Deut. med. Woch.. 1913 (39), 1944.

substances that diffuse through most cell walls are just the substances that are soluble in or dissolve these lipoids, *e. g.*, alcohol, chloroform, ether, etc., and it is probable that the anesthetic effects of many of these substances depend in some way on their fat-dissolving power and the large proportion of lipoids in nerve-cells. These observations were made first by Overton[55] and Meyer,[56] and led to the now prominent but disputed hypothesis that the permeability of cells is determined by the lipoids. Of particular interest for our purpose are Overton's observations on the effects of dyes on living cells. The best known vital stains (*i. e.*, stains that will enter the living cell without requiring or causing injury to it) are neutral red, methylene blue, toluidin blue, thionin, and safranin. If uninjured cells, *e. g.*, frog eggs, are placed in watery solutions of these dyes they soon become filled with the coloring-matter, which seems to penetrate the cell membrane quite uniformly at all points; if the dyed eggs are then placed in clear water, the stain diffuses out again, showing it to be simply absorbed, rather than chemically combined. In contrast to these stains the sulfonic acid dyes, such as indigo carmine and water-soluble indulin, nigrosin, and anilin blue, do not penetrate the living cell at all. Overton tested the solubility of dyes which are not vital stains and found them all insoluble in oils, fats, and fatty acids; but the dyes staining living cells were readily soluble in lecithin, cholesterol, "protagon," and cerebrin, the so-called cell lipoids. Furthermore, if crumbs of lecithin, "protagon," or cerebrin were placed in very dilute watery solutions of these dyes, they were found to absorb from the water the vital stains, but not the others, which indicates that stains that penetrate living cells are more soluble in lipoids than they are in water.

Many exceptions to this rule of the fat-solubility of dyes which can penetrate living cells have been found, especially by Ruhland,[57] and the universal applicability of the Overton-Meyer hypothesis has been questioned. It is at once evident that the common foodstuffs which enter the cell, such as water, sugar, amino-acids, and salts are not lipoid-soluble, hence it has been suggested that the cell membranes must have a "mosaic" structure, some of the blocks being lipoids or lipoid compounds, and others proteins without lipoids. There is, furthermore, evidence that the entire cell substance has a profound effect upon diffusion within the cell, so that it is at present impossible to say whether the osmotic phenomena of cells depend upon a cell membrane or upon the entire cell substance. Clowes[48] has advanced the plausible hypothesis that the protoplasm consists of an emulsion in which the balance between the oil-in-water and water-in-oil state is narrow, possibly varying between different parts of the same cell, or at different times in the same part of the cell, depending on the chemical and physical conditions of the environment. Emulsions of this type under experimental conditions show striking similarities to protoplasmic behavior. It may be that there are membranes or

[55] Jahrb. f. wissenschaftl. Botanik, 1900 (34), 669.
[56] Arch. f. exp. Path. u. Pharm., 1899 (42), 109.
[57] Jahrb. f. wissenschaft. Botanik, 1912 (51), 376.

surfaces within the cell, as postulated in the foam structure hypothesis of protoplasm, or that a homogeneous protoplasm develops surfaces where in contact with substances entering from the outside. However, Chambers[58] found that dyes which do not penetrate the living amoeba from outside (*e. g.*, eosin), rapidly diffuse through the cytoplasm when injected directly into the cells, but injected fatty substances do not diffuse, wherefore it seems that the surface film possesses other properties than that of the intracellular protoplasm. Possibly the cell membrane has a different electrical charge from that of the internal colloids (Heilbrunn).[59]

Many facts indicate that either the delicate external membrane of animal cells or the entire cytoplasm has the features of a semipermeable membrane, to the extent of permitting certain substances to diffuse through and not others. Had it the property of some of the artificial semipermeable membranes, of letting water pass through but holding back almost absolutely all crystalloids, the result would be the development of an enormous disproportion in the pressure between the inside and the outside of the cell. Furthermore, the exchange of nutritive material and excretion products between the blood and the cells would be impossible. But permitting some substances to pass into the cell results in their accumulation within the cell, until they are in sufficient concentration to neutralize the osmotic pressure exerted on the outside of the cell. As evidence of this elective permeability we have the fact that the proportion of certain salts within the cell is quite different from what it is in the fluids bathing them; *e. g.*, animal cells generally contain more potassium and less sodium than the fluids surrounding them. The inorganic constituents of red cells are different from those of the plasma, the corpuscles not containing any calcium at all, while the magnesium seems to enter them freely; in other words, the red corpuscle seems to be impermeable to calcium and permeable to magnesium. If the salts in a corpuscle are in smaller proportions than in the surrounding fluid, it indicates that the cell membrane is not freely permeable for them; if in greater proportion, that some constituent of the cell is holding them in combination, possibly as ion-protein compounds. Inorganic salts are probably present in the cell by virtue of both physical and chemical influences, some simply diffusing in and out, others combining with the proteins and being held chemically, while some are in excess because of the existence of a "Donnan equilibrium." (See p. 26.)

Bechhold summarizes his conception of cell walls as follows:

"Every cell at its surface possesses a membrane which is dependent upon the composition of the interior of the cell. This membrane may be visible and may have been formed through the gelatinization of the cell protoplasm at the periphery. It may, on the other hand, be so thin as to be invisible, being formed by the concentration and spreading out of such albuminous and fatty colloids as diminish the surface tension of the cell content at the interface. The cell membranes, developing as a result of

[58] "General Cytology," 1924, p. 264.
[59] Amer. Jour. Physiol., 1923 (64), 481.

the gelatinization of cell protoplasm, are at first, in youth, expansile and elastic; with increasing age these membrane colloids, depending upon their environment and upon chemical influences, or as a result of mere colloid aging phenomena, become poor in water and lose their elasticity."

The **intercellular substance** varies greatly in different tissues. In the case of the supportive tissues it is the important element, and the cells seem to exist chiefly for the purpose of forming and keeping it in repair as it is worn out. In the epithelial and secreting tissues, however, the intercellular substance is reduced to a minimum, except in so far as a cement substance is required, and the cells generally lie in almost immediate apposition. It is probable that there is a greater or less amount of cement substance, even between the most closely applied cells, and this substance seems to be related to mucin. It can generally be brought out by staining with silver nitrate, and Macallum[60] points out that this reaction is merely a micro-chemical test for chlorides, and indicates that the cement substance contains them in larger proportion than does the cytoplasm.

[60] Proceedings of the Royal Society, 1905 (76), 217.

CHAPTER II

ENZYMES

EVERY cell is constantly accomplishing an enormous number of chemical reactions of varied natures, at one and the same time; how many we do not know, but the score or more that we do know to be constantly going on in the liver cell, for example, are probably but a part of the whole. Furthermore, reactions take place between substances that show no inclination to affect each other outside the body, and proceed in directions that we find it difficult to make them take in the laboratory. Proteins are being continually broken down into urea, carbon dioxide, and water; yet to split proteins even as far as the amino-acid stage requires in the laboratory prolonged action of concentrated acids or alkalies, or superheated steam under great pressure. But all the time in the cell innumerable equally difficult changes are going on at once, within its tiny mass, always keeping the resulting heat within a fraction of a degree of constant, and the resulting products within narrow limits of concentration. We have already indicated the means used to keep the concentration of the cell products within safe limits; namely, the processes of diffusion and osmosis and their modification by the cell structure. The forces that bring about the chemical reactions reside, we say, in enzymes, although in so doing we only shift the attribute formerly conceded to the cell, to certain constituents of the cell whose nature and manner of action are equally unknown. When the only enzymes that were known were limited to those secreted from the cell, and found free in fluids, such as pepsin and trypsin, the chemical changes that went on in the cell were ascribed to its "vital acitivity." Buchner, by devising a method to crush yeast cells, and finding the expressed contents able to produce the same changes in carbohydrates that the cells themselves did, proved the existence within living cells of enzymes similar to those excreted by certain cells, and substantiated the belief of their existence that had become general before it was thus finally corroborated. Growing out from this and subsequent experiments has come a larger and larger amount of evidence that many of the chemical activities of the cells are due to the enzymes they contain, until now the point is reached where one may rightfully ask if cell life is not entirely a matter of enzyme activity. There are certain facts, however, which seem to indicate that there are some essential differences between cells and enzymes. One of the most important of these is the difference in the susceptibility to poisons of enzymes and cells.[1] Strengths of certain antiseptics that will either destroy or inhibit the action of living cells, such as alcohol, ether, salicylic acid, thymol, chloroform and toluene,

[1] See discussion by Vernon, Ergebnisse d. Physiol., 1910 (9), 234.

will harm free enzymes in solution little or not at all. This fact has been of great assistance in distinguishing between the action of enzymes and of possible contaminating bacteria in experimental work. Although this difference between enzymes and cells is characteristic, it does not finally decide that the cell actions are not enzyme actions, for it may well be that the poisons act chiefly by altering the physical conditions of the cell so that diffusion is interfered with, thus seriously interfering with the exchange of cleavage products between different parts of the cell, and checking intracellular enzyme action, which we shall see later requires free diffusion of the products for its continuance. Furthermore, it has been found that enzymes when in living cells behave as do living cells when acted on by chemicals, in that weak stimuli increase and stronger stimuli depress activity (Arndt-Schulz law), but when separated from the cells they do not follow this law.[2] At the very least we may look upon the intracellular enzymes as the most important known agents of cell metabolism, and consequently of all life manifestations, and the changes they undergo or produce in pathological conditions must be fully as fundamentally important as is their relation to physiological processes. It therefore becomes necessary for us to consider carefully—

THE NATURE OF ENZYMES AND THEIR ACTIONS[3]

Since up to the present time no ferment has been isolated in an absolutely pure condition we are entirely unfamiliar with their chemical characters, and consequently are obliged to recognize them solely by their action. As far as we know, true enzymes never occur except as the result of cell life—they are produced within the cell, and increased in amount by each new cell that is formed, and furthermore, they are present in every living cell without exception. As the same facts are equally true of the proteins it is natural to associate the enzymes with proteins, and so explain the importance of the proteins for cell life.[4] If enzymes are obtained in any of the usual ways from animal cells or secretions they are always found to give the reactions for proteins, even if repurified many times. But it is well known that whenever proteins are precipitated the other substances in the solution tend to be dragged down by the colloids, and it is possible that the enzymes are merely associated with the proteins in this way. Furthermore, enzymes are known to become so closely attached to stringy protein masses, such as fibrin and silk, that they cannot be removed by washing. Some have claimed that they have secured active preparations of pepsin and invertase that did not give protein reactions and contained very little or no ash or carbohydrate; but it has so far been impossible to secure trypsin free from protein, and diastase seems to be certainly of protein nature. Davis and Merker[5] found that the more pepsin is purified the more it approaches the character of a protein, possibly a glycoprotein, with increasing proteolytic activity.[6] The reason pepsin acts only in acid solution

[2] Hardt, Zeit. Immunität., 1924 (38), 544.

[3] It would not be profitable to discuss fully all the various theories and hypotheses that have been advanced, but the reader is referred to the following chief compilations of the entire subject: Oppenheimer, "Die Fermente und ihre Wirkungen," Leipzig; Bayliss, "The Nature of Enzyme Action," Monographs on Biochemistry, London; A. E. Taylor, "On Fermentation," Univ. of California Publications; Guggenheimer, Ergeb. inn. Med. u. Kinderhlk., 1921 (20), 281; K. G. Falk, "The Chemistry of Enzyme Actions," Amer. Chemical Society Monograph Series; 2d Ed., 1924; Euler, "Allgemeine Chemie der Enzyme."

[4] Another important point is that the closest imitations of enzymes, Bredig's "inorganic ferments" seem to owe their action to their colloidal nature.

[5] Jour. Amer. Chem. Soc., 1919 (41), 221.

[6] Bokorny (Biochem. Zeit., 1919 (100), 100) finds that the amount of formaldehyde fixed by enzymes supports the hypothesis that they are protein.

is because it is an anion, like Cl, and combines only with a positive protein ion (Northrop).[7]

Analyses of enzymes purified as completely as possible are of little value, for the "purified" enzymes are probably far from pure; however, it is of some importance that they vary greatly in the proportions of carbon, hydrogen, and nitrogen which they contain, indicating that possibly different enzymes may be of very different nature. Macallum has shown microchemically that phosphorus is closely associated with the formation of zymogen granules in cells, which seem to be started in the nucleus; and there are many other observations suggesting that certain ferments are closely related to the nucleo-proteins. This is particularly true of the oxidases, which seem also to contain iron and manganese. A final point of importance in support of the protein nature of enzymes is that pepsin destroys trypsin and diastase, while trypsin destroys pepsin.[8]

Emil Fischer's work with the sugar-splitting enzymes indicates that they owe their action to their stereochemical configuration. He prepared two sets of sugar derivatives which differed from each other solely in the arrangement of their atoms in space (*i. e.*, isomers) and found that one specific enzyme would split members of only one of the varieties, while another enzyme would act only on the variety with the opposite isomeric form. These experiments make it very probable that there must be a certain relation of geometrical structure between an enzyme and the substances it acts upon, but, as Falk says, the question may even be raised as to whether a chemical molecule, possessing a definite molecular weight and arrangement of atoms, and to which are due the enzymatic properties, is present in any enzyme preparation.

Bredig has found that *colloidal solutions of metals* have many of the properties of true enzymes, accomplishing many of the decompositions produced by enzymes, being affected by temperatures of nearly the same degree, and even being "poisoned" by substances that destroy or check enzymes.[9] The only possible explanation of these observations seems to be that the enzyme effects are brought about by *surface phenomena*. A colloidal solution of platinum, as far as is known, differs from a piece of metallic platinum solely in the enormously great amount of surface it offers in proportion to its weight, and it is well known that surfaces may affect chemical action. Hence we have the possibility that some enzyme actions, at least, may depend upon the existence of a very large surface, and since by no means all colloids are enzymes, although all enzymes seem to be colloids, that this surface must bear a certain relation in form to the surface of the body that is to be acted upon.

The Principles of Enzyme Action

The effects produced by enzymes, which at one time were considered quite unique and remarkable, have now been made comparatively plain, chiefly through the observations of Ostwald on related chemical reactions; and by the investigations of Croft Hill, Kastle and Loevenhart, and others, on enzymes, which show that enzyme action is in no way different from chemical action observed independent of enzymes. The fundamental consideration is that chemical reactions are *reversible*, that is, that their tendency is to *establish an equilibrium*, and that the change may be from either side of the equation.[10] The action of enzymes is similar to that of all catalytic agents, that is, they *increase the speed of reaction*. Such a reaction as that of NaOH and HCl is so rapid that the effect of catalyzers could hardly be noticed; but with many other substances the reaction is very slow, and without the presence of catalyzers it would go on almost or

[7] Jour. Gen. Physiol., 1920 (3), 211.

[8] Falk has obtained evidence that ester-splitting enzymes may be proteins owing their activity to the presence in the molecule of active groupings, perhaps of enol-lactim structure, —C(OH) = N—. By treating pure proteins with alkali, which favors the formation of enol-lactim groupings, the proteins were made to acquire esterase properties. (See Science, 1918 (47), 423.)

[9] See also Fischer and Hooker, J. Lab. Clin. Med., 1918 (3), 373.

[10] See Taylor, Arch. Int. Med., 1908 (2), 148.

quite imperceptibly. For example, ethyl butyrate saponifies on the addition of water according to the following equation:

$$C_2H_5-O-OC-C_3H_7 + H_2O \leftrightarrows C_2H_5OH + HOOC-C_3H_7.$$

On the other hand, if ethyl alcohol and butyric acid, the products of this reaction, are placed together, they will combine to form ethyl butyrate; in other words, the reaction is reversible, as indicated by the arrows in the equation. In any event, however, the reaction is not complete, but continues only until there exists a certain definite proportion of ethyl alcohol, butyric acid, ethyl butyrate, and water, when the change will stop, *i. e., equilibrium is established.* The time that would be required for this reaction to occur at room temperature would be extremely long, the change being hardly noticeable, but in the presence of a catalytic agent the process goes on much more rapidly. Catalytic agents, therefore, merely hasten reactions which would go on without them, and they do not initiate or change the nature of chemical reactions at all. When equilibrium is established, the reaction stops and the enzyme has nothing more to do. Furthermore, enzymes will hasten synthesis just as well as they hasten catalysis. Croft Hill first showed that maltase would synthesize glucose into maltose; Kastle and Loevenhart soon after established the synthesis of ethyl butyrate under the influence of lipase. Taylor[11] first synthesized one of the normal body fats, triolein, by the action of lipase (from the castor-oil bean) upon oleic acid and glycerol. Successful synthesis of fats by pancreatic lipase is described by Lombroso.[12] It may seem improbable at first sight that the synthesis of proteins can be accomplished by enzymes, as is the relatively very simple synthesis of carbohydrates and fats, but the improbability disappears when we recall that the products of protein cleavage are reconverted into body proteins after absorption from the intestines. Proteins manifestly are synthesized and we have not a little reason to believe that this is accomplished by enzymes, presumably by a reversal of their action in the establishment of equilibrium. Abderhalden[13] has obtained some evidence of protein formation in mixtures of amino acids derived from autolyzing tissue when acted upon by ferment-containing extracts of the same tissue. Taylor[14] was able to synthesize protamin, one of the simplest proteins, by the action of trypsin upon its cleavage products, and it has been found that the addition of proteolytic enzymes to solutions of pure albumose leads to the formation of a jelly-like, insoluble protein substance, "plastein," which seems to be the effect of a reversed action on the part of the enzymes,[15] although apparently the new-formed plastein is not the same as the original protein from which the albumose

[11] Univ. of California Publications (Pathology), 1904 (1), 33.
[12] Arch. di farmacol., 1912 (14), 429.
[13] Fermentforschung, 1914 (1), 47.
[14] Jour. Biol. Chem., 1909 (5), 381.
[15] See Micheli, Arch. ital. biol., 1906 (46), 185; Levene and Van Slyke, Biochem. Zeit, 1908 (13), 458; Taylor, Jour. Biol. Chem., 1909 (5), 399; Gay and Robertson, *ibid.* 1912 (12), 233; Abderhalden, Fermentforsch., 1914 (1), 47; v. Knaffl-Lenz and Pick, Arch. exp. Path., 1913 (71), 296, 407.

was derived.[16] However, plasteins made by different sorts of enzymes from the same albumose seem to be the same.[17]

Another well known synthesis that seems to be due to reversible ferment action is the formation of hippuric acid from benzoic acid and glycine in the kidney; the formation of glucose into glycogen and its reformation are also probably both accomplished by one and the same enzyme acting reversibly. Other reversible reactions less closely related to animal cells have also been described.

The reversible nature of enzyme action explains many problems of metabolism, and makes the whole field much clearer. The following consideration of fat metabolism on this basis may explain the manner in which chemical changes are believed to occur in the cells and fluids of the body:[18]

> In the intestines fat is split by lipase into a mixture of fat, fatty acid, and glycerol; but as the fatty acid and glycerol are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine, so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerol first enters the epithelial cells lining the intestines there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Loevenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a mixture of fat, fatty acid, and glycerol, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also appears, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerol. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to again overthrow chemical equilibrium, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, exhibit the reverse action and split the fat it has just built into fatty acid and glycerol. It is evident that these processes are all going on together, and that, as the composition of the contents of the intestines and of the blood-vessels varies, the direction of the enzyme action will also vary. In the blood-serum and in the lymphatic fluid there is also lipase, which will unite part of the fatty acid and glycerol, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.
>
> Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood-serum bathing the cells is a mixture of fat and its constituents, probably nearly in equilibrium, since lipase accompanies them. If the diffusible substances enter a cell containing lipase, *e. g.*, a liver cell, the process of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.[19]

To summarize, it may be stated that throughout the body there is constantly taking place both splitting and building of fat. Fat enters the cells, leaves them, and is utilized only in the form of its acid and alco-

[16] Hermann and Chain, Zeit. physiol. Chem., 1912 (77), 289.

[17] Hesse, Arch. f. Verdauungskr., 1923 (31), 275.

[18] See Loevenhart, Amer. Jour. of Physiol., 1902 (6), 331; Wells, Journal Amer. Med. Assoc., 1902 (38), 220. The discrepancies between the action of lipase in the tissues and *in vitro* are well explained by Taylor, Jour. Biol. Chem., 1906 (2), 103.

[19] Bradley (Jour. Biol. Chem., 1910 (8), 251; 1913 (13), 407–439) calls attention to the great concentration necessary for fat synthesis by lipase *in vitro*, and the lack of correspondence between the amount of fat and of lipase in various tissues, questioning the importance of lipase for fat synthesis in the living tissues as well as the significance of reversed enzyme reaction for biological processes in general.

hol, never as the fat itself. Fat constitutes a resting stage in its own metabolism. If proteolytic enzymes also act reversibly, then the phenomena of protein metabolism are similarly explained, for there is no doubt that every cell and body fluid contains proteolytic enzymes. All metabolism, then, may be considered as a *continuous attempt at establishment of equilibrium by enzymes, perpetuated by prevention of attainment of actual equilibrium through destruction of some of the participating substances by oxidation or other chemical processes, or by removal from the cell or entrance into it of materials which overbalance one side of the equation.*

In just what manner the enzymes accomplish their catalytic effect is yet unknown.[20] A favorite idea is that they form loose compounds with the substance to be split and with water, the resulting compound being unstable and breaking down, the water remaining attached to the components of the substance.

Enzymes do not act catalytically on all substances by any means, but show a decidedly specific nature. They affect only organic substances, and the actions are limited to two processes—hydrolysis and oxidation, or the reverse processes of dehydration and reduction.[21] The most essential difference between the enzymes and the chemicals that can accomplish hydrolysis or oxidation is this: the ordinary chemical reagents produce their effects on many sorts of substances, whereas the enzymes are specific; thus hydrochloric acid will hydrolyze starch or protein with equal facility, but pepsin will not affect starch at all.

General Properties of Enzymes.—Other properties of enzymes may be briefly mentioned. The speed of reaction they produce increases with the amount of enzymes present. Very dilute acids favor the action of nearly all ferments, and alkalies are unfavorable for all but trypsin, ptyalin, and a few others. Weak salt solutions also are more favorable than distilled water. (These facts suggest strongly the possibility that *ions* play an important rôle in the process.) Water and dilute glycerol dissolve enzymes, which always form colloidal solutions that are very slightly dialyzable; they may be precipitated from solution by alcohol, and redissolved again with but slight impairment of strength. Filtration through porcelain filters is not complete, from 10 to 25 per cent. of most enzymes being lost in each filtration, for enzymes are subject to great adsorption by surfaces, *e. g.*, charcoal, kaolin.[22] As before mentioned, many chemicals poisonous to bacteria have little influence on most enzymes, but nearly all substances when concentrated are injurious or destructive, and some enzymes are known that are more susceptible to antiseptics than are the cells that contain them. Formaldehyde is very destructive to most enzymes, even when dilute. The effect of protein-coagulating antiseptics upon enzymes is, of course, greatly modified by the amount of protein substances mingled with the enzymes; and the effects of heat and other injurious influences are greatly decreased by the presence of proteins and other impurities.

All enzymes are most active between 35° and 45° C., and it is interesting to note that Kobert found this equally true for enzymes derived from cold-blooded animals.[23] Although enzymes can stand temperatures of 100° C. or more when dry,

[20] See Euler, "Chemical Dynamics of Enzyme Reactions," Ergebnisse d. Physiol., 1910 (9), 241.

[21] Alcoholic fermentation may be an exception, the change being $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$, but it is very possibly an intramolecular oxidation.

[22] See Hedin, Ergebnisse d. Physiol., 1910 (9), 433.

[23] However, Hosaka (Mitt. med. Gesell. Tokio, 1917 (31), 1) states that frog pancreatic diastase is most active between 5° and 37°, whereas for guinea pig pancreatic diastase the optimum temperature is 27°–55°. Activity begins to be inhibited at 45° and 65° respectively.

in water they are generally destroyed somewhat below 70° C. Low temperature, even −190° C. (liquid air), does not destroy them. The loss of power through heating occurs gradually, and there is no sharp line at which their action disappears. Sunlight is harmful to enzymes in solution, but only in the presence of oxygen; this effect is augmented by the presence of fluorescent substances. Nascent oxygen is destructive to enzymes.[24] X-rays seem to have a deleterious effect upon most enzymes, and retard their rate of action,[25] but apparently, autolytic enzymes (Neuberg[26]) and tyrosinase (Willcock[27]) are not injured.[28] Ultraviolet rays are also injurious to enzymes,[29] and they can be destroyed by violent shaking (Shaklee and Meltzer[30]). Labile as enzymes are, their persistence when dry is remarkable; Kobert found active trypsin in the bodies of spiders that had been in the Nuremberg Museum for 150 years, and Sehrt[31] found that the muscle tissue of mummies contained active glycolytic ferment.

All enzymes as ordinarily prepared have the property of decomposing hydrogen peroxide, a property possessed by substances of varied nature; this effect is prevented by CNH, which does not prevent other enzyme manifestations, indicating that this property is due to an associated enzyme, *catalase*.

The retardation of enzyme action by accumulation of the products of their action is simply explained as being due to establishment of equilibrium; in some instances, however, the substances produced are of themselves harmful to the enzymes, *e. g.*, alcohol and acetic acid. Changes in *p*H, fixation of the enzyme by cleavage products, and other side reactions may also be at least partly responsible. There is a periodicity in enzyme action which makes quantitative results uncertain.[32]

Activation of Enzymes.—Within the cell, the enzymes—at least those that are excreted, such as trypsin and pepsin—exist with few exceptions in an inactive form, the *zymogen*. Their activation appears to take place normally only after they have been discharged from the cell, but after the death of an organ activation may result from the decomposition products that are formed. Under physiological conditions this activation appears to be brought about by special activating substances. In the case of the pancreas it is the *enterokinase*, which is furnished by the epithelial cells of the intestine. Enterokinase appears to unite with trypsinogen to form an active enzyme, which reminds one of the way that complement and the intermediary body unite to form hemolytic and bacteriolytic substances. *Kinases*, having the same action as enterokinase upon the trypsinogen, are found in various tissues and organs, but generally much less active than the enterokinase. It is probable that it is through this mechanism that the rate of enzyme action is modified, and perhaps it is a means of defense of the body against its own enzymes; as the prozymes are more resistant to harmful agencies than the enzymes, it also may be a method of storage. The activity of various enzymes is greatly increased by certain more or less specific substances, referred to usually as "coenzymes;" thus bile-salts act as co-enzymes for lipase (Loevenhart).

The Toxicity of Enzymes

Although present normally in greater or less amounts in all the cells in the body, when artificially isolated and injected directly into animals nearly all enzymes seem to be extremely toxic. As foreign proteins, especially extracts of tissues, are generally more or less toxic, it is difficult to state how much of the toxicity of a given enzyme-containing solution depends on the enzyme and how much on the admixt proteins. *The following statements are given at the face value placed on them by the several*

[24] See Burge, Amer. Jour. Physiol., 1914 (34), 140.
[25] See Hussey and Thompson, Jour. Gen. Physiol., 1923 (5), 647.
[26] Berl. klin. Woch., 1904 (41), 1081.
[27] Jour. of Physiol., 1906 (34), 207.
[28] Gudzent (Zeit. Strahlenther., 1914 (4), 666) denies that radium acts on enzymes.
[29] Agulhon, Ann. Inst. Pasteur, 1912 (26), 38; Burge *et al.*, Amer. Jour. Physiol., 1916 (40), 426.
[30] Amer. Jour. Physiol., 1909 (25), 81.
[31] Berl. klin. Woch., 1904 (41), 497.
[32] Groll, Nederl. Tijdschr. v. Geneesk., 1918 (1), 1085.

investigators quoted, and are subject to discount until the enzymes have been isolated and investigated in a pure condition, if such a thing should ever become possible.

The first thorough study of the toxicity of enzymes was made by Hildebrandt,[33] who found that pepsin, invertase, diastase, emulsin, myrosin, and rennin were all toxic. The symptoms produced in dogs were trembling, uneasiness, difficulty in walking, and finally coma. The anatomical changes observed were: numerous hemorrhages throughout the body, fatty degeneration of the liver and myocardium, renal congestion, and thromboses. Considerable fever results, and Mayer considers this responsible for the relative harmlessness of rennin, the action of which is impaired above 40°. That these effects are not due to contaminating bacteria is shown by Kionka and by Achalme[34] who obtained similar results with enzymes made sterile by filtration through porcelain. Wago[35] obtained also an amyloid-like degeneration widely spread in animals injected with filtered solutions of commercial trypsin, pancreatin and amylopsin. Achalme found that such sterile preparations of pancreatic juice injected subcutaneously into guinea-pigs produce a marked local pink gelatinous edema, followed by gangrene; if the animal dies, the blood is non-coagulable. Local eosinophilia has been observed.[36]

Apparently cells of nearly all types can be destroyed by *trypsin*, which may cause necrosis in one-fourth hour; however, spermatozoa and surface epithelium resist strong trypsin solutions. Intravenous injections cause death with lesions in the heart muscle and severe hemorrhages. After recovery from one injection of trypsin the animal is temporarily somewhat more resistant to another injection, and there are other resemblances to anaphylactic intoxication (Kirchheim[37]). Fiquet[38] also observed that trypsin and pepsin rendered the blood incoagulable, but after some time the coagulability of the blood is increased and thrombosis is frequent. Wells[39] found that pancreatic extracts containing very active trypsin and lipase, injected intraperitoneally, produced an acute inflammatory reaction, but no fat necrosis. Extracts containing active lipase and inactive trypsin were less toxic, but produced fat necrosis. Extracts of liver and blood serum, rich in lipase, were almost without effect on dogs and cats. Lombroso[40] found that inactive pancreatic juice was much less toxic than activated, showing that trypsin is the important toxic agent. He also found that *succus entericus* in doses of 1 to 5 c.c. is toxic, but not lethal for dogs.

Pancreatic *lipase* is hemolytic (Noguchi)[41] if activated by fats, which suggests that when this enzyme gets into the blood it may cause hemolysis.

[33] Virchow's Archiv, 1890 (121), 1.
[34] Ann. d. l'Inst. Pasteur, 1901 (15), 737.
[35] Arch. Int. Med., 1919 (23), 251.
[36] Boerner-Patzelt, Arch. exp. Path. Pharm., 1923 (99), 253.
[37] Arch. exp. Path. u. Pharm., 1911 (66), 352; 1914 (78), 99; 1913 (74), 374.
[38] Arch. d. Méd. Exper., 1899 (11), 145.
[39] Jour. Med. Research, 1903 (9), 92.
[40] Abstract in Biochem. Centralblatt, 1903 (1), 712.
[41] Biochem. Zeit., 1907 (6), 185.

Papain was found to be much more toxic than any animal enzyme, causing violent local hemorrhagic inflammation. Schepilewsky[42] also found papain much more toxic than rennin and pancreatin; repeated injection of the two latter caused amyloidosis in rabbits. Active immunity does not follow repeated injections of papain.[43]

Urease has a definite toxicity because it decomposes the urea in the blood and tissues, fatal intoxication from NH_3 poisoning resulting.[44] Hildebrandt[45] observed that enzymes were positively chemotactic, but it is probable that the products of their action on the tissues are the chief chemotactic agents.

Excretion of Enzymes.—The enzymes that are secreted into the gastro-intestinal tract seem to be chiefly destroyed, but part is eliminated in the feces, and part that is absorbed apparently reappears in the urine in very small quantities.[46] Pepsin, diastase, and rennin, all have been found in normal urine; but trypsin is present chiefly as trypsinogen, especially abundant after a meat diet.[47] Pepsin and rennin enter the urine as the zymogens, in quantities in proportion to the amount in the stomach, and are absent with gastric carcinoma (Fuld and Hirayama),[48] or with nephritis.[49] During resolution of pneumonia, leucocytic protease may appear in the urine (Bittorf[50]). Hedin[51] found in pneumonia a marked increased urinary activity on peptone and on casein at pH = 8 (trypsin and peptase) but not on casein at pH = 1.6 (pepsin).

Ferments injected subcutaneously are said seldom to be eliminated in any considerable amounts in the urine, but Opie[52] has demonstrated the presence of lipase in the urine in pancreatitis with fat necrosis, and Wago[53] found that injected trypsin is excreted rapidly and abundantly. Hildebrandt was able to prove that emulsin remained active for at least six hours after it was injected into animals subcutaneously, by its splitting amygdalin which was then injected, the CNH liberated by the cleavage of the amygdalin causing death.

Anti-enzymes

It has long been known that normal blood-serum possesses a marked resistance against the action of proteolytic enzymes, not being at all digested by dilutions of enzymes that will rapidly digest a serum that has been heated. This property seems to be shared by egg-white[54] and by

[42] Cent. f. Bakt., 1899 (25), 849.
[43] Stenitzer, Biochem. Zeit., 1908 (9), 382.
[44] See Carnot et al, Ann. Inst. Pasteur, 1921 (35), 1.
[45] Virchow's Arch., 1893 (131), 5.
[46] Falk and Kolieb, Zeit. klin. Med., 1909 (68), 156.
[47] v. Schoenborn, Zeit. f. Biol., 1910 (53), 386.
[48] Berl. klin. Woch., 1910 (47), 1062.
[49] Loeper and Baumann, Prog. Méd., 1922 (35), 205.
[50] Deut. Arch. klin. Med., 1907 (91), 212.
[51] Zeit. physiol. Chem., 1920 (112), 252.
[52] Johns Hopkins Hosp. Bull., 1902 (13), 117.
[53] Jour. Immunol., 1919 (4), 19.
[54] Sugimoto, Arch. exp. Path., 1913 (74), 14.

the tissues and organs of the body (Levene and Stookey).[55] The anti-enzyme action is easily destroyed by heat of about 70°, by the action of dilute acids, and even by prolonged standing. It is exerted not only against the secreted proteolytic enzymes, pepsin and trypsin, but also against the intracellular enzymes of various organs. It seems highly probable that the resistance of the body tissues to digestion by their own enzymes and by the enzymes of one another, depends in some way upon the presence of anti-enzymes in the cells and tissue fluids, for self-digestion of tissues is greatly impeded by serum.[56] Weiland[57] has demonstrated that certain intestinal worms contain a strong antitrypsin, to which he attributes their ability to live bathed in pancreatic juice without being digested.[58] Similar properties have been ascribed by other observers to the cells of the mucosa of the stomach[59] and intestine, and to the mucus itself.[60] The work of Bensley and Harvey[61] suggests that the absence of free acid in the gland cells and lumen is perhaps the chief protection of the stomach from pepsin. Kircheim[62] holds that the intestines are protected less by anti-enzymes than by rapid absorption and removal of the enzymes, which are really not present in any considerable excess in the intestinal contents. The anti-enzymes seem only to inhibit enzyme action, and not to destroy the enzyme itself.[63]

Dragstedt and Vaughan[64] have found that not only intestinal mucosa, but also spleen and kidney may be sewn into openings in the stomach, and suffer no injury although bathed with normal gastric juice for months. Even spleen and kidney parenchyma exposed to the gastric juice by removal of the capsule of the organ before attaching it to the stomach, continues to live with but a little surface erosion. Although these observations do not explain how the living tissues resist gastric digestion, they at least disprove all theories based on either protection by mucus, or habituation to an acid secretion. If protection depends on an anti-enzyme, this must be widespread and not specific to the digestive tract. Since protection requires an intact blood supply it seems most probable that the buffer action of the blood and the removal of excessive amounts of acid and enzymes, are the essential defensive agents.

Normal Antiproteases of Blood.—Normal anti-enzymes do not seem to be at all specific, according to v. Eisler;[65] that is, human serum is no

[55] Jour. Med. Res., 1903 (10), 217.

[56] Wells, Jour. Med. Res., 1906 (10), 149.

[57] Zeit. f. Biol., 1903 (44), 45; see also Dastre and Stassano, Compt. Rend. Soc. Biol., 1903 (55), 130 and 254; and Hamill, Jour. of Physiol., 1906 (33), 479.

[58] Burge (Jour. Parasitol., 1915 (1), 179) suggests that the protection of parasites, and perhaps of the alimentary epithelium, depends on the active oxidative properties of those tissues destroying the enzymes.

[59] See Blum and Fuld, Zeit. klin. Med., 1906 (58), 505; Langenskiöld, Skand. Arch. Physiol., 1914 (31), 1.

[60] Arch. Internat. d. physiol., 1907 (5), 297.

[61] Biological Bulletin, 1912 (23), 225.

[62] Arch. exp. Path. u. Pharm., 1912 (71), 1.

[63] Bayliss and Starling (Jour. of Physiol., 1905 (32), 129; and Meyer, Biochem. Zeit., 1909 (23), 68), oppose the view of Delezenne that the antitryptic action of the blood is due to an antikinase, and believe the antibody acts upon trypsin.

[64] Arch. of Surg., 1924 (8), 791.

[65] Ber. d. Wien. Akad., 1905 (104), 119.

more resistant to human trypsin than is pig serum—indeed, it is less so.[66] Cathcart[67] found that normal antitrypsin is associated with the "albumin fraction" of the serum, but the antitryptic activity of the serum does not vary directly with the albumin content.[68] Globulins do not possess this action, but they are not easily digested. Oppenheimer and Aron[69] consider it probable that the resistance of normal serum to trypsin digestion depends upon the configuration of the protein molecules, which perhaps, when in fresh, uninjured condition, present no suitable surfaces for attack by the ferment. Antitrypsin is found in all varieties of serum, and is destroyed by 65–70° C.[70] for one-half hour, but retains its anti-enzymatic activity after drying, and is equally effective against all sorts of proteins. The normal antitryptic activity decreases during fasting and increases during digestion (Rosenthal);[71] it is increased during pregnancy[72] and the blood of the fetus shows less than that of the mother. It is found in the urine, and in inflammatory exudates, but not in normal serous fluids, and it resists putrefaction.

Hedin attributed antitryptic action to adsorption of the enzyme by some constituent of the serum, much as charcoal inhibits tryptic digestion, but Hussey and Northrop[73] found that trypsin combines with the inhibiting agent to form an inactive but dissociable compound, in equilibrium according to the law of mass action. The antitryptic agent is not adsorbed by charcoal when free, but the trypsin-antitrypsin complex is adsorbed.[74]

As normal serum contains a tryptic enzyme as well as a substance inhibiting trypsin, the antitryptic activity is at most but a measure of the difference between these (Weil), and might depend on either lowered trypsin or increased antitrypsin content. It is now generally agreed that the active agent is not a true immune antibody.[75] Kirchheim[76] has found that the union of trypsin and antitrypsin does not follow the physico-chemical laws of a true antigen-antibody reaction. Jobling[77] and his co-workers have advanced evidence that the normal antiprotease action of serum depends on the serum lipoids,[78] which vary in activity directly with the degree of unsaturation; therefore, they were able to de-

[66] This is contradicted by Glaessner, Hofmeister's Beiträge., 1903 (4), 79.
[67] Jour. of Physiol., 1904 (31), 497; also see Kämmerer and Aubry, Biochem. Zeit., 1913 (48), 247.
[68] Beaton, Brit. Jour. Exp. Path., 1922 (5), 224.
[69] Hofmeister's Beiträge., 1903 (4), 279.
[70] Unless otherwise specified, all temperatures are given according to the Centigrade scale.
[71] Folia Serologica, 1910 (6), 285; also Franz and Jarisch, Wien. klin. Woch., 1912 (25), 1441.
[72] See Franz, Arch. f. Gyn., 1914 (102), 579.
[73] Jour. Gen. Physiol., 1923 (5), 335.
[74] Pfeiffer and Standenath, Fermentforsch., 1923 (7), 14.
[75] See Doblin, Zeit. f. Immunität., 1909 (4), 229.
[76] Arch. exp. Path., 1913 (73), 139.
[77] Series of articles in Jour. Exper. Med.; also review in Jour. Lab. and Clin. Med., 1915 (1), 172. See also Zeit. Immunität., 1914 (23), 71.
[78] Yamakawa (Jour. Exp. Med., 1918 (27), 689), however, does not believe that the anti-enzyme which prevents autolysis of serum itself is of lipoidal nature.

crease the antiferment action of serum by extracting the lipoids with fat solvents (and to restore the activity by replacing the lipoids), or by saturating the double bonds of the fatty acids with halogens,[79] or by modifying the degree of dispersion of the lipoids. Soaps of saturated fatty acids they found unable to inhibit serum protease. Other authors,[80] however, have been unable to remove the antitryptic activity of serum by extracting with fat solvents, and hence the antitrypsin cannot at this time be definitely ascribed solely to the lipoids.[81] Cohnheim, Bayliss and others have held that the antitryptic effect of serum depends solely on adsorption of the enzyme by the serum proteins, which themselves are not readily digested by trypsin, but there are difficulties in accepting this hypothesis (Beaton).[68] Zunz[82] states that normal serum acts more upon enterokinase than upon trypsin, and believes that the inhibition depends upon colloids which modify surface tension and adhere to the proteins. Red corpuscles and living unicellular organisms, including bacteria, are likewise resistant to trypsin, and normal serum also seems to contain an antirennin.[83]

Fresh and inactivated serum will prevent pepsin from digesting protein, but this is not due to a true antipepsin, according to Hamburger.[84] Nevertheless some authors have attributed gastric ulcers to a deficiency in antipepsin.[85] Normal serum does not seem to inhibit the enzymes which act upon purines. Fuld and Spiro[86] found that the natural antirennin of normal horse serum is in the pseudoglobulin fraction.

Opie[87] has found that the serum of inflammatory exudates contains an anti-enzymatic substance, destroyed at 75° and by acids; it is not present in normal cerebrospinal fluid, but appears here as in other serous cavities during inflammation (Dochez).[88] Antitrypsin has also been found in pathological urines (v. Schoenborn).[47]

The power of the blood serum to inhibit the activity of trypsin and leucocytic protease has been found to vary greatly in disease, and, as having diagnostic possibilities, this property has been considerably investigated.[89] It is especially increased in conditions associated with cell destruction, such as pneumonia and cancer, which suggests that the increased antitryptic activity results from the formation of specific antibodies for the intracellular proteases liberated during the disease,

[79] Corroborated by Slovzov and Xenophontova, Russ. Physiol. Jour., 1919 (2), 261.

[80] Rusznyák et al., Wien Arch. inn. Med., 1922 (3), 515.

[81] See Teale and Bach, Proc. Roy. Soc. Med. (Path. Sect.) 1920 (13), 5.

[82] Mem. Acad. roy. med. Belgique, 1909 (20), fasc. 5.

[83] Czapek (Ber. Deut. botan. Gesell., 1903 (21), 229) states that anti-oxidases occur normally in certain plants, strongly specific against the oxidase of the same plant species.

[84] Jour. Exper. Med., 1911 (14), 535; Arch. Int. Med., 1915 (16), 356. There seems to be no relation between the antipeptic and antitryptic powers of sera (Rubinstein, Ann. Inst. Pasteur, 1913 (27), 1074).

[85] Einstein, Med. Klin., 1924 (20), 1578.

[86] Zeit. f. physiol. Chem., 1900 (31), 132.

[87] Jour. Exp. Med., 1905 (7), 316. Review in Physiol Reviews 1922 (2), 552.

[88] Jour. Exper. Med., 1909 (11), 718.

[89] For literature and review see Wiens, Ergebnisse Physiol., 1911 (15), 1; Weil, Arch. Int. Med., 1910 (5), 109; Meyer, Folia Serologica, 1911 (7), 471.

but as yet this has not been satisfactorily established, so we do not know whether the "antitrypsin reaction" depends upon an antibody for trypsin or upon some entirely different factor. In cachexia the inhibiting effect of the serum is especially marked and it is therefore usually pronounced in cancer, but the increased inhibition is sometimes absent in cancer (10 per cent. of all cases) and often present in other conditions, so that the positive diagnostic value is slight.[90] It may also be present without cachexia and often seems to parallel the number of leucocytes in the circulating blood. Sarcoma shows it less than carcinoma, while in exophthalmic goitre and tuberculosis an antitryptic increase is said to be quite constant (Waelli).[90] In pregnancy there is usually an increase demonstrable after the fourth to sixth months, continuing until two weeks after delivery, and highest in cases of pregnancy toxemias (Ecalle).[91] Severe traumatism may also cause an increase.[92]

Attempts have been made to regulate suppurative processes by the introduction of either leucocytic proteases, or antiprotease in the form of active serum (see Wiens[89]). Whether antiprotease can be specifically developed by immunizing with leuco-protease is a matter of disagreement,[93] but no increase of antiprotease follows the enormous destruction of leucocytes caused by injecting thorium-X.[94]

Immune Antiferments.[95]—Numerous reports exist in the literature indicating that immunization with proteolytic enzymes leads to the formation of specific antiferments, but not a few investigators have been unable to confirm these positive results.

The reported work is inconclusive because: (1) The presence of inhibiting substances in varying amounts in the normal serum has not always been properly considered; (2) inadequate quantitative methods have been used for studying ferment action; (3) no proper controls have been made by immunizing with other tissue extracts not containing the enzyme under consideration; (4) the antisera usually give precipitin reactions with the proteins of the enzyme solution which may readily adsorb the enzyme independent of any specific antienzyme action; (5) in some cases the antiserum inhibits the enzyme action by altering or buffering the reaction of the solution;[96] (6) at the best, the supposed positive results have generally concerned such slight quantitative effects on the enzymes that they may readily be due to unconsidered factors; (7) several experimenters have been unable to corroborate the reputed positive results.

Similar contradictory or defective evidence is presented in respect to the production of specific antienzymes for rennin and such non-proteolytic ferments as lipase, emulsin, urease, catalase, fibrin ferment, amylase, invertin, tyrosinase and laccase or phenolase. The latest

[90] Mitt. Grenz. Med. u. Chir., 1912 (25), 184.
[91] Arch. Mens. Obst. Gyn., 1917 (6), 97.
[92] Zunz and Govaerts, Compt. Rend. Soc. Biol., 1918 (81), 146.
[93] See Bradley, Jour. Hyg., 1910 (12), 209.
[94] G. Rosenow and Färber, Zeit. exp. Med., 1914 (3), 377.
[95] Bibliography given in Wells, Chemical Aspects of Immunity, 1924, p. 45.
[96] See also Lorber, Biochem. Zeit., 1924 (148), 49.

investigation of the antigenic activity of these enzymes by Abderhalden and Wertheimer[97] gave entirely negative results.[98]

Resemblances of Enzymes and Toxins.—It is evident from the foregoing, that despite many points of resemblance, the enzymes are quite different from the toxins in respect to their antigenic capacity, since this is certainly very slight if not altogether lacking in all the preparations of enzymes so far investigated. The chief points of resemblance are the following: According to some experiments, the enzymes behave much as if they possessed a haptophore and a toxophore group, the former of which combines with the substance that is to be acted upon; and immunity appears to be produced by the development of receptors that combine the haptophore groups, these receptors constituting the antiferments. The oxidizing enzymes especially, with their complex relationship of substrate, combining body (peroxides) and enzyme, present striking analogies to immune reactions (Moore),[99] and the proteolytic substances of the blood resemble the lysins in certain respects (Dick).[1] Enzymes and toxins also resemble one another in being readily adsorbed by membranes, precipitates, and highly developed surfaces in general.[2] Finally, there is much reason to believe that the hemolytic toxin of cobra venom is a lipase, which acts by splitting lecithin into hemolytic substances (Coca).[3]

THE INTRACELLULAR ENZYMES[4]

With few exceptions the familiar enzymes of the digestive fluids are without influence in pathological processes, since they seem to be but little absorbed, and rarely enter the blood or tissues in any other way. But with the intracellular enzymes, many of which are present in every cell,[5] the relation to pathology is very intimate. These intracellular enzymes, as we now know them, and their chief properties, are as follows:

OXIDIZING ENZYMES[6]

Although oxidation of organic compounds is the chief source of energy in the animal body, yet the way in which it is accomplished is very little

[97] Fermentforsch., 1922 (6), 286.

[98] Lüers and Albrecht (Fermentforsch., 1924 (8), 52) believe they have produced a true antiamylase.

[99] Biochem. Jour., 1909 (4), 165.

[1] Jour. Infect. Dis., 1911 (9), 282.

[2] See Porter, Quart. Jour. Exp. Physiol., 1910 (3), 375.

[3] Jour. Infect. Dis., 1915 (17), 351.

[4] See Vernon, Ergebnisse d. Physiol., 1910 (9), 138; also his monograph, "Intracellular Enzymes," London, 1908.

[5] Herlitzka (Arch. ital. biol., 1907 (48), 119) and others have shown that the different enzymes appear one by one in the development of the ovum. Their activity is modified considerably by infections (Sieber, Biochem. Zeit., 1911 (32), 108) and other diseases (Grossmann, *ibid.*, 1912 (41), 181).

[6] Complete bibliography and discussion by Kastle, Bull. Hygienic Lab., No. 59; by Loele, Ergeb. allg. Path., 1912 (16, Pt. 2), 760; and by Battelli and Stern, Ergebnisse d. Physiol., 1912 (12), 96. Concerning the chemistry of vital oxidations see Dakin, "Oxidations and Reductions in the Animal Body," Monographs on Biochemistry, London. Review by v. Fürth, "Chemistry of Metabolism," Chaps. 22 and 23; translated by A. J. Smith, Philadelphia, 1916.

understood. We only know that it is brought about within the cells, and that substances that outside the body are oxidized with difficulty, are completely oxidized to carbon dioxide and water within the cells, and that this is done with just such a degree of rapidity that the heat produced is in exactly the amount necessary for the wants of the body. There can be little question that this oxidation is accomplished through catalytic agents acting within the cells, and certain of them have been obtained in a condition permitting of study. As yet their exact relations to intracellular oxidation are not clearly defined, but for the present they may be grouped provisionally as oxidizing enzymes. That some of them are highly specific is shown by those disorders, such as alkaptonuria and diabetes, in which the body loses the power to oxidize a certain chemical substance while retaining the normal power to oxidize innumerable other substances. Of the oxidizing "enzymes" as yet identified none can be considered as of importance in the energy-producing oxidations of the body (Battelli and Stern),[6] all the enzymes of this class yet known being apparently concerned with less essential oxidizing processes; it is indeed possible that the essential oxidation of food-stuffs may not be dependent on enzymes.

According to Lillie[7] the oxidative processes in cells take place most actively in relation to the membrane surfaces (or phase boundaries) of the cells. The studies of Meyerhof[8] and Warburg indicate that intracellular respiration occurs on the structural surfaces of heterogeneous systems containing iron, *i. e.*, by surface catalysis. It is also possible that substances capable of taking up H from water may be responsible for oxidation, because of the coincident setting free of available oxygen,[9] and these substances with such an "oxidoreductase" effect may not be enzymes but relatively simple compounds.[10]

Closely related to the oxidizing enzymes is—

Catalase.[11]—It has long been known that most enzymes possess the power of decomposing hydrogen peroxide, with liberation of oxygen; but it was not until 1901 that it was finally demonstrated by Loew that this property was due to a separate enzyme and was independent of the specific properties of the various other enzymes. This ferment is very wide-spread, and so is generally obtained along with the other enzymes when attempts are made to isolate them from the cell. It was named *catalase* by Loew, and he described two forms, *α-catalase*, which was thought to be a nucleoprotein,[12] and *β-catalase*, which has the properties of an albumose. It has been demonstrated by Bach and Chodat that peroxides are contained in plant cells, and they also occur in animal cells. According to Golodetz and Unna[13] the catalases are held in the cytoplasm

[7] Jour. Biol. Chem., 1913 (15), 237.
[8] Lancet, 1923, Feb. 17, p. 323.
[9] See Dakin, Physiol. Rev., 1921 (1), 394; and "Oxidations and Reductions in the Animal Body," Biochemical Monograph Series.
[10] See Hopkins, Johns Hopkins Hosp. Bull., 1921 (32), 321; Lancet, June 23, 1923, p. 1251.
[11] Review by Morgulis, Ergeb. Physiol., 1924 (23), 308.
[12] Not corroborated by Waentig and Gierisch, Fermentforsch., 1914 (1), 165.
[13] Berl. klin. Woch., 1912 (49), 1134.

of the cells while the peroxidases are in the nucleus. Just what function the catalase performs is at present merely a matter of speculation, but that it serves an important purpose is maintained by Burge[14] who claims the amount of catalase in tissues varies directly with their activity. Becht[15] and others question the validity of the evidence so far brought forward in support of the hypothesis that catalase is essentially responsible for tissue oxidation.[16] In favor of the view that catalase does play a rôle in cellular oxidation is the fact pointed out by Kluyver[17] that bacteria generally contain catalase except when they nourish themselves exclusively by fermentation, strict anaerobes being devoid of catalase.

Loew considers that it destroys peroxides formed in metabolism, which are very poisonous to cell life. Shaffer has found evidence that under the influence of catalase the oxygen liberated is in the molecular form, O_2, and therefore relatively inert; whereas when peroxides spontaneously decompose, they liberate atomic oxygen which is an active oxidizing agent. He found that uric acid is oxidized by H_2O_2, but when catalase is present, this oxidation is prevented. According to this the function of catalase is rather to prevent dangerous forms of oxidation than to help in normal oxidative processes, and it is known that bacteria which produce catalase are more resistant to peroxides.

Occurrence of Catalase under Normal and Pathological Conditions.[18]—Battelli any Stern found that the catalytic power of the tissues endures many hours after death. Its abundance is different for different organs of the same animal, but remarkably constant for the same organ in the same species. In general the order in decreasing strength is: liver, kidney, blood, spleen, gastro-intestinal mucosa, salivary glands, lung, pancreas, testicle, heart, muscle, brain; but this order varies in different species. In the erythrocytes the catalase seems to be attached to the hemoglobin rather than to the stroma. Catalase is abundant even in the early embryo (Mendel and Leavenworth) and in sea urchin eggs it increases rapidly after they are fertilized (Lyon).[19] Leucocytes contain little, most of that in the blood being in the stroma of the red blood-corpuscles. The body fluids contain little or none. Injected intravenously, catalase (of the liver) is destroyed rapidly, and does not appear in the urine; it does not cause any toxic effects, nor does it increase resistance to poisoning by venoms. The tissues also contain *anti-catalases*, and still further a substance which protects the catalase from the anti-catalase; this protective substance is called the *philocatalase* by Battelli and Stern. Injection of animals with catalase solutions does not cause the formation of specific anticatalase (Burnett and Schmidt).[20]

The gas evolved by the action of pus on H_2O_2 was found by Marshall[21] to be pure oxygen, each c.c. of a certain sample of pus examined liberating 133.9 c.c. of gas. The active constituent of pus, he states, is contained in the serum and not in the corpuscles. Catalase is abundant in the tissues of lower animal forms, *e. g.*, *Ascaris*.[22] Substances decomposing H_2O_2 have been found also in bacterial cultures (See p. 107.) Loewenstein[23] found that the addition of H_2O_2 to a diphtheria toxin-anti-

[14] Amer. Jour. Physiol., 1916 (41), 153; 1917 (42), 373; 1919 (48), 133. See also Alvarez and Starkweather, *ibid.;* 1918 (47), 60; Dutcher, Jour. Biol. Chem., 1918 (36), 63.

[15] Amer. Jour. Physiol., 1919 (48), 171.

[16] See also Stehle, Jour. Biol. Chem., 1919 (39), 403; Dakin, loc. cit.[9]

[17] Zeit. physiol. Chem., 1924 (183), 100.

[18] Concerning the catalase of lower animals see Zieger, Biochem. Zeit., 1915 (69), 39.

[19] Amer. Jour. Physiol., 1909 (25), 199.

[20] Jour. Immunol., 1921 (6), 255.

[21] Univ. of Penn. Med. Bull., 1902 (15), 366.

[22] Magath, Jour. Biol. Chem. 1918 (33), 395.

[23] Wien. klin. Woch., 1903 (16), 1393.

toxin mixture destroyed the toxin, leaving the antitoxin free. A similar destruction of tetanus toxin by peroxides, first demonstrated by Sieber, can occur without the catalase.

Winternitz[24] and his associates, found that all tissues have reduced catalase activity in chronic nephritis, in proportion to the severity of the condition, and experimental nephritis in animals has the same effect; the blood shows great reduction in catalase in uremia, and less reduction with less severe nephritic manifestations. Eclampsia shows little or much reduction of catalase in the blood in proportion to the amount of renal involvement; normal pregnancy and labor have no effect. Cardiac disease has no effect if the kidneys are normal. Acute peritonitis causes a rise in blood catalase; diabetes, leukemia and jaundice are without effect. In hyperthyreosis the catalase tends to increase, in hypothyreosis to decrease; complete removal of the thyroid causes a decrease which disappears on feeding thyroid. Intravenous injection of salts, acids and alkalies decreases the catalytic activity of the blood. They found all anemias associated with a decrease in catalase, but numerous others report that in pernicious anemia the catalase content of the blood is high in proportion to the number of red cells.[25] In shock, blood catalase is decreased.[26] Normal individuals show considerable variations in the catalase activity of the blood, but for each individual it is remarkably constant; age has very little influence, but catalase is decreased in cachexia.[27]

In the tissues *post mortem* change causes but slight reduction in catalase. Extirpation of large amounts of kidney or liver tissue has little effect, but removal of the spleen, ovaries, or testicles causes a transient decrease in the catalase of the blood. In diabetes and starvation, tissue catalase is said to be decreased.[28] If the red corpuscles are prevented from laking, the catalase activity manifested by the blood *in vitro* is reduced (Strauss)[29] and iodides increase the catalase activity of the blood. Normal spinal fluid contains no catalase, but in meningitis the activity parallels the amount of cells or coagulum.[30] Catalase and anticatalase have been found in pathological urine, in both acute and chronic nephritis (Primavera).[31] Kahn and Brim[32] also found traces of catalase in normal urine, greatly increased in urine containing blood, bile, or acetone, normal in cancer, high in diabetic acidosis, Hodgkin's disease, septic infections and typhoid. Grossman[33] found that bacterial poisons generally increase the catalase content of the various viscera, and Rosenthal[34] observed a great decrease in the liver and blood of mice receiving intraperitoneal inoculations of cancer. The catalase activity of the non-cancerous organs of cancer patients is not affected, except slightly lowered by cachexia (Colwell),[35] however, the liver tissue between cancer nodules may show less catalase than normal liver.[36] In phosphorus poisoning the catalase content of the liver, heart and blood is decreased (Burge).[37]

But it is to be borne in mind that *the questionable accuracy of our existing methods*[38] *of determining quantitatively the amount or activity of catalase in tissues makes the foregoing statements of uncertain value.* Very possibly what is being measured is not the catalase activity but the amount of acceleration or retardation of catalase action by other substances in the tissue extract.[39]

True Oxidizing Enzymes.—While it is by no means certain that catalase is active in causing intracellular oxidations, there are other enzymes

[24] Review in Arch. Int. Med., 1911 (7), 624.
[25] Strauss and Rammelt, Biochem. Zeit., 1921 (122), 137; Nissen, Zeit. klin. Med., 1921 (92), 1; Segall and Händel, Deut. Arch. klin. Med., 1922 (138), 243.
[26] Burge and Neill, Amer. Jour. Physiol., 1918 (45), 286).
[27] Magat, Zeit. ges. exp. Med., 1924 (42), 95.
[28] Burge, Science, 1918 (47), 347.
[29] Bull. Johns Hopkins Hosp., 1912 (23), 120.
[30] Levinson and Becht, Jour. Amer. Med. Assoc., 1920 (74), 1310.
[31] Riforma Med., 1906 (12), 1266.
[32] Amer. Jour. Obst., 1915 (71), 39.
[33] Biochem, Zeit., 1912 (41), 181.
[34] Deut. med. Woch., 1912 (38), 2270.
[35] Arch. Middlesex Hosp., 1910 (19), 64.
[36] Blumenthal and Brahn, Zeit. Krebsforsch., 1910 (8), 436.
[37] Amer. Jour. Physiol., 1917 (43), 545.
[38] See Morgulis, Jour. Biol. Chem., 1921 (47), 341.
[39] See Steppuhn and Timofejewa, Biochem. Zeit., 1923 (136), 213.

or enzyme-like substances that come more properly under the head of oxidases or oxidizing enzymes. Battelli and Stern[6] contend that the only real oxidases which have been completely established are: 1. Polyphenoloxidases[40] (oxidizing phenols and their amino compounds, but not tyrosine); 2. Tyrosinase; 3. Alcohol oxidase; 4. Xanthine oxidase; 5. Uricase. Chodat and Bach believe that the enzymes which are designated above as polyphenoloxidases have a complex structure, consisting of peroxidase and oxygenase.[41] Mathews[42] holds that "under the term oxidases there have been confused two classes of substances, one which activates the oxygen; the other the more important class, which activates, by dissociation, the reducing substances. The latter are specific, the former not." This view has received support by Bach.

Peroxidase.—This name is given to an enzyme that is believed to cause oxidation by activating peroxides, and is quite distinct from catalase and from the other oxidases. The peroxide on which it chiefly acts in the cell is supposed to be the so-called "oxygenase."

Oxygenase.—This can also act as an oxidizer independent of the peroxidase, in the presence of certain manganese compounds. Loevenhart and Kastle question the true enzyme nature of this and other "oxidases," which they look upon as organic peroxides, behaving like other peroxides rather than as catalyzers. For example, in plant tissues it has been found[43] that catechol, or substances containing the catechol grouping, may function as the oxygenase. Practically the existence of "oxygenases" is demonstrated by their power to turn tincture of guaiac blue, and they are, therefore, present in pus as shown by the guaiac reaction.

Von Fürth[6] sums up the situation in these words: "In the tissues active catalytic agents, the peroxidases, are widely distributed, and seem, just like the coloring matter of the blood, to be capable of conveying the oxygen from peroxides to very readily oxidizable substances. We find, too, in the statements bearing upon the oxygenases, the aldehydases, and indophenoloxidases, occasion for assuming that there are substances in the tissues charged with oxygen which are able to give this off to easily oxidizable matter; and these we may in a measure regard as peroxides. But that is all. We do not know whether the peroxidases are ferments or not."

By their conception of oxygenase and peroxidase Chodat and Bach would displace entirely the idea of enzymes oxidizing directly, the true "oxidases," which they consider mixtures of oxygenase and peroxidase. There have been, in any event, a number of ferments described that seem to possess distinct oxidative powers. As each is quite specific in its action, oxidizing but one substance, or one group of related substances, they are generally designated by the name of the substances upon which they act. Most studied of these are aldehydase and tyrosinase.

Aldehydase,[44] which is characterized by oxidizing aldehydes, particularly salicyl-aldehyde. According to Jacquet, this enzyme is so intimately bound with the cell that it cannot be obtained in extracts until after the cells are dead, but is present in expressed cell-juices. It can be isolated by the usual methods, is destroyed by boiling, acts best when no free oxygen is present, and its action is inhibited by CNH. It has been demonstrated in nearly all organs and tissues except pancreas, muscle. marrow, and mammary gland; it is present in the blood in small amounts, but not at all in the bile. It is most abundant in the liver[45] and spleen, and is present in pig embryos, 9 cm. long, but not in those 2–3 cm. long. In cancerous livers the tumor nodules cannot oxidize salicylaldehyde, and the liver tissue itself shows a reduced activity (Brahn).[46] Jacoby has obtained a body with the properties of aldehydase

[40] Full review on the phenol-oxidizing agents of cells given by Loele, Folia Hematol., 1920 (25), 190.

[41] See also Onslow, Biochem. Jour. 1919 (13), 1.

[42] Jour. Biol. Chem., 1909 (6), 1.

[43] Onslow, Biochem. Jour., 1920 (14), 535.

[44] Battelli and Stern do not include aldehydase among the oxidizing enzymes, on the ground that its action is not oxidative but hydrolytic.

[45] Battelli and Stern, Biochem. Zeit., 1910 (29), 130.

[46] Zeit. Krebsforsch., 1920 (17), 417.

which did not give protein reactions. It is a true enzyme, since it oxidizes aldehydes without itself being used up. Its range of action is limited, for Jacoby found it without effect upon acetic acid and stearic acid.

Tyrosinase.—This enzyme, which is found both in animal and plant tissues, is particularly interesting in relation to the formation of pigments. Bertrand found that the transformation of the juice of lac-yielding plants into the black lacquer was brought about by the action of an oxidizing ferment, *laccase*, upon an easily oxidized substance, *laccol*, which is a member of the aromatic series. He later found in a number of plants an enzyme acting on tyrosine, distinct from the laccase, which he named *tyrosinase*. Biederman later found tyrosinase in the intestinal fluid of meal worms. v. Fürth and Schneider found a similar enzyme in the hemolymph of insects and arthropods, which explains its darkening when exposed to air. This enzyme, as obtained from different sources, is not always specific for tyrosine,frequently oxidizing other substances. As yet the chemical processes and end results of the oxidation of tyrosine by tyrosinase are unknown. Bach[47] obtained evidence that tyrosinase is not a specific oxidizing enzyme, but consists of an aminoacidase, which disintegrates the tyrosine and makes it susceptible to the action of phenolase which is the oxidizing agent. Onslow[48] extends this hypothesis and regards tyrosinase as a mixture of (a) a reductase or deaminase; (b) a carboxylase; and (c), an oxidase consisting of oxygenase, aromatic substances and peroxidase. v. Fürth and Schneider found the product of oxidation of tyrosine by animal tyrosinase related to certain of the *melanins* of animal tissues, and believe that tyrosinase is responsible for the production of many normal pigments.[49] In the ink-sacs of the squid, which eject an inky fluid containing melanin-like pigment, tyrosinase was also found, corroborating this hypothesis, and it is probable that tyrosinase in the skins of animals is responsible for their pigmentation.[50] Bacteria also contain tyrosinase,[51] and this or similar enzymes seem to be present in melano-sarcomas.[52]

Gonnermann[53] found that tyrosinase from beet-root produced *homogentisic acid* by acting on tyrosine, which is of interest in connection with the congenital hereditary disease, *alkaptonuria* (*q. v.*), in which the urine becomes dark upon exposure because of the presence of homogentisic acid. The action of tyrosinase upon the aromatic radicals of proteins is of great importance in the study of both physiological and pathological pigment formation, and hence has received extensive study, which will be found fully described in the monograph by Kastle (*loc. cit.*)[6] and under the appropriate subjects in subsequent chapters.

Other Oxidizing Enzymes.—Of the great number of other less studied oxidizing enzymes little can be definitely stated. Some consider that they are largely different manifestations of the action of one oxidizing ferment, but against this view Jacoby mentions that they occur distributed unequally in different organs, can be separated from each other, and they cause different reactions. For the catalase and for laccase (which produces the Japanese lacquer by an oxidizing process) and perhaps for other oxidizing ferments, iron and manganese may be essential constituents. Of particular significance for pathology are the enzymes which accomplish the oxidation of purines to uric acid and the subsequent destruction of uric acid. These are discussed in Chapter XXV. Also the enzymatic oxidation and reduction of β-oxybutyric acid and aceto-acetic acid in the liver, as studied by Dakin and Wakeman,[54] are of great importance in acidosis (*q. v.*).

Reducing enzymes have not yet been satisfactorily demonstrated. It is possible that they do not exist, and that the intracellular reductions that are carried on within the cells are brought about by simple chemical reactions independent of catalysis. The best known intracellular reduction is that of methylene blue,[55] which

[47] Biochem. Zeit., 1914 (60), 221; see also Haehn, ibid., 1920 (105), 169.

[48] Biochem. Jour., 1923 (17), 216.

[49] Bloch (Zeit. physiol. Chem., 1917 (100), 226) describes under the name *dopa-oxidase* an enzyme present in the protoplasm of the basal epidermal and hair follicle cells, which act specifically on 3, 4—dihydroxyphenylalanine, causing oxidation and condensation with formation of a dark brown or black pigment.

[50] Meirowsky, Cent. f. Path., 1909 (20), 301.

[51] Stapp, Biochem. Zeit., 1923 (141), 42.

[52] Alsberg, Jour. Med. Res., 1907 (16), 117; Neuberg, Virchow's Archiv., 1908 (192), 514; Gessard, Compt. Rend. Soc. Biol., 1902 (54), 1305.

[53] Pflüger's Arch., 1900 (82), 289.

[54] Jour. Amer. Med. Assoc., 1910 (54), 1441.

[55] See Thunberg, Skand. Arch. Physiol., 1917 (35), 163; Rosling, *ibid.*, 1924 (45), 132.

can be readily studied experimentally because the blue color disappears on reduction of the dye. It is not now considered that this particular reduction is due to a reducing enzyme. According to Ricketts[56] the reduction depends upon two bodies, one thermostabile, the other thermolabile, recalling the reaction of complement and amboceptor. Strassner[57] found evidence that the SH groups of the tissues are responsible for the reduction of methylene blue; their activity is impaired by heating, but a thermostable element of tissues augments the reducing activity of SH compounds, thus corroborating and explaining the observations of Ricketts. The importance of the SH groups is emphasized by Hopkins,[58] who has isolated from tissues a dipeptid of cysteine and glutamic acid, called *glutathione*, which reduces methylene blue, and by taking on a hydrogen ion presumably renders oxygen available for oxidative processes, thus becoming an agent of possible fundamental importance in cell metabolism.[59]

An enzyme has been found in the liver, muscle and kidney which transforms acetoacetic acid into l-β-oxybutyric acid, and called *ketoreductase* (Friedmann and Maase).[60]

Oxidizing "Enzymes" in Pathological Processes.—These agents may play an important part in pathological conditions, but satisfactory methods for their quantitative study are lacking. Using aldehyde oxidation as an index, Jacoby found that they did not disappear from the degenerated liver in phosphorus poisoning or in diabetes, or when the liver undergoes self-digestion, which speaks against Spitzer's contention that oxidase is a nucleoprotein.[61] Schlesinger[62] found that it is less in amount in livers of children dead from gastro-intestinal diseases than in normal livers, as also did Brüning.[63] I am inclined to believe that fatty metamorphosis, when brought about by poisons, is often due to inhibition of oxidation (*v.* fatty metamorphosis), although I found that livers exhibiting most profound fatty degeneration showed no evident impairment of their power to oxidize xanthine and uric acid.[64] Buxton[65] failed to find in *tumors* any enzyme giving the guaiac test alone, but found enzymes that did so in the presence of H_2O_2 (peroxidases). Catalase was present, but no very positive reactions for oxidizing enzymes were obtained by the indo-phenol reaction, the hydrochinon reaction, or with tyrosine for tyrosinase. Fürth and Jerusalem[66] have found evidence that the melanin of melanotic tumors of horses is produced by tyrosinase. Peroxidase has been demonstrated in the granules of pus cells (Fischel).[67]

Meyer[68] found that leucocytes, whether from pus or from leukemic or pneumonic blood, contained a substance oxidizing guaiac directly, without the presence of H_2O_2, which is not liberated until the cells are destroyed. By microchemical reactions oxidases have been found present in the

[56] Jour. of Infectious Diseases, 1904 (1), 590.
[57] Biochem. Zeit., 1910 (29), 295.
[58] See Lancet, June 23, 1923, p. 1251 for a review of reduction processes.
[59] Hopkins, Biochem. Jour., 1921 (15), 286.
[60] Biochem. Zeit., 1912 (27), 474; 1913 (55), 458.
[61] Duccheschi and Almagia (Arch. ital. Biol., 1903 (39), 29) also found the aldehydase in livers of phosphorus poisoning usually no less abundant than in normal livers.
[62] Hofmeister's Beitr., 1903 (4), 87.
[63] Monat. f. Kinderheilk., 1903 (2), 129.
[64] Jour. Exper. Med., 1910 (12), 607.
[65] Jour. Med. Research, 1903 (9), 356.
[66] Hofmeister's Beitr., 1907 (10), 131.
[67] Wien. klin. Woch., 1910 (23), 1557.
[68] Münch. med. Woch., 1903 (50), 1489.

myelocytes and nucleated erythrocytes in leukemia, being absent from the polynuclear cells.[69] The observation of Natalie Sieber[70] that oxidases of the blood and of vegetable origin destroy diphtheria toxin rapidly, and also tetanus toxin and ricin, has been confirmed by Loewenstein[23] as far as destruction by peroxide, with or without the presence of catalase, is concerned. Oxidation is undoubtedly an important process in defending the body against other forms of poisons, and Battelli and Stern consider that all the oxidizing enzymes so far definitely identified are concerned only in protective processes. Schmidt[71] has found that liver extracts render certain morphine derivatives non-poisonous by oxidation. Oxalic acid and poisonous fatty acids are also oxidized into harmless substances; phosphorus and sulphur are oxidized into their acids, which are then neutralized. Indole and skatole are oxidized into less harmful substances.

The Indophenol Reaction.[72]—Alpha-naphthol and dimethyl-para-phenylendiamine, when brought together in alkaline solution, become oxidized in the presence of air and form an insoluble blue dye, indophenol. This reaction is greatly accelerated by oxidizing agents, and it has been found that certain tissues possess this property, hence the indophenol synthesis has been used for microchemical study of the presence and distribution of oxidizing enzymes in cells. As the intracellular agent which causes this reaction is, however, so resistant to heat and chemicals that it can be demonstrated in sections fixed in formalin and prepared by the ordinary paraffin imbedding method (Dunn), there is room for much doubt as to whether it represents a true enzyme, although it has been considered identical with phenolase.[73] In the presence of small amounts of peroxide the granules of leucocytes and myelocytes are stained with alphanaphthol alone, which Graham interprets as oxidation by an enzyme of the peroxidase type. By using α-naphthol and paraphenylenediamine and staining for long periods, Menten[74] has obtained positive reactions in all tissues, and has observed a similar effect with cholesterol esters. Oxidation always depends on the acquisition by the oxidized body of positive electrical charges, and the reaction is due fundamentally to a transfer of these charges from the cellular constituents to the dissociated particles of phenol which they have taken up. The evidence obtained indicates that the oxidation is not determined by enzymes but apparently is dependent on adsorption phenomena taking place on intracellular surfaces.

Loele[75] says that the oxidases are of two sorts, labile and stable. The former behave as protective colloids against certain injurious agents, and are of unknown nature. The stable oxidases, at least those giving the phenol reaction which is characterized by the coloring of an alkaline α-naphthol solution violet to black, are amines, probably cyclic aldehydes, which he calls aldamines. These aldamines are found especially in cells serving a protective function, and may be divided into the following groups:

I. Primary aldamines; (a) giving positive naphthol reaction without formalin fixation; (b) giving positive reactions only after formalin fixation.

II. Secondary aldamines; (a) giving granule reactions; (b) giving nucleoli reactions. These are manifested only in tissues that have first been treated with extracts containing primary aldamines.

The nature of the granules that exhibit the stain is unknown, but as indophenol blue is a good fat stain it is probable that the stained granules are lipoidal, and it

[69] Fiessinger and Roudowska, Arch. de med. exper., 1912 (24), 585.
[70] Zeit. physiol. Chem., 1901 (32), 573.
[71] Dissertation, Heidelberg, 1901.
[72] Literature given by Dunn, Jour. Path. and Bact., 1910 (15), 20; Gräff, Frankfürter Zeit. f. Path., 1912 (12), 358; Rosenthal, Arch. Int. Med., 1917 (20), 185. Loele, "Die Phenolreaktion und ihre Bedeutung," Klinkhardt, Leipzig.
[73] Bach and Maryanovitsch, Biochem. Zeit., 1912 (42), 417.
[74] Jour. Med. Res., 1919 (40), 433; Brit. Jour. Exp. Path., 1920 (1), 225.
[75] Cent. f. Path., 1920 (30), 614.

may well be that they are not the site of the oxidative action, but merely selectively stained lipoids in cells capable of forming the indophenol blue. When animals are exposed to *x*-rays the stable granules are destroyed sooner than the labile granules. The relation of the granules to other cell granules is undetermined, and their distribution is not identical with the granules that take the vital stains. Katsunuma[76] considers that they are probably not permanent specific structures, but transitional alterations produced in functional activity of the protoplasm. Gräff[77] believes the indophenol blue reaction to depend on the presence of catalytically active iron and to be a real index of the vital oxidative capacity of the cells.

The indophenol reaction is observed best in the granules of neutrophile leucocytes of blood and in myeloid cells of bone marrow, leukemic blood and fetal organs; eosinophiles and basophile leucocytes also give reactions, but not lymphocytes, platelets, megakarocytes, plasma cells, mature erythrocytes, or most fixt tissue cells,[78] except certain large mononuclear cells of the blood and splenic pulp.[79] By using alkali-free, unfixt tissues Gierke found granules present in tissue cells generally, and Gräff[72] states that they occur in proportion to the metabolic activity of the cells; they are abundant in carcinomas, scanty in sarcoma and connective tissue growths generally, are not destroyed in cloudy swelling or fatty changes, but disappear in infarcts and autolyzing tissues, and in tissues asphyxiated with illuminating gas.[80] Lung tissue is especially poor in this form of oxidative activity,[81] but giant cells of tubercles contain oxidase granules.[82] During experimental pneumococcus septicemia the indophenol oxidase reaction is decreased in the tissues.[83] It is said to increase with immunization.[84]

Glucolytic Enzymes.[85]—The oxidation of sugar by the tissues, which is one of the chief sources of energy in the animal body, presumably takes place through several steps. Of these, it was once believed by some that the first is the formation of glucuronic acid—

$$\underset{\text{(glucose)}}{CH_2OH-(CHOH)_4\overset{\overset{O}{\|}}{C}-H} + O_2 = \underset{\text{(glucuronic acid)}}{COOH-(CHOH)_4\overset{\overset{O}{\|}}{C}-H} + H_2O,$$

But as glucuronic acid is excreted almost quantitatively when injected into animals, it is probably not a main intermediate in carbohydrate metabolism. The possibility that pyruvic aldehyde, glyceric aldehyde and dihydroxyacetone are intermediates has been much discussed and investigated, but Dakin[86] considers that their rôle has not yet been established. He investigated "glyoxalases" which convert pyruvic aldehyde (methyl glyoxal) into lactic acid, which may be of importance in carbohydrate oxidation. Attempts to isolate from various organs an enzyme oxidizing glucose, particularly from the pancreas, muscle, and liver, have led to varying results and much dissension, but it is probable, because of these failures, that no such enzyme exists in quantities sufficient to account for the amount of sugar combustion that is normally accomplished. O. Cohnheim[87] attempted to explain the failures by his observation that the pancreas produces a substance that activates an inactive glycolytic enzyme in the muscles, liver, and probably in other organs. This work is not generally accepted, so we are still in the dark as to how the carbohydrate oxidations are accomplished, even since the production of insulin has stimulated research in carbohydrate metabolism. (See Chapter XXVI.)

[76] Verh. Jap. Path. Ges., 1916 (6), 76.
[77] Beitr. path. Anat., 1922 (70), 1.
[78] See Dunn, Quart. Jour. Med., 1913 (6), 293.
[79] McJunkin, Anat. Record, 1922 (24), 67.
[80] See Klopfer, Zeit, exp. Pharm., 1912 (11), 467.
[81] Weiss, Wien, klin. Woch., 1912 (25), 697.
[82] Makino, Verh. Japan. Path. Gesell., 1915 (5), 71.
[83] Medigreceanu, Jour. Exp. Med., 1914 (19), 303.
[84] Horacio, Anales inst. mod. clin. méd., 1919 (4), 210.
[85] Also discussed under "Diabetes," chap. XXVI. As glycolysis by blood and tissues can occur without oxygen, Battelli and Stern exclude the glycolytic from the oxidizing enzymes.
[86] "Oxidations and Reductions in the Animal Body," 1922, p. 113.
[87] Zeit. physiol. Chem., 1903 (39), 336; also see Simpson, Biochem. Jour., 1910, (5), 126.

LIPASE[88]

Lipase is probably present in greater or less amount in all cells. In the discussion of the reversible action of enzymes, (see page 39) the modern conception of fat metabolism has been explained, which considers it to depend upon the existence of lipase in the cells and fluids throughout the body. On account of the technical difficulties in the way of using higher fats, such as triolein, in experimental work, the esters of lower fatty acids have generally been used, particularly ethyl butyrate, tributyrin salicylic acid esters, and glycerol triacetate. Enzymes splitting ethyl butyrate, and other esters (*esterases*), have been demonstrated in practically all tissues examined.[89]

What the relation of these esterases may be to the enzyme splitting fats, the true lipase, is not yet known. However, Fanconi[90] found that human serum does split neutral fats as well as cholesterol esters.

Much of the work so far reported on the occurrence of lipase in tissues is of questionable value, especially as to quantitative results, because of faulty methods. Saxl[91] points out and avoids some of these errors, and finds that during autolysis of tissues the splitting of the natural fats present in the cells is but slight; simple esters are attacked more, especially amyl-salicylate; muscle and blood are the least active tissues.[92] Bergel has reported that lymphocytes are especially lipolytic, but his observations have failed of confirmation.[93] In the serum of normal individuals the esterase content seems to be quite constant,[94] and Quinan[95] found the tissue content also constant, the liver containing about twice as much as the kidney and over three times as much as the muscle. He states that different parts of the brain have characteristic lipase activity (butyrase).[96] Thiele[97] has found that blood, chyle, and various tissues also contain an enzyme which can hydrolyze lecithin, but except in the pancreas it does not hydrolyze neutral fats. The brain contains enzymes hydrolyzing mono- and triacetin, lecithin and cephalin.[98] Red corpuscles are said to contain an enzyme splitting cholesterol esters, "*choles-*

[88] For literature on lipase see Connstein, Ergebnisse Physiol., 1904 (3, Abt. 1), 194; concerning the behavior of lipase see Taylor, Jour. Biol. Chem., 1906 (2), 103; Falk, Proc. Natl. Acad., 1915 (1), 136; Science, 1918 (47), 423.

[89] Enzymes splitting glycerophosphates (glycerophosphatase) are widely distributed in epithelial tissues (See Forrai, Biochem. Zeit., 1923 (142), 282). Neuberg has described a sulfatase, which splits ethereal sulfates (Naturwissensch., 1924 (12), 797).

[90] Fermentforsch., 1924 (7), 307.

[91] Biochem. Zeit., 1908 (12), 343.

[92] The distribution of lipases in different species of animals and their various organs has been investigated by Porter, Münch. med. Woch., 1914 (61), 1774.

[93] See Reed, Amer. Rev. Tuberc., 1923 (7), 105; Aschoff and Kamiya, Deut. med. Woch., 1922 (48), 794.

[94] Sagal, Jour. Med. Res., 1916 (34), 231.

[95] *Ibid.*, 1915 (32), 45.

[96] *Ibid.*, 1916 (35), 79.

[97] Biochem. Jour., 1913 (7), 275.

[98] English and MacArthur (Jour. Amer. Chem. Soc., 1915 (37), 653), who have also found in sheep brain, erepsin, amylase, catalase, enzymes decomposing arbutin and salol, probably pepsin and trypsin, but not peroxidase, oxidase, reductase, guanase, urease, or rennin.

terase."[99] In leucocytes a "*lipoidase*" has been found by Fiessinger and Clogne[1] that splits choline out of lecithin.

Little is known about the part played by lipase in pathological conditions. According to Achard and Clerc,[2] the amount of splitting of ethyl butyrate by the blood-serum is lessened in most diseases, and increases and decreases with the health of the patient; according to Pribram[3] and Sagal[94] it is increased in the blood during fevers. It must be recognized, however, that the supposed variations in lipase activity may merely mean variations in inhibiting or stimulating factors also present in the fluids and tissues.[4] Clerc[5] found that acute arsenic, phosphorus, and diphtheria-toxin poisoning increased this property of the serum, while chronic poisoning and staphylococcus intoxication lowered it. Somewhat similar results were obtained by Grossmann,[6] but Saxl found no increased activity in phosphorus poisoning. Using the ethyl butyrate test, Winternitz and Meloy[7] found that the more nearly normal an organ is the more cleavage of the ester; lipolytic activity is low at birth, increases rapidly during the first few days of life, and does not decrease in old age. There is a decline in activity of tissues in diabetes, tuberculosis, and the toxemia of pregnancy, in the livers of passive congestion and fatty degeneration, in the pneumonic lung and the cirrhotic liver. Cachexia from various causes leads to a reduced blood esterase, and in tuberculosis it is diminished commensurate with the extent of the disease.[8] After taking food there is a slight increase in esterase, reaching a maximum in three hours.[9] Whipple[10] finds the blood lipase (butyrase) increased whenever there is injury to the liver, such as in chloroform anesthesia and puerperal eclampsia; it is lowered in cirrhosis. Poulain[11] found that the butyric-splitting power of lymph-glands draining infected areas was decreased. Fischer[12] observed, in a case of extreme lipemia in diabetes, that the lipolytic power of the blood was absent. The lipase of lipomas presents no demonstrable difference from that of ordinary fatty areolar tissues.[13]

Fat necrosis, resulting from the escape of pancreatic juice into the peripancreatic tissues and abdominal cavity, undoubtedly is largely the result of lipase action. (See "Fat Necrosis," Chapter XVII, for complete consideration.)

[99] See Cytronberg, Biochem. Zeit., 1912 (45), 281.
[1] Compt. Rend. Acad. Sci., 1917 (165), 730.
[2] Compt. Rend. Soc. Biol., 1902 (54), 1144.
[3] Cent. inn. Med., 1908 (29), 81.
[4] See Bayer, Zeit. Biol., 1919 (69), 365.
[5] Compt. Rend. Soc. Biol., 1901 (53), 1131.
[6] Biochem. Zeit., 1912 (41), 181.
[7] Jour. Med. Res., 1910 (22), 107.
[8] Woolley, Amer. Rev. Tuberc., 1924 (9), 32.
[9] Jobling *et al.*, Jour. Exp. Med., 1915 (22), 129.
[10] Whipple *et al.*, Bull. Johns Hopkins Hosp., 1913 (24), 207 and 357.
[11] Compt. Rend. Soc. Biol., 1901 (53), 786.
[12] Virchow's Arch., 1903 (172), 218.
[13] Wells, Arch. Int. Med., 1912 (10), 297.

Lipase has also been demonstrated in pus by a number of observers,[14] who agree that there is more in exudates than in transudates. Zeri[15] found lipase in the urine only when pus or blood was also present, but Pribram and Loewy[16] found it in nephritis, congestion, polyuria and other conditions. Lorenzini,[17] however, reports that in albuminuria the lipase content of the urine is reduced, in common with other enzymes, there being a simultaneous accumulation of enzymes in the blood.

Fiessinger and Marie[18] and Bergell[19] contend that the lymphocytes of exudates are the chief source of lipase, and suggest that this may be of effect in defense against the fatty tubercle bacilli, an hypothesis now of doubtful merit (Reed).[93] Caro[20] found no relation between the lipase activity of the blood or exudates and their lymphocyte content. Toxins were found by Pesci[21] to increase the butyrase but not the other lipases of liver tissue. In syphilis the lipolytic activity of the serum is said to be increased.[22] Jobling and Bull[23] state that a specific serum lipase increase occurs in animals immunized to red corpuscles, and that this lipase has to do with hemolysis; but Mendel[24] found no evidence that hemolysis by ricin is related to lipase. Abderhalden and Rona[25] reported that excess feeding of fats leads to an increase in the lipase of the blood. Caro[20] found a decrease in all cases of cachexia, but there was no relation between the lipolytic enzyme and the blood picture.

The part played by lipase in fatty degeneration may be of importance, but as yet it has been little considered, except that Loevenhart, and Duccheschi and Almagia[61] found no appreciable difference in the lipase content of normal and phosphorus-poisoned livers, but in chloroform poisoning Quinan[26] found a decrease in the butyrase of the liver, although it was increased in the kidneys and muscles. This question will be considered more fully in discussing fatty metamorphosis.

An improved method of testing for lipase action has been devised by Rona and Michaelis, by measuring the change in surface tension caused by hydrolysis of a soluble ester, usually tributyrin. Using this, Bauer[27] found that every human serum contains fat-splitting enzymes, which are greatly decreased in carcinoma and advanced phthisis, somewhat decreased in syphilis and exophthalmic goitre, and increased in early pulmonary

[14] Achalme, Comp. Rend. Soc. Biol., 1899 (51), 568; Zeri, Il Policlinico, 1903 (10), 433; Memmi, Clin. Med. Ital., 1905 (44), 129.
[15] Il Policlinico, 1905 (12), 733.
[16] Zeit. physiol. Chem., 1912 (76), 489.
[17] Policlinico, 1915 (22), 358.
[18] Compt. Rend. Soc. Biol., 1909 (68), 177.
[19] Münch. med. Woch., 1909 (56), 64. See also Resch, Deut. Arch. klin. Med., 1915 (118), 179.
[20] Zeit. klin. Med., 1913 (78), 286; 1920 (89), 49.
[21] Pathologica, 1912 (3), 207.
[22] Citron and Reicher, Berl., klin. Woch., 1908 (45), 1398.
[23] Jour. Exp. Med., 1912 (16), 483.
[24] Arch. fisiol., 1909 (7), 168.
[25] Zeit. physiol. Chem., 1911 (75), 30.
[26] Jour. Med. Res., 1915 (32), 73.
[27] Wien. klin. Woch., 1912 (25), 1376 (bibliography).

tuberculosis. With tuberculosis the lipase falls with the progress of the disease, becoming very low shortly before death.[28]

Rona[29] has also reported that the lipases of different tissues may be distinguished by their differing resistance to various poisons; *e. g.*, lipase of serum and pancreas is inhibited by quinine, whereas liver and kidney lipase resists quinine; pancreas lipase resists atoxyl, which inhibits liver and serum lipase. He suggests that by this method tissue lipases may be detected when liberated into the blood by injury to the several viscera, but Krömeke[30] found that injury of liver and lymphocytes by Roentgen rays as well as of tissues by other measures, did not lead to the appearance in the blood of added amounts of tissue lipase (quinine resistant). On the other hand, some observers have obtained results confirming those of Rona.[31] Thus, Simon[32] reports that atoyxl-resistant lipase, like that of the pancreas, is found in the blood only in some affections of the pancreas, (and in pernicious anemia), although not in diabetes; he examined no cases of acute pancreatitis. Meyer and Jahr[33] found quinine-resistant lipase present in the serum of patients with liver disease and negative in the controls. Broekmeyer[34] not only doubts that pancreatic lipase can be identified by this procedure, but also found that normal serum contains thermostable agents inhibiting pancreatic lipase, quite analogous to antitrypsin. Block and Einstein[35] report that kidney lipase is found in the urine with injury to the renal epithelium, especially in nephrosis, while serum lipase is found in the urine with almost all types of renal injury. Simon[36] found that the red corpuscles contain a lipase distinct from that of the serum.

AMYLASE OR DIASTASE[37]

Although under ordinary conditions starch does not enter the blood stream and tissues, yet all tissues and body fluids are capable of hydrolyzing starch. Apparently the amylase is derived from the pancreas and salivary glands, and possibly from many or all other tissues, but it is not quantitatively related to the amount of carbohydrate in the diet of a species or an individual (Carlson and Luckhardt). In the blood it occurs in the albumin fraction.[38] There is disagreement in the literature as to the variations in amount of amylase in the blood during disease, and little

[28] Frisch and Kollert, Beitr. Klin. Tuberk., 1921 (47), 146.
[29] See Klin. Woch., 1922 (1), 2367; Biochem. Zeit., 1924 (146), 144.
[30] Arch. exp. Path. u. Pharm., 1923 (100), 77.
[31] See Petow and Schrieber, Klin. Woch., 1923 (2), 1248; Block, *ibid.*, 1923 (2), 1793.
[32] Ibid., 1924 (3), 674.
[33] Klin. Woch., 1924 (3), 2056.
[34] Ibid., 1924 (3), 1526.
[35] Klin. Woch., 1924 (3), 1230.
[36] Zeit. ges. exp. Med., 1924 (39), 407.
[37] Literature given by Stocks, Quart. Jour. Med., 1916 (9), 216; McClure and Pratt, Arch. Int. Med., 1917 (19), 568; Winslow, Hospitalstindende, 1918 (61), 832; Block, Zeit. klin. Med., 1922 (93), 381.
[38] Satta, Arch. Sci. Med., 1915 (39), 46.

information concerning its distribution in the tissues. Normally the kidneys and liver seem to be most active and Winslow says that all glycogen-containing organs produce diastase. McGuigan[39] finds the amount to vary directly with the activity and vigor of the tissue, and the amount in the blood falls with cachexia. When the pancreas is removed the diastase in the blood may be increased[40] as it also is by asphyxia, but other authors find a decrease after pancreas extirpation and maintain that the blood diastase comes chiefly, if not solely, from the pancreas.[41] During acute infections the blood amylase is increased, presumably coming from the leucocytes (King). It is greatly increased when the pancreas is acutely inflamed or injured (Stocks).[37] In diabetes it is ordinarily increased,[42] (but not in syphilitic diabetes),[43] and the amount of blood diastase does not bear any constant relation to the blood sugar content.[44] Intravenous or subcutaneous injection of starch is said to increase the blood and urinary amylase[45] presumably as a defensive reaction (Abderhalden). There appears to be no normal antiamylase in the blood.[46] Starch granules taken up by phagocytes show a glycogen reaction after some time, suggesting that these cells have intracellular diastases.[47]

Because of possible diagnostic significance, the amylolytic activity of the urine has been particularly studied, and found normally to be approximately constant for 24 hour specimens of the same individual.[48] Cammidge[49] says that the amount in the urine depends on the functional activity of the liver, (from which amylase largely comes), the integrity of pancreatic control, (for pancreatic injury increases amylase excretion), and the permeability of the kidneys. Anything impairing the excretory capacity of the kidney decreases the urinary amylase,[50] although sometimes when the urine contains blood, pus, or much albumen, there may be an increased amylase excretion in spite of diminished functional activity. Especially in the toxemias of pregnancy the urinary diastase is high.[51] There may be an increase in the amylase in the blood when the urinary amylase is decreased, but with normal kidneys increase of the blood amylase causes an incease in the urine;[45] hence, acute pancreatic diseases cause an increased urinary amylase (Stocks), but this is not constant (McClure and Pratt). In diabetic urine it is said to be usually decreased,

[39] Science, 1921 (53), 145.
[40] Karsner *et al.*,[40] Jour. Exp. Med., 1921 (34), 349.
[41] See Davis and Ross, Amer. Jour. Physiol., 1921 (56), 22.
[42] Myers, Jour. Lab. Clin. Med., 1920 (5), 640.
[43] De Niord and Schreiner, Arch. Int. Med., 1919 (23), 484.
[44] Karsner *et al.*,[40] also Lewis and Mason, Jour. Biol. Chem., 1920 (44), 455.
[45] King, Proc. Soc. Exp. Biol., 1917 (15), 101.
[46] Maltase is said to be lacking in the blood of man although present in many other mammals (Compton, Biochem. Jour., 1921 (15), 681).
[47] Okazaki, Sei-I-Kwai Med. Jour., 1917 (46), 101.
[48] In infants the urine amylase is low (McClure and Chancellor, Zeit. Kinderheilk., 1914 (11), 483). Fetal blood contains much less than the maternal blood (Kito, Amer. Jour. Physiol., 1919 (48), 481).
[49] Proc. Royal Soc. Med., Urol. Sect., 1922 (15), 37.
[50] See Sladden, Lancet, 1922 (2), 68; Cameron, Jour. Metabol. Res., 1923 (3), 756.
[51] Wallis, Brit. Med. Jour., 1920 (2), 273.

but this is mostly accounted for by the dilution of the urine. Block[37] concludes from an extensive study of the literature, that only in pancreatic disease is the variation from the wide normal limits sufficiently great to make the diastase content of blood and urine of diagnostic value. In malignant diseases of the pancreas the amount of urinary diastase may be normal or decreased (Cameron).[50]

CHAPTER III

ENZYMES (Continued)

INTRACELLULAR PROTEASES[1] (PROTEOLYTIC ENZYMES), INCLUDING A CONSIDERATION OF AUTOLYSIS

To what extent synthesis of proteins goes on in the body is still a problem; still more uncertain is the part played by reversible action of proteases. There is evidence enough that somewhere in the body the amino-acids can be rebuilt into protein, for several investigators have succeeded in keeping animals in nitrogenous equilibrium by feeding them products of proteolysis that contained no proteins whatever, and as the proteins of the animal body are being broken down incessantly, it must be that they were replaced by synthesis of the non-protein material fed to the animals. In addition, Cohnheim found that in the intestinal epithelium there is an enzyme, *erepsin*, capable of splitting albumoses and peptones into the amino-acids, which enzyme presumably exists for the purpose of securing complete cleavage of all ingested proteins into their ultimate "building stones." This may be looked upon as a provision to reduce all varieties of proteins to their common elements, so that the body by quantitative selection can resynthesize them into its own types of protein, for, as is well known, foreign proteins (*e. g.* egg-albumin) introduced directly into the blood stream cannot be utilized, but are excreted unaltered in the urine.[2] As was shown for lipase, the assumption that such synthesis occurs as a normal physiological process by reverse enzyme action, requires that the proper enzymes be present in the cells throughout the body, and it has been abundantly demonstrated that such is the case.

For over half a century it has been known that amebæ digest solid proteins within their bodies, but it is only within a comparatively few years that proteolytic enzymes have been definitely isolated from them. It has been much the same with the intracellular proteases of the higher organisms. In 1871 Hoppe-Seyler referred to the liquefaction of dead tissues within the body which occurred without putrefaction, and, as he noted, resembled the effects of the digestive ferments. It was nearly twenty years later that Salkowski[3] showed definitely that this softening of dead

[1] As the possibility exists that ferments which digest proteins may be able to perform a certain amount of synthesis of proteins, the term "proteolytic enzyme" seems to be less suitable than the term "protease," which merely means an enzyme acting on proteins, and does not compel us to accept any particular view as to what the action is.

[2] According to Austin and Eisenbrey (Arch. Int. Med., 1912 (10), 305), dogs on a nitrogen-free diet can utilize horse serum injected intravenously.

[3] Zeit. f. klin. Med., 1890, supplement to Bd. 17, p. 77.

tissues was really brought about through a true digestion by intracellular enzymes, which produced the same splitting products that were at that time considered characteristic for tryptic digestion (leucine and tyrosine). The process he named "*autodigestion.*" This important observation remained almost unnoticed for ten years more, when Jacoby,[4] in 1900, took up the investigation of this matter of cellular self-digestion, and after this the importance of the principles involved became for the first time generally appreciated. Jacoby rechristened the process "*autolysis,*" by which name it is now commonly known.

AUTOLYSIS[5]

Autolysis is generally studied by the method used by Salkowski which depends upon the difference in the susceptibility of bacteria and of enzymes to antiseptics. The organs are ground to a pulp, placed in flasks with or without the addition of water or dilute acids, and bacterial action is prevented by the addition of antiseptics that are not poisonous to enzymes—toluene and chloroform are most commonly used. It is possible also to secure organs in an aseptic condition and to permit them to undergo autolysis without the use of antiseptics, but the practical difficulties are such that this method is seldom used—it is sometimes designated as "*aseptic autolysis,*" in contradistinction to antiseptic autolysis by the Salkowski method. In a short time it can be seen that digestive changes have taken place, particularly if comparisons are made with control flasks in which the enzymes have been destroyed by boiling. To determine the rate of autolysis the amount of nitrogen that remains in the form of coagulable compounds, and that which is converted into soluble, non-coagulable compounds (albumoses, peptones, ammonia compounds, amino-acids, etc.), are compared. The method may be illustrated by a concrete example: A given specimen of emulsionized liver tissue was permitted to digest itself for twenty-two days. At the end of that time 39.4 per cent. of the nitrogen was still contained in the compounds that remained insoluble or became so after the autolysis was stopped by boiling, while 60.6 per cent. of the nitrogen was in a soluble form. A control specimen from the same liver was boiled while fresh to kill the enzymes, and then let stand under the same conditions. In this specimen 90.4 per cent. of the nitrogen was in an insoluble form, and 9.6 per cent. was soluble. Therefore, over half of all the protein of the liver had been changed into non-coagulable substances in the course of about three weeks (at 37° C.). Complete disintegration of the proteins with liberation of all the amino-acid complexes is probably never reached.[6] For example, of 45.8 grams of amino-acids present in 100 grams

[4] Zeit. f. physiol. Chem., 1900 (30), 149.

[5] Résumé of literature by Salkowski, Deutsche Klinik, 1903 (11), 147; von Fürth, "Chemistry of Metabolism," Amer. Transl., 1916; Bradley, Physiol. Reviews, 1922 (2), 415.

[6] The connective tissues (Bradley)[5] and elastic tissue (Tritchkowitch, Compt. Rend. Soc. Biol., 1922 (87), 1135), especially resisting autolysis.

of liver in the form of proteins, in ten days' autolysis there had been set free but 1.85 gm., after 30 days 10.1 gm., and after 50 days but 29.1 gm. (Abderhalden and Prym.)[7] Undoubtedly the accumulation of the products of hydrolysis tends to check the rate and degree of hydrolysis, for if autolysis is carried out in a dialyzing sack as much as 90 per cent. of the tissue may be disintegrated.[8]

By determining the freezing point and conductivity of autolyzing mixtures, valuable evidence can be obtained as to the rate of change, which, in some cases, is much more significant than the usual estimation of soluble and insoluble nitrogen (Benson and Wells[9]). Titration of the free amino-acids by the formaldehyde method, together with the estimation of proteose and peptone nitrogen, also furnish valuable information, while the Van Slyke method of determining free amino-acids is especially useful for this purpose.

Since Jacoby's paper appeared, the field has been invaded by many workers, who have examined practically every tissue in the mammalian body, as well as simple forms, including plants and unicellular organisms, and found that all possess the power of self-digestion; or, in other words, *proteases are present in every cell.*[10] The rate of digestion is very different in different organs, however, liver, kidney and spleen digesting rapidly while brain and muscle tissue digest much more slowly and the autolytic activity varies under different conditions;[11] thus, fever causes a great increase in the proteolytic activity of the muscles,[12] but only about 15 per cent. of striated muscle proteins can be autolyzed under the most favorable circumstances.[13] Probably the slight autolysis of brain tissue corresponds to its low protein content, for it does undergo autolysis to some degree and attacks added proteins.[14]

It is improbable that the intracellular enzymes are merely pancreatic enzymes taken out of the blood by the cells, because autolysis occurs best at H ion concentrations corresponding to pH = 6 or even lower, and almost not at all at the reaction favorable for trypsin. Furthermore, Matthes[15] found that the liver retained its autolytic power after the pancreas had been extirpated (in dogs), and that the autolytic degeneration of cut peripheral nerves went on just the same.

Whenever tissues are disintegrated in any considerable quantities, as after extensive burns, peptolytic enzymes become demonstrable in the blood and urine, and presumably these are related to the cell autolysis.[16] They are noticeably increased in most infectious diseases in which

[7] Zeit. physiol. Chem., 1907 (53), 320.
[8] Rona and Mislowitzer, Biochem. Zeit., 1923 (140), 517.
[9] Jour. Biol. Chem., 1910 (8), 61.
[10] Except, perhaps, the red corpuscles (Pincussohn and Roques, Biochem. Zeit., 1914 (64), 1; Morse, Jour. Biol. Chem., 1921 (46), xlvi).
[11] Concerning autolysis of skin, see Sexsmith and Petersen, Jour. Exp. Med., 1917 (27), 273.
[12] Aronshon and Blumenthal, Zeit. klin. Med., 1908 (65), 1.
[13] Chen and Bradley, Jour. Biol. Chem., 1924 (59), 151.
[14] Gibson, Umbreit and Bradley, Jour. Biol. Chem., 1921 (47), 333.
[15] Arch. f. exp. Path. u. Pharm., 1904 (51), 442.
[16] See Pfeiffer, Münch. med. Woch., 1914 (51), 1099, 1329.

the reaction between the body defenses and the infecting organism takes place in the blood stream (Falls).[17] Also in the premortal state a similar increase in peptolytic enzyme in the serum is associated with a high non-protein nitrogen figure for the serum.[18] The relation of the autolytic enzymes to the increased proteolytic power of serum in pregnancy, as shown in the Abderhalden reaction (*q. v.*) has not yet been determined,[19] but Falls finds evidence of their correlation.[17] Blood proteases are also increased in pregnancy. They bear no constant relation to the leucocyte count. Autodigestion of serum is normally prevented by the presence of a specific antienzyme, which latter can be inhibited by chloroform and various saturated monovalent ketones and alcohols (Yamakawa).[20]

Influence of H-ion Concentration on Autolysis.—The character of the antiseptic used modifies greatly the rate, salicylic and benzoic acids giving the most rapid autolysis, while of non-acid antiseptics toluene is perhaps the least inhibitory. One of the most important factors in modifying the rate of autolysis is the H-ion concentration developing in the tissues.[21] Acidity acts, partly, at least, by so modifying the substrate that the enzymes can attack it, and a very small excess of acid will destroy the enzymes; Bradley[22] estimates this destructive acidity at about that concentration of H-ions which is indicated by methyl orange and Congo red, the maximum autolysis being obtained with an acidity at about pH = 6.00. A reaction approximating that of blood (pH = 7.4 – 7.8) reduces autolysis to a minimum, which Bradley explains by the assumption that at this pH the tissue proteins exist as salts with bases, which salts are not digestible by the tissue enzymes.[23] The tissue enzymes are present at the normal pH of about 7.4 in the form of a stable base-protein-enzyme combination which can only be liberated by a rise of the H-ion concentration.

A latent period has been observed before autolysis *in vitro* seems to begin, part of which time may be occupied in the development of sufficient acidity to permit of autolysis, although Bradley's[24] results indicate that it can be accounted for largely by the time required for proteolysis to proceed far enough to be detected by chemical means. In this connection it is significant that the tissues develop an acidity of pH 6.6–6.7 immediately after death, probably from anoxemia.[25] According to the observations of Bradley and his colleagues[26] following death the H-ion concentration of liver cells (pig) increases very rapidly, then more slowly to

[17] Jour. Infect. Dis., 1915 (16), 466; also Peterson and Short, Jour. Infect. Dis., 1918 (22), 147.

[18] See Schulz, Münch. med. Woch., 1913 (60), 2512; Mandelbaum, *ibid.*, 1914 (61), 461.

[19] See Sloan, Amer. Jour. Physiol., 1915 (39), 9.

[20] Jour. Exp. Med., 1918 (27), 689.

[21] See Morse, Jour. Biol. Chem., 1916 (24), 163.

[22] Jour. Biol. Chem., 1915 (22), 113; 1916 (25), 261.

[23] Jour. Biol. Chem., 1924 (61), 275; (62), 231.

[24] Jour. Biol. Chem., 1916 (25), 363.

[25] Koehler, Morse, Jour. Biol. Chem., 1923 (55), viii.

[26] Severinghaus, Koehler and Bradley, Jour. Biol. Chem., 1923 (57), 163.

a pH of about 6 within 4 to 48 hours. The acidity then falls to a pH of 6.6 in 10 days and then slowly increases again. If acid is added the initial high H-ion concentration sinks slightly for a few days until the bulk of the proteins are converted into amino acids; thereafter, there is little change. The greater part of the acidity produced is due to H_3PO_4, but fatty acids are also produced and the addition of fats makes the mixture more acid and increases autolysis.

Dernby[27] finds that in several tissues studied, including leucocytes, there are two intracellular proteases, one resembling pepsin[28] in carrying digestion only to the peptone stage and in requiring an acid medium, optimum pH = 4.5 to 3.5; the other resembling ereptase, splitting only peptones and peptids into amino-acids, with optimum reaction pH = 7.8 and not inhibited by acid reaction above pH = 3 (Bradley).[5] Autolysis of tissues proceeds farthest in a pH range between 5 and 6, presumably because in this condition both enzymes can act.[29] Bradley has found no evidence that enzymes exactly corresponding to either trypsin or pepsin occur in tissues other than pancreas and stomach. He confirms Dernby's view that the first step in autolytic proteolysis is a partial disintegration by an enzyme active between pH = 7 to 3, the products of which are then carried to the amino-acid stage by an ereptase-like enzyme.[30] Dernby[31] classifies the autolytic proteases as follows:

Name	Substances digested	Products	Optimum activity
Primary proteases (pepsinases)	Native protein (not peptones)	Peptones	Acid
Secondary proteases (tryptases)	Denatured proteins and peptones	Amino acids or peptides	Alk. or neutral
Tertiary proteases (ereptases)	Peptides	Amino acids	Alk. or neutral

From these facts it is evident that *quantitative studies of rates of autolysis are valueless if the H-ion concentration is not taken into consideration.*

Chemical Changes of Autolysis.—It was once maintained that the cleavage products resulting from tissue autolysis contain a much larger proportion of the nitrogen in the form of ammonia and its compounds than is the case with simple tryptic digestion, because of the pres-

[27] Jour. Biol. Chem., 1918 (35), 179.

[28] It differs from pepsin in being destroyed at a pH less than 1.5, which is the optimum concentration for pepsin action (Bradley, Jour. Biol. Chem., 1922 (52), 467).

[29] Corroborated by Shima, Jour. Biochem., Japan, 1922 (2), 1. Hedin (Jour. Biol. Chem., 1922 (54), 177) maintains that spleen tissue contains another enzyme which is most active at pH = 8.8. Morse (Jour. Biol. Chem., 1917 (31), 303 considers this enzyme to be heterolytic, derived from the white cells, and Opie found that there is more autolysis by spleen in an alkaline medium than in the other tissues except bone marrow, which, like the leucocytes, contains an enzyme most active at about pH = 8. (See Opie, Physiol. Reviews, 1922 (2), 552).

[30] Jour. Biol. Chem., 1922 (52), 467.

[31] Biochem. Zeit., 1922 (133), 432.

ence in tissues of deaminizing enzymes which split the NH_2 groups out of the amino-acids and purines, but most of these results probably depend on bacterial action. Dakin[32] has shown that there is very little deaminization if putrefaction is excluded. According to Bostock[33] the greater the acidity the less NH_3 is formed. It is quite probable that in tissue autolysis several intracellular enzymes are in action which may not be present in pancreatic or gastric juice; for example, in the liver is an enzyme, *arginase*, which splits the urea radical out of the arginine of the proteins (Kossel and Dakin),[34] and the enzymes which disintegrate purines are also absent from the digestive juices. On the whole, however, the products are quite similar to those obtained by tryptic digestion. To give a concrete example, Dakin[32] detected in the products of autolysis by the kidney in acid solution, the following substances: Ammonia, alanine, α-aminovalerianic acid, leucine, α-pyrollidine carboxylic acid, phenylalanine, tyrosine, lysine, histidine, cystine, hypoxanthine, and indole derivatives, including probably tryptophan.[35] The cleavage of simple *peptids* by different tissues shows characteristic differences, the distribution of the enzyme which splits glycyl-tryptophan having been most studied. During life the cells retain this enzyme, and hence it appears in the body fluids only when the tissues are being rapidly disintegrated (Mandelbaum).[36]

During autolysis the changes are by no means limited to the proteins. Glycogen is split into glucose very early, and the sugar undergoes further changes. Fats are also split by the lipase, fatty acids being found in autolyzed organs. Reducing substances appear, and as before mentioned, numerous volatile fatty acids are said to be produced. Much doubt exists concerning the supposed formation of volatile fatty acids and gases during autolysis since it was shown by Wolbach, Saiki and Jackson[37] that anaerobic bacteria are almost invariably present in aseptically removed dog livers, for control of autolysis by anaerobic cultures has seldom been carried out. However, there is much evidence that lactic acid is formed, and perhaps partially destroyed, in autolysis (Türkel,[38] Ssobolew[39]). Carefully controlled experiments by Lindemann[40] seem to show that even in the absence of bacteria, autolyzing liver and heart can produce volatile acids, CO_2 and hydrogen. The increase in fat described by some authors is probably only apparent, and due rather to the liberation of the fat from its combination with the proteins so that it is free and

[32] Jour. of Physiology, 1903 (30), 84.
[33] Biochem. Jour., 1912 (6), 388.
[34] Zeit. physiol. Chem., 1904 (42), 181; Fuchs, Zeit. physiol. Chem., 1921 (114), 101.
[35] The results of autolysis by different tissues are said to be quite dissimilar (Kashiwabara, Zeit. physiol. Chem., 1913 (85), 161).
[36] Münch. med. Woch., 1914 (61), 461.
[37] Jour. Med. Res. 1909 (21), 267.
[38] Biochem. Zeit., 1909 (20), 431.
[39] *Ibid.*, 1912 (47), 367. See also v. Stein and Salkowski, Biochem. Zeit., 1913 (40), 486.
[40] Zeit. f. Biol., 1910 (55), 36.

not "masked," as in normal organs.[41] Lecithin is decomposed, yielding choline, but cholesterol remains unchanged except for some hydrolysis of cholesterol esters.[42] Creatine is changed to creatinine in autolyzing muscle, and apparently both are formed in autolysis of blood and liver.[43]

The *nucleo-proteins* seem to be attacked by the autolytic enzymes, as the purine bases are prominent among the products of autolysis, and in quite different proportions from those obtaining in digestion of the same tissues by other means. Apparently autolytic enzymes attack the protein group of the nucleoproteins, liberating the nucleic acids. These in turn are attacked by specific enzymes, *nucleases*,[44] which liberate the purine bases, which are further decomposed by specific enzymes, *guanase*, *adenase*, etc. (See Chap. XXV.)

Influence of Chemicals on Autolysis.—As a general rule the addition of antiseptics to tissues to prevent bacterial action reduces the rate of autolysis, but as most of the results of "aseptic" autolysis so far reported are open to question, there is a reasonable doubt as to just how much depression of autolysis there is. Yoshimoto[45] found that of the antiseptics ordinarily used, salicylic acid, boric acid, and mustard oil (one-eighth saturated solution) permit the greatest autolysis; but it is probable that the acidity of the first two antiseptics plays an important part, hence the value of the results obtained in autolysis with these acids is questionable. However, sodium salicylate and benzoate are said to favor autolysis (Laqueur).[46] Toluene seems to interfere much less with autolysis than chloroform or thymol (Benson and Wells[47]), and bromides are less harmful than toluene (Laqueur). Toluene vapor, acting on solid aseptic tissues, seems to cause more depression of autolysis than is usually observed in autolysis in solution.[48] Dorothy Court[49] found the only satisfactory antiseptics to be chloroform, formaldehyde, benzoic and salicylic acids, and HNC; she emphasizes the fact that for different sorts of materials the different antiseptics give variable results, so that the antiseptic used should be selected with reference to the material. Autolysis proceeds rapidly in weak ethyl alcohol, 5 per cent. being the minimum strength that will prevent putrefaction; for complete suppression of autolysis by alcohol the strength must be at least 90 per cent. net, after allowing for the water content of the tissues (Wells and Caldwell).[50]

Certain inorganic substances in proper concentrations have been reported as increasing the rate of autolysis[51] [mercury and silver, (colloidal or salts)], yellow phosphorus, iodides, arsenic, $CaCl_2$, salts of Fe, Mg, and Co, as well as salts of Se, Te, and Mn, but a distinctly increased rate of autolysis has been observed in liver tissue after exposure to Roentgen rays.[52] *These results are of doubtful value, since in most of the experiments the influence of the pH was not adequately considered*, and many of the substances described as accelerators are either acids or hydrolyze in water to form acids. The results with colloidal metals could not be confirmed by Bradley.[5]

Because of their effect on tissue *p*H, CO_2 increases and oxygen decreases autolysis[53] *in vitro* (Laqueur). There is disagreement as to whether radium rays augment auto-

[41] See Krontowski and Poleff, Beitr. Path. Anat., 1914 (58), 407.

[42] Corper, Jour. Biol. Chem., 1912 (11), 37; Kondo, Biochem. Zeit., 1910 (27), 427; Gardner and Fox, Proc. Royal Soc., London, 1922 (93*B*), 486.

[43] Myers and Fine, Jour. Biol. Chem., 1915 (21), 583; Hoagland and McBryde, Jour. Agric. Res., 1916 (6), 535.

[44] Sachs, Zeit. physiol. Chem., 1905 (46), 337; Jones *ibid.*, 1903 (41), 101, and 1906 (48), 110.

[45] Zeit. physiol. Chem., 1908 (58), 341.

[46] Zeit. physiol. Chem., 1912 (79), 38 and 65.

[47] Jour. Biol. Chem., 1910 (8), 61.

[48] Cruickshank, Jour. Path. and Bact. 1911 (16), 167.

[49] Proc. Roy. Soc., Edinburgh, 1912 (32), 251.

[50] Jour. Biol. Chem., 1914 (19), 57.

[51] Full bibliography given by Bradley.[5]

[52] Hajos and Hofhauser, Biochem. Zeit., 1924 (146), 204.

[53] M. Morse found oxygen without effect on autolysis. Biochem. Bullet., 1915 (5), 143.

lysis.[54] Injection of iodids into animals is said to increase the postmortem autolysis of their tissues (Stookey, Kepinow), as also do iron salts,[55] while large doses of salicylates decrease it (Laqueur). It has been reported that addition of tuberculin to tissues at first delays and then increases the autolysis (Pesci[56]), and diphtheria toxin in small amounts increases autolysis (Barlocco,[57] Bertolini[58]), neutralization by antitoxin not preventing this effect. Lipoids also accelerate autolysis (Satta and Fasiani[59]). According to Soula[60] narcotic poisons decrease, and convulsive poisons increase the rate of autolysis of nervous tissue. After phosphorus poisoning the liver shows an increased rate of autolysis because of greater acidity, but even if this is neutralized the protein cleavage goes farther than in normal livers.[61] Glucose in one per cent. concentration decreases autolysis, and this may be related to the "protein-sparing action of carbohydrates."[62] A high glycogen content in a tissue is associated with slower autolysis.[63] Extracts of various ductless glands, or removal of these glands from animals, seem to have but slight effect on autolysis.[64]

In considering the foregoing statements allowance must be made for the fact that in most of the work cited there has been no proper consideration of H-ion concentration in the autolyzing mixtures, and that it is highly probable that most of the effects observed depend on the changes in this respect, although some effects have been observed that cannot be attributed solely to changes in the *p*H.[65]

Relation of Autolysis To Metabolism

It having been shown that proteases are present in all cells, the next question to be considered is, do they act only to destroy tissues after death, or are they of importance in metabolism? Since it is presumably necessary for proteins to be split into diffusible and easily oxidized forms in order that they may enter the cell and be built up into the cell proteins, or be decomposed with the liberation of energy, the autolytic proteases may be assumed to be of prime importance in protein metabolism; but to prove it is another matter. Jacoby found that if he ligated off a portion of the liver and let it remain *in situ* in the animal the necrotic tissues showed an accumulation of leucine, tyrosine, and other cleavage products of the proteins. This suggested that these same bodies are being formed in the liver constantly, but that they are as constantly removed from the normal organs by the circulating blood, or are undergoing further alterations which cease when the circulation is checked; but this conclusion is not justified for in the asphyxiated liver tissue the *p*H reaches a much lower level than in normal liver, and initiates autolysis not present at the normal *p*H. However, the influence of various chemicals upon nitrogen

[54] See Loewenthal and Edelstein, Biochem. Zeit., 1908 (14), 485; Brown, Arch. Int. Med., 1912 (10), 405.
[55] Kottmann, Zeit. exp. Path., 1912 (11), 355.
[56] Cent. f. Bakt., 1911 (59), 71 and 186.
[57] Cent. f. Bakt., 1911 (60), 43.
[58] Biochem. Zeit., 1913 (48), 448.
[59] Berl. klin. Woch., 1910 (47), 1500.
[60] Compt. Rend. Soc. Biol., 1913 (73), 297.
[61] Rona and Mislowitzer, Biochem. Zeit., 1924 (146), 26.
[62] Shaffer, Proc. Soc. Biol. Chem., 1915 (8), xlii.
[63] Simonds *et al.* Jour. Med. Res., 1921 (42), 455.
[64] Izar and Fagiuoli, Sperimentale, 1916 (70), 265.
[65] See also Rona, Biochem. Zeit., 1924 (146), 1.

elimination seems to correspond to their effect on autolysis (Izar,[66] Laqueur[67]). Also, the histologic changes of starvation are similar in many respects to those of autolysis (Casa-Bianchi[67a]), and in starvation the least change is seen in tissues best protected against anoxemic acidosis, the brain and heart. A marked reduction in oxygen supply to an animal results in excessive nitrogen elimination, presumably through increased tissue acidosis because of the anoxemia.[68]

Among other observations possibly bearing on the same question are those of Hildebrandt,[69] who found that autolysis in the functionating mammary gland is much more active than in the resting gland; and of Schlesinger,[70] who found that autolysis was at its maximum (in rabbits) in new-born animals, decreasing rapidly in the first few months of life, and that in conditions associated with emaciation the rate of autolysis varied directly with the degree of emaciation. Wells[71] sought for a possible influence on autolysis by thyroid extract, which increases protein metabolism, but could demonstrate none *in vitro;* Schryver,[72] however, reported that autolysis was more rapid in the liver of dogs fed thyroid extract for some days before death than it was in control animals. The results of the former observer, but not those of the latter, have been confirmed by Morse.[73] There is every reason to believe that the breakdown of tissues is a function of autolysis, and, as Bradley says, it is safe to assume that every increase of mass, as in hypertrophy and growth, involves the same mechanism operating in the reverse direction. Furthermore, Gazz[74] has found that the products of autolysis may have an important function in stimulating regenerative proliferation.

Defense of the Cells against Their Autolytic Enzymes

The question of why the autolytic ferments do not destroy the cells until after death is a revival of the old problem of "why the stomach does not digest itself," and the answer that satisfies some is that dead protoplasm is essentially different from living protoplasm. More specific replies are suggested by Wiener's studies on the relation of the reaction of the tissues to their autolysis. He found that autolysis does not begin in an organ until the original alkalinity is neutralized by the acids which are formed in all dead and dying cells. If enough alkali is added to the material from time to time to neutralize the acidity as it develops, autolysis does not take place. The studies of Bradley,[5] Dernby[27] and others have corroborated and extended these views, until now it seems probable

[66] Internat. Beitr. Ernährungstör., 1910 (1), 287.
[67] Zeit. physiol. Chem., 1912 (79), 1 *et seq.*
[67a] Frankfurter Zeit. Pathol., 1909(3), 723.
[68] Brunquist, Schneller and Loevenhart., Jour. Biol. Chem., 1924 (62), 93.
[69] Hofmeister's Beiträge, 1904 (5), 463; see also Grimmer, Biochem. Zeit., 1913 (53), 429.
[70] Hofmeister's Beitr., 1903 (4), 87.
[71] Amer. Jour. of Physiol., 1904 (11), 351; corroborated by Kottmann, Zeit. klin. Med., 1910 (71), 369.
[72] Jour. of Physiol., 1905 (32), 159.
[73] Jour. Biol. Chem., 1915 (22), 125.
[74] Arch. klin, Chir., 1922 (121), 378.

that autolysis is entirely dependent on the occurrence of an H-ion concentration considerably greater than that normally present in the tissues. Organic acids are formed in autolysis of the tissues, and the latent period between the time of the removal of an organ from the body and the appearance of autolysis may be explained partly by the time required for the neutralization of tissue alkalinity. Bradley[22] has also obtained evidence that the acid renders the substrate susceptible to digestion by the proteases. Dernby's[27] demonstration of the existence of pepsin-like and erepsin-like enzymes suggests that there must be developed enough acidity to permit the peptase to form peptones before the ereptases can begin their further cleavage. The old observation that rigor mortis disappears most rapidly in muscles that have been exhausted just before death is probably explained by the greater amount of acid in such muscles. Before the significance of the *p*H of the tissues was recognized, it was thought that self digestion of the tissue cells was prevented by the existence of *specific antienzymes.* Just as the serum contains antitrypsin, so it seems to contain substances antagonistic to the autolytic enzymes. Levene and Stookey found that tissue juices show a resistance to digestion, Yamakawa[20] found that serum autolysis is prevented by an antienzyme, and Opie found that the serum of inflammatory exudates retards the action of the autolytic enzymes that are contained within the leucocytes. Serum also inhibits autolysis of the tissues, and of fibrin,[75] so it is possible that continuance of the circulation may provide antibodies to the tissues to hold the intracellular enzymes in check, possibly without interfering with their action on other proteins than those of the cell structure.[76] (See Antienzymes.) However, it is highly probable that serum checks autolysis at least in part by virtue of its "buffer" function, which interferes with the development of acidity. Lack of oxygen cannot be held solely responsible for autolysis, according to the studies of Morse,[77] who found that autolysis occurs in muscles with divided nerves but intact blood supply. Nevertheless, reduced blood supply results in increased H-ion concentration which greatly facilitates autolysis, and it cannot be denied that autolysis is observed chiefly if not solely in asphyxiated tissues.

Jacoby,[78] found that to a certain degree the autolytic enzymes of each organ are specific for that organ. Liver extract will not split lung tissue, although it will split the proteoses that are formed in lung autolysis, possibly because these proteoses are less specific than the proteins from which they arise, or perhaps because of the erepsin the extract contains. Leucocytic proteases, however, seem capable of splitting foreign proteins of all sorts. Richet[79] states that the protease of liver tissue does not attack either muscle tissue or liver tissue that has been coagulated.

[75] See Rosemann, Biochem. Zeit., 1922 (129), 101; Klin. Woch., 1923 (2), 450.

[76] According to Guggenheimer (Deut. Arch. klin. Med., 1913 (112), 248; Deut. med. Woch., 1914 (40), 63), the serum in various diseases has a characteristic stimulating or inhibiting effect on *in vitro* tissue autolysis, but the conditions of such experiments are so complex as to make their significance doubtful.

[77] Amer. Jour. Physiol., 1915 (36), 147.

[78] Hofmeister's Beitr., 1903 (3), 446.

[79] Compt. Rend. Soc. Biol., 1903 (55), 656.

Possibly the enzymes exist in the cells in their inactive zymogen form, and are changed into the active form as needed, and inhibited or changed back again when their work is temporarily finished. A rhythmical change of this nature might be imagined as occurring and accounting for interaction by the enzymes, particularly since rhythmical changes in metabolism are known to occur (*e. g.*,) rhythmical production of carbon dioxide (Lyon[80]), and enzyme action *in vitro* may show rhythmic variations (Groll).[81]

With unicellular organisms the supply of food-stuff is of importance in determining autolytic changes, for it has been found by Conradi,[82] Rettger,[83] and Effront[84] that bacteria and yeasts begin to undergo autolysis when they are placed in distilled water or salt solution, which they do not do, to any such extent at least, when in nutrient media. Autolysis is not marked so long as the bacteria are supplied with nourishment, but when nutrient material is lacking, autolytic decomposition is no longer repaired and the bacteria disintegrate. Tissue enzymes are also capable of digesting bacteria (Turró).[85]

Autolysis in Pathological Processes

Absorption of dead or injured tissues, and of organic foreign bodies, seems to be accomplished by means of digestion by the enzymes of the cells and tissue fluids. We may distinguish between the digestion brought about by the enzymes of the digested tissue itself, or *autolysis*, and digestion by enzymes from other cells or tissue fluids, or *heterolysis* (Jacoby). Heterolysis is accomplished particularly by the leucocytes, which contain ferments capable of digesting not only leucocytic proteins but apparently every other sort,[86] from serum-albumin to catgut ligatures. The heterolysis may be intracellular when the material to be digested has first been taken up by the cells (phagocytosis); or extra-cellular, either by enzymes normally contained in the blood plasma and tissue fluids, or by enzymes liberated by the leucocytes and fixed tissue cells. On death and dissolution of a cell the intracellular enzymes are released,[87]

[80] Science, 1904 (19), 350.
[81] Nederl. Tijdschr. v. Geneesk., 1918 (1), 1085.
[82] Deut. med. Woch., 1903 (29), 26.
[83] Jour. Med. Research, 1904 (13), 79.
[84] Bull. Soc. Chim., 1905 (33), 847.
[85] Cent. f. Bakt., 1902 (32), 105.
[86] It has been suggested that the leucocytes merely carry enzymes from one organ, particularly the pancreas, to another, and that these enzymes are not formed by the leucocyte itself. Opie (Jour. Exp. Med., 1905 (7), 759) has shown, however, that the bone-marrow contains proteolytic enzymes which are like those of the leucocytes in that they act best in an alkaline medium, whereas the autolytic enzymes of the lymphatic glands and most other tissues act best in an acid medium. This leaves little room for doubt that the leucocytes are equipped with their characteristic enzymes when they leave the bone-marrow, and that they are not obtained later in the pancreas or elsewhere.
[87] Peptolytic enzymes appear in the urine after severe superficial burning, presumably coming from the disintegrated cells. (Pfeiffer, Münch. med. Woch., 1914 (61), 1329.)

but it is not known to what extent the enzymes may be secreted from intact living cells. As far as pathological processes show, the amount of liberation of enzymes from normal cells is very slight, if any, and the digestive enzymes of the blood plasma seem to be very feeble, but this is perhaps because they are largely held in check by the anti-enzymatic substances or the buffer action of the serum. Pathological autolysis and heterolysis, therefore, are brought about chiefly by enzymes liberated from dead or injured cells. Bacteria, however, can multiply upon a medium of coagulated protein, which suggests that they also secrete proteolytic substances. In pathological conditions digestion of degenerated tissues seems usually to be the result of both autolysis and heterolysis. An infarct softens because the intracellular enzymes digest the dead cells, exactly as they do when the tissue is removed from the body, ground up, and put in the incubator under toluene. In addition leucocytes wander in, disintegrate, and their liberated enzymes help in the process, as also do, to a less degree, the enzymes of the blood plasma. It is because of the heterolysis by leucocytic enzymes that a septic infarct becomes softened so much more rapidly than does a sterile infarct, and by comparing the rate of softening in septic and aseptic infarcts we see that the cellular autolysis is a very slow process as compared to the heterolysis accomplished by the leucocytes. The explanation of this may lie in the fact that most intracellular proteases act best in an acid medium, while leucocytic proteases act best in an alkaline medium, and the infarcts of small size are seeped through by alkaline blood fluids. When an infarct is large, we find it undergoing central softening while the periphery remains firm; this corroborates our hypothesis, for acids are developed during autolysis (Magnus-Levy), which at the periphery are neutralized by the blood plasma, so that only at the center is autolysis active. The inhibiting action of the serum also has a similar effect, limiting autolysis at the periphery. Necrotic areas of any kind are absorbed by similar processes.

Apparently all varieties of cells are subject to autolysis or heterolysis whenever they are killed or sufficiently injured. Involution of the uterus probably depends upon autolysis, which is much more active in the puerperal uterus (Ferroni[88]), and creatine is found in the urine when such autolysis occurs,[89] although A. Morse[88] considers this to be independent of the uterine autolysis. Bradley suggests that uterine involution results from local acidosis caused by shutting down of the blood supply, and a similar explanation is advanced for the atrophy of the lactating mammary gland produced by binding or unrelieved secretion pressure. The wasting of anemia and senility may be explained on a similar basis. Atrophy may be looked upon as an autolysis in the normal course of catabolism, not met by a corresponding building up of the proteins.

The solution of fibrin by tissues, *fibrinolysis*, is considered to be distinct from tissue autolysis by Fleisher and Loeb.[90] Rosenmann[75] says that the

[88] Ann. di Ostetrica e Ginecol., 1906 (2), 553; see also Slemons, Bull. Johns Hopkins Hosp., 1914 (25), 195; Arthur Morse, Jour. Amer. Med. Assoc., 1915 (65), 1613.

[89] Shaffer, Amer. Jour. Physiol. 1908 (23), 1.

[90] Jour. Biol. Chem., 1915 (21), 477.

fibrinolysin is contained in the globulin fraction of the plasma, the filtrate from the globulin being antifibrinolytic. In atrophic cirrhosis the fibrinolytic activity of the blood is increased, which may explain the hemorrhagic tendency of this disease.[91]

In the case of septic softening the action of the bacteria needs also to be taken into consideration, since they produce proteolytic ferments, but their effect seems to be relatively small as compared with leucocytic digestion.[91a] Phagocytosis and intracellular digestion of necrotic tissue by leucocytes seem also to be relatively unimportant. Suppuration, therefore, must be considered as the result of digestion of dead tissue by enzymes derived from the leucocytes, the plasma, the bacteria, and the destroyed cells themselves. A tubercle does not ordinarily suppurate, because the tubercle bacillus and the substances it produces are not strongly chemotactic, and hence not enough leucocytes enter the necrotic area to produce a digestive softening.

The products of autolysis may of themselves be toxic; albumoses and peptones certainly are, and the other cleavage products are probably not altogether innocuous. (See "Autointoxication.") Some of the symptoms of suppuration, particularly the fever and chills, have been ascribed to the autolytic products rather than to the bacterial poisons, particularly as aseptic suppuration is accompanied by fever. Jochmann[92] has found evidence that the protease of leucocytes can cause fever and also reduce the coagulability of the blood. The work of Vaughan and other students of the reaction to foreign proteins, shows that typical fevers can be produced by the enzymatic disintegration of proteins in the body.[93] Aseptic resorption of a ligated kidney is said to lead to toxic injury of the whole organism, most pronounced in the other kidney (Walthard).[94] Degenerative changes in nervous tissue are associated with autolytic decomposition of the lecithin (Noll[95]) and the liberated choline, or its more toxic derivatives, may be a source of intoxication.[96] In all conditions associated with autolysis, such as resolving pneumonic exudates, large abscesses, softening tumors, etc., albumoses (and peptones?) may appear in the urine. Autolytic products may also be hemolytic (Levaditi[97]), and they may prevent clotting of the blood (Conradi[98]). It is probable that among the products of autolysis are bactericidal substances,[99] although it is doubtful if the concentration is often sufficient for them to be of influence except in well walled areas.

[91] Goodpasture, Bull. Johns Hopkins Hosp., 1914 (25), 330.

[91a] The enzymes of staphylococcus are much more strongly proteolytic than those of streptococcus (Knapp, Zeit. f. Heilk. (Chir.), 1902 (23), 236) which may be one reason why the latter so much more frequently produces lesions without suppuration than does the former.

[92] Virchow's Arch., 1908 (194), 342.

[93] See Vaughan, "Protein Split Products," Philadelphia, 1913.

[94] Zeit. urol. chir., 1924 (15), 263.

[95] Zeit. physiol. Chemie, 1899 (27), 380.

[96] See Halliburton, Ergebnisse der Physiol., 1904 (4), 24.

[97] Ann. d. l'Inst. Pasteur, 1903 (17), 187; also Fukuhara, Zeit. f. exp. Path. u. Pharm., 1907 (4), 658.

[98] Hofmeister's Beitr., 1901 (1), 136.

[99] See Bilancioni, Arch. farmacol., 1911 (1), 491.

There is also much evidence that after extensive traumatism, the products of the tissue autolysis may be responsible for serious intoxication and possibly for conditions interpreted at times as *shock*.[1] The observations made in experimental anaphylaxis suggest that it is especially the slightly altered proteins, perhaps only changed in their colloidal properties, that are most likely to be responsible for these shock-like intoxications. However, it is also possible that amines derived from the amino-acids may be of importance in producing shock whenever tissues are injured.[2] Methyl guanidine may also be formed from disintegrating tissues and has considerable toxicity.

AUTOLYSIS IN DISEASE

Work has been reported upon autolytic processes in a number of pathological conditions, which may be discussed briefly as follows:

Autolysis in Exudates.—The presence of leucine, tyrosine, proteoses, and peptones in pus has been known for many years, and the reason for their appearance is now clear. Müller[3] long ago, observed that purulent sputum digested fibrin, but that non-purulent sputum did not have this property. Achalme[4] found that pus would dissolve gelatin, fibrin, and egg-albumen. Ascoli and Mareschi[5] detected autolysis in sterile exudates obtained experimentally. Umber[6] found that ascitic fluid exhibited autolytic changes, which observation could not be confirmed by Schütz[7] in pleural exudates and ascitic fluids. Zak[8] found that autolysis was inconstant in various exudates. The differences in these results are explained by Opie's[9] observation that in experimental inflammatory exudates the leucocytes are capable of marked autolysis, whereas the serum contains an inhibiting agent which holds this autolysis in check; if the inhibitor is destroyed by heat, then the serum proteins are also digested by the leucocytic enzymes. This inhibitor seems to be contained normally in the albumin of the blood-serum, and is apparently not a true antibody. In old exudates the inhibition is decreased, and autolysis then occurs, explaining the variable results of Umber, Schütz and Zak. The intracellular proteases of the polynuclear leucocytes act best in an alkaline medium; those of the mononuclears, in acid medium. If the proportion of serum to leucocytes is high, then there is no autolysis, as in serous exudates; but if the leucocytes are abundant, then the antibody is overcome and we get autolysis, as in ordinary suppurative exudates. Animals with but little protease in their leucocytes (*e. g.*, rabbits), do not ordinarily produce a liquid pus (Opie). Exudates produced by bac-

[1] Review by Cannon, Arch. Surg., 1922 (4), 1.
[2] See Abel and Kubota, Jour. Pharm. Exp. Ther., 1919 (13), 243.
[3] Kossel, Zeit. f. klin. Med., 1888 (13), 149.
[4] Compt. Rend. Soc. Biol., 1899 (51), 568.
[5] See Maly's Jahresbericht, 1902 (32), 568.
[6] Münch. med. Woch., 1902 (49), 1169.
[7] Cent. f. inn. Med., 1902 (23), 1161.
[8] Wien. klin. Woch., 1905 (18), 376.
[9] Full review in Physiol. Reviews, 1922 (2), 552.

terial infection also seem to possess the properties above described. Galdi[10] found autolysis greater in exudates than in transudates, but observed no constant relation between the number of leucocytes, or the amount of chlorides, and the rate of autolysis. All exudates, according to Lenk and Pollak,[11] contain enzymes splitting glycyl-glycine (peptolytic enzymes); the most active exudates are those of cancer and tuberculosis, the least active are passive congestion fluids; pleural exudates contain more active enzymes than peritoneal exudates of similar character.

Knapp[91a] holds that in pus the cocci and the enzymes they produce are responsible for much of the digestion. Pus cells alone do not undergo digestion so rapidly as when bacteria are present, and digestion is more rapid if the bacteria are alive then when inhibited or killed by antiseptics. Streptococcus is almost inactive, staphylococcus is quite active, and *B. coli* still more so. However, pus corpuscles free from bacteria are highly proteolytic, causing digestion in serum plates in dilutions of 1–700 (Jochmann). Knapp could find no relation between the autolytic power of the pus and the severity of the infection from which it resulted. A constant constituent of pus is d-lactic acid,[12] and it increases during autolysis; this may well modify the rate of autolysis of pus. (See also the discussion of the "Chemistry of Pus," Chap. XIII).

Proteolytic Enzymes of the Leucocytes.[13]—By the introduction of the plate method[14] of testing the proteolytic activity of leucocytes, Müller and Jochmann brought the study of this particular vital activity into the range of clinical laboratories, and aroused general interest in what had previously concerned only a few pathologists, especially E. L. Opie. The principle is that of permitting the leucocytes or other cells to act upon a blood serum plate at a temperature of 55°, which prevents bacterial action but permits the proteolytic enzymes of the cells to digest the coagulated serum, forming depressions in the surface ("Dellbildung"). This proteolytic activity is, of course, heterolysis rather than autolysis, and the method is much less accurate than the direct chemical determination of proteolysis by microchemical procedures.

Many modifications of the plate method have been introduced (such as using casein-agar), but the principle involved is the same, and they are fully explained and discussed by Wiens.[14] Normal blood does not contain enough leucocytes to cause observable digestion, but myelogenous leukemia blood causes distinct digestion while lymphatic leukemia does not, showing that it is the polynuclears and myelocytes that are responsible. Other observations fasten the proteolytic activity upon the neutrophile granules. The leucoprotease resembles trypsin in many respects, but also exhibits some differences. The large mononuclear macrophages

[10] See Folia Hemat., 1905 (2), 529.

[11] Deut. Arch. klin. Med., 1913 (109), 350. See also Wiener, Biochem. Zeit. 1912 (41), 149; Mandelbaum, Münch. med. Woch., 1914 (61), 461.

[12] Ito, Jour. Biol. Chem., 1916 (26), 173.

[13] See review by Opie, Physiol. Reviews, 1922 (2), 552.

[14] Full bibliography by Wiens, Ergebnisse Physiol., 1911 (15), 1; also Jochmann, Kolle and Wassermann's Handbuch, 1912 (2), 1301.

contain proteolytic enzymes active only in an acid medium (Opie), thus differing from the polymorphonuclear leucocytes. Apparently the blood plasma contains minute amounts of both types of proteases.

Leucocytes of normal human blood will, if concentrated enough, cause digestion of serum plates, as also, of course, will pus. The leucocytes of rabbits, guinea pigs, and practically all animals except man, apes and monkeys, are devoid of proteolytic activity demonstrable by the plate method. Normal serum, both homologous and heterologous, exercises a strong inhibition on this digestion, so that it is necessary to have an excess of leucocytes present to obtain the reaction. The activity of leucocytic autolysis is indicated by the observation that in drawn cerebrospinal fluid the leucocytes all disappear in from three to sixty days, and in 24 hours the count has been observed to drop from 392 to 6.[15] The leucocytic enzymes seem to be very resistant to chemicals, especially formaldehyde, so that museum specimens of leukemic tissues preserved in formalin for years are still proteolytic. Liver tissue is but slightly proteolytic by this test, spleen more so, and leucocyte-containing fluids, such as saliva and colostrum, are much more active. Pancreas tissue, has, of course, strong proteolytic action, but it is shown to be distinct from the leucocytic protease by being inhibited by certain sera that do not inhibit the leucocytic protease. In general, tissues do not cause much proteolysis of serum plates unless they are invaded by many leucocytes, which applies also to tumors, including multiple myelomas.

Besides proteases, leucocytes contain other enzymes.[16] To quote the summary by Morris and Boggs[17] "it has been shown that the normal and pathological neutrophile leucocytes and myeloblasts contain an oxidase and probably a lipase and an amylase; myeloblasts contain an amylase. In lymphoid tissue two proteases and a lipase have been shown to exist. In leukemia, leukoprotease has been demonstrated in the myeloid variety of the disease, while it has not been found in chronic lymphoid leukemia. Lipase has been demonstrated in two cases of myeloid leukemia, and oxidase in all myeloid cases observed in which the neutrophilic cells were present in excess." Jobling and Strouse,[18] confirming Opie's observation of two distinct proteases in leucocytes, find also evidence of an ereptic enzyme acting in either acid or alkaline fluids.[19]

Pneumonia.—In the stage of resolution, lobar pneumonia presents a striking example of autolysis. The often-remarked phenomenon that the lung tissue itself is not in the least affected, while the dense contents

[15] Bonaba, Anales de la Facultad de Med., 1919 (4), 111.

[16] According to Tschernoruzki (Zeit. physiol. Chem., 1911 (75), 216) amylase, diastase, catalase, peroxidase, and nuclease, but not lipase. I also found uricase absent from dog leucocytes (Jour. Biol. Chem., 1909 (6), 321). Fiessinger and Marie (Compt. Rend. Soc. Biol., 1909 (67), 177) state that the lymphocytes contain lipase, although myeloid cells do not. (See also Resch,. Deut. Arch. klin. Med., 1915 (118), 179). Leucocytes are also said to contain a "lipoidase" splitting choline from lecithin (Fiessinger and Clogne, Compt. Rend. Acad. Sci., 1917 (165), 730.

[17] Arch. Int. Med., 1911 (8), 806.

[18] Jour. Exp. Med., 1912 (16), 269.

[19] Concerning enzymes of normal leucocytes see also Fiessinger and Clogne, Ann. de Méd., 1917 (4), 445; Parker and Franke, Jour. Med. Res., 1917 (37), 345.

of the alveoli are rapidly dissolved and removed is explained by the invariable immunity of living cells to digestive enzymes. Except for some slight possible assistance by the alveolar epithelium and the enzymes of the serum, the enormous and rapid digestion of pneumonic exudates is accomplished by the leucocytic enzymes. The rapid rate of digestion may be accounted for by the absence of circulation within the alveolar contents, which permits the leucocytes to act unimpeded by the inhibitors of the blood plasma. Digestion of the exudate continues after death, accounting for the marked diffuse softening observed in pneumonic lungs in bodies kept some days before autopsy. As long ago as 1888, Kossel[20] mentioned that Fr. Müller had found that glycerol extracts of purulent sputum exhibited a digestive action upon fibrin and coagulated protein, whereas non-purulent sputum did not possess this property. In 1877 Filehne extracted ferments in the same way from the sputum in gangrene of the lung; Stolniknow, in 1878, found a similar ferment in pneumonic sputa, and Escherich in 1885 showed that the proteolytic action of tuberculous sputum was independent of putrefaction. Other early observations of similar nature are reviewed by Simon,[21] who demonstrated the presence of leucine and tyrosine in the autolyzed lungs. In a later work Müller reports finding three grams of leucine and tyrosine in a pneumonic lung, as well as lysine, histidine, and purine bases from the decomposed nucleoproteins. The appearance of free purines during autolysis of pneumonic lungs has been investigated by Mayeda,[22] Long and Wells.[23] Boehm[24] isolated histidine and arginine from similar material. Rietschel and Langstein[25] found 0.32 gm. leucine in the urine of a pneumonic child.

Flexner[26] noted that autolysis, while very rapid in the gray stage, is but slight in the red stage (because of paucity of leucocytes) and also in unresolved pneumonia, which he considers as due to some interference with autolysis. Silvestrini[27] found that in gray hepatization the reaction was strongly acid, in red, faintly so; the gray hepatization showed more peptone, and leucine and lactic acid were both demonstrable. A fibrin-digesting enzyme was isolated,[28] and milk was coagulated. Weiss[29] has reported finding a toxic albumose in gray pneumonic lungs.

Lord[30] has found in pneumonic lungs a proteolytic enzyme active at *p*H 7.3 to 6.7, but inactive at higher acidity; also an enzyme splitting peptone to amino acids and active at *p*H 8.0 to 4.8, but most active at 6.3 to 5.2. Since the enzymes of normal lung tissue are active only at about *p*H 4, it is probable that the enzymes concerned with lysis in pneumonia

[20] Zeit. f. klin. Med., 1888 (13), 149.
[21] Deut. Arch. klin. Med., 1901 (70), 604.
[22] Deut. Arch. klin. Med., 1910 (98), 587.
[23] *Ibid.*, 1914 (115), 377.
[24] *Ibid.*, 1910 (98), 583.
[25] Biochem. Zeit., 1906 (1), 75.
[26] Univ. of Penn. Med. Bull., 1903 (16), 185.
[27] Bull. del. Soc. Eustachiana, 1903, abst. in Biochem. Centralbl., 1903 (1), 713.
[28] Rosenmann (Biochem. Zeit., 1920 (112), 98) has also obtained a fibrinolytic enzyme from pneumonic lungs which he believes differs from leucocytic protease.
[29] Arch. Int. Med., 1919 (23), 395.
[30] Jour. Exp. Med., 1919 (30), 379

are largely derived from the leucocytes.[31] Lord, pictures resolution of pneumonic exudates as occurring in two stages: First, proteolysis while the reaction is nearly neutral, and later as the acidity increases the cleavage of the peptone increases. He also finds that pneumococci cannot long survive a reaction more acid than *p*H 6.8, and their dissolution takes place at reaction from 6.0 to 5.0, which is of significance in view of the observation that pneumonic lungs are more acid than normal organs, acidity as high as *p*H 6.0 to 5.4 having been found.[32]

Rzentkowski[33] found an increase of non-coagulable nitrogen in the blood of pneumonics, probably resulting from autolysis in the exudate.[34] According to Dick[35] the blood serum after the crisis contains an enzyme which acts specifically on the pneumococcus proteins. Petersen and Short[36] found an increase of serum ereptase in the blood preceding or accompanying crisis or lysis, and suggest that it may have a function in attacking the toxic protein fragments. In the liver from experimental pneumococcus septicemia, autolysis is said to be increased in rate.[37] Almagia[38] suggests that the bactericidal action of the products of fibrinolysis in pneumonia may be of importance in checking the disease.

Necrotic Areas.—Jacoby[39] found that if a portion of a dog's liver was ligated off and the animal kept alive for some time, the necrotic tissue contained the same products that he had obtained in experimental autolysis. The absorption of necrotic tissues generally is ascribable to either autolysis or heterolysis. Presumably there is no great difference in the self-digestion of an organ which is necrotic because its blood supply is cut off, and of a similar organ removed from the body aseptically and allowed to undergo aseptic autolysis in an incubator. At the periphery there might be some effects produced *in vivo* by the inhibitive action of the serum or the digestive action of the leucocytes, but beyond that no marked differences are to be expected. In both cases asphyxia is present, leading to increased acidity, without which little if any autolysis can occur. It has been found that in experimental infarction of the kidney there develops sufficient acidity to permit of autolysis.

A study of the relation of autolysis to the histological changes that occur in necrotic areas by Wells[40] gave evidence that there occurs early a decomposition of the nucleoproteins of the nuclei which is probably brought about by the intracellular autolytic enzymes. The liberation of the nucleic acid and the reduction in the bulk of nuclear material through the digestion away of the protein is probably the cause of the *pycnosis* observed in necrotic areas. Later the nucleic acids are further

[31] Nye, Jour. Exp. Med., 1922 (35), 153.
[32] Jour. Amer. Med. Assoc., 1919 (72), 1364.
[33] Virchow's Arch., 1905 (179), 405.
[34] Corroborated by Killian, Proc. New York Path. Soc., 1922 (22), 72.
[35] Jour. Infect. Dis., 1912 (10), 383.
[36] Jour. Infect. Dis., 1918 (22), 147.
[37] Medigreceanu, Jour. Exp. Med., 1914 (19), 31.
[38] Festschr. for Celli, Torino, 1913, p. 459.
[39] Zeit. physiol. Chem., 1900 (30), 149.
[40] Jour. Med. Research, 1906 (15), 149.

decomposed through the special enzymes described by Jones, Sachs, and others, the "nucleases." This is presumably the cause of the loss of nuclear staining so characteristic of necrosis. That these changes are due to the intracellular enzymes was shown by implanting in animals pieces of sterile tissues, the enzymes of which had been destroyed by heating; these were found to undergo alterations only after several weeks, and then as the result of the action upon them of invading leucocytes. The slow rate of autolysis that occurs in infarcts and other aseptic areas is presumably due in part to the action of the inhibitors of the serum, for it was found, experimentally, that the histological changes of autolysis when the tissues are placed in heated serum proceed about twice as rapidly as when they are placed in fresh serum. Chemotactic substances do not seem to be formed in aseptic dead tissues, but the slow absorption of such tissues is, however, finally accomplished by the leucocytes acting from the periphery, there being little actual autolysis of the dead cells by their own enzymes. The rapidity with which autolytic changes occur in different organs, as indicated by the disappearance of nuclear staining, seems to be about as follows: (1) Liver, kidney (epithelium of convoluted tubules); (2) spleen, pancreas; (3) kidney (collecting tubules, straight tubules, glomerules); (4) lung (alveolar and bronchial epithelium); (5) thyroid; (6) myocardium; (7) voluntary muscle; (8) skin (epithelium); (9) brain (cortical cells.) Stroma cells seem to be attacked chiefly by enzymes from the parenchyma cells. Of all cellular elements, the endothelium of the vessels seems to have the greatest resistance to both autolysis and heterolysis. The length of cell life after removal of tissues does not correspond to these degrees of autolysis, for Lewis and McCoy[41] found that brain cells lived but one hour, while tissue macrophages survived 240 hours and renal epithelium was relatively long lived.

The finer structural changes of aseptic autolysis of liver in salt solution, have been carefully studied by Launoy,[42] who notes a period of relative latency (20 to 24 hours at 38°), followed by rapid changes in both cytoplasm and nucleus, associated with the appearance of myelin forms. Dyson[43] describes loss of the Altmann's granules in autolyzing cells. Cruickshank[44] states that aseptic autolysis of tissues kept in a moist chamber is slower, and there is less solution of the cytoplasm, but in general the results are much the same. No fat could be found by special stains. Fetuses that have undergone aseptic autolysis in the uterus show complete loss of nuclei in 5 to 6 days, a stage corresponding to 8 to 15 days autolysis in the moist chamber. In experimental nephritis Simons[45] observed a decreased autolysis of the kidneys.

Degenerated *nervous tissue* also undergoes a slow autolysis which, according to Noll,[46] results in the splitting of "protagon" with liberation

[41] Bull. Johns Hopkins Hosp., 1922 (33), 284.
[42] Ann. Inst. Pasteur, 1909 (23), 1.
[43] Jour. Path. and Bact., 1912 (17), 12; also Aschoff, Verh. deut. Path. Gesellsch., 1914 (17), 109, and Oka, Virchows Arch., 1920 (228), 200.
[44] Jour. Path. and Bact., 1911 (16), 167.
[45] Biochem. Zeit., 1914 (67), 483.
[46] Zeit. physiol. Chem., 1899 (27), 390.

of lecithin. Mott, Halliburton,[47] Donath, and others have shown that in nerve destruction lecithin is split up with liberation of choline (see "Choline"). Koch and Goodson[48] found that degenerated nervous tissue is characterized, chemically, by containing a relatively increased amount of nucleo-proteins, with an absolute decrease in solid constituents, while the lecithins are greatly altered.

In *caseation*,[49] autolysis is very slight, as is shown by the persistence of the caseous material for long periods of time without absorption. Presumably the toxin of tuberculosis destroys the autolytic ferments of the cells it kills,[50] and as there is little chemotactic influence, leucocytes do not enter the caseous area. Jobling and Petersen[51] find evidence that the soaps of unsaturated fatty acids present in tubercles are responsible for the inhibition of digestion. Spiethoff[52] found that pure caseous material is usually free from even traces of albumose and peptone, but the caseous material at the periphery mixed with tissue elements contains them in very small quantities, suggesting that at the periphery of caseous areas some slight autolysis does occur. The fact that *B. tuberculosis* is, itself, very poor in proteolytic enzymes as compared with most other bacteria may be another factor. When leucocytes are attracted into a tuberculous focus softening goes on rapidly, showing that there is no loss of digestibility of the caseous material, but merely a lack of enzymes. Pus from a cold tuberculous abscess will not digest fibrin, but if iodoform is injected, leucocytes enter in great numbers, softening is rapid, and the pus will then digest fibrin (Heile[53]). On serum plates tuberculous pus produces no digestion unless a secondary infection or other cause has resulted in a local accumulation of leucocytes.[14] Tuberculous material contains, like the lymphocytes, an enzyme which is proteolytic in acid media and which is inhibited by normal serum (Opie and Barker[54]).

Correlation of Histological and Chemical Changes.—A careful study of the relationship of the chemical changes produced by autolysis, to the histological changes of necrosis and autolysis, has been made by H. J. Corper,[55] and colored plates published together with analytical figures make it possible to correlate the structural and chemical changes of necrobiosis. Corper found that in the early stages, characterized by a high grade of pycnosis but no further nuclear changes, the nucleins are still intact; but with well developed karyorrhexis and beginning karyolysis some 10% of the nuclein nitrogen has become soluble in the form of purine bases. When karyolysis is completed so that no more nuclei remain in a stainable condition, only 28% of the nucleo-proteins was found to have been decomposed to free purine bases,[56] the remaining 72% being intact although unstainable. This observation indicates that the stainable chromatin represents but about one-fourth of the nucleins

[47] General résumé in Ergebnisse der Physiol., 1904 (4), 24.

[48] Amer. Jour. Physiol., 1906 (15), 272.

[49] More fully discussed in "Chemistry of Tuberculosis," Wells, DeWitt and Long, Baltimore, 1923.

[50] However, Pesci (Pathologica, 1912 (3), 144) states that tuberculin increases autolysis *in vitro*.

[51] Jour. Exp. Med., 1914 (19), 383.

[52] Cent. f. inn. Med., 1904 (25), 481.

[53] Zeit. klin. Med., 1904 (55), 508.

[54] Jour. Exper. Med., 1909 (11), 686.

[55] Jour. Exper. Med., 1912 (15), 429.

[56] Marshall (Jour. Biol. Chem., 1913 (15), 81) has also found that much of the nucleic acid remains unaltered in autolysis of thymus.

of the cell, which is in accord with the views of Hammarsten and others. The lecithin disintegrates somewhat more completely, about one-half or two-thirds being disintegrated by the time nuclear destruction is complete, after which this and all other autolytic change is slow. The change from coagulable to non-coagulable forms of nitrogen was as follows: Normal spleen, non-coagulable nitrogen, 5.7 per cent. of the total; stage of marked pycnosis, without rhexis or lysis, 7.4 per cent.; stage of karyorrhexis and early karyolysis, 26.5 per cent.; stage of complete karyolysis, 30.3 per cent. That is, when nuclear structures in the spleen have lost their staining properties entirely through autolysis, about 72 per cent. of the nuclein nitrogen, 50 per cent. of the insoluble phosphorus compounds, 70 per cent. of the coagulable nitrogen and about two-thirds of the lecithin are still intact.

Liver Degenerations.—The relation of the disintegration observed in *phosphorus-poisoning* and *acute yellow atrophy* to the experimental autolysis of the liver has been the object of much study. Salkowski originally pointed out that the same products were found in the blood, urine, and liver tissue in acute yellow atrophy as are produced in autolysis. Jacoby[57] found that the livers of dogs, taken just as the animals were dying of phosphorus-poisoning, contained free leucine and tyrosine; also, he found that the rate of autolysis of such livers after removal from the body was much geater than in normal livers. The oxidizing ferments (aldehydase) are not destroyed by the process. He found that addition of minute amounts of phosphorus to liver enzymes did not increase their proteolytic power; nevertheless, he seems inclined to assume that in phosphorus-poisoning alteration in the autolytic enzymes is an important factor in the liver degeneration. It would seem much more probable that phosphorus is a poison that kills cells and does not destroy their autolytic enzymes, hence favoring autolysis. The liver degeneration following chloroform poisoning may, perhaps, be explained in a similar way, the cells behaving exactly as bacteria would do under the same conditions. Taylor[58] has analyzed several livers in degenerative conditions for amino-acids and found them in only one liver, which showed necrosis probably due to chloroform poisoning, and which was from a case clinically resembling acute yellow atrophy. Here he obtained 4 gm. of leucine, 2.2 gm. of tyrosine, and 2.3 gm. of arginine nitrate. Waldvogel and Tintemann,[59] in phosphorus livers, found an increase in protagon, jecorin, fatty acids, cholesterol, and neutral fat, while lecithin was decreased. Wakeman[60] found arginine, histidine, and lysine decreased in phosphorus livers in proportion to the total nitrogen, indicating that the protein-splitting enzyme in this condition either picks out certain varieties of proteins first, or removes the nitrogen-rich constituents most rapidly.[61]

[57] Zeit. f. physiol. Chem., 1900 (30), 174.
[58] Univ. of Calif. Public. (pathol.), 1904 (1), 43.
[59] Cent. f. Path., 1904 (15), 97.
[60] Berl. klin. Woch., 1904 (41), 1067.
[61] Considerable quantities of amino-acids of various sorts have been isolated from the liver in acute yellow atrophy and chloroform necrosis by Wells (Jour. Exper. Med., 1907 (9), 627; Jour. Biol. Chem., 1908 (5), 129); but the value of these figures is questionable because it is possible that the alcohol in which the tissues were kept before analysis was not strong enough entirely to prevent autolysis (Wells and Caldwell, Jour. Biol. Chem., 1914 (19), 57).

It is probable that many poisons may injure the liver cells to such an extent that they cannot maintain their normal chemical equilibrium, but without destroying the autolytic enzymes. When this occurs, the liver undergoes autolysis, and we get marked degenerative changes with appearance of amino-acids in the blood and urine, reduction in coagulability of the blood and numerous hemorrhages, giving a picture both clinically and anatomically more or less like that of typical acute yellow atrophy. Chloroform is a poison that stops cell activities without destroying the proteolytic enzymes, hence the cells undergo autolysis, and, as a result, we have many cases of what appears to be acute yellow atrophy following chloroform anesthesia. The liberation of HCl in the liver cells during chloroform poisoning, as demonstrated by Evarts Graham,[62] may be largely responsible for the rapid disintegration of the liver in this condition.[63] (See "Acute Yellow Atrophy," Chap. XXII.) Probably the liver changes in puerperal eclampsia, and in streptococcus and other septicemias are of a similar nature.[64] Autolysis of fatty livers in tuberculosis is said to yield more lactic acid than the livers from other conditions (Youssouf),[65] and a high glycogen content seems to protect livers against autolysis.[66]

Postmortem changes are undoubtedly due to two factors, bacterial action and autolysis. Postmortem autolysis presumably depends on the asphyxial acidity which begins rapidly after death.[67]

In tissues kept at a low enough temperature to exclude bacterial action, but not so low as absolutely to stop enzyme action,[68] there occurs a slow autolysis; this constitutes the "ripening" process of meat. Fish flesh may also ripen when made sterile in saturated salt solutions, as Schmidt-Nielsen[69] has shown occurs with salted herrings, oxy-acids and xanthine bases being prominent among the products. The softening of muscles in rigor mortis is probably also an autolytic manifestation, as muscles contain proteases acting best in acid medium, and the muscle is known to become increasingly acid after circulation ceases within it. The short duration of rigor mortis when the body is kept warm, and its early disappearance when death has been preceded by muscular exhaustion (which increases the acidity), agree with this view. The early postmortem softening of many organs in pathological conditions is also probably an autolytic manifestation. Flexner[26] has called attention to this in relation to the softening of the parenchymatous organs in acute infectious diseases, such as typhoid and septicemia. Schumm noted great autolytic activity in a swollen spleen from a case of perityphlitis.

[62] Jour. Exp. Med., 1915 (22), 48.

[63] Quinan (Jour. Med., Res., 1915 (32), 73) found no change in the rate of *in vitro* autolysis of liver tissue from experimental chloroform poisoning. It was found increased by phlohrizin (Satta and Fasiani, Arch. di. Fisiol., 1913 (11), 391).

[64] Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

[65] Virchow's Arch., 1912 (207), 374.

[66] Simonds *et al.*, Jour. Med. Res., 1921 (42), 455.

[67] Sevringhaus *et al.*, Jour. Biol. Chem., 1923 (57), 163.

[68] Some chemical change may take place at temperatures as low as −2 and −14 (Costantino, Arch. farm. sper., 1917 (24), 255).

[69] Hofmeister's Beiträge, 1903 (3), 267.

Histological changes are produced by autolysis in the organs after death that are, as might be expected, much like those seen in necrotic areas.[70] At first the changes resemble those of parenchymatous degeneration (cloudy swelling), and often there is an apparent increase in fat, which is probably due to liberation of masked fat through the destruction of the protein.[71] Nuclear staining is lost (karyolysis), and eventually even cell forms become indistinguishable.

Still-born children that have been carried for some time after death usually show considerable disintegration of the viscera, especially the liver. This is undoubtedly due to autolysis, which Schlesinger[72] has shown can begin before birth if the fetus dies *in utero*.

Autolysis in Relation to Infection.—According to Conradi[73] the substances produced in tissue autolysis have a decided inhibiting effect upon bacteria, which apparently depends upon the antiseptic properties of the aromatic derivatives that are split out of the protein molecule in autolysis. This action is manifested not only *in vitro*, but the autolytic products will also render harmless lethal doses of certain bacteria, if they are injected simultaneously with the bacteria into an animal. One specific class of products of autolysis which is strongly bactericidal is the soaps.[74] It may well be questioned, however, whether enough of these substances ever accumulates in infected tissues during *intra vitam* autolysis to have much effect upon the infecting bacteria; yet this property may possibly explain the sterilization of old pus collections and similar infected localized accumulations within the body, including tubercles.[75] The bacteria themselves also produce autolytic products that are powerfully bactericidal. (See "Bacteria," Chap. IV.)

It is quite possible that bacterial toxins may be destroyed by autolytic enzymes, for Baldwin and Levene[76] have shown that trypsin, pepsin, and papain destroy tetanus and diphtheria toxin, while tuberculin is destroyed by trypsin, but not readily by pepsin, possibly because it is of a nucleoprotein nature. The leucocytic proteases, however, seem not to attack either toxins or living bacteria (Jochmann). Bertolini[77] states that autolyzing liver will destroy diphtheria toxin. On the other hand, there are many pathogenic bacteria which do not secrete their toxic materials, but store them up within the cell body, *e. g.*, typhoid, cholera, and, indeed, the majority of pathogenic forms. These *endotoxins* are probably liberated from the bacteria only through digestion of their cells, either by their own autolytic enzymes or by the enzymes of the infected tissues and leucocytes.

[70] More fully discussed by Wells, Jour. Med. Research, 1906 (15), 149.

[71] Siegert (Hofmeister's Beitr., 1901 (1), 114) found no actual increase in fats and fatty acids in autolysis even when an increase was apparent histologically, although ether-soluble materials of other nature than fat may be increased. See also Hess and Saxl, Virchow's Arch., 1910 (202), 149.

[72] Hofmeister's Beitr., 1903 (4), 87.

[73] Hofmeister's Beitr., 1901 (1), 193. See also Bilancioni[99] and Almagia[38].

[74] See Lamar, Jour. Exp. Med., 1911 (13), 1.

[75] See Corper *et al.*, Amer. Jour. Hyg., 1924 (4), 77.

[76] Jour. Med. Research, 1901 (6), 120. See also Blum, Hofmeister's Beitr., 1904 (5), 142.

[77] Biochem. Zeit., 1913 (48), 448.

Leukemia.—The abundant elimination of uric acid and other purine bodies in the urine in leukemia testifies to the great amount of destruction of nucleoprotein that is going on in the autolysis of leucocytes, which perhaps depends on the relatively large proportion of leucocytes to serum. Schumm[78] has found that leukemic spleens and bone marrow autolyze rapidly and completely, and he isolated many of the cleavage products of protein digestion from such autolysates. Leucocytes from myeloid leukemia liquefy alkaline gelatin vigorously, but those from lymphatic leukemia do not; the liquefaction is inhibited by normal serum (Stern and Eppenstein).[79] By the plate method this observation has been much extended, and the heterolytic action of the leucocytes has been found limited to the neutrophile granules. In neutral media, evidence is obtained of the presence of protease in the lymphocytes of chronic lymphatic leukemia and the leucocytes of acute and chronic myeloid leukemia; maltase, lipase and amylase are found in both types of cells, and oxidase in the granular cells derived from the marrow (Morris and Boggs).[80] v. Jaksch,[81] Erben,[82] and others have noted the occurrence of peptones and albumoses in leukemic blood, particularly if removed postmortem. The improvement in leukemia that follows *x*-ray treatment is associated with an increased nitrogen elimination, probably due to autolysis of disintegrating cells,[83] although *x*-rays have no appreciable effect upon the leucocytic proteases *in vitro* (Müller and Jochmann). (See also "Leukemia," Chap. XV.)

Tumors.—Probably because of the great amount of necrosis that is constantly going on in all malignant growths, with subsequent digestion of the dead cells, autolytic products are present in them in very considerable amounts. This was first demonstrated by Petry,[84] who found that carcinomas of the breast contained much of their nitrogen in compounds not coagulated by heat, while in the normal gland practically all is coagulable. He also demonstrated an autolytic property in tumor tissue, showing that tumor cells do not differ in this respect from normal cells. Beebe[85] found products of autolysis constantly present in several tumors; namely, a carcinoma of the broad ligament, a hypernephroma, an angiosarcoma, and a round-cell sarcoma.

Neuberg[86] found that while, according to other observers, most enzymes, as well as bacteria, are very susceptible to the action of radium rays, the autolytic enzymes of cancer cells are an exception, for cancer

[78] Hofmeister's Beitr., 1903 (3), 576; 1905 (7), 175.

[79] See discussion of leucocytic enzymes, p. 79. Longcope and Donhauser (Jour. Exper. Med., 1908 (10), 618) found proteases in the large lymphocytes in acute leukemia, which were most active in an alkaline medium.

[80] Arch. Int. Med., 1911 (8), 806.

[81] Zeit. f. physiol. Chem., 1892 (16), 243.

[82] Zeit. f. klin. Med., 1900 (40), 282; Zeit. f. Heilkunde, 1903 (24), 70; Hofmeister's Beitr., 1904 (5), 461.

[83] Musser and Edsall, Univ. Penn. Med. Bull., 1905 (18), 174.

[84] Zeit. f. physiol. Chem., 1899 (27), 398; Hofmeister's Beitr., 1902 (2), 94.

[85] Amer. Jour. Physiol., 1904 (11), 139.

[86] Zeit. f. Krebsforschung, 1904 (2), 171; Berlin. klin. Woch., 1904 (41), 1081; *ibid.*, 1905 (42), 118; Arb. Path. Inst. Berlin. 1906, p. 593.

tissue exposed to radium undergoes autolysis much faster than cancer tissue not exposed to radium; *x*-rays are less active in this respect. He attributes the effects of radium on cancer to its deleterious effects on the oxidizing and other enzymes of the cells, inhibiting their activities, which results in destruction of the cells by the autolytic enzymes.[87] A cancer of the stomach was found to contain autolytic enzymes capable of digesting lung tissue (pepsin was excluded) and autolyzed cancers yielded much pentose. Blumenthal and Wolf[88] believe that tumor tissues have particularly active autolytic enzymes, since liver tissue added to tumor tissue underwent autolysis much more rapidly than normal; but tumors do not cause digestion of serum plates unless many leucocytes are present (Müller and Kolaczek).[89] Active ereptase has been found in tumors.[90] Cancer extracts digest peptids in ways different from normal tissues, which seems to indicate some fundamental abnormality in their metabolism (Abderhalden,[91] Neuberg).[92] The almost constant presence of ereptases hydrolyzing proteoses and peptids, in gastric juice of patients with carcinoma of the stomach, is generally attributed to the disintegration of the cancer with liberation of these enzymes.[93] Tumors also contain nuclease[94] to disintegrate their nucleic acid, and the same outfit of purine-splitting enzymes as normal tissues,[95] so that in regard to the nucleoproteins of tumors, autolysis follows the same course as in normal tissues.

The non-cancerous livers of cancerous patients were found by Youssouf[65] to produce more lactic acid during antiseptic autolysis than did livers in other conditions. Autolysis of organs of cancer patients is about as rapid as normal (Colwell).[96] Several observations have suggested that tumor tissues might contain proteolytic enzymes differing from those of normal tissues especially in their ability to digest heterologous normal tissues, but at present this work needs confirmation and amplification before it can carry the weight of speculation which has been heaped upon it.[97]

Micheli and Donati[98] attribute the hemolytic properties possessed by extracts of malignant tumors to the products of autolysis that are present,

[87] Wohlgemuth, (Berl. klin. Woch., 1904 (41), 704), found that autolysis in tuberculous lung tissue was three or four times more rapid when exposed to radium rays. Heile (Arch. klin. Chir., 1905 (77), 107) looks upon the favorable effects of *x*-rays as partly produced by their liberation of autolytic enzymes from the leucocytes.

[88] Med. Klinik, 1905 (1), 166.

[89] Müller and Kolaczek, Münch. med. Woch., 1907 (54), 354; Hess and Saxl, Wien. klin. Woch., 1908 (21), 1183; Kepinow, Zeit. f. Krebsforsch., 1909 (7), 517.

[90] Loeper *et al.*, Compt. Rend. Soc. Biol., 1920 (83), 993.

[91] Zeit. physiol. Chem., 1910 (66), 277.

[92] Biochem. Zeit., 1910 (26), 344.

[93] See Jacques and Woodyatt, Arch. Int. Med., 1912 (10), 560; Hamburger, Jour. Amer. Med. Assoc., 1912 (59), 847.

[94] Goodman, Jour. Exp. Med., 1912 (15), 477.

[95] Wells and Long, Zeit. Krebforsch., 1913 (12), 598.

[96] Arch. Middlesex Hosp., 1910 (19), 55. The autolysis of lung tissue is not appreciably altered by the presence of cancer either in the lung or elsewhere in the body. (Walch-Sorgdrager, Inaug. Diss. Amsterdam, 1918.)

[97] See, for example, Rulf, Zeit. Krebsforsch., 1906 (4), 417; Müller, Cent. inn. Med., 1909 (30), 89.

[98] Riforma med., 1903 (19), 1037.

which Petry has also demonstrated to produce hemolysis. Emerson[99] attributed the disappearance of HCl from the gastric juice in carcinoma of the stomach to neutralization by basic products of autolysis, an hypothesis that may well be questioned. (See also "Tumors," Chap. XXI.)

Various other intracellular enzymes have been described, which for the most part have as yet no significance in pathology. An exception is *fibrin ferment*, which will be considered fully in discussing thrombosis. Ferments coagulating milk seem to be widely spread in the tissues. The precipitation of plastein from proteose solution by organ extracts (Nürnberg) may be either the effect of a coagulating ferment or due to reverse action of the proteases. Ferments splitting specifically maltose, lactose, sucrose, glucosides, and nucleoproteins have been described, and the glycogenolytic ferment is probably nearly universally present. Other enzymes decomposing amino-acids into ammonium compounds may also exist. The enzymes acting specifically upon the nucleic acids and the purine bodies are discussed in Chapter XXV.

[99] Deut. Arch. klin. Med., 1902 (72), 415.

CHAPTER IV

THE CHEMISTRY OF BACTERIA AND THEIR PRODUCTS

STRUCTURE AND PHYSICAL PROPERTIES[1]

In structure, as in nearly all other respects, bacterial cells stand intermediate between the cells of ordinary plant and animal tissues. Their cell wall seems to be generally more highly developed than that of animal cells, and less so than the wall of most plant cells. The much vexed question as to the existence or non-existence of a nucleus seems to be best answered by Zettnow, who considers that the portion of the bacterial cell usually made evident by ordinary staining methods consists of a mixture of nuclear substance (*chromatin*) with non-chromatic substance (*endoplasm*); the outer membrane, which requires special methods for its satisfactory demonstration, consists of a modified cytoplasm (*ectoplasm*). Some bacteria consist chiefly of chromatin (*e. g. vibrios*), but the proportion of the different elements varies greatly, not only in different varieties, but also in the same variety under different conditions. The fact that the chromatin is not aggregated into the usual nuclear form may be ascribed to the low stage of development reached by bacteria in the scale of evolution; or, as Vejdovosky has suggested, to the extremely rapid rate of cell division in the bacteria which prevents the chromatin from appearing in the resting stage which a nucleus constitutes. Putter[2] found that the electrophoretic behavior of bacteria corresponds closely to that of the nuclei of animal cells. Finer structures within the bacterial cell have as yet been only imperfectly discerned. Nevertheless, it must be recognized that a bacterial cell is a complete living creature, carrying out all the complicated processes of life, growth, and reproduction, apparently exhibiting both youth and old age,[3] and therefore presumably of more complex structure and composition than it seems to be. An important feature in all bacterial life processes is the relatively enormous ratio of surface to volume,[4] so that they exhibit some of the properties of suspension colloids.

The thickness of the ectoplasm varies greatly even in the same species, being generally greatest in older cultures. In some forms the ectoplasm

[1] In this chapter references will not generally be given that can be found by consulting Kolle and Wassermann's Handbuch. The early literature of the Biology of the Bacteria, including references to the effects of light, heat, osmotic pressure, etc., is given by Müller, Ergb. der Physiol., 1904 (4), 138; concerning their chemistry see H. Fischer, Lafar's Handbuch der Technischen Mykologie, 1908 (1), 222.

[2] Zeit. f. Immunität., 1921 (32), 538.

[3] See Sherman and Albus, Jour. Bact., 1923 (8), 127.

[4] See review on Bacterial Metabolism by Kendall, Physiol. Reviews, 1923 (3), 438.

may constitute one-half of the total mass of the cells. The capsule seems to arise through a swelling of the ectoplasm, and is probably present in at least a rudimentary stage in all bacteria (Migula). In common with other cells, bacteria increase the resistance to the electric current offered by the fluid in which they are suspended. This is somewhat decreased when the bacteria are killed, because of exosmosis of salts into the fluid and because of a decrease in the size of the cells.[5]

Plasmolysis and Plasmoptysis.—Under conditions of altered osmotic pressure the bacterial cell behaves quite similarly to the plant cell.[6] If placed suddenly in a solution of higher osmotic pressure than the one in which it has been, the cell contents shrink away from the cell wall (*plasmolysis*) indicating that there exists a semipermeable membrane through which water passes more rapidly than salts. If the change in osmotic pressure is gradual, the bacteria accomodate themselves to it by the slow diffusion of the salts through the cell membrane, indicating that it is not absolutely semipermeable. Different bacteria behave differently, some bacteria not being plasmolyzed by solutions that plasmolyze others. As a rule, old bacteria plasmolyze more rapidly than young, and in some varieties there seems to be a spontaneous plasmolysis, to which has been attributed the irregular staining of diphtheria and tubercle bacilli, the polar staining of plague bacilli, etc. Plasmolysis occurs only in living bacilli, but does not necessarily cause death. The Gram-staining bacteria cannot generally be plasmolyzed, and contain more water.[7]

When bacteria pass from solutions of higher osmotic concentration into solutions of lower concentration, the phenomenon of *plasmoptysis* is produced. The cell contents swell until the cell wall gives way at some point, and then exude as glistening drops, which may become detached from the wall and escape free into the fluid. Plasmoptysis is shown best by bacteria that have been grown on salt-rich media before being placed in the salt-free fluid. Not all varieties of bacteria can be made to undergo this change, depending probably upon the degree of permeability of their cell membranes for salts. The exposure of the naked cell contents to the hypotonic fluid outside the cells makes plasmoptysis more serious for bacterial life than plasmolysis, but how often, if ever, either process plays a part in the resistance of infected animals against bacteria is unknown. The resistance of bacteria to direct pressure is striking; spore bearers may not be killed under direct pressure of 12,000 atmospheres for 14 hours, and non-spored bacteria resist 3,000 but not 6,000 atmospheres.[8]

Chemotaxis.[9]—Just as with unicellular animal organisms, bacteria respond to chemotactic influences, in general being attracted by substances favorable for food, such as peptone, amino acids,[10] dilute potas-

[5] Green and Larson, Jour. Infect. Dis., 1922 (30), 550.
[6] Literature, see Gotschlich, Kolle and Wassermann's Handbuch, vol. 1.
[7] Nicolle and Alilaire, Ann. Inst. Pasteur, 1909 (23), 547.
[8] Larson, Hartzell and Diehl, Jour. Infect. Dis., 1918 (22), 271.
[9] Concerning the adsorption of bacteria see Bechhold, Kolloid-Zeitschr., 1918 (23), 35.
[10] Pringsheim and Ernst, Zeit. physiol. Chem., 1916 (97), 176.

sium salts, etc., and being repelled by harmful substances, such as strong acids and alkalies. Attempts have been made to separate different organisms in mixed cultures by means of their response to chemotaxis, but without striking success. It is possible that chemotaxis may play a part in the localization of bacteria from the blood stream in favorable localities, just as leucocytes are attracted to points of injury, but this has not be demonstrated.[11] (The chemotactic influence of bacteria upon leucocytes is discussed in Chapter XIII.)

CHEMICAL COMPOSITION

This varies greatly, not only between different species, but even in the same species grown on different media;[12] in this respect bacteria are much more modified by their environment than are higher organisms. On the other hand, they can develop in solutions containing only a few of the simplest organic and inorganic compounds and synthesize the complex components of their cells, as well as enzymes, toxins, and pigments.[13] They usually contain between 80 and 90 per cent. of water. Grown on a salt-rich medium they yield much ash;[14] grown on a peptone-rich medium they contain much protein; grown on a fat-rich medium they contain much material soluble in ether. Cholera vibrios grown on a bouillon medium contained 69.25 per cent. of protein, and 25.87 per cent. of ash, whereas the same organism grown on Uschinsky's medium, which contains no proteins but only various simple chemical compounds, contained but 35.75 per cent. of protein and 13.7 per cent. of ash (Cramer). Even in the same medium two different strains of the same organism may show equally great differences: Two strains of cholera vibrios grown on the same medium showed respectively 65.63 per cent. and 34.37 per cent. of protein. It is evident, therefore, that quantitative analyses of bacteria show nothing as to their nature, and on account of the extreme limits of their variation are practically valueless. The specific gravity of bacteria, generally between 1.12 and 1.345, also varies with media and age.[15] In an electric field they move towards the anode,[16] they can be discharged by strong acids and the charge is reversible only by trivalent cations (Putter),[17] wherein they behave electrically quite like the nuclei of higher organisms. According to Winslow, Falk, and Caulfield[18] the isoelectric point is at about *p*H 3, the migration becom-

[11] Concerning the surface tension and other physical properties of bacteria, see Gildemeister, Cent. f. Bakt., 1919 (83), 497; Rideal, Brit. Assoc. Adv. Sci., 1923, p. 31; Tinti, Zeit. Immunität., 1923 (37), 337.

[12] See Dawson, Jour. Bact., 1919 (4), 133.

[13] Concerning fundamentals of nutrition of bacilli see Koser and Rettger, Jour. Infect. Dis., 1919 (24), 301; Long, Amer. Rev. Tuberc., 1919 (3), 86.

[14] Concerning the relation of bacteria to inorganic salts see review by Falk, Abstr. of Bact., 1923 (71), 33.

[15] Stigell, Cent. f. Bakt., 1907 (43), 487.

[16] Buxton, Zeit. physikal. Chem., 1906 (57), 47; Girard and Audubert, Compt. Rend. Acad. Sci., 1918 (167), 301. Concerning the *electrical conductivity* of bacteria see Thornton, Proc. Royal Soc., London, Sec. B., 1913 (85), 331.

[17] Zeit. f. Immunität., 1921 (32), 538.

[18] Jour. Gen. Physiol., 1923 (6), 177.

ing reversed at greater acidity until it disappears at *p*H 1; at reactions less acid than *p*H 3 the velocity is towards the anode, increasing with increasing alkalinity to *p*H 10. Falk has also found that the electrophoretic charge is generally greater in young than in old cultures.

Qualitatively the variations in composition are not great—all bacteria contain proteins, lipoid substances, and salts, of which phosphates are most prominent in the ash. According to Guillemin and Larson[19] part of the salts is fixed in the protoplasm in approximately constant quantities, and part is free, serving to modify the osmotic pressure, and varying much with the composition of the surrounding fluids. The character of the proteins and fats of bacteria grown on peptone bouillon is quite the same as when they are grown on protein-free media.[20] The older analyses of bacterial constituents are of little value. Later studies suggest that the chief constituent of the cell contents is a true nucleoprotein (Iwanoff[21]) containing some sulfur and iron; probably many of the "pyogenetic proteins," "bacterial toxalbumins," "bacterial caseins" of earlier investigators are either nucleoproteins,[22] or phosphoproteins, for in tubercle bacilli Johnson and Brown found much phosphorus in the protein fraction free from nucleic acid. The stainable substance of anthrax bacilli behaves as if it were a chromatin, while the spores resemble linin (Růžička).[23] Schumacher[24] found evidence that the polar granules of diphtheria bacilli are composed of free nucleic acid. Mary Leach[25] reported that the colon bacillus is largely made up of nuclein or glyco-nucleoproteins, but contains no cellulose.

The predominance of nuclein compounds in bacteria was also suggested by Ruppel's summary of the composition of dried tubercle bacilli; namely, in per cent., tuberculonucleic acid, 8.5; nucleo-protamine, 24.5; nucleo-protein, 26.5; fatty matter, 26.5; inorganic, 9.2; insoluble "proteinoid" residue, 8.3. Toenniessen[26] also found that 10 per cent. of the total nitrogen is purine nitrogen. However, Long's[27] analysis of tubercle bacilli showed a surprisingly low purine content, indicating that the nucleic acid content is less than had been supposed, amounting to only 2 to 3.5 per cent. Presumably much of the relatively high phosphorus content of bacteria must be in other forms than nucleic acid. In a water bacillus, Nishimura found xanthine, guanine, and adenine, indicating the presence of nucleoprotein; others have found that bacterial nucleoproteins split off pentoses, as do the nucleoproteins of higher cells.

[19] Jour. Infect. Dis., 1922 (31), 349.
[20] Tamura, Zeit. physiol. Chem., 1913 (88), 190.
[21] Hofmeister's Beitr., 1902 (1), 524; bibliography by Lustig, Kolle and Wassermann's Handbuch, 1913 (II), 1362.
[22] The purity of many of the preparations worked with as bacterial nucleoproteins, is very doubtful. (See Wells, Zeit. Immunität., 1913 (19), 599.)
[23] Arch. Entwicklungsmk., 1906 (21), 306.
[24] Cent. f. Bakt., 1922 (88), 362.
[25] Jour. Biol. Chem., 1906 (1), 463. Full bibliography on Chemistry of Bacteria. See also Vaughan, "Protein Split Products in Relation to Immunity and Disease," Philadelphia, 1913.
[26] Münch. med. Woch., 1919 (66), 1412.
[27] Amer. Review Tuberc., 1921 (4), 842.

A study of the nucleic acid in the colon bacillus has been made by Schaffer, Folkoff, and Bayne-Jones,[28] who used a method that has been found suitable for isolation of nucleic acid in yeast, but does not lead to the isolation of nucleic acid from animal tissue. The product thus obtained contains about the amount of phosphorus required for plant nucleic acid, half of which is easily split and half firmly bound. It contains guanine, but no pentose, so that it does not correspond fully to plant nucleic acids. They have not studied the tubercle bacillus by this method, and in this organism Bendix[29] believed that he had demonstrated the presence of pentose. If it is true that bacterial nucleo-proteins contain pentose, it ranks them with the plant nucleo-proteins, for animal nucleic acids contain hexose. Johnson and Brown[30] found that tubercle bacilli contain the two pyrimidines which are considered characteristic of animal nucleo-proteins, namely, thymine and cytosine, but none of the uracil which is said to be characteristic of plant nucleic acid. They found the total amount of nucleic acid even lower than Long's analyses had suggested. This author has reviewed the evidence on the relation of bacteria to animals and plants,[31] and finds that they exhibit recognized plant tendencies and recognized animal characteristics, so that at present they cannot be definitely located with either kingdom, but certainly their remarkable powers of synthesis resemble those of plants.[32]

Albumins and globulins have been described in bacteria, but the investigation of their nature has been far from complete. Proteins containing a sugar molecule are certainly present but have been little studied. Published figures indicate that about half of the dry weight of the tubercle bacillus is of protein nature. Thomson[33] says that the solubility of different bacteria in alkalies varies inversely with the amount of lipoidal material.

The complete amino-acid content of bacterial protein does not seem to have been worked out, although the workers in Vaughan's laboratory have identified many of the usual amino-acids of proteins among the products of hydrolysis of bacteria.[34] Analysis of *B. mesentericus* shows it to be deficient in diamino-acids, tyrosine, glycine, and to contain 16.6 per cent. of glutamic acid.[35] Tamura[36] found phenylalanine and valine high in tubercle bacilli and very low in *B. diphtheriæ*, in which tyrosine is more abundant. In an azobacterium, lysine has been found especially abundant.[37] Cystine has been lacking in several analyses,

[28] Johns Hop. Hosp. Bull., 1922 (33), 151.
[29] Deut. med. Woch., 1900 (27), 180. A.
[30] Jour. Biol. Chem., 1922 (54), 731.
[31] Amer. Review Tuberc., 1923 (8), 195.
[32] Jones and Perkins (Jour. Biol. Chem., 1924 (62), 291) have found that all the nucleotids of yeast nucleic acid can be prepared from mammalian pancreas, which makes it probable that the supposed distinction between animal and plant nucleic acid may not rest on safe grounds.
[33] Lancet, 1921 (1), 795.
[34] See Wheeler, Jour. Biol. Chem., 1909 (6), 509.
[35] Horowitz-Wlassowa, Arch. Sci. Biologique, 1910 (15), 40.
[36] Zeit. physiol. Chem., 1913 (87), 85; 1914 (89), 289.
[37] Omeliansky and Sieber, Zeit. physiol. Chem., 1913 (88), 445.

but Salkowski[38] extracted from putrefactive bacilli an albumose and a globulin, both of which contain sulfur and lack tryptophan.

Johnson and Brown, and also Campbell[39] have analyzed the protein residue left after removing nucleic acid from tubercle bacilli, and the latter summarizes the results of these analyses, together with those of Tamura, as follows:

DISTRIBUTION OF NITROGEN IN PROTEIN OF THE TUBERCLE BACILLUS

	Tamura	Johnson and Brown	Campbell
Total nitrogen	100.00%	100.00%	100.00%
Histidine nitrogen	1.53	11.48	3.41
Arginine nitrogen	7.64	10.63	27.87
Lysine nitrogen	1.49	3.69	1.71
Glutamic acid nitrogen	.....	.....	3.05
Tyrosine nitrogen	Present	.....	.73
Tryptophan	.....	Present	Present
Melanin nitrogen	15.79	4.11	9.13
Cystine nitrogen	.....	1.26	1.26
Non amino nitrogen	7.47	9.34	9.34
Nitrogen accounted for	33.92%	40.51%	56.50%

Tamura[40] found that bacteria can synthesize from simple nonprotein media the purines, phosphatids, and typical proteins containing the aromatic amino-acids. Nitrogen-fixing bacteria, indeed, can form all the components of nucleic acid from atmospheric nitrogen even when grown on N-free media.[41] This synthetic activity of bacteria, in view of the large quantity of bacterial substances in feces, may possibly be of importance in metabolism studies, leading to erroneous conclusions as to utilization or synthesis of proteins by the subject.[42]

In common with other forms of cellular life, bacteria require certain specific substances, "vitamins," to permit their growth;[43] also they possibly produce substances with the va'ue of vitamins.[44] Müller[45] has found that at least certain of the unknown substances essential for growth of bacteria are adsorbed by animal charcoal, but has not been able to determine their exact nature, beyond that known amino acids will not

[38] Zeit. physiol. Chem., 1920 (109), 45.
[39] Amer. Rev. Tuberc., 1925 (11), 452.
[40] Zeit. physiol. Chem., 1913 (88), 190.
[41] Mockeridge, Biochem. Jour., 1924 (18), 550.
[42] Osborne and Mendel. Jour. Biol. Chem., 1913 (18), 177.
[43] See Davis, Jour. Infect. Dis., 1917 (21), 392; Kligler, Jour. Exp. Med., 1919 (30), 31.
[44] Pacini and Russell, Jour. Biol. Chem., 1918 (34), 43. Damon, Jour. Biol. Chem., 1921 (48), 379.
[45] Jour. Bact., 1922 (7), 309.

replace them. With some "hemophilic" bacteria, such as *B. influenzæ*, at least traces of hemoglobin or hematin must be present.[46]

The slimy material produced in cultures by some varieties of bacteria is, at least for certain forms, a body closely related to, or identical with, true mucin,[47] but in certain cases (*B. radicicola*) it is a gum related to the dextrans and free from nitrogen (Buchanan).[48] This finding has been corroborated by Kramer,[49] who also found the capsule of the Friedländer bacillus to be a galactan,[50] but the capsule of *B. anthracis* seems to be a glucoprotein containing sulfur but no phosphorus. Tubercle bacilli grown for many years on artificial media may produce a true mucin (Weleminsky).[51] Heim[52] considers that anthrax bacilli also produce mucin. Some nonpathogenic bacteria contain granules of sulfur in their protoplasm, and others have noteworthy quantities of iron in the sheath.

Bacterial Carbohydrates.[53]—The earlier descriptions of *cellulose* or *hemicellulose* in the cell membrane of bacteria have been contested.[54] Numerous investigators have reported that the insoluble bacterial cell wall consists chiefly of *chitin*, which on being split with acids yields 80 to 90 per cent. of the nitrogenous carbohydrate, *glucosamin*.[55] The distinction is a very important one, since cellulose is a typically vegetable product, while chitin is equally typically animal in origin, being found chiefly in the shells of lobsters and crabs, the wings and coverings of flies, beetles, etc., although it is described in some plant tissues, *e. g.*, algæ.[56] Chitin seems to be a polymeric form of glucosamine,[57] an amino-carbohydrate, just as cellulose is a polymer of a simpler carbohydrate. Other carbohydrates seem to be scanty in the bacterial cell, but Tamura[58] does not accept the chitinous nature of bacterial carbohydrate, finding in tubercle and diphtheria bacilli a hemicellulose, apparently a pentosan yielding l-arabinose on hydrolysis. As mentioned previously pentoses have been found in bacteria by some observers but not by others. Long considers, however, that the presence of glucosamine or of cellulose in bacteria is still unproved.

Wester[59] found no chitin in several varieties of bacteria, and cellulose only in *B. xylinum;* he therefore considers it probable that bacterial

[46] See Fildes, Brit. Jour. Exp. Path., 1921 (2), 16; Thjötta and Avery, Jour. Exp. Med., 1921 (34), 97.

[47] Rettger, Jour. Med. Research, 1903 (10), 101.

[48] Cent. f. Bakt., II Abt., 1909 (22), 371.

[49] Cent. f. Bakt., 1922 (87), 401.

[50] Corroborating Toenniessen, Cent. f. Bakt., 1920 (85), 225.

[51] Berl. klin. Woch., 1912 (49), 1302.

[52] Münch. med. Woch., 1904 (51), 426.

[53] Full bibliography by Long, "Chemistry of Tuberculosis," 1923, p. 28.

[54] However, Dreyer (Zeit. ges. Brauw., 1913 (36), 201) states that the cell wall of yeasts contains a hemicellulose and a manno-dextran. See also Kozniewski, Zeit. physiol. Chem., 1914 (90), 208.

[55] See Viehofer, Ber. Deut. Chem. Ges., 1912 (30), 443.

[56] Wurdack, Ohio Jour. Sci., 1923 (23), 181.

[57] Morgulis states that chitin consists of two parts, one containing all the glucose and amino groups, the other being a stable nitrogenous compound yielding no glucose. (Science, 1916 (44), 866.)

[58] Zeit. physiol. Chem., 1914 (89), 304.

[59] Pharm. Weekblad, 1916 (53), 1183.

cell walls do not always consist of the same substance. Cramer could find no glucose in any variety, although there are some bacteria that contain material reacting like starch with iodin. Levene,[60] however, found in *B. tuberculosis* a substance with some of the properties of glycogen. This is corroborated by Warkany,[61] who has reviewed the literature and made a study of the carbohydrates of tubercle bacilli, finding no evidence of the presence of chitin, gums (pectin), hemicellulose or difficultly hydrolyzed pentosans. He did find a total of 15.35 per cent. of the dry weight to be carbohydrate, of which 7.1 per cent. was cellulose, 4.1 per cent. glycogen and 4.15 per cent. unidentified, easily hydrolyzed carbohydrate giving the furfural reaction for pentoses. In pneumococci a polysaccharide built up of glucose molecules, which probably is an important component of the capsule, has been analyzed by Heidelberger and Avery.[62]

Bacterial Fats.—By staining methods, fats have been recognized in many species, and by extraction with fat solvents lecithin, cholesterol, simple fats, and specific bacterial fats have been isolated. Numerous studies of these fats of *B. tuberculosis* have been made and Long[63] summarizes his review of the literature on this subject as follows:

> Approximately 40 per cent. of the dry weight of the bacillus consists of fat-like substance;—substance, that is to say, extractable with the common fat solvents such as ether, chloroform, benzene, toluene, and xylene. Evidence has been brought forth to show that the amount varies somewhat with the strain of bacillus, the bovine type being richer in lipin than the human type, and that it varies also with the composition of the medium. Accepting Frouin's results on the relatively low fat content of bacilli grown on glycerol-free media we are perhaps warranted in believing that the reported analyses of the large crops of bacilli grown on glycerol broth do not represent accurately the condition of the parasite within the tissues of the animal body, where the concentration of glycerol is by no means so high as in the usual glycerol broth. Even if this be so, basing our notions on the lowest figures reported for tubercle bacilli grown on media containing any glycerol at all, we must, however, conceive of this organism as distinguished from most other bacteria by its exceptionally high lipin content.
>
> The lipin is apparently not true fat for the most part, but an ester, or mixture of esters, of the group of waxes.[64] Because of the difficult saponifiability of this wax, reports on the saponification number of the "crude fat" of the bacillus have been rather inaccurate, but it is apparently in the neighborhood of 130, relatively low, that is to say, as compared with true fats, and an indication that the ester combination is one of compounds of high molecular weight. Prolonged alkaline hydrolysis liberates a waxy substance of the general group of sterols or solid alcohols. The closest approximation to purity in this substance has probably been attained by Tamura, who assigns the empirical formula $C_{29}H_{55}OH$ to it on the basis of combustion analysis for carbon and hydrogen, and on the amount of bromine and benzoic acid absorbed on bromination and benzoylation.
>
> Much discussion has centered on the problem of the presence of cholesterol. The best analyses have failed to give any indication of its occurrence, either by the usual color tests or the digitonin precipitation method of Windaus. Furthermore, the melting points of the waxy residues after saponification differ greatly from that of cholesterol.

[60] Jour. Med. Research, 1901 (6), 135.
[61] Zeit. f. Tuberk., 1925 (42), 184.
[62] Jour. Exp. Med., 1923 (38), 73.
[63] "Chemistry of Tuberculosis," Wells, DeWitt, and Long, 1923, p. 35.
[64] Koganei (Jour. Biochem., Japan, 1922 (1), 353; 1923 (2), 495) reports finding in tubercle bacilli phrenosin, kerasin, sphingomyelin, and cephalin, to the last of which he attributes acid fastness, but there is reason to doubt the validity of these results.

About 40 per cent. of the total lipin, or approximately 16 per cent. of the dry weight of the bacillus, consists of a solid alcohol, which is a relatively inert substance. The ester combination with various fatty acids, as it actually exists within the bacillus, makes up 60 per cent. and upwards of the total lipin, or 15 to 25 per cent. of the dry weight of the microörganism, and is also relatively inert from the point of view of metabolic change. One is naturally led to speculate on the meaning of this storing of a non-labile substance. There is much value in Bürger's conception of its protective nature and his comparison with the similar conditions in the walls of xerophytic plants. Whether applied as a coating to the surface of the bacillus, or diffused uniformly through the protoplasmic mass, it must render the germ less permeable to destructive agencies and therefore more resistant to intracorporeal lysis. The essential foreign-body nature of the host's response to the presence of the tubercle bacillus, which constitutes the pathological process, tuberculosis, is unquestionably largely dependent upon the organism's high content of this non-labile material.

From all that may be gathered from the literature, true, neutral fats make up no great proportion of the total lipin of the bacillus. In fact the presence of glycerol, as indicated by the acrolein test, has been recorded but once or twice. On the other hand the presence of free fatty acids has several times been emphasized. If we may believe the results of Aronson, Kresling, and Goris, the "fat" of the tubercle bacillus is already a rancid fat, containing about 15 per cent. of its weight as free fatty acids. This is perhaps to be correlated with Ruppel's finding of a high free nucleic acid content also in its protoplasm.

Reliable quantitative results on the phosphatids are not at hand, but Tamura's work gives us some conception of their type. They seem to resemble lecithin in the general property of insolubility in acetone, but differ in that the ratio of phosphorus to nitrogen is 1 to 2, not 1 to 1. Tamura believed that they belonged to the diamino-monophosphatids.

The bulk of the lipin is thus of the nature of wax, consisting largely of esters of alcohols of high molecular weight, chiefly one of the composition $C_{29}H_{55}OH$, with various fatty acids, apparently largely lauric and palmitic acids. In addition to this there seems to be an appreciable amount of free fatty acid, a small amount of neutral fat, an undetermined but not large amount of phosphatid and a small, undetermined proportion of glucolipins. Substances of still less understood composition, like the "hyalinol" of Goris, are also present.

The high content in unsaturated fatty acids gives acid-fast bacteria a high antitryptic power, which may be concerned in the defense of the bacteria and also in the persistence of caseous material in tubercles (Jobling and Petersen).[65] The oily material obtained by extracting tubercle bacilli with cold ether is non-toxic, while the waxy material extracted with hot alcohol produces foreign body tubercles (Morse and Stott).[66] Ray and Shipman[67] found that not only tubercle bacillus lipins but the lipins of grass bacilli and colon bacilli, and the defatted bacilli as well, also produce similar tubercles, but always without caseation.

By staining with Sudan III, Sata[68] demonstrated fats, not only in the acid-fast bacilli, but also in anthrax, *Staphylococcus aureus*, *B. mucosus*, and actinomyces; but not in diphtheria, pseudo-diphtheria, plague, cholera, and chicken cholera bacilli, or in members of the colon group.[69] Ritchie[70] obtained positive fat staining in *B. diphtheriæ* and *anthracis*,

[65] Jour. Exp. Med., 1914 (19), 239.
[66] Jour. Lab. Clin. Med., 1916 (2), 159.
[67] Amer. Rev. Tuberc., 1923 (7), 88.
[68] Cent. f. allg. Path., 1900 (11), 97.
[69] Auclair (Arch. Méd. Exper., 1903 (15), 725) contends that the ether and chloroform extracts of many pathogenic bacteria contain important toxic substances. Holmes (Guy's Hosp. Reports, 1905 (59), 155) states that injection of fatty acids from tubercle bacilli into rabbits causes a lymphocytosis.
[70] Jour. Pathol. and Bact., 1905 (10), 334.

but not in *S. pyogenes aureus* or *M. tetragenus*, although these last forms contain chemically demonstrable lipins. Tamura[71] also reported finding lecithin and the high molecular alcohol, "mycol," in *B. diphtheriæ*. According to Larson[72] carbohydrates and glycerol are converted into lipins only when they are not fermented, and these lipins have a marked effect on the surface tension of the bacteria. Analyses of different bacteria show a relatively low content of lipins as compared with tubercle bacilli, varying from 1.7 per cent. in *B. subtilis* to 8.5 per cent. in staphylococci (Jobling and Petersen).[73] However, the degree of unsaturation of the fatty acids is less with tubercle bacilli than with other bacteria examined by these authors. Extensive studies of bacterial fat stains are reported by Eisenberg,[74] but practically nothing is known of the character of the fatty or lipoid constituents of bacteria outside the acid-fast group.[75]

Acid-fastness.—There is still much disagreement as to whether the acid-fastness of tubercle bacilli depends upon waxes, alcohols, fatty acids, or lipoid-protein compounds. It must be admitted that a high content of fatty materials is regularly present in acid-fast bacilli; thus, in an acid-fast bacillus isolated from leprous lesions, 34.7 per cent. of fats, fatty acids, and cholesterol, and 1.7 per cent. of lecithin were found by Gurd and Denis.[76] Miller[77] attributes the unstained, spore-like areas of tubercle bacilli to oleins, as bacilli grown on olive and sperm oil show a marked decrease in acid-fast areas. Tamura[71] ascribes acid- and Gram-fastness to a high molecular alcohol, "mykol." In a gram-negative bacillus[78] he found lecithin, but no cholesterol or mykol. Apparently the fats of tubercle bacilli resemble in character and complexity the "waxes" of plants which are called "cerolipoids" by Czapek. By growing tubercle bacilli on suitable media, they can be made to lose their acid-fast property, although still Gram-positive (Wherry).[79] The observation of Hope Sherman,[80] that tubercle bacilli are almost absolutely impermeable to fat-soluble dyes which stain their isolated fats well,[81] and her corroboration of Benians' demonstration that acid-fastness depends on the integrity of the bacillary envelope, makes the rôle of the fatty substances uncertain.

Long[82] has reviewed exhaustively the literature on acid fastness and concludes:

[71] Zeit. physiol. Chem., 1913 (87), 85; 1914 (89), 289.
[72] Jour. Infect. Dis., 1922 (31), 407.
[73] Jour. Exp. Med., 1914 (20), 456.
[74] Virchow's Archiv., 1910 (199), 502.
[75] Yeast fat contains palmitic, oleic, linoleic, and probably lauric and arachidic acids, and about 20 per cent. of sterol which seems to contain three double bonds (MacLean and Thomas, Biochem. Jour., 1920 (14), 483).
[76] Jour. Exper. Med., 1911 (14), 606.
[77] Jour. Path. and Bact., 1916 (21), 41; see also McJunkin, Amer. Rev. Tuberc., 1923 (8), 393.
[78] Zeit. physiol. Chem., 1914 (90), 286.
[79] Jour. Infect. Dis., 1913 (13), 144.
[80] Jour. Infect. Dis., 1913 (12), 249.
[81] See also Gutstein, Zeit. Tuberk., 1924 (39), 436.
[82] "Chemistry of Tuberculosis, Wells, DeWitt, and Long, 1923, p. 85.

Several irrefutable facts stand out as the result of the investigations to date. The most conspicuous of these are perhaps the following:

1. Disintegration of the cell destroys acid- fastness.
2. Extraction of the cell body with fat solvents until no more lipin is removed, leaves the bacilli acid-fast, provided they have not been mechanically disintegrated, for example, by trituration, in the process.
3. The wax of the bacillus is acid-fast.

He has attempted to reconcile the two chief conflicting explanations of acid-fastness, viz., that based on the conception of a relatively impermeable membrane, and that which credits the acid-fastness of the whole bacillus to its content of an acid-fast wax. It was found that although any amount of extraction with simple fat solvents leaves them acid-fast, they also continue to retain some of the lipins. However, if treatment with acid is added to the extraction until all the lipin is removed, the acid-fastness is removed. Apparently, then, the acid-fastness depends on a relatively small part of the lipin, which is probably firmly held as an emulsion in a protein substrate.[83] Some bacteria, such as *B. subtilis*, also hold lipins resistant to extraction, but are not acid-fast because the lipin is disposed differently from the lipins in the tubercle bacilli. Whereas prolonged vigorous grinding is necessary to deprive intact bacilli of their acid-fastness, as in the early experiments of Koch and later ones of Benians and Sherman, less severe pressure between glass slides is sufficient to turn defatted bacilli into a formless mass which takes the blue, not the fuchsin, in the method of Ziehl and Neelsen. No chemical change, probably, has taken place. If chemical affinity rather than manner of disposition is to account for acid-fastness, then in the instance just cited loss of acid-fastness may be explained only by a finer emulsification of the acid fast constituent.

Spores differ from their parent bacteria in containing a much greater proportion of the solid constituents and less water. In molds, Drymont found that the spores contained over 60 per cent. of dry substance, and almost all the water was so held as to resist drying by temperatures below boiling; the dry substance is very rich in protein and poor in salts. As the spores may lose their chromatin content without loss of capacity to propagate, it would seem that this is not a nuclear chromatin but merely a reserve food supply.[84] The wall of the spore consists of a "cellulose-like" substance and a very hygroscopic extractive matter. The great resistance of spores to drying and to heat can be readily understood in view of these facts. They contain, and perhaps secrete, active enzymes (Effront).[85] Flagella also seem to be composed of a relatively condensed protein.

Staining Reactions.—The staining reactions of bacterial cells are much as if the bacteria consisted entirely of chromatin, so that at one time the theory prevailed that bacteria consisted merely of a nucleus and a cell wall, without any true cytoplasm. The demonstration of abundant nucleoprotein in the contents of bacterial cells explains their staining affinity for basic aniline dyes. Owing to some unknown differences in composition, not all bacteria are stained equally well by the same basic dyes.[86] Although the staining of bacteria depends upon a chemical

[83] See also Browning and Gulbransen, Jour. Path. Bact., 1924 (27), 326.

[84] Ruzicka, Cent. f. Bakt., 1914 (41), 641.

[85] Mon. sc. Quesneville, 1907, p. 81

[86] The presence of serum interferes with staining, probably from protective colloid action (Fleisher, Jour. Med. Res., 1917 (36), 31).

reaction between the nucleoproteins and the basic dye, yet the combination is not usually a firm one, being readily broken by weak acids in most cases. That the decolorization of bacteria depends upon dissociation of the dye-protein compound, is shown by the fact that absolutely water-free alcohol will not decolorize dry bacteria, nor do water-free alcoholic solutions of dyes stain dehydrated bacteria. There seems to be a marked difference in the accessibility of dead and living bacteria to stains; thus, only dead bacteria stain with $AgNO_3$.[87] The amount of dye taken up by bacteria and the firmness with which it is held undoubtedly depends on the H-ion concentration of the bacterial protein,[88] since in general when on the acid side of the isoelectric point proteins combine with acids and on the alkaline side with bases (Loeb). Such influences may account for the fact that cultures may change from Gram-positive to Gram-negative with age.

Gram's Method[89] of staining has been ascribed to the formation of an iodin-pararosaniline-protein compound which is not easily dissociated by water in the case of bacteria that stain by this method, and which is readily dissociated and dissolved out in the case of bacteria that do not retain the stain. Only pararosaniline dyes (gentian violet, methyl violet, victoria blue) form such combinations, the rosaniline dyes not being suitable.[90]

The relation of bacterial protein to Gram staining is shown by the fact that trypsin will digest killed bacteria which are Gram-negative, but not Gram-positive forms; gastric juice attacks only a few Gram-positive bacteria.[91] They are also more resistant to alkalies, 1 per cent. KOH dissolving only the Gram-negative bacteria. Brundy[92] considers that they are more permeable to iodine, so that a more central iodine dye precipitate occurs, and Eisenberg[93] suggests that lipoid-protein compounds in the bacterial surface are important, in support of which is the observation that ether extraction of staphylococci renders them negative to Gram's method, while colon bacilli treated with lecithin become positive.[94] Gram-positive bacteria are also more readily adsorbed by adsorbents because of the surface lipins,[95] and on the other hand they adsorb halogens less strongly.[96] Churchman[97] has observed that Gram-positive organisms are particularly susceptible to the bactericidal action of gentian violet, and has applied this principle to therapeutics.

Jobling and Petersen[73] have also found the lipoids of Gram-positive bacteria more resistant to extraction by fat solvents than lipoids of Gram-negative bacteria, and Tamura[98] found that the lipoid extract contains the bacterial element responsible for Gram staining. The first-named authors suggest a relation between the high content in unsaturated fatty acids, with the high affinity for iodine and the positive Gram staining. On the other hand, Hottinger[99] attributes Gram staining solely to the degree of dispersion of the nucleo-proteins, which he believes to be higher in the

[87] Nyfeldt, Nordiskt Med. Arkiv., 1917 (50), 184.
[88] Stearn, Jour. Bact., 1923 (8), 567.
[89] Full review by Deussen, Zeit. Hyg., 1918 (85), 235; 1921 (93), 512; also, Smith, Amer. Jour. Hyg., 1922 (2), 607.
[90] Any metallic iodide may be substituted for KI (Leidy, Jour. Lab. Clin. Med., 1919 (4), 354). It is probable, especially from the observations of Deussen,[89] that the nucleoproteins are the essential cell constituents, and other cells than bacteria (*e. g.*, sperm) may be Gram-positive.
[91] Bürgers, Schermann and Schreiber, Zeit. f. Hyg., 1911 (70), 119; Weinkoff, Zeit. Immunität., 1912 (11), 1.
[92] Cent. f. Bakt. II Abt., 1908 (21), 62.
[93] Cent. f. Bakt., 1910 (56), 193.
[94] Jour. Path. and Bact., 1911 (16), 146.
[95] Eisenberg, Cent. f. Bakt., 1918 (81), 72.
[96] Breinl, Zeit. f. Immunität., 1920 (29), 343.
[97] See Bull. Johns Hop. Hosp., 1921 (32), 225.
[98] Zeit. physiol. Chem., 1914 (89), 289.
[99] Cent. f. Bakt., 1916 (76), 367.

Gram-negative forms. Benians[1] has found that crushed Gram-positive bacteria are promptly decolorized, indicating that the dye and the cell contents do not form an insoluble compound, but that the bacterial cell wall is the chief factor in determining Gram positiveness; presumably the iodine renders the cell membrane impermeable to alcohol. This important contribution has been confirmed, as far as the staining of tubercle bacilli is concerned, by Hope Sherman,[2] who corroborates the finding of Benians that if the bacilli are not intact they are neither acid-fast nor Gram positive. The same is true of yeast cells (Henrici),[3] but Deussen[89] states that press juice from yeast (Buchner's zymase) contains Gram-positive granules.

Burke[4] has corroborated Benians' explanation by demonstrating that the reverse staining effect may be demonstrated, *i.e.*, an alcohol solution of the iodine dye complex stains Gram-negative but not Gram-positive bacteria. Evidently Gram-positive cells are characterized by permitting entrance of the water-soluble dye but not the egress of the alcohol-soluble iodine-dye complex, while the opposite condition characterizes the Gram-negative forms.

In general, Gram-positive bacteria are sensitive to all weak electrolytes (Smith),[89] although more resistant to most other agents, and they usually exhibit optimum growth at a higher H-ion concentration than the Gram-negative forms. The Stearns[5] have found that bacteria exhibit the ampholyte character common to proteins, and tend to retain acid stains when in acid solution and basic stains when in alkaline solution. They found that Gram-positive bacteria can be rendered Gram-negative by increasing the acidity, and the reverse effect is produced by alkali; at the iso-electric range there is little staining, and this range generally is wider with Gram-negative than with Gram-positive bacteria. Gram-positive organisms have a limited iso-electric range centered about *p*H 2–3; Gram-negative organisms have a wider range centered about *p*H 5. Mordanting of bacteria seems to be a mild oxidation which increases the acidity and hence the affinity for basic dyes. They suggest that Gram staining depends on the presence in the compound lipo-proteins of bacteria, of unsaturated fatty acids, which are partially oxidized by the iodine or other mordant used in the stain, with intensification of the acid properties of the lipo-protein and a resulting increased affinity for basic dyes.

Bacterial Enzymes[6]

The metabolic processes of bacteria seem to be closely dependent upon enzyme action, just as with higher cells.[7] Liquefaction of gelatin is a familiar example of the enzyme action of bacteria; and since the filtered cultures of liquefactive bacteria are also capable of digesting gelatin, the enzymes are evidently excreted from the cells. Dead bacteria, killed by thymol of by other antiseptics that do not destroy proteolytic enzymes, will also digest gelatin. Numerous investigations have established the wide-spread occurrence of many soluble enzymes both in bacteria and in their secretions, indicating that bacterial cells are as dependent on enzymes for the production of their metabolic activities as are higher types of cells, and that these enzymes are not only present as intracellular constituents, but that they also escape from the cells. Even the spores of bacteria and molds contain active enzymes.[8] A striking property of bacteria is their reducing power, which has led to the introduc-

[1] Jour. Path. and Bact., 1912 (17) 199; ibid., 1920 (23), 401.
[2] Jour. Infect. Dis., 1913 (12), 249.
[3] Jour. Med. Res., 1914 (30), 409.
[4] Jour. Bact., 1922 (7), 159.
[5] Jour. Bact., 1924 (9), 463, 479; 1925 (10), 13.
[6] Complete bibliography by Waksman, Abstr. Bact., 1922 (6), 265.
[7] Review on bacterial metabolism given by Kendall, Physiol. Reviews, 1923 (3), 438.
[8] Effront, Mon. sc. Quesneville, 1907, p. 81; Kopeloff, Jour. Agric. Res., 1919 (18), 195; Ruehle, Jour. Bact., 1923 (8), 487.

tion of selenium and tellurium salts, which are reduced to the metals, as an index of bacterial life and activity (Gosio).

The diffusion method of Wijsman, or, as it is more frequently called, *auxanographic* method of Beijerinck, offers a relatively simple means of detecting the presence of extracellular bacterial enzymes. Eijkman[9] in particular has used this method, which consists of mixing agar with milk, or starch, or whatever material is to serve as the indicator of the enzyme action; the agar is then inoculated with bacteria and plated (or else the bacteria are inoculated as a streak on the surface of the agar). About each colony there will appear a zone of clearing in the medium if it produces enzymes digesting the admixed substance. By this means Eijkman found that all bacteria that produce enzymes digesting gelatin also digest casein, and those that do not digest gelatin are equally without effect on casein; therefore, it is probably the same enzyme that digests both. As the hemolytic action of bacteria is not constantly related to their gelatin-dissolving property, bacterial hemolysis probably is produced by other means than the proteolytic enzymes.[10] A few pathogenic bacteria (anthrax, cholera, and some strains of hemolytic streptococci[11]) digest starch[12] and *B. pyocyaneus*, *Staphylococcus pyogenes aureus*, and *B. prodigiosus* all produce fat-splitting enzymes demonstrable by this method.[13] Some bacteria split neutral fats, some split lecithin, and some split both.[14] The hydrolysis of ethyl butyrate by hemolytic streptococci does not increase with increase of virulence.[15] According to Avery and Cullen,[16] pneumococci contain esterase, invertase, amylase, and inulase, but Michaelis[17] could demonstrate no splitting of tributyrin by pneumococci, *B. coli* or *B. diphtheriae*, although tubercle bacilli and pyogenic cocci were active. In hemolytic streptococci have been demonstrated lipase invertase and protease.[18] According to Tongs,[19] different groups of streptococci differ in their ability to digest casein and starch. The lipolysis produced by staphylococci may lead to hemolysis.[20] Cholera vibrios are said to split starch (amylase) with particular vigor.[21]

Rennin is produced by many bacteria, as is shown by their coagulating milk, independent of any acid reaction,[22] and protease from *pyocyaneus*

[9] Cent. f. Bakt., 1901 (29), 841.
[10] See Jordan, Biol. Studies by the pupils of W. T. Sedgwick, 1906, p. 124.
[11] Tongs, Jour. Amer. Med. Assoc., 1919 (73), 1277.
[12] In relation to carbohydrate enzymes, the extensive studies of Kendall (Jour. Biol. Chem., 1912, vol. 12) should be consulted. He emphasizes especially that as a rule bacteria ferment carbohydrates in preference to attacking proteins when both foodstuffs are available.
[13] See Buxton (American Med., 1903 (6), 137) concerning enzymes of numerous bacteria.
[14] Kraay and Wolff, Proc. Acad. Sci. Amsterdam, 1923 (26), 436.
[15] Thompson and Meleney, Jour. Exp. Med., 1924 (40), 233.
[16] Jour. Exp. Med., 1920 (32), 583.
[17] Zeit. Immunität., 1923 (36), 449.
[18] Stevens and West, Jour. Exp. Med., 1922 (35), 823.
[19] Jour. Amer. Med. Assoc., 1919 (73), 1277.
[20] Orcutt and Howe, Jour. Exp. Med., 1922 (35), 409.
[21] Kodama and Takeda, Cent. f. Bakt., 1922 (88), 513.
[22] Contradicted by DeWaele, Cent. f. Bakt., 1905 (39), 353.

causes "plastein" formation in albumose solutions (Zak).[23] Bacteria which give negative results by the plate method may contain active *lipase* demonstrable in killed bacteria by direct action upon fats and esters, these lipases behaving exactly like the lipase of animal tissues (Wells and Corper).[24] *Staphylococcus* and *pyocyaneus* are more actively lipolytic than *B. coli*, *B. dysenteriæ* and *B. tuberculosis*. Urease seems to be widespread.[25] Tubercle bacilli contain enzymes resembling lipase, trypsin, erepsin, nuclease and urease, but not amylase, elastase or invertase.[26] Eijkman found, that *B. pyocyaneus* digested elastic tissue readily,[27] as also did a bacillus resembling *B. subtilis* obtained from the tissue of a gangrenous lung.

Schmailowitsch[28] stated that the amount and nature of enzymes produced by bacteria is modified by the amount and nature of their food, but Jordan found that gelatinase is produced by bacteria growing on non-protein media; he failed entirely to support the statement of Abbott and Gildersleeve[29] that bacteria grown on gelatin produce much more active gelatin-dissolving enzyme than do bacteria grown on bouillon. Diehl[30] found that bacteria grown on media containing no organic nitrogen produce no proteolytic enzymes, and the enzyme content of bacteria is much modified by the composition of the media, depending on the character of the amino-acids present rather than the proteins themselves. Jacoby[31] has made extensive studies on the requirements for the production of urease on Uschinski's medium, and finds that while bacteria will grow if the sodium asparaginate is present, no urease is formed unless leucine or isolucine is also added. There does not seem to be any important relation between enzyme production and pathogenicity.[32]

In general, bacterial proteolytic enzymes resemble trypsin more closely than they do pepsin, acting best in an alkaline medium; but the enzymes extracted from bacterial cultures are very feeble as compared with pancreatic trypsin. It is probable that there are several proteolytic enzymes in bacterial cells, gelatinase being a distinct protease (Jordan).[33] Abbott and Gildersleeve[29] found that the gelatin-dissolving enzyme of bacteria resists a temperature of 100° C. for as long as fifteen to thirty minutes, but Jordan found that the reaction of the medium modifies greatly this heat resistance. Schmailowitsch[34] states that some bacteria produce

[23] Hofmeister's Beitr., 1907 (10), 287.
[24] Jour. Infect. Dis., 1912 (11), 388; literature on bacterial lipases. See also Kendall.[12]
[25] See Jacoby, Biochem. Zeit., 1917 (80), 357.
[26] Corper and Sweany, Jour. Bact., 1918 (3), 129.
[27] Cent. f. Bakt., 1903 (35), 1.
[28] Wratschebnaja Gazetta, 1902, p. 52.
[29] Jour. Med. Research, 1903 (10), 42.
[30] Jour. Infect. Dis., 1919 (24), 347.
[31] Biochem. Zeit., 1917 (83), 74; Deut. med. Woch., 1923 (49), 1541.
[32] Rosenthal and Patai, Cent. f. Bakt., 1914 (73), 406; (74), 369; also Thompson and Meleney.[15]
[33] Corroborated by Bertiau, Cent. f. Bakt., 1914 (75), 374.
[34] Abt. in Biochem. Centr., 1903 (1), 230; see also DeWaele, Cent. f. Bakt., 1905 (39), 353.

an enzyme acting in acid medium upon gelatin but not albumin, and this enzyme carries the digestion only as far as the gelatin-peptone stage, whereas the enzymes acting in an alkaline medium carry the splitting through to leucine, tyrosine, etc. Kendall and Walker[35] state that the proteolytic enzymes of *B. proteus* are not formed when the bacteria have enough carbohydrate supplied so that they need not depend on proteins for their energy requirements; deaminization is independent of proteolysis and represents intracellular enzyme action.

Arginase has been described in *B. pyocyaneus* and *fluorescens*, but it could not be demonstrated in *B. typhosus*, streptococci, and several related organisms (Hino).[36] Plenge[37] suggests that there is a special enzyme digesting nucleoproteins (nuclease). Bacteria are able to split nucleic acids and to convert amino-purines into oxypurines, but they do not carry the oxidation to uric acid; putrefactive bacteria can slowly destroy uric acid (Schittenhelm),[38] and *B. coli* destroys purines.[39]

Cacace[40] investigated the cleavage products of gelatin and coagulated blood when digested by *B. anthracis*, *Staph. pyogenes aureus*, and *Sarcina aurantiaca*, and found that proteoses and peptone are produced, which disappear in the later stages of digestion. Rettger[41] found leucine, tyrosine, tryptophan, as well as phenols, skatole, indole, aromatic oxy-acids, and mercaptan, among the products of bacterial decomposition of egg-albumen and meat; proteoses and peptones appear in the early stages, but later disappear, as also eventually do the leucine, tyrosine, etc. Choline has also been found in the products of autolysis.[42] Molliard[43] reports that *Isaria densa* produces large masses of crystals of glycine, even when grown on proteins that contain little or no glycine.

The digestive power of the filtrates of cultures and of killed bacteria is far less than that of the living bacteria (Knapp).[44] Streptococci digest proteins of exudates feebly, staphylococci more rapidly, and colon bacilli are still more active. He could find no relation between the proteolytic power of the bacteria and the severity of the infection from which they came. Reduction of virulence of pneumococci also has no effect on their enzyme activities.[45] Staphylococci can cause coagulation of plasma[46] and then dissolve the coagulum, showing the presence of two enzymes, *staphylokinase* and *fibrinolysin* (Kleinschmidt).[47] Dernby[48]

[35] Jour. Infect. Dis., 1915 (17), 442. See also Berman and Rettger, Jour. Bact., 1918 (3), 367.
[36] Zeit. physiol.Chem., 1924 (133), 100.
[37] Zeit. f. physiol. Chem., 1903 (39), 190.
[38] Zeit. physiol. Chem., 1908 (57), 21.
[39] Siven, Zeit. physiol.Chem., 1914 (91), 336; Morris and Ecker, Jour. Infect. Dis., 1924 (34), 592.
[40] Cent. f. Bakt., 1901 (30), 244.
[41] Amer. Jour. of Physiol., 1903 (8), 284.
[42] Kutscher and Lohmann, Zeit. physiol. Chem., 1903 (39), 313.
[43] Compt. Rend. Acad. Sci., 1918 (167), 786.
[44] Zeit. f. Heilk. (Chir. Abt.), 1902 (23), 236.
[45] Avery and Cullen, Jour. Exp. Med., 1920 (32), 547.
[46] See Gratia, Arch. internat. physiol., 1921 (18), 355.
[47] Zeit. Immunität., 1909 (3), 516.
[48] Biochem. Zeit., 1921 (126), 105; 1922 (134), 3.

could find no proteolytic enzymes in the filtrates of *B. diphtheriæ*, *B. tuberculosis*, *B. tetani*, and pathogenic cocci, although several liquifying bacteria secrete enzymes which are most active at *p*H 6–7. Sperry and Rettger,[49] however, found that even the most actively putrefactive bacteria are unable to attack or grow upon carefully purified proteins, although the presence of small amounts of amino acids or other available nutrient makes the proteins available to the bacteria; apparently they must have some nutrient more available than intact protein molecules to enable them to grow sufficiently to produce enough free enzymes to attack the proteins. By virtue of their proteolytic enzymes, filtrates of bacteria that liquefy gelatin also can digest hardened liver, kidney, and other tissue elements *in vitro*, the changes resembling those of necrobiosis.[50]

Oxidizing Enzymes.—*Catalase* is demonstrable in bacteria, the anaerobic forms showing the least activity (Rywosch),[51] but practically no species is entirely inactive (Jorns);[52] it may exist as either endo- or ecto-enzyme. Hahn obtained catalase activity with extracts of tubercle bacilli, and many other organisms have also given positive results.[53] *B. proteus* synthesizes catalase even when grown on a simple synthetic medium containing, besides inorganic salts, sodium lactate and alanine or aspartic acid (Jacoby).[54] Presumably catalase formation by bacteria is a protective mechanism against peroxides, for M'Leod and Gordon[55] found that anaerobes which do not produce catalase are particularly susceptible to H_2O_2, the chief catalase producers being facultative anaerobes. Pneumococci and streptococci seem to be the most active formers of peroxide, the former producing it even under conditions that do not permit of growth.[56] The peroxides thus formed account for the production of pigments through oxidation of aromatic amines, which is characterisitc of streptococci and pneumococci.[57] Lactic acid bacilli are peculiar in being aerobic bacteria which do not produce catalase.[58]

Pneumococci, streptococcus viridans, and hemolyticus, seem to produce an oxidase under suitable conditions.[59] Some of the enzymes of pneumococci (especially sucrase, raffinase, inulase, and amylase) are destroyed by oxidizing agents, presumably peroxides, formed on exposure of bacterial extracts to air.[60] Certain bacteria and actinomyces exhibit oxidative effects, resembling *tyrosinase*,[61] but such an enzyme could not be extracted by Lehmann and Sano.[62] Tsudji,[63] however, not only observed oxidation of tyrosine, but states furthermore that proteus produces always a d-oxyacid product and subtilis the l-oxyacid type, regardless of whether they have oxidized d-, l-, or dl-tyrosine.

Immunity against bacterial enzymes has been described, but as with other enzymes, the degree of antienzyme activity is so slight as to leave doubt that it represents a true antibody formation. Abbott and Gilder-

[49] Jour. Biol. Chem., 1915 (20), 445; Jour. Bact., 1916 (1), 15.
[50] Bittrolff, Ziegler's Beitr., 1915 (60), 337.
[51] Cent. f. Bakt., 1907 (44), 295.
[52] Arch. f. Hyg., 1908 (67), 134.
[53] Lowenstein, Wien. klin. Woch., 1903 (16), 1393; Ohtsubo, Kitasato Arch. Exp. Med., 1923 (6), 61.
[54] Biochem. Zeit., 1918 (88), 35 and (89), 350; 1919 (95), 124.
[55] Jour. Path. and Bact., 1923 (26), 326; see also Schlunk, Cent. f. Bakt., 1924 (192), 116.
[56] Morgan and Avery, Jour. Exp. Med., 1924 (39), 335, 347, 357.
[57] Penfold, Med. Jour. Australia, 1922 (2), 120.
[58] See Kluyver, Zeit. physiol. Chem., 1924 (183), 100.
[59] Felton, Jour. Exp. Med., 1923 (38), 291.
[60] Neill and Avery, Jour. Exp. Med., 1924 (40), 405.
[61] Stapp, Biochem. Zeit., 1923 (141), 42.
[62] Arch. f. Hyg., 1908 (67), 99.
[63] Acta Scholae Med. Univ. Kioto, 1918 (2), 115.

sleeve[29] found that by injections into animals of proteolytic bacterial filtrates which were only slightly toxic, the serum of the animals acquired a slight but specific increase in resistance to the proteolytic enzymes of the filtrates.[64] Normal serum contains a certain amount of enzyme-resisting substance.[65] Other observers have found that immunization against living or dead bacteria leads to the production of substances antagonistic to their enzymes, but the degree of resistance acquired is never great, and very possibly depends on precipitins throwing down the enzymes along with the bacterial proteins in the solution tested.[66] v. Dungern,[67] however, found that the serum of animals infected with various bacteria prevented digestion of gelatin by the enzymes obtained from cultures of the same species of bacteria. He applied this fact to the diagnosis of infectious conditions, finding that the serum of a patient with osteomyelitis was over twenty times as strongly inhibitory to staphylococcus enzymes as was serum of normal persons; the reaction is specific, cholera vibrio enzymes not being inhibited to any corresponding degree. This observation does not seem to have been repeated by others.

Kantorowicz[68] and de Waele[69] state that bacteria contain an intracellular *anti-protease* which, with most bacteria, holds in check the proteolytic action; only with the liquefying bacteria are the proteases in excess. Bacteria grow well in strong solutions of enzymes, and without destroying the enzymes (Fermi).[70] After Gram-negative bacteria have been heated to 80° they are readily digested by trypsin, pepsin, or leucocytic proteases; but Gram-positive bacteria are resistant even after heating. This is ascribed by Jobling and Petersen[71] to the unsaturated fatty acids, which are present in greater amounts in Gram-positive bacteria. Therefore, Gram-positive bacteria may be killed by agents which do not destroy the protective lipoidal covering, such as weak antiseptics or low temperature, and still retain their resistance to proteolytic enzymes, nor are they rendered more digestible by the action of specific immune serum (Dukes).[72]

Autolysis of Bacteria.[73]—Autolysis occurs also in bacteria, their proteolytic enzymes digesting the cell substance whenever the organisms are killed by agents (chloroform, toluene, etc.) that do not destroy these enzymes, and which, being fat solvents, may facilitate digestion by removing the inhibitory lipoids. Even the absence of food leads to autolysis, which occurs when bacteria are kept in salt solution or distilled water. Undernourished bacteria exhibit marked alterations in form and other

[64] Antigelatinase has also been obtained by Bertiau, Cent. f. Bakt., 1914 (74), 374, and by Launoy, Ann. Inst. Pasteur, 1920 (34), 249.
[65] Launoy, Ann. Inst. Pasteur, 1919 (33), 657.
[66] See Dukes, Jour. Path. and Bact., 1922 (25), 258.
[67] Münch. med. Woch., 1898 (45), 1040.
[68] Münch. med. Woch., 1909 (56), 897.
[69] Cent. f. Bakt., 1909 (50), 40.
[70] Arch. Farmacol., 1909 (8), 481.
[71] Jour. Exp. Med., 1914 (20), 321.
[72] Brit. Med. Jour., Mar. 18, 1922, p. 430.
[73] Bibliography and Review by Sturges and Rettger, Jour. Bact., 1922 (7), 551.

properties, similar changes being produced by germicides in sublethal amounts, so that apparently these agents affect bacterial nourishment.[74] Possibly with bacteria as with tissue cells, autolysis is initiated by increased H-ion concentration, which seems to be an important factor in the death of bacteria in cultures.[75]

Although it had been known for many years that yeast cells digest one another when there is nothing else for them to live upon, the first definite study of bacterial autolysis seems to have been made by Levy and Pfersdorff[76] and Conradi.[77] The former digested anthrax bacilli (in whose bodies are contained rennin, lipase and protease) under toluene for several weeks and obtained a slightly toxic product. Conradi permitted dysentery bacilli and typhoid bacilli to digest themselves in normal salt solution for twenty-four to forty-eight hours at 37° C., and obtained in this way the soluble, highly poisonous endotoxins of the bacteria, which are liberated by the destruction of the bacterial structure by the autolytic enzymes. Longer autolysis results in the destruction by the enzymes of the endotoxins themselves. Rettger[78] found among the autolytic products of bacteria, leucine, tyrosine, basic substances, and phosphoric acid. Under favorable conditions complete autolysis can occur in two to ten days. Brieger and Mayer[79] found that at room temperature (15° C.) practically no autolysis occurs with typhoid bacilli in distilled water, and the soluble products thus obtained are quite non-toxic, although if injected into animals they give rise to the production of agglutinins and bacteriolysins. Bertarelli[80] has used the products of autolysis of cholera vibrios successfully in the production of immunity, and states that the products of autolysis consist largely of nucleins. It is probable that in every culture bacteria are constantly being destroyed, either by their own enzymes or by the proteolytic enzymes of the other bacteria. Some bacteria are much more rapidly autolyzed than others.

Sturges and Rettger[73] found that proteolytic bacteria of the type of *B. prodigiosus*, *B. pyocyaneus*, and *B. subtilis* autolyze rapidly. *B. coli* undergo slight changes which may be autolytic in nature, but which at most involve only a small part of the complex nitrogenous constituent or constituents of the cells. The pathogenic cocci undergo an actual autolysis with a breaking down of the protein or protein-like substances of the bacterial cells. During autolysis the electrical conductivity increases, but not in proportion to the rise in free amino nitrogen. They found that, at least with *B. prodigiosus*, the most rapid digestion occurred at *p*H 7.0. Non-liquefying bacteria, such as pneumococci, do not digest albumin, casein, or gelatin. As a general rule, Gram-negative organisms resist autolysis less than Gram-positive, but pneumococci autolyze readily.

[74] Braun, Zeit. allg. Physiol., 1921 (19), 1.
[75] See Chambers, Ann. Missouri Bot. Gardens, 1920 (7), 249.
[76] Deut. med. Woch., 1902 (28), 879.
[77] *Ibid.*, 1903 (29), 26.
[78] Jour. Med. Research, 1904 (13), 79.
[79] Deut. med. Woch., 1904 (30), 980.
[80] Cent. f. Bakt., 1905 (38), 584.

With pneumococci, at least, autolysis seems to be most rapid at *p*H 5.0 to 7.0, fresh human serum having an inhibitory action.[81] Pneumococcus proteolytic enzymes may attack killed pneumococci of all strains and also some related cocci, but not the less closely related cocci, and have no effect on living organisms.[82] In this respect pneumococcus protease differs from pyocyanase, an enzyme which is said to cause lysis of many varieties of living bacteria.[83] Staphylococci and streptococci do not exhibit autolysis under the conditions which produce it with pneumococci.[81]

It has been found by Turro[84] that extracts from various tissues containing autolytic enzymes can digest bacterial cells. It is very possible that the endotoxins contained within such pathogenic bacteria as typhoid and cholera are liberated through digestion of the bacteria, either by autolysis or by the enzymes of the leucocytes and tissues of the organism that they have infected. These, and a number of other bacteria, produce no soluble toxins that diffuse from the cells as do diphtheria and tetanus toxin, and it is difficult to explain the toxic effects these bacteria produce without assuming that their intracellular toxins are liberated in some such way. It is also quite probable that the enzymes found in filtrates from bacterial cultures are liberated from the bacterial cells only when these have been autolyzed. With the possible exception just mentioned, there is little evidence that the bacterial enzymes play any important röle in infectious diseases. They may be a slight factor in the digestion of tissue and exudates in suppuration, but as compared with the leucocytic enzymes their influence is probably minute; beyond this they have no apparent influence upon their host, and are chiefly concerned in the metabolism of the bacteria. The proteoses and peptones produced by bacterial action and isolated from cultures do not seem to be any more toxic than those produced by pepsin and trypsin, but violent poisons may be liberated from bacteria during autolysis, as Rosenow[85] has shown for the pneumococcus and other bacteria; these poisons seem similar to or identical with the so-called *anaphylatoxin* which is supposedly formed by the digestion of bacteria with serum complement, and presumably they are proteoses or polypeptids, but their exact nature is not known. (See Anaphylatoxin, Chap. X.)

Conradi,[85a] who has shown that certain products of autolysis of tissues are bactericidal, believes that also in cultures powerfully bactericidal substances are produced through autolysis of the bacteria. This he thinks, accounts for the decrease in numbers of living bacteria that always sets in after a short period of growth on artificial media; but there is much doubt as to these substances being of any considerable importance in

[81] Lord and Nye, Jour. Exp. Med., 1922 (35), 685.
[82] Avery and Cullen, Jour. Exp. Med., 1923 (38), 199.
[83] Emmerich and Loew, Zeit. f. Hyg., 1899 (31), 1.
[84] Cent. f. Bakt., 1902 (32), 105.
[85] Jour. Infect. Dis., 1912 (10), 113; (11), 94, 235 and 480.
[85a] Münch. med. Wochenschr., 1905 (52), 1761.

infections within the body,[86] except perhaps in the autosterilization of closed pus pockets. Pneumococci produce in cultures substances inhibiting not only their own growth but that of other bacteria; these inhibiting agents are thermolabile and may be enzymatic (McLeod and Govenlock).[87]

The "bacteriophage" activity observed in some cultures by Twort and by d'Herelle[88] is believed by some, especially Bordet, to represent merely an augmented autolysis of the affected bacteria, although d'Herelle believes it to be a specific infectious agent attacking the bacteria. Antibodies can be developed against the lytic agent,[89] but it is still undetermined whether the bacteriophage is an autolytic enzyme or not. The solution of the bacteria seems to be a simple lysis rather than a true proteolysis.[90]

POISONOUS BACTERIAL PRODUCTS

Almost without exception all the harm that bacteria do is brought about by means of the chemical substances produced in one way or another by their metabolic processes. Animal parasites may do harm mechanically, but with the possible exception of the effects of capillary emboli (especially with anthrax), bacteria produce all their effects through chemical means. The poisonous chemical substances produced by bacteria are commonly grouped into four classes:

I. Products of the decomposition of the media upon which the bacteria are growing; among these the best known are the *ptomaines.*

II. Soluble, specific, antigenic poisons manufactured by the bacteria, and secreted from the cell into its surrounding media—the true *toxins.*

III. Poisons manufactured by the bacteria which do not escape from the normal cell, which are not antigenic and not so specific in their poisonous properties as the true toxins; because of their intracellular situation they are called *endotoxins.*

IV. Poisonous protein constituents of the bacterial cell which form part of the cell protoplasm, but which are not soluble, and the poisonous effects of which are not specific and not usually responsible for the disease; these are called *bacterial proteins.*

The nature of the products of bacterial growth is greatly modified by the composition of the culture media, even in respect to specific toxin or enzyme production, for Kendall has found that the presence of an abundance of carbohydrates may largely inhibit the formation of the various characteristic nitrogenous compounds, presumably because the bacteria draw their energy from carbohydrate if it is available in pref-

[86] See Eijkman, Cent. f. Bakt., 1906 (41), 367; Passini, Wien. klin. Woch., 1906 (19), 627.

[87] Lancet, 1921, Apr. 30, p. 900; full review on bactericidal action of bacterial products.

[88] Bibliography by Kuttner, Jour. Bact., 1923 (8), 49; Bull, Physiol. Reviews, 1925 (5), 95.

[89] See Weiss and Arnold, Jour. Immunol., 1924 (34), 317.

[90] Ionescu-Mihaiesti, Jour. Exp. Med., 1924 (40), 317.

erence to utilizing proteins which yield more complex products.[91] This is illustrated by the following table:

Organism	Significant product in protein medium	Significant change in the carbohydrate-protein medium
Diphtheria bacillus	Soluble diphtheria toxin	Lactic acid. No toxin
Shiga bacillus	Soluble Shiga toxin	Lactic acid. No toxin
Bacillus coli	Indol	Lactic acid. No indol
Bacillus proteus	Soluble proteolytic enzyme	Lactic acid. No enzyme

PTOMAINES

Ptomaines, the soluble basic nitrogenous substances that are found in the medium in which bacteria have been growing, were the first bacterial products that were recognized, and for some time it was believed that it was through the production of such alkaloid-like substances that bacteria caused disease, just as poisonous plants owe their effects to poisonous alkaloids. It was soon found, however, that the ptomaines that could be isolated from cultures of pathogenic bacteria were insufficient by themselves to cause the poisonous effects that such cultures produced when injected into animals. The isolated ptomaines were not only far less poisonous than the original culture, but furthermore they did not produce the symptoms and anatomical changes characteristic of the diseases that the pathogenic organism caused. Moreover, the majority of ptomaines are not very poisonous, and highly poisonous ptomaines may be produced by non-pathogenic bacteria. As a result, the work on ptomaines, which once occupied many laboratories and promised to reveal the entire chemistry of bacterial intoxication, has now been almost completely dropped. The interest in ptomaines is by no means entirely historical, however, for it is possible that poisonous ptomaines at times do enter the body and cause illness, perhaps even death. The close chemical resemblance to vegetable alkaloids of some of the ptomaines that may arise in decomposing corpses, makes them of great importance to chemists searching for the cause of death in cases of supposed poisoning. Therefore the most essential features of the ptomaines and their chief known relations to intoxications will be briefly discussed, referring the reader for a full consideration to Vaughan and Novy's "Cellular Toxins," Barger's "The Simpler Natural Bases," and Guggenheim's "Die Biogenen Amine."

The ptomaines owe their basic character to nitrogen-containing radicals, principally amino-groups, and hence are formed from nitrogenous substances, chiefly proteins, which contain their nitrogen in the amino form. Probably most ptomaines arise from the decomposition of the protein medium upon which the bacteria grow, although undoubtedly

[91] Full résumé in Physiol. Reviews, 1923 (3), 438.

part of the ptomaines is also formed from the destruction of the bacterial cells themselves; how large a part of the ptomaines is formed by intracellular bacterial processes and how much by cleavage of the proteins of the media by extracellular bacterial enzymes is unknown. The structure of the ptomaines shows them to be very closely related to the amino-acids obtained by cleavage of the protein molecule by enzymes and other hydrolytic agencies. Most of the ptomaines are free from or poor in oxygen, hence reduction processes, or lack of sufficient oxygen for oxidation are probably important in their production. The poisonous ptomaines, which are decidedly in the minority among the entire group, are themselves subject to decomposition, being most abundant in the cultures after a certain period of time, and then decreasing in amount. Very old cultures show almost none of the higher molecular forms of nitrogen, such as ptomaines, these substances having been changed into ammonium and nitrate compounds. In sharp contradistinction to the toxins, *the ptomaines are not specific.* No matter upon what medium diphtheria bacilli grow, the toxin produced has qualitatively the same properties, whereas the nature of the ptomaines depends not only upon the nature of the bacteria producing them, but also even more upon the sort of soil upon which the bacteria are grown, the temperature, the duration of the process, and the quantity of oxygen furnished. The same organism may produce totally different ptomaines when grown on different media or under different conditions. Another essential difference is that we cannot obtain an immune serum, antagonizing the action of ptomaines, by injecting ptomaines into animals; *i. e.*, they are not antigenic.

If ptomaines do cause intoxications, presumably it is when they are taken in with food in which they have been produced by bacterial decomposition. Besides this food poisoning, it is also possible that ptomaines may be formed by putrefaction within the gastrointestinal tract. Another possible source of ptomaines is in decomposing tissues in gangrene. It is doubtful if ptomaines are produced in sufficient quantities by pathogenic bacteria infecting living tissue to be of any importance. Food poisoning is by no means uncommon, but we do not know how often it is due to ptomaines; it may be the result of poisonous materials contained abnormally in the food, that are not ptomaines, *e. g.*, botulism; or it may be due to an infection of the animal from which the meat came with pathogenic organisms, particularly the *B. enteritidis* of Gaertner and other bacteria related to the colon-typhoid group; or in other ways food ordinarily wholesome may become poisonous.[92] The commonest sources of ptomaine poisoning are supposed to be imperfectly preserved canned meats, sausages, decomposing fish, cheese, ice-cream, and milk.[93]

Chemical Composition of Ptomaines.—To indicate the composition and nature of ptomaines a few of the more important ones will be described. As illustrative of the simpler forms may be mentioned:

[92] See Jordan, "Food Poisoning," University of Chicago Press, 1917.

[93] All these matters are discussed at length by Vaughan and Novy, to whose book the reader is referred.

Methyl amine, $CH_3 - NH_2$.
Di-methyl amine, $CH_3 - NH - CH_3$.
Tri-methyl amine, $CH_3 - N(CH_3) - CH_3$.

These bodies, which are commonly found in decomposing proteins, are but very slightly toxic, and of little pathological importance.

The source of the ptomaines in the various amino-acids is usually easily traced through their chemical structure, and Ackermann and Kutscher[94] have classified them in this relation under the name "*aporrhegma.*"

When we examine the structural formulæ of some of the larger ptomaine molecules and compare them with the formulæ of the amino-acids that form the protein molecule, the relation is apparent, *e. g.*, compare iso-amylamine with leucine.

$(CH_3)_2CH - CH_2 - CH_2 - NH_2$ (iso-amylamine)

$(CH_3)_2CH - CH_2 - CH(NH_2) - COOH$ (leucine).

Putrescine, $C_4H_{12}N_2$, structural formula,

$$NH_2 - CH_2 - CH_2 - CH_2 - CH_2 - NH_2,$$

and *cadaverine*, $C_5H_{14}N_2$, structural formula,

$$NH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - NH_2,$$

are of interest because they have been found in the intestinal contents, arising from putrefaction of proteins, and also are sometimes present in the urine in *cystinuria*.[95] They are closely related to the diamino-acids, lysine and ornithine. They are but slightly toxic, although capable of causing local necrosis when injected subcutaneously. Ethylenediamine, $NH_2 - CH_2 - NH_2$, which is a homologue of putrescine and cadaverine, is also of low toxicity.[96] (See further discussion on these and the Pressor Bases in Chap. XXIII.)

The Choline Group.—Another group of ptomaines, including choline and closely related substances, is also of interest. These ptomaines are:

Choline, $CH_2OH-CH_2-N(CH_3)_3-OH$
Neurine, $CH_2=CH-N(CH_3)_3-OH$
Muscarine, $CH(OH)_2-CH_2-N(CH_3)_3-OH$
Betaine, $COOH-CH_2-N(CH_3)_3-OH$

The first point of importance is that choline is present in every cell normally, forming the nitrogenous portion of the lecithin molecule. Its source in putrefaction of tissues is, therefore, plain, and it is further discussed in Chapter XXIII.

The Pressor Bases.—By decarboxylation of amino acids, amines are obtained, and some of them, notably those derived from leucine, tyrosine, phenylalanine, and histidine, have a marked effect on non-striated muscle. These are discussed in Chapter XXIII.

Toxins

Certain bacteria produce soluble poisons by synthetic processes, which poisons are secreted into the surrounding medium and represent the chief poisonous products of the bacteria, being capable of causing most or all of the symptoms attributed to infection by the specific bacteria that have manufactured them. To this class of soluble poisons the term *toxin* has now become limited (for reasons that will be mentioned below),[97] including not only toxins of bacterial origin, but also poisons of similar nature produced by animals (snake venoms, eel serum, etc.) and by plants (ricin, abrin, crotin). The chief bacteria secreting true

[94] Zeit. physiol. Chem., 1910 (69), 265.
[95] Udránsky and Baumann, Zeit. physiol. Chem., 1889 (13), 562; 1889 (15), 77.
[96] Barbour and Hjort, Jour. Lab. Clin. Med., 1920 (5), 3.
[97] See Couch, Amer. Jour. Pharm., 1924 (96), 746.

toxins are *B. diphtheriæ*, *B. tetani*, *B. pyocyaneus*, and *B. botulinus*. Dysentery bacilli, *B. pyocyaneus*, the bacillus of symptomatic anthrax, the anaerobes of gas gangrene, and a few other pathogens also secrete toxins, and the hemolytic poisons produced by many bacteria seem to be true toxins.[98] It will be seen that the term toxin has been greatly narrowed since the time when all ptomaines and other poisonous bacterial products were called toxins, until now it has come to include the specific poisons of but a few of the great group of pathogenic bacteria.

Chemical Properties of Toxins.—The chemical nature of the toxins is entirely unknown. By various precipitation methods they may be carried down, but included with them are masses of impurities, chiefly proteins. They behave like electro-positive colloids,[99] but diffuse faster than proteins. They are precipitated by heavy metals which precipitate proteins, are adsorbed by animal charcoal, pass slowly through dialyzing membranes but not through filters permeable only to ultramicroscopic particles of fine dimensions, and diphtheria toxin at least is precipitated by weak acetic acid.[1]

It is not certain that toxins are not proteins, for although certain investigators report that by purification processes very active toxins have been obtained that did not give the protein reactions, yet the toxins are attacked by proteolytic enzymes, and, like proteins, are precipitated by nucleic acid (Kossel). Furthermore, experience with immunological processes adds doubt as to the possibility of antibody formation being incited by anything but proteins. It is possible that a toxin is a protein to which is attached a toxic radical, which may or may not be an integral part of the protein molecule. This toxic radical of itself is not necessarily antigenic, and the production of antibodies is dependent entirely upon the colloidal protein radical which itself may not be toxic. Thus, the venoms of different snakes of a single group (*e. g.*, the vipers) may produce identical physiological and anatomical effects, which fact suggests that the poisonous element is the same in each; but on the other hand, the immune serum against each, shows specific differences, which indicates that the protein radical of each venom is different.[2] Particularly supporting this view of the complex structure of toxin are the observations of Landsteiner,[3] that proteins combined with various organic compounds act as antigens which produce antibodies reacting with any sort of protein to which the same or similar chemical groups are attached. The simple chemical radical when alone does not act as an antigen in the sense of stimulating the production of specific antibodies, although it will unite specifically with the antibodies engendered by the protein complex.

[98] The Dicks attribute the rash of scarlet fever to a toxin produced by a hemolytic streptococcus, (Jour. Amer. Med. Assoc., 1924 (82), 1246). A toxin seems to be produced by *Staphyl. aureus* (Julia T. Parker, Jour. Exp. Med., 1924 (40), 761).

[99] Field and Teague, Jour. Exper. Med., 1907 (9), 86.

[1] Watson and Wallace, Jour. Path. and Bact., 1924 (27), 289.

[2] See Nicolle, Jour. State Med., 1920 (28), 293.

[3] Biochem. Zeit., 1919 (93), 106; ibid., 1920 (104), 280.

A characteristic difference between the action of toxins and many simpler chemical poisons is, that while with the latter the effects are produced in a very short time after injection, there is a *latent period* of several hours before symptoms appear after injecting toxins. What occurs during this latent period is not fully known, but that there is a latent period suggests a resemblance to enzyme action. An alkaloidal or other chemical poison enters the cell, and its harm is done at once. A toxin combines with the cell, and then, if it produces its effects by an enzymatic alteration of the cellular structure, some time must elapse before the changes are great enough to cause the appearance of symptoms.

Resemblance of Toxins to Enzymes.—Oppenheimer says of the toxins, "we must be contented to assume that they are large molecular complexes, probably related to the proteins, corresponding to them in certain properties, but standing even nearer to the equally mysterious enzymes with whose properties they show the most extended analogies both in their reactions and in their activities." First of all we meet the same difficulty in isolating toxins that we do in isolating enzymes. "A pure toxin is as unknown as a pure enzyme" (Oppenheimer). At first both were believed to be proteins; now both are considered by many not to be proteins, but molecular complexes of nearly equally great dimensions. That toxins, like enzymes, are colloids, has been abundantly demonstrated.[4] Both pass through porcelain filters, but both lose much of their strength in the process, and they are almost entirely held back by dialyzing membranes. They behave similarly as regards adsorption by suspensions,[5] and have similar effects on the physical properties of their solutions (Zunz).[6] Neither will withstand boiling, and most forms are destroyed at 80° instantly or in a very short time; on the whole, however, toxins are more susceptible to heat, as well as to most other injurious agencies. Both stand dry heat over 100°, and extremely low temperature, without much injury. Left standing in solution for some time they gradually lose their specific properties, and in each case this seems to be due to an alteration in the portion of the molecule that produces the destructive effects (*toxophore* or *zymophore group*), while the portion of the molecule that unites with the substance that is to be attacked (*haptophore* group) remains uninjured, the toxin becoming a *toxoid*, the enzyme a *fermentoid*. On the other hand, enzymes and toxins seem to produce their effects according to different laws:—A small amount of enzyme can in course of time produce an almost indefinite amount of effect, whereas toxins act more nearly quantitatively. It seems as if the enzyme were bound to the body upon which it acts, as is the toxin, but that after it has destroyed this body it is set free in a still active form, ready to accomplish further work, whereas the toxin is either not set free, or it becomes inactive after it has once been combined. Furthermore, as pointed out in Chap. VII, enzymes have little if any capacity to incite the formation in animals of specific antienzymes which neutralize their actions.

Agencies Destroying or Modifying Toxins.—Toxins are very susceptible to light, direct sunlight soon destroying the power of toxin solutions. Fluorescent substances destroy toxins both *in vitro* and in the body.[7] They are generally destroyed by moist heat of 80°, but resist 100° when dry. Oxygen, even dilute as in air, is harmful; and all oxidizing agents destroy them quickly.[8] Like enzymes, they withstand such antiseptics as chloroform, toluene, etc., and are precipitated by the

[4] See Zangger, Cent. f. Bakt. (ref.), 1905 (36), 239.

[5] By flocculation of the colloids bearing adsorbed toxins it may be possible to secure them in comparatively pure condition (London, Compt. Rend. Soc. Biol., 1917 (80), 756).

[6] Arch. di Fisiol., 1909 (7), 137.

[7] Literature given by Noguchi, Jour. Exper. Med., 1906 (8), 263.

[8] According to Pitini (Biochem. Zeit., 1910 (25), 257) toxins cause their harmful effects by reducing the oxidizing capacity of the tissues.

heavy metals; contact with polished silver or copper destroys them.[9] Some agencies seem to attack only the toxophore portion of the molecule, *e. g.*, iodine,[10] carbon disulfide (Ehrlich). Formalin and iodine also change toxins into toxoids or "anatoxins"[11] of high immunizing capacity.[12] The fact that the plant toxins such as abrin, which seem definitely to be proteins, are also converted into anatoxins by the same procedures,[13] is strong support for the view that toxins are protein. Certain toxins (diphtheria, dysentery) can be converted into non-toxic modifications by acids, the original toxicity being restored by bases (Doerr),[14] which fact, Pick maintains, is in support of the protein nature of toxins. Salts of monovalent metals have no effect on toxins, but bivalent and trivalent salts are injurious to them, tetanus toxin being more sensitive than diphtheria toxin. *X*-rays are said to weaken them.[15]

Introduced into the gastro-intestinal tract, most bacterial toxins are not absorbed (botulinus toxin excepted), cause no symptoms, and do not reappear in the feces; they are probably destroyed by the contents of the tract, pepsin, pancreatic juice, and bile all being capable of destroying toxins.[16] They may, however, when injected subcutaneously, circulate unimpaired in the blood of non-susceptible animals, gradually disappearing, more through slow processes of destruction than by elimination. When injected into susceptible animals they soon disappear from the blood, being fixed in the organs that they attack. Toxins are also bound by lipoids, fats, and similar substances, which accounts, at least in part, for the affinity of tetanus toxin for nervous tissues.[17] In common with other colloids they are adsorbed by surfaces, such as charcoal, kaolin, etc.; such adsorption is accompanied by little change in any of the physical properties of the solution, except an increase in surface tension (Zunz).

Differences from Ptomaines.—While ptomaines are formed by cleavage processes from the medium upon which the bacteria grow, and the same ptomaines can be produced by several different kinds of bacteria, the *toxins are synthetic products of absolutely specific nature.* However, the toxins seem to be produced little if at all by growing the bacteria on Uschinsky's or similar media, which contain no proteins, carbohydrates, or fats, but merely simple organic and inorganic salts of known composition.[18] Nevertheless, diphtheria toxin is essentially the same no matter on what sort of medium the bacteria are grown, whereas ptomaines vary with the nature of the substance from which they are produced. Toxins are true secretions of bac-

[9] Erdstein and Fürth, Biochem. Zeit., 1921 (118), 256.

[10] See Teale and Embleton, Jour. Path. and Bact., 1919 (23), 61.

[11] Ramon, Ann. Inst. Past., 1924 (38), 1.

[12] Glenny and Hopkins, Brit. Jour. Exp. Path., 1923 (4), 283; Jour. Path. and Bact., 1924 (27), 261; Weinberg and Goy, Compt. Rend. Soc. Biol., 1924 (91), 1140.

[13] Ramon, Ann. Inst. Past., 1925 (39), 1.

[14] Wien. klin. Woch., 1907 (20), 5. See also de Potter, Bull. Acad. roy. med. Belg., 1923 (3), 394.

[15] Gerhartz, Berl. klin. Woch., 1909 (46), 1800.

[16] Baldwin and Levene (Jour. Med. Research, 1901 (6), 120) found that diphtheria and tetanus toxin are both destroyed, apparently through digestion, by pepsin, trypsin and papain acting for several days. Review of Literature by Lust, Hofmeister's Beitr., 1904 (6), 132. See Vincent, Ann. Inst. Pasteur, 1908 (22), 341.

[17] Loewe, Biochem. Zeit., 1911 (33), 225, and (34), 495.

[18] See Rettger and Robinson, Jour. Med. Res., 1917 (38), 357.

terial cells, just as trypsin is of pancreatic cells, or thyroiodin of thyroid cells. Antibodies can be produced against toxins, but not against ptomaines.

Ehrlich's Conception of the Nature of Toxins.—Chemical studies of toxins being impossible, we have been obliged to study them through their physiological effects, just as we have obtained information concerning enzymes through their specific actions. In this way Ehrlich developed definite ideas concerning the structure of toxins, as well as the manner in which they act, which may be briefly summarized as follows: Each toxin molecule consists of a large number of organic complexes, grouped, as in other organic compounds, as side-chains about a central chain or radical. One or more of these complexes has a chemical affinity for certain chemical constituents of the tissues of susceptible animals, with which the toxin molecule unites; this binding group is called the *haptophore* (meaning "bearing a bond"). Another side-chain or group of side-chains exerts the injurious effects upon the tissue to which the molecule has been bound by the haptophore, and cannot produce these injurious effects unless it has been so bound. This injury-working group is called the *toxophore*. An animal is susceptible to a toxin only when its cells contain substances which possess a chemical affinity for the haptophore groups of the toxin, and also substances which can be harmfully influenced by the toxophore groups. Tetanus toxin, for example, owes its effects to the fact that nervous tissues contain chemical substances having a strong affinity for the haptophore group of tetanus toxin, and also substances that can be attacked with serious results by the toxophore group of the toxin. The nature of the changes brought about by the toxophore groups of toxins is not understood; there are many resemblances to the action of enzymes, as mentioned previously, but the analogy is by no means complete. We find perhaps the closest analogy to the enzymes in the toxic substances that destroy red corpuscles and bacteria (*hemolysins, bacteriolysins*), which will be considered in another place. The immunity against toxins was believed by Ehrlich to be produced by an overproduction of the cellular constituents (*receptors*) which bind the haptophore groups to the cells, these excessive receptors being secreted into the blood, where they combine with the toxin so that it cannot enter into combination with the cells. This "side chain theory" of Ehrlich has been a useful working hypothesis, although it is becoming highly probable that it does not picture the exact method of toxin and antitoxin action.[19]

Endotoxins[20]

By far the greater number of pathogenic bacteria do not secrete their poisons as toxins into the surrounding medium, although they manifestly cause disease by poisoning their host. Among them are such organisms as the typhoid bacillus, pneumococcus, the pus cocci, cholera vibrios, and many others. If cultures of these organisms are filtered, the filtrate will be found to be but slightly toxic (except for the hemolytic poisons), although the bodies of the bacteria, after they have been killed by chloroform or other antiseptics, are highly poisonous if injected into an animal. These bacteria,

[19] Full review by Wells in "Chemical Aspects of Immunity," 1924, Chap. V.
[20] See general review by Pfeiffer, Jahresber. d. Immunitätsforsch., 1910 (6), 13.

then, produce poisons which do not escape from the cells into the culture-medium, but are firmly held within them. By using various means these intracellular toxins, or *endotoxins*, can be obtained independent of the bacterial cells. One of these is to grind up the cells, which can be particularly well done if they are first made brittle by freezing at the temperature of liquid air (MacFadyen's method). By very great pressure in the Buchner press the cellular contents can be expressed. They may also be obtained by letting the bacteria autolyze themselves for a short time in non-nutrient fluids (Conradi,[21] *et al.*), or they may be liberated when bacteria are digested with trypsin after preliminary extraction with acetone.[22]

Endotoxins obtained in this way are soluble and highly poisonous, and it is undoubtedly through their action that the characteristic diseases are produced by the bacteria that contain them. Presumably the endotoxins are liberated in the body either by autolysis, or by heterolysis by the enzymes of the body cells and fluids, and there is some question as to whether they are preformed specific constituents of the bacteria, or merely the poisonous product of enzymatic disintegration of the bacterial proteins, similar to the "anaphylatoxins."[23]

Endotoxins differ from the true toxins in one important respect: namely, *it is difficult or impossible to obtain an antitoxin for endotoxins by immunization of animals.*[24] Animals immunized against endotoxins develop in their serum substances that are bactericidal and agglutinative to the bacteria from which the poisons are derived, but according to many observers the serum will not neutralize the endotoxins. The chemical nature of the endotoxins is also unknown, for they are always obtained mixed with the other constituents of the bacteria.[25]

Tuberculin,[26] once supposed to be an albumose, is produced even when the bacilli are grown on a protein-free medium, and in the active solution no albumose or other protein is then found. Hence it has been thought that tuberculin is of the nature of a polypeptid, which gives no biuret reaction but is destroyed by pepsin and trypsin, according to Loevenstein and Pick,[27] but not by erepsin (Pfeiffer)[28]. The isoelectric point of tuberculin is about 4.1,[29] and it behaves like proteins in this as in other respects. Whether tuberculin should be considered an endotoxin liberated by the disintegration of the bacilli in the cultures is unknown; Pick looks upon it as a secretion of the bacilli, and closely related to the true toxins. It has no appreciable toxicity of itself, and hence cannot be classed among the toxins. Only in the animal infected with tuberculosis does it exhibit any appreciable local or systemic toxicity, and hence it resembles the protein antigens in the anaphylaxis reactions. According to Zinsser[30]

[21] Deut. med. Woch., 1903 (29), 26.

[22] Douglas, Brit. Jour. Exp. Path., 1921 (2), 175.

[23] See Dold and Hanau, Zeit. Immunität., 1913 (19), 31; Zinsser, "Infection and Resistance," N. Y., 1923, Chap. XIX; Teale, Lancet, Aug. 7, 1920, p. 279.

[24] Positive results are claimed by Besredka (Ann. Inst. Pasteur, 1906 (20), 304), and some others; see Kraus, Wien. klin. (Woch., 1906 (19), 655; Zeit. Immunität., 1909 (3), 646. It is suggested by Wassermann (Kolle and Wassermann's Handbuch, 1912 (2), 246) that this difficulty in obtaining antiendotoxins depends on the large size of the molecule,—the small diffusible toxin molecule is so altered in its physical condition through union with the antibody that its properties are much altered, whereas the large endotoxin molecule must be digested by complement before its toxicity is destroyed. Douglas[22] suggests that antitoxin formation depends on the bacterial digestion reaching the proper toxic phase, only agglutinins and opsonins being formed against the antigens produced in early stages of bacteriolysis.

[25] The *Aggressins* of Bail, to which he ascribes the pathogenicity of bacteria, are too little established to permit of a discussion from the chemical standpoint. By many they are believed to be nothing more than endotoxins, but Ohtsubo (Kitasito Arch. Exp. Med., 1923 (6), 82) believes aggressins to be metabolic secretion products of the bacteria. (Literature given by Müller, Oppenheimer's Handb. d. Biochem., 1909 (II (1)), 681; Dudgeon, Lancet, 1912 (182), 1673). According to Ingravelle (Ann. d' ig. sperim., 1910 (20), 483), typhoid aggressins are found in the albumins.

[26] See review by Long, "Chemistry of Tuberculosis," Wells, DeWitt and Long, 1924, p. 70.

[27] Biochem. Zeit., 1911 (31), 142.

[28] Wien. klin. Woch., 1911 (24), 1115; see also Lockmann, Zeit. physiol. Chem., 1911 (73), 389.

[29] Gabbe, Biochem. Zeit., 1923 (141), 523.

[30] Jour. Exp. Med., 1921 (34), 495.

there are two fundamental types of sensitization to the products of the tubercle bacillus. One is the ordinary anaphylactic reaction to the proteins of the bacillus present in most preparations of tuberculin. The other, the typical skin reaction, is given even by preparations of tuberculin freed as far as possible from proteins removable by heat coagulation, the active solutions containing no proteins demonstrable by ordinary tests. That such preparations of tuberculin are entirely free from protein has not been proved, and it is quite certain that often they are not, since heat coagulation is never complete.[31] Neither has it been shown that such preparations are antigenic to the extent of inducing the formation of specific antibodies in non-tuberculous animals.[32] Hence our knowledge of tuberculin does not permit us to say that it does or does not contain a non-protein antigen.

Poisonous Bacterial Proteins

If we filter a bouillon culture of diphtheria bacilli through porcelain, wash thoroughly with salt solution the bacteria remaining, and collect them thus freed from their secretion products, it will be found that extracts of the bacterial substance or the bodies of the killed bacteria themselves are quite free from the typical toxin. This indicates that the toxin is eliminated from the bacteria as fast as it is formed, and no considerable quantity is retained within the cell. The bacterial substance, however, or proteins isolated from it, is found to produce severe local changes when injected into the bodies of animals, necrosis and a strong inflammatory reaction with pus-formation being the chief features. This local effect is not a specific property of the diphtheria bacillus, for other bacterial proteins, including proteins from non-pathogenic bacteria, will produce the same changes; indeed, many proteins that are derived from vegetable and animal sources have equally marked pyogenic properties. All foreign proteins when introduced into the circulation of animals are more or less toxic, and the toxic effects of the bacterial proteins are, for the most part, neither specific nor particularly striking. There are a few pathogenic organisms, however, which seem to produce neither true toxins nor endotoxins, notably the tubercle bacillus and the anthrax bacillus, and with these there may be a relation between their protein constituents and their specific effects.

Numerous protein substances have been extracted from bacterial cells, particularly nucleoproteins, but also proteins resembling albumins, nucleo-albumin, and globulins. That the chief proteins of the bacterial cell are nuclein or phosphoprotein compounds, is indicated by their nuclear staining.

Some of the earlier observations on the toxicity of bacterial proteins were erroneous because impure proteins, containing toxins, endo-toxins, and ptomaines, were used. Schittenhelm and Weichardt[33] have found, however, that bacterial proteins are much more toxic than any ordinary proteins, as indicated by loss of nitrogen, temperature changes and alterations in the leucocytes of injected animals. Furthermore, there are few other proteins that produce so much inflammatory reaction as the bacterial proteins.

Vaughan and his students have been able to split off from the bodies of various pathogenic bacteria toxic materials which are stated to resemble in some respects the protamins,[34] although they do not all give a satisfactory biuret test. These toxic materials are evidently quite different from either the true soluble toxins or the endotoxins, since they resist heating for ten minutes, at 110° in the autoclave with 1 per cent. sulfuric acid, this being a method used for securing the substance. Since the sarcinæ and *B. prodigiosus* also yield similar toxic products, they cannot be considered as the specific toxic substances of the pathogenic bacteria, but apparently are common to all proteins of whatever origin. With some bacteria the splitting process with sulfuric acid separates completely the toxic from the non-toxic insoluble bacterial substance,[35] *e. g., B. coli communis;* with others a toxic portion remains insol-

[31] The failure to obtain the biuret reaction with tuberculin may depend on the fact that this test is inhibited by the glycerol in the preparations, as shown by Seibert and Long, (Jour. Biol. Chem., 1925 (64), 229.

[32] See Adler, Wien. Arch. inn. Med., 1923 (7), 27; Markert, Zeit. Immun., 1924 (40), 172.

[33] Münch. med. Woch., 1911 (58), 841.

[34] A full review of this work is given in Vaughan's "Protein Split Products," Philadelphia, 1913; and in Jour. Lab. Clin. Med., 1916, Vols. 1 and 2.

[35] Wheeler, Jour. Amer. Med. Assoc., 1905 (44), 1271.

uble. The colon bacillus protein gives all the protein reactions, is synthesized on Uschinsky's medium, and does not yield a reducing carbohydrate. From *B. typhosus* about 10 per cent. by weight of protein can be split off by dilute acid, of which at least a part seems to be a phosphorized glycoprotein.[36] Poisonous substances have also been obtained from *B. diphtheriæ*, *B. anthracis*, *B. tuberculosis*[37] and *B. pyocyaneus*. They produce death without the usual latent period observed with toxins, and are very toxic, a few (10–20) milligrams of colon bacillus poison killing guinea-pigs in less than ten minutes.[38] A certain degree of immunity can be obtained against them.[39] Their relation to endotoxins and anaphylatoxins has yet to be determined.

Bacterial Pigments[40]

The formation of pigment by bacteria seems to be, for the most part an adventitious, unessential property. There are a few bacteria which possess pigments of the nature of chlorophyll,[41] or allied to it, and this pigment is undoubtedly of great importance in the life processes of these particular forms. Other varieties of pigment-forming bacteria, of which but very few are pathogenic (*Bacillus pyocyaneus*, *B. proteus fluorescenes*, *S. pyogenes aureus* and *citreus*, *M. cereus flavus*), seem to produce pigment as a waste product which is excreted from the cell as fast as formed. Generally the pigments are produced in a colorless form (*leuco-base*) which is oxidized by the air into the pigment, *e. g.*, in *pyocyaneus* infections the soiled dressings are most colored about the portions most exposed to air. Since pigment-forming bacteria produce pigments only under certain conditions, and can grow abundantly without producing any pigment, it is evident that the pigment formation is no very essential part of their metabolism. It is possible to modify pigment production almost at will, and even to develop races of bacteria that do not produce pigment at all from races that ordinarily are pigment-producers.

Of numerous classifications of pigment-forming bacteria, all faulty because of our slight knowledge of the chemistry of the process, that of Migula seems the best; it is based on the solubility of the pigments formed, as follows:

(1) **Pigments Soluble in Water.**—This includes the pigments of all fluorescent bacteria, as well as those giving a red or brown color to gelatin media. Most important among these is *Bacillus pyocyaneus*, whose pigments have been considerably studied. There seem to be two pigments, one, *pyocyanin*, characteristic for this organism; and a fluorescent pigment which numerous other organisms also produce. Pyocyanin has been analyzed by Ledderhose, who found it to be a ptomaine-like body, a derivative of the aromatic series, probably related to the anthracenes. It can be reduced to a colorless leuco-base, in which form it is probably produced by the bacteria, and then is oxidized in the air into the pigment. Wrede and Strack[42] have separated pyocyanin in the form of dark blue needles, and give to it the formula $C_{26}H_{24}N_4O_2$. This is readily split into two molecules of hemipyocyanin ($C_{13}H_{12}N_2O$) in which the oxygen is in an OH group, but further than this the chemical constitution

[36] *Ibid.*, 1904 (42), 1000.
[37] See White and Avery, Jour. Med. Res., 1912 (26), 317.
[38] Jour. Amer. Med. Assoc., 1905 (44), 1340; American Medicine, 1905 (10), 145.
[39] Vaughan (Jr.), Jour. of Med. Research, 1905 (14), 67.
[40] For complete bibliography and résumé see Sullivan, Jour. Med. Research, 1905 (14), 109.
[41] See Metzner, Ber. botan. Ges., 1922 (40), 125.
[42] Zeit. physiol. Chem., 1924 (140), 1.

has not yet been worked out. Probably the nitrogen is in the imide form.[43] The fluorescent pigment is insoluble in alcohol and chloroform, and can thus be separated from pyocyanin, which is soluble in chloroform. Although related to the ptomaines, pyocyanin seems to be altogether non-poisonous to animals.[43]

Jordan[44] and Sullivan[40] have studied the conditions under which pigments are formed, and found that pyocyanin can be produced in protein-free media, and without the presence of either phosphates or sulfates; but both sulfur and phosphorus must be present to produce the fluorescent pigment. As pigments can be produced on media containing only ammonium salts of succinic, lactic, or aspartic acid, or asparagin, they are evidently formed synthetically, and not by cleavage of the media.

(2) **Pigments Soluble in Alcohol and Insoluble in Water.**—The most important bacteria of this group are the *Staphylococcus pyogenes aureus* and *citreus*. Their pigment is of a fatty nature, a *lipochrome*, which lies among the bacteria in the form of dendritic crystals. Being a fat, it can be saponified, and when decomposed it gives the acrolein reactions and odor, from the breaking down of the glycerol of the fat molecule. Acted upon by strong sulfuric acid, the yellow pigment changes into blue granules and crystals (*lipocyanin* reaction). The lipochromes are soluble in the usual fat solvents, and form fat spots on paper.

(3) **Pigments Insoluble in Water and in Alcohol.**—The pigment of *Micrococcus cereus flavus* belongs to this class; its nature is quite unknown.

[43] Analysis of pyocyanin-HCl by Madinaveitia (Anales soc. espan. fis. quim., 1916 (14), 263) gave $C_{60}H_{59}N_{10}Cl_5O_3$, but the physical properties indicated a lower molecular weight.

[44] Jour. Exper. Med., 1899 (4), 627.

CHAPTER V

CHEMISTRY OF THE ANIMAL PARASITES[1]

THIS subject has received much less consideration than its importance deserves, and we are quite in the dark as to how much of the effects produced by animal parasites are not merely mechanical, but are due to soluble poisons that they may secrete or excrete. Some of the parasites probably cause harm mechanically and in no other way, but with most of them there is more or less evidence of the formation of poisonous substances. The composition of the bodies of the animal parasites is an almost unexplored field, but we have no reason to believe that the composition of the cells of invertebrates differs essentially from that of the cells of higher organisms. Perhaps the most characteristic constituent observed in many forms is *chitin*, which forms a large part of the outer covering of the encysted forms, and probably of many of the worms. *Glycogen* is usually abundant in the invertebrates, and the animal parasites form no exception,[2] this carbohydrate having been found in their bodies by many observers.

Eosinophilia.—One of the most characteristic features of the animal parasites is that they exert a positive chemotaxis for eosinophile leucocytes.[3]

An increase in the number of these cells in the blood, as well as a local accumulation in the tissues nearest the parasite, has been observed in infection with practically all the animal parasites.[4] Of these, infection with *Trichinella spiralis* causes the most pronounced eosinophilia, presumably because of the great number of parasites present in the tissues at once. That the eosinophilia is due to the action of the soluble products or constituents of the parasites has been shown by experimental injection into animals of extracts from the bodies of the parasites. The substances which cause chemotaxis of eosinophiles are not destroyed by preservation of the parasites in alcohol or formalin and may be extracted with either water or alcohol;[5] apparently they are constituents of the parasites rather than products of their metabolism. Calamida has found that extracts of dog tapeworms also, when placed in the tissues in a capillary tube, cause an accumulation of eosinophile cells in the tube.[6] Experimental infection with excessive numbers of trichinella causes a rapid diminution in the number of eosinophile leucocytes, which also show evidences of disintegration in the bone-marrow and lymph nodes. Such large infections are fatal, which suggests that the eosinophilia has a protective influence. In favor of this view is the observation of Milian,[7] who found that sarcosporidia in beef are destroyed by a violent leucocytic reaction, the prevailing cell being the eosinophile. As the eosinophile

[1] General references to this subject will be found in v. Fürth's "Vergleichende chemische Physiologie der niederen Tiere," Jena, 1903; Faust's "Tierische Gifte," Braunschweig, 1906; Koch, Ergebnisse Pathol., 1910 (XIV(1)), 41.

[2] See Pflüger, Pflügers Arch., 1903 (96), 153.

[3] Literature by Schwarz, Ergeb. allg. Pathol., 1914 (17_1), 138.

[4] Literature by Bruns, Liefmann and Mäckel, Münch. med. Woch., 1905 (52). 253; Vallillo, Arch. wiss. u. prakt. Tierhk., 1908 (34), 505.

[5] Homma, Arch. path. Anat., 1921 (233), 11.

[6] Negative results were obtained with extracts of *Sclerostoma equinum* by Grosso (Folia Hematol., 1912 (14), 18).

[7] Bull. et mem. soc. anat., 1901 (Ser. 6, T. 3), 323.

increase does not occur until several days after the infected flesh is eaten, the chemotactic substance is not liberated from the encapsulated trichinellæ when their capsules are digested off in the gastric juice, but comes either from the free larvæ, or from the degenerated muscles in which they burrow. Coincident bacterial infection may reduce the number of eosinophiles. Herrick[8] finds that extracts of *Ascaris lumbricoides* cause a notable eosinophilia, but only when the animal has been sensitized previously with the same extract, the active agent of which is a protein; this suggests a relationship between parasitic and anaphylactic eosinophilia.[9] That the eosinophiles play a part in the immunity reactions observed in the hosts of animal parasites is indicated by the observation that hydatid fluid loses its antigenic properties when in contact with eosinophiles.[10]

PROTOZOA

These unicellular forms possess all the chemical characters of the cells of higher forms, even to the more specialized constituents.[11] Thus it has been demonstrated that protozoa contain proteolytic enzymes,[12] and that they secrete an acid into their digestive vacuoles.[13] Whether the *Endameba histolytica* produces any toxic materials, specific or nonspecific, has not yet been determined, but the necrosis that it produces in liver abscesses, when bacterial coöperation can often be excluded by culture, strongly indicates the production of necrogenic substances. Apparently these substances are not chemotactic, in view of the absence of leucocytic accumulation in the lesions of amebic dysentery. There is also no evidence, clinical or experimental, that amebic infection causes the formation of anti-substances of any kind in the body of the host, except that precipitins for *Endameba histolytica* have been found in the blood of cats infected with this organism.[14] The serum of rabbits immunized against amebæ was found by Sellards[15] to be cytolytic for the same amebæ, but no antibodies could be found in the blood of patients with amebic dysentery. The spontaneous clinical recovery from amebic and other protozoan infections, however, may be considered as indicating the development of an *immunity* against these organisms.[16]

Numerous observers have suggested the possibility of obtaining artificial immunity against protozoa, and Rössle[17] has obtained immune sera against infusoria. Glaessner[18] has obtained an antiserum by immun-

[8] Arch. Int. Med., 1913 (11), 165.

[9] Supported by Paulian, Presse Méd., 1915 (23), 403.

[10] Weinberg and Séguin, Ann. Inst. Pasteur, 1916 (30), 323.

[11] However, on the basis of microchemical reactions, Unna and Tielemann (Cent. f. Bakt., 1917 (80), 66) maintain that *Amœbæ* from hay infusion possess no nucleins, a conclusion so improbable that it will require verification before acceptance. These authors (Arch. f. Schiffshyg. 1919 (23), 37) also maintain that the nucleus of trypanosomes contains histone, while the chief constituent of the ameba nucleus they believe to be a protamine.

[12] Mouton, Compt. Rend. Soc. Biol., 1901 (53), 801. However, Glaessner[18] could demonstrate no proteolytic activity with supsensions of *Balantidium coli* which did exhibit diastase and hemolysins.

[13] Le Dantec, Ann. Inst. Pasteur, 1890 (4), 776; Greenwood and Saunders, Jour. of Physiol., 1894 (16), 441.

[14] Wagener, Univ. Calif. Publ. Zool., 1924 (26), 15.

[15] Philippine Jour. Sci., 1911 (6), 281.

[16] Concerning immunity to protozoan infections see Schilling, Kolle and Wassermann's Handbuch, 1913 (7), 566.

[17] Arch. f. Hyg., 1905 (54), 1; full review of this topic.

[18] Cent. f. Bakt., 1908 (47), 351.

izing rabbits with *Balantidium coli* and Wagener[19] has observed skin reactions in rabbits infected with *Leishmania*. Novy[20] has obtained immunity against trypanosomes, but the serum of immune animals will not confer passive immunity. Braun and Teichmann,[21] however, claim positive results with immune serum from rabbits; they found no poisonous agent in trypanosome substance.[22] Taliaferro[23] has found that rats infected with *T. lewisi* develop in the blood some agent which inhibits reproduction of the trypanosomes without killing them. A lysin is formed later which kills the parasites, but this persists for a much shorter time than the agent which inhibits reproduction. In human serum a trypanocidal agent has been found associated with the globulins,[24] and Rosenthal[25] considers the reticulo-endothelial system to be particularly important in producing this agent.

The fact that trypanosomes themselves readily become immune to various trypanocidal chemicals has been demonstrated and extensively studied in Ehrlich's laboratory.[26] Gonder[27] has made the interesting observation that trypanosomes which can be stained by certain vital stains, become unstainable while alive if immune to arsenic compounds, suggesting that this immunity is associated with considerable structural or chemical changes. Neuschlosz[28] found that *Paramecium* becomes resistant to quinine through developing the capacity to destroy it, while when immunized to arsenic or antimony this organism protects itself by changing the trivalent poison to the less toxic pentavalent form.

Malarial plasmodia undoubtedly produce toxic substances, which seem to be of such a nature that they do not diffuse from the red corpuscle, but are only liberated when the corpuscle breaks up on the maturation of the parasite. In this way the characteristic paroxysmal manifestations of the disease are produced. The nature of the poison or poisons is unknown, but we have evidence that it is hemolytic, since malarial serum may hemolyze normal corpuscles,[29] and extracts of the parasites are strongly hemolytic (Brem[30]); probably the malarial hemoglobinuria is caused by this hemolysis. Sudden release in the blood stream of a large amount of foreign protein might also explain the paroxysm. Presumably malarial poisons are not extremely toxic for parenchymatous cells, since the parenchymatous lesions in malaria seem to be relatively slight as

[19] Univ. of Calif. Publ. Zool., 1923 (20), 477.
[20] Jour. Infec. Dis., 1912 (11), 411.
[21] Zeit. Immunität., Ref., 1912 (6), 465.
[22] Hintze (Zeit. f. Hyg., 1915 (80), 377) obtained little immunity with *T. brucei*, but Schilling and Rondoni (Zeit. Immunität., 1913 (18), 651) obtained a poison from Nagana trypanosomes which produced active immunity in mice. When trypanosomes are killed by weak electric currents they may liberate an active poison (Uhlenhuth and Seyderhelm, Zeit. Immunität., 1914 (21), 366).
[23] Jour. Exp. Med., 1924 (39), 171.
[24] Rosenthal and Freund, Zeit. f. Immunität., 1923 (37), 48.
[25] Klin. Woch., 1924 (3), 1657.
[26] See Voegtlin *et al.*, Jour. Pharm., 1924 (23), 55.
[27] Zeit. Immunität., 1913 (15), 257.
[28] Arch. ges. Physiol., 1919 (176), 223; 1920 (178), 61, 69.
[29] See Regnault, Revue de Méd., 1903 (23), 729.
[30] Arch. Int. Med., 1912 (9), 129.

compared with the intensity and duration of the intoxication. Some authors state that the toxicity of the urine is increased after the paroxysm,[31] which, however, does not necessarily indicate that a poison formed by the parasites is excreted in the urine. Immunity seems to be seldom developed against the malarial poison or against the parasite itself, although some persons seem to be naturally immune, while some acquire immunity through previous infection.[32] The blood of persons with malaria exhibits no demonstrable antibodies for the parasite (Ferrannini),[33] although it seems to have some antihemolytic power (Brem). (Concerning the pigment present in the malarial parasites see "Pigmentation," Chap. XX.)

Sarcosporidia of sheep yield aqueous and glycerol extracts that are highly toxic for rabbits (Pfeiffer), the poisonous constituent of which was called *sarcocystin* by Laveran and Mesnil.[34] This is so highly toxic that 0.0001 gm. is fatal to rabbits (per kilo), other animals being less susceptible. It loses its toxicity on heating at 85° for twenty minutes, and is impaired at 55–57° for two hours. It produces pruritis and other anaphylactic symptoms, and although the serum of sheep with this parasite does not confer passive anaphylaxis to sarcosporidia, yet it does give positive complement fixation.[35] That it is a true toxin is shown by Teichmann and Braun,[36] who produced an effective antitoxin by immunizing rabbits; only rabbits seem to be susceptible to the toxin. The sarcosporidia contain also a distinct thermostable agglutinin. The lethal dose of dried substance of sarcosporidia is, for rabbits, but 0.0002 gm., and the poison seems to unite with the lipoids of the nervous system (Teichmann).[37] It is probable that the pathogenic protozoa, at least in some instances, have a semipermeable membrane about them, for Goebel[38] found that trypanosomes are very susceptible to changes in osmotic conditions.

CESTODES

Tænia echinococcus has been by far the most studied, its abundant fluid content furnishing suitable material for investigation. That this fluid is toxic has been repeatedly observed when, through rupture or puncture, the fluid has escaped into the body cavities; such accidents are often followed by violent intoxication, sometimes by death.[39] As long as the cyst is unopened no toxic manifestations are observed. The most constant symptoms are local irritation and inflammation, accompanied by urticaria, which may also be produced experimentally in man

[31] Quoted from Blanchard, Arch. d. Parasitol., 1905 (10), 83; this article gives a résumé of the subject of the toxic substances produced by the animal parasites.
[32] See Celli, Cent. f. Bakt., 1900 (27), 107.
[33] Riforma Med., 1911 (27), 177.
[34] Compt. Rend. Soc. Biol., 1899 (51), 311.
[35] McGowan, Jour. Path. and Bact., 1913 (18), 125.
[36] Arch. f. Protistenk., 1911 (22), 351.
[37] *Ibid.*, 1910 (20), 96; see also Knebel, Cent. f. Bakt., 1912 (66), 523.
[38] Ann. Soc. Méd. d. le Gand, 1906 (86), 11.
[39] See Achard, Arch. gén. de méd., 1887 (22), 410 and 572.

if the cyst contents are injected subcutaneously. The symptoms are so strikingly similar to those of anaphylactic intoxication, that it is now generally believed that they are the result of such a reaction in a person sensitized by absorption of antigenic substances from the cyst.[40]

Carriers of echinococcus cysts have been found to have in their blood antibodies giving precipitin[41] and complement fixation[42] reactions with extracts of echinococcus, and sometimes with other tænia.[43] The antigen of the echinococcus is believed by some to be a lipoid;[44] in the case of *Tænia saginata*, at least, it seems to be associated with the lecithin (Meyer[43]). Graetz,[45] however, states that the protein of the hydatid cyst is derived from the host, and that it is therefore incapable of causing anaphylaxis in that host, but it may undergo alterations in the cyst so that it is toxic after the order of anaphylatoxins (*q. v.*). However, intracutaneous reactions are given by infected individuals injected with hydatid fluid or with the proteins, especially the globulins, isolated from the cyst fluid (Botteri.)[46] Hydatid fluids from human sources contain more protein than those from bovine cysts. The complement fixation reaction with echinococcus fluid has been found quite reliable in the clinic, 93 per cent. of positive reactions having been obtained in 500 cases collected by Zapelloni,[47] while controls were always negative.

The *fluid* of the echinococcus cysts has generally a specific gravity of 1005–1015, and contains 1.4–2 per cent. of solids. It resembles somewhat the blood plasma in respect to its *p*H, viscosity and other physical properties,[48] as well as in its content of such diffusible constituents as urea, non-protein N and NaCl, but there is less sugar and no creatinine.[49] Flössner[50] reports finding glycogen, betaine, lactic and succinic acids and purines. The presence of betaine, if verified, suggests that metabolism in these parasites approaches more that of plants than animals. Contrary to Botteri, he says that proteins are not present, but that creatinine is found in about half the specimens. Cholesterol is often abundant, while inosite, creatin, and succinic acid may be present. Clerc has found traces of lipase, but other enzymes seem to be absent. Proteins are present only in traces, unless inflammation has occurred. Schilling[51] found the molecular concentration of the cyst fluid to be quite the same as that of the patient's blood. The fluid is said not to be toxic to laboratory

[40] See Boidin and Laroche, Presse Méd., 1910 (18), 329; Ghedini and Zamorani, Cent. f. Bakt., 1910 (55), 49.

[41] Welch, *et al.*, Lancet, 1909, Apr. 17; Brice *et al.*, Austral. Jour. Exp. Biol., 1924 (1), 77.

[42] Kreuter, Münch. med. Woch., 1909 (56), 1828; Weinberg, Ann. Inst. Pasteur 1909 (23), 472.

[43] Meyer, Berl. klin. Woch., 1910 (47), 1316; Zeit. Immunität., 1910 (7), 732.

[44] Israel, Zeit. Hyg., 1910 (66), 487; Meyer, Zeit. Immunität., 1911 (9), 530; Hoeden, Münch. med. Woch., 1924 (71), 77.

[45] Zeit. Immunität., 1912 (15), 60; general review.

[46] Zeit. ges. exp. Med., 1923 (37), 175.

[47] Policlinico, Surg., 1915 (22), Nos. 6–11.

[48] Wernicke and Savino, Compt. Rend. Soc. Biol., 1923 (88), 343.

[49] Mazzocco, Compt. Rend. Soc. Biol., 1923 (88), 342.

[50] Zeit. f. Biol., 1924 (80), 255; Münch. med. Woch., 1924 (71), 1427.

[51] Cent. inn. Med., 1904 (25), 833.

animals,[52] but intravenous injection produces shock.[53] According to Brice[41] hydatid fluid contains a substance with an ergotoxin-like action on plain muscle.

The *cyst wall* consists of a hyaline substance which seems to stand between chitin and protein, and probably consists of a mixture of both. Because of the chitin it yields about 50 per cent. of a reducing, sugar-like body when boiled with acid. Schmiedeberg[54] says that the carbohydrate complex of the cyst wall, which he calls hyaloidin, is the same compound as that present in such gluco-proteins as ovomucoid, having an empirical formula of $C_{26}H_{46}N_2O_{20}$, and is very firmly bound to the protein. Flössner describes it as glucosamine. According to Cameron and Fitzpatrick[55] microchemical tests show an abundance of K, Na, and Ca in both the ectocyst and endocyst walls, Fe, P, Cl, and I being less abundant. As the cyst grows older the Na and K decrease while the Ca and fatty substances increase. The scolices resemble the cyst wall microchemically. Glycogen was found throughout the whole cyst wall,[56] distributed like the lipins.

Other cestodes, when in the cystic form, contain fluids which are more or less toxic. Thus Moursou and Schlagdenhauffen[57] found a "leucomaïn" in the cysticercus of *Tænia marginata*, which causes urticaria and other toxic symptoms when injected into animals (thus resembling histamine). The fluids of *Cysticercus pisiformis* (the common cestode of rabbits) have been found toxic for frogs, and Vaullegeard[58] has determined the presence of an "alkaloid" and a "ferment toxin" in this fluid. The fluids of the cysts of *Cœnurus cerebralis*, *Cœnurus serialis*, and *Echinococcus polymorphus*, have all been found toxic, and it is probable that this is a general rule with the cestodes[59] but human forms other than the echinococcus seem not to have been investigated;[60] according to Jammes and Mandoul, extracts of tænia are bactericidal.[61]

Diphyllobothrium latum frequently causes anemia, which has been attributed to a poison liberated by the parasite when it undergoes disintegration and possibly as a secretion of the living worm.[62] All the intestinal cestodes are equipped with a well-developed excretory apparatus, and it is easy to imagine that their excretory products may be toxic to the animal into whose intestine they are excreted. Faust and Tallqvist[63] have made extensive studies of bothriocephalus, which revealed the presence of an active hemolytic agent in the lipoid of the parasites,

[52] Graetz, Cent. f. Bakt., 1910 (55), 234; Zeit. Immunität, 1912 (15), 60.
[53] Giusti and Hug, Compt. Rend. Soc. Biol., 1923 (88), 344.
[54] Arch. exp. Path. u. Pharm., 1920 (87), 1.
[55] Amer. Jour. Path., 1925 (1), 225.
[56] Brault and Loeper reported it as limited to the germinating membrane (Jour. phys. path. gén., 1904 (6), 295).
[57] Compt. Rend. Soc. Biol., 1882 (95), 791.
[58] Bull. Soc. linnéenne de Normandie, 1901 (4), 84.
[59] Blanchard., *loc. cit.*[23]
[60] Semaine méd., 1905 (25), 55.
[61] See also Joyeux, Arch. d. Parasitol., 1907 (11), 409.
[62] Literature by Blanchard, *loc. cit.*
[63] Zeit. klin. Med., 1907 (61), 427.

presumably a cholesterol ester of oleic acid.[64] The proglottides contain a a proteolytic enzyme, which apparently digests the substance of dead segments, liberating the hemolytic lipoid, which constitutes about ten per cent. of the solids of the parasite. There is also a hemagglutinin, which, unlike the hemolytic substance, is thermolabile, and causes the appearance of an antibody in immunized animals. In common with other parasites, antitryptic and antipeptic effects are exhibited by extracts.

The importance of the lipoid in producing anemia is not known, for since oleic acid and its esters are normal constituents of blood, and form a large part of our daily food, their significance as a cause of anemia in parasitic infections is dubious.[65] Syderhelm[66] found that the lipoid fraction of this parasite, although hemolytic *in vitro*, has very little effect when injected into animals, but there is also another toxic material, not a lipoid, which produces in animals a picture simulating pernicious anemia, although this poison is not hemolytic *in vitro*. Two similar poisons, a lipoid hemolytic *in vitro* but not producing anemia *in vivo*, and a poison producing profound anemia although not hemolytic *in vitro*, were also found in the *Gastrophilus* larvæ of *Œstrus equi*, which parasite causes in horses a condition closely simulating pernicious anemia.

Rosenqvist[67] has studied the metabolism of twenty-one cases of bothriocephalus anemia, and found evidence in nearly all of a toxogenic destruction of protein, which ceases promptly when the worms are removed. He has found that these worms produce a poison which is globulicidal, and, probably also, generally cytotoxic, since in the anemias that they produce, the elimination of purine bodies of tissue origin (endogenous purine) is increased. The nitrogenous metabolism is quite the same in pernicious anemia and in bothriocephalus anemia. Isaac and v. d. Velden[68] state that the blood of patients infected with this parasite gives a precipitin reaction with autolytic fluid obtained from bothriocephalus, and that rabbits immunized with such autolytic fluids developed a precipitin. Complement fixation reactions may be demonstrated in human infections with bothriocephalus or other tænia (Jerlov).[69] However, only a very small proportion of the persons infested with this parasite exhibit marked anemia, so that evidently the responsible agent is absorbed in significant amounts only in exceptional cases.

Other Tænia.—There is much less evidence that other forms of tænia produce toxic substances which injure their host, although the clinical manifestations observed in persons harboring tænia are often of such a nature as to indicate strongly an intoxication. Jammes and Mandoul[70] found no toxic manifestations produced by extracts of *Tænia saginata*, which negative finding is supported by Cao,[71] Tallqvist, and Boycott,[72] using various sorts of tænia. These results contradict the earlier

[64] Faust and Tallqvist, Arch. exp. path. u. Pharm., 1907 (57), 367.
[65] See Beumer, Biochem. Zeit., 1919 (95), 239.
[66] Deut. Arch. klin. Med., 1918 (126), 95.
[67] Zeit. klin. Med., 1903 (49), 193.
[68] Deut. med. Woch., 1904 (30), 982.
[69] Zeit. Immunität., 1919 (28), 489.
[70] Compt. Rend. Acad. Sci., 1904 (138), 1734.
[71] Riforma med., 1901 (3), 795.
[72] Jour. Pathol. and Bacteriol., 1905 (10), 383.

positive findings of Messineo and Calamida,[73] who found extracts of tænia from dogs to be hemolytic, chemotactic (especially for eosinophiles), and to cause local fatty degeneration in the liver. Extracts of *T. perfoliata* and *plicata* (of the horse) were found highly toxic for guinea-pigs by Pomella,[74] the hematopoietic organs being greatly stimulated. Bedson[75] found that extracts of all sorts of helminths produced similar effects on guinea-pigs, the chief lesions being in the adrenals and thyroid. Possibly these differences in results are due to the fact that different parasites were studied by different investigators; furthermore, tests of toxicity of human parasites upon rabbits and guinea-pigs can hardly be considered conclusive. Le Dantec did not find a precipitin for *Tænia saginata* extracts in the blood of persons harboring this parasite, and negative results with several other tænia were obtained by Langer,[76] but complement fixation reactions may be given.[77]

Picou and Ramond[78] state that tænia extracts undergo putrefraction very slowly, and attribute this to a bactericidal property, which was observed with several forms of tænia by Allesandrini. Weinland[79] has found that many intestinal parasites exhibit *antitryptic* properties,[80] but in a study of the histological changes of autolysis I observed a tænia in the intestine of a dog undergo more rapid karyolytic changes than did the intestinal epithelium. Dastre and Stessano[81] state that extracts of *Tænia serrata* act upon enterokinase rather than on trypsinogen.

NEMATODES

Ascaris.[82]—The toxicity of members of this group has been a matter of dispute, although, as with the *Tænia*, there have been observed in patients symptoms that were more easily explained as due to chemical substances than to mechanical irritation. Miram, while studying *Ascaris megalocephala*, suffered from attacks of sneezing, lachrymation, itching, and swelling of the fingers. v. Linstow suffered from a severe attack of conjunctivitis with chemosis after touching his eye with a finger that had been in contact with one of these worms. Others have had similar experiences, and it has been found that the fluid from these worms is toxic to rabbits. In man it seems to affect especially those who have been sensitized by previous poisoning, some persons being entirely insusceptible. Ransom *et al.*,[83] found *Ascaris* sensitization a not infrequent condition, independent of evident infestation with this parasite, and although the active agent was absent from the globulin fraction and present in the albumin fraction of extracts from *Ascaris*, they were unable to determine whether it is a protein or not.

An extensive investigation of ascaris from both the chemical and toxicological standpoint has been made by Flury,[88] which indicates the source and nature of these toxic substances. Because of the practically anaerobic conditions under which the worms live, Flury believes, the products of their metabolism are characterized by being incompletely oxidized, and

[73] Cent. f. Bakt., 1901 (30), 346 and 374.
[74] Compt. Rend. Soc. Biol., 1912 (73), 445.
[75] Ann. Inst. Pasteur, 1913 (27), 682.
[76] Münch. med. Woch., 1905 (52), 1665.
[77] Meyer, Zeit. Immunität., 1910 (7), 732.
[78] Compt. Rend. Soc. Biol., 1899 (51), 176.
[79] Zeit. f. Biol., 1902 (44), 1 and 45.
[80] Corroborated for *Tænia saginata* by Fetteroli (Univ. of Pennsylvania Med. Bull., 1907 (20), 94).
[81] Compt. Rend. Soc. Biol., 1903 (55), 130.
[82] The metabolism of *Ascaris* has been studied by Krummacher, Zeit. Biol., 1919 (69), 293; and Fischer, Biochem. Zeit., 1924 (144), 224.
[83] Ransom, Harrison and Couch, Jour. Agric. Res., 1924 (28), 577.

resemble the products of anaerobic bacteria. Most important of these are volatile aldehydes and fatty acids, especially valerianic and butyric acids, in less quantities formic, acrylic, and propionic acids. The toxicologic action of these volatile substances is of such a character as fully to explain the severe irritation of skin and mucous membranes observed in persons handling these parasites; aldehydes are notoriously inclined to produce conditions of hypersensitiveness, *e. g.*, formaldehyde. It is quite possible that the severe constitutional symptoms observed occasionally in persons infected with ascaris, are produced by these substances or by poisonous substances set free through disintegration of worms which have died and remained in the bowel. A capillary poison resembling sepsin, poisonous bases acting like atropine and coniine, and hemolytic unsaturated fatty acids were also found, among other less toxic substances produced by ascaris, and the sum of their action is certainly adequate to account for anything ascribed to these parasites. Paulian,[84] however, would attribute the chief effect to anaphylaxis from absorbed proteins, while Brinda[85] believes that ascaris produces an active toxalbumin. This, he found, causes a tetany-like type of respiration, and a similar symptom is often noticed in children with ascarides. An actively toxic mixture of proteoses and peptones has been obtained from several species of ascaris, and designated as "askaron," by Shimamura and Fujii.[86] Horses can be immunized to withstand 400 lethal doses. Ether and alcohol extracts of ascaris are not poisonous in large doses, although they are hemolytic.

Schwartz[87] found the hemolytic agents of several worms, including *Ascaris*, *Ankylostoma*, species of *Trichuris* and other forms, to be soluble in alcohol, inactive at 8° C., non-specific for the type of blood hemolyzed, and thermolabile. Fractions of the worms freed from ether-soluble substances are hemolytic, so that evidently fats and fatty acids are not responsible.

Analysis by Flury of a great quantity of ascaris from horse and hog gave as the chief results, the following:[88] They differ much in composition from the higher animals. About half the ash is water-soluble; and of the dry substance about half is protein or related substances, from which the usual amino-acids and purines can be isolated. Uric acid and creatinine were lacking. The superficial layer does not consist of chitin, but of an albuminoid rich in sulfur and free from carbohydrates, resembling keratin. They have abundant and active enzymes of many kinds. Glycogen is the chief carbohydrate, but there are also glucoproteins and glucose. The ascaris differs from higher animals especially in the ether-soluble substances, which consist chiefly of free fatty acids, many of which are volatile. Also found were lecithin, aldehydes and neutral fats, but little glycerol, no cholesterol, and an "ascaryl alcohol" ($C_{32}H_{64}O_2$) which probably substitutes for both glycerol and cholesterol.

Trichinella spiralis has been investigated from the chemical standpoint by Flury,[89] who found that the infected muscles of experimental animals differed from normal muscles in having more water because of edema, an increase in extractives, ammonia compounds, lactic acid, and volatile acids, with fluctuating values in both creatine and purines.

[84] Compt. Rend. Soc. Biol., 1915 (78), 73.
[85] Arch. de méd., 1915 (17), 801.
[86] Japanese Jour. Bact. (Saikingaku Zassi), June 10, 1916.
[87] Arch. Int. Med., 1920 (26), 431; Jour. Agric. Res., 1921 (22), 379.
[88] Arch. exp. Path. u. Pharm., 1912 (67), 275 (literature).
[89] Arch. exp. Path. u. Pharm., 1913 (73), 164 and 214.

Glycogen is decreased not only in the infected muscle but also in the liver and kidneys. The parasites themselves are remarkably resistant to strong acids, perhaps because of the lipoid content of their surface covering, in which keratin could not be positively identified. The blood of infected animals shows an excess of nuclein material, and may give albumose and diazo reactions; the red corpuscles have a lowered resistance to hemolysis by hypotonic solutions. Trichinous muscle contains substances that produce marked local tissue irritation, which may be purines; a curare-like poison was also found, which was believed to be a guanidine derivative, as well as a "fatigue poison" which probably consists of the lactic acid and other muscle extractives. The location of trichinella in muscle may be ascribed to their need for glycogen for nourishment, and the fact that their metabolism is carried out anaerobically may account for the character of the products (fatty acids, etc.).

The intoxication of trichinosis probably is the combined result of the products of the metabolism of the parasites, the products of muscle disintegration, and perhaps also of anaphylactic reaction to the proteins of the parasite and the altered muscle proteins. As evidence of the anaphylactic condition is the conspicuous eosinophilia, which we know is often the result of anaphylactic intoxication.[90] Metabolism studies show a preliminary nitrogen, creatinine, and purine retention, followed by excessive loss of all three. There is also an intense diazo reaction, and increased excretion of lactic and organic acids. The hypothesis that bacterial invasion is responsible for the intoxication of trichinosis does not seem to be well supported (Herrick, Gruber), and Gläser[91] has been able to extract a toxic material from trichinella.

The serum of infected animals is not toxic, and does not protect against infection with trichinella (Gruber[92]). Salzer,[93] however, found that the serum of recovered patients had a curative effect in persons acutely intoxicated with trichiniasis, and also a marked prophylactic effect in experimental animals;[94] it removed the eosinophilia both in men and animals. He also observed evidence of a reduction of the bilirubin of the feces by the trichinæ, so that the stools were clay colored without icterus. Positive complement fixation reactions are given by the serum of trichinella-infected persons.[95]

Ankylostoma duodenale (**Uncinaria**) which has for its chief effect the production of a severe anemia, seems to cause this anemia by producing repeated small hemorrhages rather than by any toxic action. The abundance of this loss of blood is explained by L. Loeb[96] as due to the presence,

[90] See Herrick, Jour. Amer. Med. Assoc., 1915 (65), 1870; Schwartz, Ergeb. allg. Pathol., 1914 (17), 136.

[91] Arbeit. Reichsgesundh., 1920 (52), 573.

[92] Münch. med. Woch., 1914 (61), 645.

[93] Jour. Amer. Med. Assoc., 1916 (67), 579.

[94] Not corroborated by Schwartz, Jour. Amer. Med. Assoc., 1917 (69), 884; or Hall and Wigdor, Arch. Int. Med., 1918 (22), 601.

[95] Stroebel, Münch. med. Woch., 1911 (58), 672.

[96] Cent. f. Bakt., 1904 (37), 93; 1906 (40), 740; Loeb and Fleischer, Jour. Infec. Dis., 1910 (7), 625.

in the anterior portion of the parasite (they studied *Ankylostoma caninum*), of a substance that inhibits the coagulation of the blood. However, Preti[97] would ascribe importance to a lipoidal or lipoid-like hemolytic constituent of the parasitic tissues of the European ankylostoma, but Whipple,[98] who has observed a weak hemolysin in the American hook worm, considers it too ineffective to be of practical importance. In *Sclerostoma equinum*, however, Bondonoy[99] found active hemolytic agents, ascribed by him to lipase; also a ptomaïn, an alkaloid and other substances. Corresponding to Flury's analyses of ascaris, he found that the cuticle is albuminoid and not chitinous, and that the parasite produces volatile fatty acids, especially butyric; both lecithin and cholesterol were absent. The dermatitis produced by uncinaria larvæ is ascribed by C. A. Smith[1] to an alcohol-soluble substance. Watery extracts of *Sclerostoma* were found by Grosso[2] to cause but slight chemotaxis, without eosinophilia.

Filaria seem not to produce any appreciable amount of toxic material, if we may judge by the slight evidence of intoxication shown by infected individuals. An exception may be made in the case of the guinea-worm (*Dracunculus* or *F. medinensis*). This parasite causes chiefly mechanical injury unless its body is ruptured, which may happen in attempting to remove it forcibly; this accident is followed by violent local inflammation or gangrene, which indicates that some powerfully irritant substance is liberated from the torn body of the worm.[3]

TREMATODES

The common liver flukes of sheep (*Fasciola hepatica*) apparently secrete a toxic material, in view of the inflammatory changes produced in the liver tissue about the parasites. Infested animals exhibit a specific antibody in the serum which gives complement fixation reactions with the extracts of the parasites as antigens.[4] Similarly, *Schistosomum hæmatobium* and *S. mansoni* produce toxic effects in human tissues, with eosinophilia, epithelial proliferation, and the development in the serum of complement-fixing antibodies.[5] They produce a pigment which Dew considers identical with malarial pigment. The nature of the antigen giving complement fixation tests in Bilharziosis has been investigated by LeBas[6] who believes it to be a protein or some closely associated substance, rather than a lipoid.

[97] Münch. med. Woch., 1908 (55), 436.
[98] Jour. Exp. Med., 1909 (11), 331.
[99] Arch. Parasitol., 1910 (14), 5; see also Ashcroft, Compt. Rend. Soc. Biol., 1914 (77), 442.
[1] Jour. Amer. Med. Assoc., 1906 (47), 1693.
[2] Folia Hematol., 1912 (14), 18.
[3] Earthworms are said by Yagi (Arch. internat. pharmacodyn., 1911 (21), 105) to contain a hemolytic substance, "lumbricin," the properties of which he describes. Nukada and Tenaka (Mitt. med. Fakult., Tokio, 1915 (14), 1), found an antipyretic agent which seems to be derived from tyrosine.
[4] See Fairley and Williams, Jour. Path. and Bact., 1923 (26), 19.
[5] See Dew, Jour. Path. and Bact., 1923 (26), 27; also Faust and Meleney, Amer. Jour. Hyg., 1924; Monograph No. 3 in respect to *S. japonicum*.
[6] Jour. Trop. Med., 1922 (25), 49.

CHAPTER VI

PHYTOTOXINS AND ZOOTOXINS

THE production of substances possessing the essential features of true toxins is by no means limited to the bacterial cell. In the plant kingdom such substances are formed, and called *phytotoxins.*[1] Among the toxins of animal origin, *zootoxins*, are the venoms of poisonous snakes, lizards, spiders and scorpions, and the serum of eels and snakes.

PHYTOTOXINS[2]

The chief known phytotoxins are the following:
Ricin, from the castor-oil bean (*Ricinus communis*).
Abrin, from the seeds of *Abrus precatorius.*
Crotin, from the seeds of *Croton tiglium.*
Robin, from the leaves and bark of the locust, *Robinia pseudoacacia.*
Curcin, from the seeds of *Jatropha curcus.*

In their general properties, all these substances are very similar and may be considered together. They resemble proteins in many respects, for they can be salted out of solutions in definite fractions of the precipitate, are precipitated by alcohol, and are slowly destroyed by proteolytic enzymes. For some time they were referred to in the literature as toxalbumins, but Jacoby stated that, by combining the salting-out method with trypsin digestion, he was able to secure preparations of ricin and abrin that did not give the protein reactions. This seemed to place them in the same category with bacterial toxins and enzymes, *i. e.*, large molecular colloids, closely resembling the proteins with which they are associated, but still not giving the usual protein reactions. Because of their great similarity to bacterial toxins this seemed a very probable description, but the studies of Osborne, Mendel, and Harris[3] did not support Jacoby's contention. They found the toxic properties of ricin associated inseparably with the coagulable albumin of the castor beans, and were able to isolate it in such purity that one one-thousandth of a milligram (0.000001 gram) was fatal *per kilo* of rabbit, and solutions of 0.001 per cent. would agglutinate red corpuscles. The toxicity was also impaired or destroyed by tryptic digestion. They suggest that probably, because of its extremely great toxicity, Jacoby was able to get active preparations that

[1] The poison in certain peas, especially *Lathyrus saturis*, which causes severe peripheral paralysis, (*lathyrism*) is believed to be an alkaloid. (Full discussion by Stockman, Edinb. Med. Jour., 1917 (19), 277).

[2] Résumé of literature by Ford, Cent. f. Bakt., 1913 (58), 129; Jacoby, Kolle and Wassermann's Handbuch, 1913 (2), 1453.

[3] Amer. Jour. of Physiol., 1905 (14), 259.

contained too little active substance to give the protein reactions. As they remark: "If one-thousandth of a milligram of a compound giving on analysis every indication of being a relatively pure protein, is physiologically active in the degree characterized by our experiments, the toxicity of any impurity must be infinitely greater than that of any known toxins." Against the claim that the toxic principle is simply associated with the protein is the fact that it does not come down in the first fraction that is precipitated, the globulin, which usually carries with it all impurities. All the ricin comes down between the limits of one-fifth and one-third saturation with ammonium sulfate, exactly as does the albumin. During germination of the castor bean the ricin disappears with the albumin.[4] Later investigators[5] have also found the toxic agent inseparable from the albumin, and even when a sample was digested with trypsin until there remained but 5–10 per cent. of the original albumin this residue exhibited the same degree of toxicity as an equal weight of undigested ricin.

Field[6] has found evidence that the hemagglutinin and toxin of pure ricin are separable, but Reid[6] believes them identical. Of 21 varieties of ricinus seeds examined by Agulhon,[7] all yielded hemagglutinins. The agglutinating agent can be extracted quantitatively from a ricin solution by red corpuscles,[8] and crotin is readily adsorbed by kaolin, etc.[9] Ricin also agglutinates tissue cells of all sorts, and causes precipitates in normal serum.[10] Curcin alone seems to have no hemagglutinative action.[11]

Immunity.—The phytotoxins have been of service in the study of immunity, since they act like bacterial toxins and can be handled in more definite quantities. By their use Ehrlich first determined that toxin and antitoxin act quantitatively. They seem to possess haptophore and toxophore groups, and immunity is readily obtained against them, not only by subcutaneous injection, but by dropping into the conjunctival sac, and also by feeding, showing their direct absorbability and their resistance to digestion. The antitoxin is present in the milk of the immunized mother and immunizes the suckling; but little is carried through the placenta into the fetal blood. The immunity is specific, ricin antitoxin, for example, not protecting against abrin (although it is said to protect against robin). That the phytotoxins are fundamentally similar to the bacterial toxins is shown by the observation of Ramon[12] that by treatment with formaldehyde they form "anatoxins," similar to diphtheria anatoxin in being nontoxic but highly antigenic. Roemer found that in animals immunized by conjunctival application, the eye so used became immune to the local action of the poison before the other eye did, indicat-

[4] Agulhon, Ann. Inst. Pasteur, 1915 (29), 237.
[5] Karrer *et al.*, Zeit. physiol. Chem., 1924 (135), 129.
[6] Jour. Exper. Med., 1910 (12), 551; Reid, Landwirtsch. Versuchst., 1913 (82), 393.
[7] Ann. Inst. Pasteur, 1914 (28), 819.
[8] P. Hirsch, Fermentforsch., 1919 (2), 269 and 290.
[9] Fujiwara, Biochem. Zeit., 1923 (140), 132.
[10] Michaelis and Steindorff, Biochem. Zeit., 1906 (2), 43.
[11] Felke, Landwirts. Versuchst., 1913 (82), 427.
[12] Ann. Inst. Pasteur, 1925 (39), 1.

ing a local development of immune substance. In general immunization the immune substance appears first in the spleen and bone-marrow. Normal serum gives a precipitate with ricin, but immune serum gives a much heavier one and apparently the ricin is quantitatively precipitated (P. Hirsch).[8] Antiricin, like other antitoxins, is inseparable from the proteins of the serum and exhibits also an antiagglutinative effect.

Physiological Action.—Their poisonous actions are manifold, most prominent being agglutination of the erythrocytes, local cellular destruction, and, to a less extent, hemolysis. The agglutinative reaction, according to Gunn,[13] may be at least partly non-specific, being merely the precipitation of one colloid by another of opposite charge, for ricin agglutinates suspensions of cholesterol, carmine and other negatively charged particles, and the agglutinated particles are found to have lost their negative charges. By long action of pepsin-HCl upon ricin, Jacoby secured a preparation with all the other properties of ricin except that it was inactive against erythrocytes; the same result could not be obtained with abrin. Heating to 65° or 70° does not destroy the toxicity of phytotoxins, but boiling does. There is a latent period of several hours after injection of the poison, the onset of symptoms being sudden; death rarely occurs in less than fifteen to eighteen hours (Osborne *et al.*).

Flexner[13a] has studied particularly the histological changes produced by ricin and abrin poisoning in animals. Both act alike, affecting the tissues much as bacterial toxins do (diphtheria). Fever, albuminuria, and convulsions are followed by exhaustion and lowered temperature. Punctiform hemorrhages are found beneath the serous surfaces, with fluid in the peritoneal cavity. At least in the case of ricin the hemorrhages are not due to blood changes, but to a special toxin destroying the endothelial cells.[14] There occur general lymphatic enlargement and marked changes in the intestinal mucosa, with swelling of the Peyer's patches. The spleen is swollen and dark in color, as also is the liver, which shows much focal necrosis. The glycogen content of the liver is decreased in abrin poisoning.[15] Subcutaneous injection causes local edematous inflammation without suppuration. Histologically, in the most affected organs are found much cellular necrosis and disintegration, especially of lymphoid and epithelial cells. Changes in the capillary endothelium, fibrinous thrombi, and abundant hemorrhagic extravasations are widespread. Probably agglutinative thrombosis by red corpuscles plays an important part in these intoxications (Ehrlich), but Aschoff[16] ascribes the thrombosis to the fragments of disintegrated marrow and blood cells. The great amount of intestinal injury probably depends upon the fact that these poisons are largely eliminated through the intestinal mucosa.

[13] Jour. Physiol., Proc., 1921 (54), lxxxviii.
[13a] Jour. Exper. Med., 1897 (2), 197.
[14] Amer. Jour. Med. Sci., 1903 (126), 206.
[15] Doyon, Compt. Rend. Soc. Biol., 1909 (67), 30.
[16] Arch. Int. Med., 1913 (12), 503.

There are also severe changes in the bone marrow, accompanied by the appearance of nucleated erythrocytes in the blood.[17]

Mushroom Poisons.[18]—The poisons of the three chief poisonous mushrooms, *Amanita muscaria*, *Helvella esculentia*, and *Amanita phalloides*, differ essentially from one another. The poisonous principles of the first and second, muscarine and helvellic acid, are non-protein substances, of known chemical composition, which are discussed elsewhere; but the *Amanita phalloides*, the most important of the three, owes its toxic properties to at least two poisonous constituents. One is powerfully hemolytic, is destroyed by heating thirty minutes at 65°, and acts directly upon red corpuscles without the presence of serum.[19]

The studies of Ford[20] and his associates have shown that this hemolysin is a glucoside, yielding on hydrolysis pentose and volatile bases, and yet capable of acting as an antigen, since antihemolytic sera can be produced by immunizing animals. This substance corresponds to the *phallin* of Kobert. Probably this hemolytic poison is not the important agent in poisoning by *Amanita*, as it is easily destroyed by heat and the digestive fluids. The thermostable poison, *Amanita-toxin*, gives no reactions for either glucosides or proteins,[21] and does not confer any considerable antitoxic property on the blood of immunized animals. The toxin kills acutely, the animals dying in 24—48 hours, and showing no changes beyond a fatty degeneration of the internal organs. The hemolysin kills slowly in 3—10 days, causing local edema and hemoglobinuria.

Amanita muscaria contains a heat-resistant agglutinin which also seems to be a glucoside, but it is not toxic nor antigenic.

An extensive study of many fungi by Ford[22] led him to classify the toxic action in three groups: (1) nerve poisons, *e. g.*, muscarine; (2) those causing structural changes in the viscera, *e. g.*, *A. phalloides*, causing fatty degeneration; (3) gastro-intestinal irritants, *e. g.*, *Lactarius torminosus*.

The poison of *Rhus toxicodendron*[23] was believed by Acree and Syme[24] to be a glucoside,[25] and Adelung[26] held that the same is true of the poison oak, *Rhus diversi-loba*, which has no antigenic properties. McNair,[27] however, believes that the active agent of poison oak is a polyhydrophenol, which he has named *lobinol*. So, too, Brown[28] has obtained from poison ivy what seems to be a phenol, and so-called poison oak and poison ivy are practically identical plants, differing only in a slight difference in the leaf shape.[29] Immunization of man against *Rhus* has been reported.[30]

(The effects of the phytotoxins on the blood are discussed under "Hemolysis" in Chapter XI. Vegetable hemolytic poisons that do not resemble the toxins, *e. g.*, glucosides, etc., will also be found discussed under the same heading.)

[17] Bunting, Jour. Exper. Med., 1906 (8), 625.

[18] Résumé by Mörner, Upsala Läkaref. Förh., 1919 (24), 1. Pathological anatomy described by Prym, Virchow's Archiv., 1919 (226), 229; also Klemperer, ibid., 1922 (237), 401.

[19] The hemagglutinin of *Agaricus campestris* is precipitated at a H-ion concentration of 2.6×10^{-4} (Brossa, Arch. sci. med., 1915 (39), 241).

[20] See Jour. of Pharm., 1910 (2), 145; 1913 (4), 235, 241, and 321.

[21] Rabe (Zeit. exp. Path., 1911 (9), 352) considers it to be an alkaloid.

[22] Jour. of Pharm., 1911 (2), 285.

[23] Full discussion by McNair, in "Rhus Dermatitis," Univ. Chicago Press, 1923.

[24] Jour. Biol. Chem., 1907 (2), 547.

[25] Questioned by McNair, Jour. Amer. Chem. Soc., 1916 (38), 1417.

[26] Arch. Int. Med., 1913 (11), 148.

[27] Jour. Amer. Chem. Soc., 1921 (43), 159.

[28] Proc. Soc. Exp. Biol. Med., 1921 (19), 136.

[29] The irritant of nettles seems to be formic acid, although other agents may also perhaps be present, Dobbin, Proc. Roy. Soc. Edinb., 1918 (39), 137).

[30] Schamberg, Jour. Amer. Med. Assoc., 1919 (73), 1213.

ZOOTOXINS[31]

Snake Venoms[32]

This important class of poisons, first thoroughly investigated by Weir Mitchell (1860), and Mitchell and Reichert (1883), has more recently aroused great interest through its relations to bacterial toxins and the problems of immunity. The poisons of different species of snakes seem to have much in common, whether derived from the *Elaperine* snakes (cobras and numerous other Indian and Australian snakes), or *Viperidæ* (including most poisonous American snakes), or *Hydrophinæ* (the poisonous sea-snakes), although very characteristic differences exist between each.

The essential anatomical differences between the different classes of snakes are as follows: *Colubridæ*, which include all the non-poisonous snakes, have no mechanism for injecting poisons into their victims. *Colubridæ venenosæ* are venomous snakes resembling in many particulars the harmless Colubrines, but having short poison fangs, firmly fastened to the maxilla in an erect position; in this class are included the cobra and the venomous snakes of Australia. *Viperidæ*, or vipers, are characterized by a highly specialized apparatus for injecting the poison; their poison fangs are very long, and the maxillary bone, to which they are fastened, is so articulated that it rotates about a quarter of a circle when the snake strikes, bringing the fangs into an erect position. The fangs are canalized and pointed at the end like a hypodermic needle, and the poison is forced through them under considerable pressure by a large muscle that contracts over the salivary gland. Accessory fangs in various stages of development are also present to replace any fang lost in action. All the poisonous snakes of North America, with one insignificant exception, belong to the vipers, and to a special class known as the "pit vipers," because of the presence of a deep pit of unknown function above the maxilla. The exception mentioned is the "coral snake" found on the coast of Florida, around the Gulf of Mexico, and in the south-eastern states; it is a member of the colubrine poisonous snakes, of small size, and seldom causes serious poisoning. The poisonous vipers are the rattlesnakes (*Crotalus*), of which there are some ten to twelve or more species, and *Sistrurus*, of which there are two species; the copperhead adder (*Ancistrodon controtrix*) and the water moccasin (*Ancistrodon piscivorus*).

The classification used above is the one followed in most publications on poisonous snakes; a more modern classification divides the snakes (*Ophidia*) into several series, one of these including all poisonous snakes under the title of *Proteroglypha*, and dividing this series into the three families: (1) *Elapinæ*, including cobras, coral snakes, etc.; (2) *Hydrophivæ*, the poisonous sea-snakes; (3) *Viperidæ*, including all snakes with erectile fangs.[33]

The source of the venom is probably in part the blood, since snake blood has been found to contain poisons very similar to some of those

[31] Full review and literature given by Faust, "Die tierischen Gifte," Braunschweig, 1906; also in Abderhalden's Handbuch, Vol. II. Sachs, in Kolle and Wassermann's Handbuch, 1913 (2), 1407.

[32] Elaborate review and bibliography given by Noguchi, Carnegie Institution Publications, 1909 No. 111; also by Calmette, "Les vénins, les animaux vénimaux et la sérotherapie antivénimeuse," Paris, Masson, 1907; Calmette, Kolle and Wassermann's Handbuch, Vol. II, p. 1381; "Animaux Venimaux et Venins," Marie Phisalix, Masson et Cie, Paris, 1922. With reference to North American snakes, see Prentiss Willson, Arch. Int. Med., 1908 (1), 516.

[33] For a full discussion of the characteristics of the poisonous snakes of North America, see the monograph with that title by Stejneger, Report of U. S. National Museum, 1893, Washington. A good summary is also given by Langmann, Reference Handbook of Medical Sciences. Concerning poisonous sea-snakes, *Hydrophidia*, see Boulanger, Natural Science, 1892 (1), 44. The poisonous snakes of India are described by Fayrer, in "The Thanatophidia of India," London, 1874.

in the venom; therefore these are presumably simply filtered out by the venom glands, and not manufactured by them.[34] Other poisonous constituents of venom are not found in snake serum, and therefore are probably manufactured by the venom gland. Apparently many of the harmless snakes produce a poisonous saliva, since extracts of their glands are said by Blanchard[35] to possess the properties of the venoms, and if so these snakes are harmless chiefly because they lack an apparatus for injecting the poison.[36] As a rule, however, the venom glands are much more highly developed in the poisonous snakes, and are connected with a specialized injection apparatus; in structure they are compound racemose glands.

Properties of Venom.—As ejected, the venom is weakly acid or neutral in reaction, and free from bacteria, contrary to earlier ideas (Langmann). Its specific gravity is 1.030 to 1.077, and it contains a large amount of solids, generally 20 to 40 per cent. by weight. These are precipitated by alcohol, ether, tannin, and iodin, but do not adhere to precipitates of phosphates as do enzymes and toxins (Calmette). They do not diffuse through dialyzing membranes. When dried, the venom can be kept almost indefinitely without losing its strength, specimens over twenty years old having been found unimpaired. Glycerol and alcohol also seem not to injure it, but oxidizing agents of all kinds are very destructive. Light impairs the power of venoms, as also does radium (Phisalix).[37] Eosin and erythrosin also reduce the power of venom through their photodynamic action, affecting the neurotoxic properties less than the hematotoxic components (Noguchi).[38] Cobra venom withstands even 100° for a short time, but crotaline venoms are destroyed at 80–85°.

Much work has been done upon the nature of the constituents of venom. As early as 1843, Prince Lucien Bonaparte found that there were proteins in the venom, which was corroborated by Mitchell in 1861. In 1883 Mitchell and Reichert described two poisonous protein constituents of venom, one of which was coagulable by heat and seemed to be a globulin; the other resembled the proteoses (they called it "peptone," according to the nomenclature of that time). To the globulin they ascribed the local, irritating properties of venom; to the albumose, the systemic intoxication. Corresponding to their action, venoms of different serpents were found to vary greatly in the proportions of these proteins. Cobra venom, which acts chiefly systemically, contains 98 per cent. of albumose and but 2 per cent. of globulin; rattlesnake venom, with its marked local effects, contains 25 per cent. of the irritating globulin; moccasin venom contains 8 per cent. of globulin. Several other observers soon corroborated the main facts of Mitchell and Reichert's

[34] Contradicted by Arthus, Arch. internat. physiol, 1912 (12), 162.
[35] Compt. Rend. Soc. Biol., 1894 (46), 35.
[36] See also Kraus, Münch. med. Woch., 1922 (69), 1277; Med. Klin., 1924 (20), 771.
[37] Compt. Rend. Soc. Biol., 1904 (56), 327.
[38] Jour. Exper. Med., 1906 (8), 252.

report; but, as has been seen in connection with the consideration of the composition of enzymes, toxins, etc., the fact that a substance is carried down with a protein is no proof that it is itself a protein. What has been established is merely that the irritating component of venom can be destroyed by heat, and is removed with the globulin in fractional separation;[39] while there remains a substance not destroyed by boiling, which comes down at least in part with the albumoses of the venom, and causes chiefly systemic manifestations.

Since venoms act as antigens and stimulate the formation of specific antibodies, it is to be presumed that the poisonous principles are proteins, or toxalbumins, although this conclusion does not necessarily follow. Faust[40] believes the poison of venoms not to be proteins, but glucosides, free from nitrogen, resembling very much quillajic acid, and therefore belonging to the saponin group of hemolytic agents. He has isolated such a substance from cobra venom, which he calls *ophiotoxin* ($C_{17}H_{26}O_{10}$), and from rattlesnake venom, a substance which seems to be a polymer of the ophiotoxin, ($C_{34}H_{54}O_{21}$). Possibly these glucosides are bound to proteins, forming compound proteins which act as specific antigens. According to this work, the snake venoms and the dermal poisons of toads and frogs are all closely related substances.

In favor of the view that snake venoms are compounds of a toxic radical with a protein is the fact, emphasized by Nicolle,[41] that according to pharmacological and pathological evidence the poisonous elements of several different sorts of *snake venoms* must be the same or similar, despite the fact that the protein-rich venom is immunologically specific, the antiserum for one venom failing to protect against another. This may be explained by the assumption that the poisonous element in these venoms is the same, but attached to different and antigenically specific proteins.

Enzymes in Venoms.—As venom causes rapid liquefaction of tissues into which it is injected, Flexner and Noguchi[42] tested crotalus and cobra venom for proteases, and found that they digested muscle rapidly, and also gelatin and unboiled fibrin; whereas boiled fibrin and boiled egg-albumen were undigested.[43] Kinases and nucleases are also present in venoms (Delezenne).[44] Wehrmann[45] found that venom digests fibrin and inverts saccharose, but does not digest starch. Martin[46] found fibrin ferments in various venoms, which are probably important agents in causing thrombosis. Some venoms cause blood coagulation by their own coagulating activity or by stimulating thrombin production, while others inhibit coagulation by destroying the cytozyme or by precipitating the fibrinogen.[47] There are also active lipases in venoms, to which many of the effects, especially hemolysis and fatty degeneration of the tissues, may be at least partly due (Noguchi), and the hemolysin of cobra venom seems to be a lipase that splits lecithin into hemolytic substances (Coca).[48]

[39] See also Welker, Jour. Lab. Clin. Med., 1925 (10), 298.
[40] Arch. exp. Path. u. Pharm., 1907 (56), 236; 1911 (64), 244.
[41] Jour. State Med., 1920 (28), 293.
[42] Univ. of Penn. Med. Bull., 1902 (15), 360.
[43] See also Houssay and Negrete, Revista de l'inst. bact. Buenos Aires, 1918 (1), 431.
[44] Ann. Inst. Pasteur, 1919 (33), 68.
[45] Ann. d. l'Inst. Pasteur, 1898 (12), 510.
[46] Jour. of Physiol., 1905 (32), 207.
[47] Houssay and Sordelli, Arch. internat. physiol., 1920 (25), 378.
[48] Jour. Infect. Dis., 1915 (17), 351.

Delezenne[44] found zinc always present in venom and attributes to it a relation to the enzyme activity.

Toxicity.[49]—Calmette has determined the toxicity of several venoms, and gives the following figures:

1 gm. *cobra* or *aspis* kills	4,000 kgm. of rabbit.
1 gm. *hoplocephalus* kills	3,450 kgm. of rabbit.
1 gm. *fer de lance* or *pseudechis* kills	800 kgm. of rabbit.
1 gm. *Crotalus horridus* kills	600 kgm. of rabbit.
1 gm. *Pelias berus* kills	250 kgm. of rabbit.

The danger of the bite depends not only upon the difference in the strength of the venom of different varieties of serpents, but also upon the size of the snake, the time of year and condition of hunger or plenty, and particularly upon whether the entire discharge is injected successfully or not. The fatal dose of cobra venom for an adult man is variously estimated at from 0.01 to 0.03 gm., while the venom of Hydrophiinæ is about ten times as toxic; for crotalus venom the lethal dose is probably 0.15 to 0.3 gm. (Noguchi). Probably in the majority of strikes, by no means all the fluid ejected by both fangs is injected beneath the skin of the victim. A large diamond rattler may eject as much as a half teaspoonful of venom at one discharge and such a dose would usually be fatal. Repeated ejections decrease the strength of the venom rapidly, until it may have almost no toxicity. In general, venom is most active in warm weather and immediately after the snake has fed; in winter its toxicity is slight.

The mortality in America from snake-bites is very hard to ascertain, various authors giving figures at wide variance. The extensive studies of Willson[50] show about ten per cent. mortality from all venomous snake-bites in this country, the different species giving figures as follows: Coral snakes, twenty to fifty per cent.; water moccasins, seventeen per cent.; large rattlesnakes, eleven to twelve per cent.; copperheads and ground rattlers, no mortality except in children or in cases of complications. The mortality in children is at least double that in adults. Many deaths from snake-bites of all kinds are due to the treatment rather than to the bite. The poisonous snakes of Australia, although numerous, are not very virulent, and the mortality is given as about seven per cent. A full charge of venom from the cobra and many other Indian snakes is inevitably fatal (Fayrer). The crotaline snakes of the tropics are more venomous than those of the north, *Lachesis lanceolatus* of Central America and Mexico being nearly as dangerous as the cobra. The cobra of the Philippines, *Naja philippinensis*, produces a venom said to be even more toxic than that of the cobra of India.[51]

When venom is taken into the stomach in the intervals of digestion, enough may be absorbed to produce death, especially in the case of those venoms which contain a large proportion of the albumose, which is dialyz-

[49] Concerning use of venoms and other agents as *arrow poisons*, see L. Lewin, "Die Pfeilgifte," Barth, Leipsig, 1923.

[50] Arch. Int. Med., 1908 (1), 516.

[51] Monserrat, Philip. Jour. Sci., 1920 (17), 59.

able; but during active digestion the venom undergoes alteration and is rendered harmless. It has been found experimentally in animals that cobra venom placed in the stomach causes ordinarily no harm whatever, but if a loop of the intestine is isolated, a fistula established and allowed to heal, venom introduced through this opening always produces death. It is probably not so much the pepsin and hydrochloric acid that destroys the venom, as the trypsin. If the bile-duct is ligated, the venom is destroyed just the same. Much of the venom seems to be eliminated into the stomach, no matter how it is introduced into the system, and apparently it is also partly excreted by the kidneys. Rattlesnake venom seems not to be absorbed through mucous membranes.

Physiological Action.—As indicated in the preceding paragraph, the effects of the bites of different classes of snakes are quite different. Langmann describes the symptoms as follows:

Cobra Poisoning.—"Within an hour, on an average, the first constitutional symptoms appear: a pronounced vertigo, quickly followed by weakness of the legs, which is increased to paraplegia, ptosis, falling of the jaw with paralysis of the tongue and epiglottis; at the same time there exists an inability to speak and swallow, with fully preserved sensorium. The symptoms thus resemble those of an acute bulbar paralysis. The pulse is of moderate strength until a few minutes after the cessation of respiration; the latter becomes slower, labored, and more and more superficial until it dies out almost inperceptibly. Death occurs at the latest within fifteen hours; in 32 per cent. of all cases in three hours. There are very few local changes." Cushny[52] finds that cobra venom produces paralysis of the motor nerve terminations of muscle, resembling the action of curare; the central nervous system is not directly involved. Death results from failure of the motor nerve ends in the respiratory muscles to transmit impulses to the muscles. Alkaloids that are antagonistic to curare (physostigmine, guanidine) are not effective in cobra poisoning, but are themselves rendered inactive.[53]

Viper Poisoning.—"After the bite of a viper the local changes are most pronounced; there are violent pains in the bleeding wound, hemorrhagic discoloration of its surroundings, bloody exudations on all the mucous membranes, and hemoglobinuria. Usually somewhat later than in cobra poisoning constitutional symptoms develop; viz., great prostration with nausea and vomiting, blood pressure falls continuously, and respiration grows slow and stertorous. After a temporary increase in reflexes, paresis supervenes, with paraplegia of the lower extremities, extending in an upward direction and ending in a complete paralysis. It therefore resembles an acute ascending spinal paralysis. If the patient recovers from the paralysis, a septic fever may develop; not rarely there remain suppurating gangrenous wounds, which heal poorly."

It will be noticed that there is lacking the usual period of incubation that follows injection of bacterial toxins, and if it happens that the venom has been injected directly into one of the veins, death may occur within a few minutes. When recovery occurs, the disappearance of symptoms is remarkably abrupt, within a few hours a desperately sick person becoming almost entirely free from all evidences of the intoxication.

Pathological Anatomy.—*Postmortem examination* shows changes varying with the nature of the poisonous snake that has caused death. In the case of a cobra bite, according to Martin, the areolar tissue about the wound is infiltrated with pinkish

[52] Trans. Roy. Soc., London (B), 1916 (208), 1.

[53] The "habu" snake of Japan is related to, but less poisonous than the cobra, and the pharmacological action of its venom is discussed by Ishiwara, Arch. exp. Path. Pharm., 1924 (103), 219.

fluid; the blood is often fluid; the veins of the pia are congested, and the ventricles often contain turbid fluid; the kidneys may show much congestion. When death occurs in a few minutes, enormous general intravascular clotting is found, which seems to be the cause of death. After death from a viper bite the site of the wound is the seat of intense edema and extravasation of blood; if in the muscles, these are much softened and disorganized. Hemorrhages are found in all organs and in the intestinal tract. If death occurs after several days it is generally because of sepsis, and shows the usual changes of this condition, in addition, as a rule, to marked gangrenous, ulcerative, and sloughing processes at the site of the bite.

Histologically there are found, in addition to innumerable hemorrhages in nearly all the organs, many vessels plugged with thrombi composed of more or less hemolyzed, agglutinated erythrocytes. The changes produced in the nervous tissue by the Australian tiger snake are described by Kilvington,[54] who found marked chromatolysis, the Nissl bodies breaking into dust-like particles, and eventually all stainable substance disappearing from the cytoplasm; the nucleus retains its central position, but often loses its outline and may disappear. The cells around the central canal of the cord are most affected. There are no inflammatory changes in the nervous system, and if death occurs very quickly there may be no microscopic alterations. Hunter[55] found similar changes in the Nissl bodies in both krait and cobra poisoning; in the medullated fibers he found the myelin sheath converted into ordinary fat. The venom of sea snakes (*Enhydrina valakadien*) has a severe action on the nervous tissues, while *Daboia* has none (Lamb and Hunter[56]). Nowak[57] studied experimental animals, and found much fatty change in the livers, even if death occurred one-half hour after poisoning; also focal necrosis in the liver, acute parenchymatous alterations in the kidney, and pneumonic patches in the lungs.

Effects of the Blood.—There has been much discussion concerning the part played by the abundant and prominent intravascular clotting in causing death after snake-bite. Lamb[58] states that when venoms are slowly absorbed the coagulability of the blood is decreased and it is found fluid after death, but when a fatal dose of venom (viper) is rapidly absorbed, clotting is increased and thrombosis is the chief cause of death. Martin[46] has demonstrated very active fibrin ferments in snake venom. It is highly probable, however, that many of the thrombi of venom poisoning are not produced by coagulation of fibrin, but by agglutination of the red corpuscles, which Flexner[59] has shown can cause large clots in the heart and great vessels, as well as "hyalin" thrombi in the small vessels. Houssay[60] states that most snake venoms destroy the cytozyme (which combines with serozyme and calcium to form thrombin), so that the blood becomes incoagulable. The Argentine crotalus and lachesis venoms, however, coagulate even citrated blood. Arthus[61] finds that there are coagulating and anticoagulating types of venoms, the former containing thrombin or else favoring the production of thrombin from prothrombin; the anticoagulating venoms either stimulate the formation of antithrombin *in vivo*, or prevent thrombin formation *in vitro*.

Nature of Venoms.—The varied effects produced by venoms have been found to be due to a number of poisonous elements which they contain, and which have been distinguished and separated from one another by Flexner and Noguchi.[62] These are *hemotoxins* (*hemolysins and hemagglutinins*), *leucocytolysins*, *neurotoxins*, and *endotheliotoxins* (*hemorrhagin*), but it must be taken into consideration that Faust[63] believes that the single glucosidal poison which he has found in rattlesnake venom is responsible for all the effects of the venom, except the hemagglutination. (In another place (see "Hemolysis") the nature of

[54] Jour. of Physiol., 1902 (28), 426.
[55] Glasgow. Med. Jour., 1903 (59), 98.
[56] Lancet, 1907 (ii), 1017.
[57] Ann. d. l'Inst. Pasteur, 1898 (12), 369.
[58] Indian Medical Gazette, Dec., 1901.
[59] Univ. of Penn. Med. Bull., 1902 (15), 324.
[60] Prensa Med. Argentina, 1919 (6), 133; Jour. physiol. path. gen., 1920 (18), 781.
[61] Arch. internat. physiol., 1919 (15), 203.
[62] Jour. Exp. Med., 1903 (9) 257; Univ. Penn. Med. Ball.,1902 (15) 345.

the hemolytic agent is discussed.) Venom agglutinin is quite independent of the hemolysin, for it is destroyed by heating to 75°–80°, whereas the hemolysin is destroyed only partly at 100°. Agglutinin acts in the absence of serum complement, and therefore is not an amboceptor; it is apparently more like the toxins in its nature. The agglutination of the corpuscles does not interfere with their subsequent hemolysis, and may be merely the effect of the venoms in neutralizing the negative charges of the corpuscles (Gunn).[63] Michel states that the agglutinin of cobra venom can be separated from the hemolysin and the toxin by means of ultrafiltration through collodion membranes, as the agglutinin exists in larger molecular aggregates.[64] The hemolytic element may also be separated from the element that unites with antihemolysin, by adsorption with animal charcoal.[65]

The leucocytotoxins were found by Flexner and Noguchi to be quite distinct from the hemolysins, for after saturating all the hemolysin with red corpuscles, the venom still shows its effects on the leucocytes, which effects consist in cessation of motility and disintegration, affecting particularly the granular cells. The leucocytotoxin, however, resembles the hemolysin in that it appears to be an amboceptor. Leucocytes are also agglutinated by venom, possibly by the same agglutinin that acts on the red corpuscles. Serum complement is inactivated *in vitro* by cobra venom, through changes in the globulins brought about by the venoms.[66] By saturating venom with either red corpuscles or nerve-cells it was found by Flexner and Noguchi that the toxic principle for each is distinct and separate.[68] Other sorts of cells, however, are able to combine, or at least remove some parts of the toxic elements, but to a much less degree. The neurotoxin, like the hemolysin, resembles an amboceptor, and since venom contains no complement, the neurotoxin has first to be supplied with complement by the victim's blood or tissues before it can harm the cells.[67] The venoms are not only toxic for mammalian cells, but also for simple unicellular organisms, including bacteria; tadpoles are paralyzed in solutions containing one part of cobra venom per million.[68]

The pronounced hemorrhage-producing property of serums, particularly that of the rattlesnake, is due to a specific toxin acting on the endothelium of the capillaries and small veins, and not to the changes in the blood itself, as was formerly thought. This endotheliotoxin, which Flexner and Noguchi call "hemorrhagin," is quite distinct from the other toxic substances, being destroyed at 75°, a temperature that leaves the neurotoxin and hemolysin uninjured. Its endotheliolytic action is shown in the glomerular capillaries, where it causes hemorrhage and

[63] Arch. Exper. Path. u. Pharm. 1911 (64), 244.

[64] Compt. Rend. Soc. Biol., 1916 (77), 150.

[65] de Houssay, Revista Inst. Bact., Buenos Aires, 1921 (2), 197.

[66] Hirschfeld and Klinger, Biochem. Zeit., 1915 (70), 398.

[67] The production of hemolytic complement by the liver under the influence of cobra venom has been observed. (Hahn and Skramlik, Biochem. Zeit., 1919 (98), 120).

[68] Bang and Overton, Biochem. Zeit., 1911 (31), 243, state that corpuscles can take up the neurotoxin, which is soluble in fats and lipoids.

hematuria (Pearce).[69] Cobra venom seems also to have the property of destroying antithrombin and interferes with its formation;[70] after snake bite the blood may be incoagulable.

Variations in Venoms.—In distribution among the various poisonous reptiles these toxins seem also quite distinct from one another, which explains the difference in the effects of bites of snakes of various kinds. Cobra venom contains chiefly neurotoxin, hence the symptoms of cobra bite are largely of nervous origin, with but little local tissue change. Rattlesnake venom owes its effects chiefly to hemorrhagin, hence the marked local necrosis and extravasations of the blood, and the generalized hemorrhages; the nervous effects following viper bite are probably, in part, due to hemorrhages in the nervous tissue. Cobra venom produces great hemolysis and little agglutination. Rattlesnake venom has relatively little agglutinative or hemolytic power. Water moccasin and copperhead venoms are more agglutinative than either, and intermediate in hemolytic strength; they cause much local tissue destruction. Many venoms have been found to cause vascular hypertension, often with bradycardia.[71]

The exact action of cobra venom on various centers and organs has been studied by Elliot.[72] It raises blood pressure when in dilution of 1:10,000,000, by contracting vessels and stimulating the heart; low lethal doses kill by paralyzing the respiratory center. Cobra venom is remarkably cumulative, even one-fifth of the lethal dose being fatal when given repeatedly (Cushny),[73] and evidence of persistence of the poison in the tissues may sometimes be obtained for a month after the injection.

Krait (*Bungarus cærutus*) venom acts similarly, but less powerfully, and cannot be neutralized by Calmette's antivenin.[74]

Sea-snake venoms are by far the most poisonous of all. For *Enhydrina valakadien* the lethal dose for rabbits is 0.00006 gram per kilo body weight. It acts by vagus stimulation and paralysis of respiratory centers and of motor nerve endings.[75]

Russell's viper (*Daboia Russellii*) owes its effects chiefly to intravascular clotting, according to Lamb and Hanna,[76] and contains no neurotoxin. It is not neutralized by Calmette's antivenin. The clots are due to agglutination and contain no fibrin (Flexner).

The "Gila Monster" (*Heloderma suspectum*) seldom causes serious poisoning in man, but may kill small animals, such as frogs.[77] Its poison is only slightly hemolytic, but produces degenerative changes in the nervous system (Langmann). The hemolysin is activated by lecithin (Cooke and Loeb). Gila venom kills guinea pigs in doses of 0.05 cc., or 5 mg. dried substance, acting mainly on the respiratory centers. Gila monsters are resistant to their own poison, but not to snake venom, and snakes are poisoned by Gila venom. But slight immunity can be produced in rabbits. An elaborate series of studies by Leo Loeb and his associates give all the known facts concerning the Gila Monster.[78]

[69] Jour. Exper. Med., 1909 (11), 532.

[70] Rabinovich, Anales Asoc. quim. Argentina, 1922 (10), 335, 380; Compt. Rend. Soc. Biol., 1923 (88), 369.

[71] Pacella, Compt. Rend. Soc. Biol., 1923 (88), 366.

[72] Lancet, 1904 (i), 715.

[73] Arch. Pharm. Exp. Ther. 1922 (20), 233.

[74] Elliot, Sillar, and Carmichael, Lancet, 1904 (ii), 142.

[75] Fraser and Elliot, Lancet, 1904 (ii), 141; also Rogers, Jour. of Physiol., 1903 (30), iv. The above are also given completely in the Philosophical Transactions of the Royal Society, 1904–5, vol. 187.

[76] Jour. of Path. and Bact., 1902 (8), 1.

[77] Van Denburgh and Wright, Amer. Jour. of Physiol., 1900 (4), 209.

[78] Carnegie Inst. Publication No. 177, 1913.

Loss of Bactericidal Powers.—The frequency of marked and persistent sloughing and suppuration at the site of snake-bites, particularly bites of vipers, and the common termination in sepsis, was attributed by Welch and Ewing[79] to a loss of bactericidal power of the blood, which they found followed experimental venom poisoning. This has been ascribed by Flexner and Noguchi to saturation of serum complement by the amboceptors of the venoms, so that no complement is left for the serum to use against the bacteria. In serum whose complement does not combine with the venom amboceptors (*e. g., Necturus*) the normal bactericidal powers are not in the least impaired by the addition of venom. Morgenroth and Kaya ascribe the loss of complement to a destruction by some agent in the venom.

Snake Serum.—The *serum* of serpents is also toxic for other animals,[80] even when the serpent is not a venomous one; *e. g.*, the harmless pine snake (*Pityophis cateniferis*). The toxicity of snake serum seems to depend chiefly upon its hemotoxic effects (hemagglutination and hemolysis), the toxic substances resembling amboceptors and being similar to, but not altogether identical with, the amboceptor of the venoms. Crotalus tissues also produce poisoning in proportion to the blood they contain, but are without toxic effects of their own (Flexner and Noguchi). Buglia[81] finds evidence that the hemolytic effect of extracts of snake serum, skin, and tissues may depend on soaps, but the general toxic effects probably depend on something else.

Antivenin.—Snake venom has the essential property of all true toxins of immunizing, with the appearance of an antitoxin in the blood. The first successful immunizations seem to have been made by Sewall,[82] but the practical production of antitoxic serum was first accomplished by Calmette[83] and by Fraser.[84] At first it was believed that cobra antivenin neutralizes the neurotoxins and hemolysins of venoms of *any* origin, and also of snake serums, and, therefore, should be quite effective against cobra and similar venoms which produce chiefly neurotoxic and hemolytic changes. This implies that these toxic substances are of identical nature in all snakes, no matter how dissimilar the snakes may be, but various investigators, especially Lamb, have found sufficient specificity exhibited by different venoms and antivenoms to indicate the necessity of employing the specific antiserum in each case of snake bite. The extensive investigations of de Houssay with South American snakes have served to emphasize the specificity of the antivenins.[85] A special antitoxin against rattlesnake venom and its hemorrhagic toxin has been successfully prepared by Noguchi.[86] This crotalus antivenin also neutralizes hemolysins of various venoms, and also of snake serums.

[79] Lancet, 1894 (1), 1236; Ewing, Med. Record, 1894 (45), 663.
[80] Questioned by Welker and Marshall, Jour. Pharmacol., 1915 (6), 563.
[81] Arch. ital. Biol., 1923 (72), 81.
[82] Jour. of Physiol., 1887 (8), 203.
[83] Ann. d. l'Inst. Pasteur, 1894 (6), 257; also subsequent articles in 1897 (11), 214; 1898 (12), 343.
[84] British Med. Jour., 1895 (i), 1309.
[85] Revista Assoc. med. Argentina, 1922 (35), 649; Compt. Rend. Soc. Biol., 1923 (89), 454.
[86] Univ. of Penn. Med. Bull., 1904 (17), 154; Jour. Exper. Med., 1906 (8), 614.

During immunization a certain amount of cellular immunity may appear independent of the humoral immunity,[87] and even caterpillars have been successfully immunized.[88]

Presumably antivenin neutralizes venoms in exactly the same way that antitoxin neutralizes toxins; *i. e.*, cell receptors are thrown off from the injured cells during immunization, which combine with venom amboceptors in the blood, and thus prevent their combining with the cells. Antivenin also prevents the inhibiting action of venom on bactericidal serum, indicating that it prevents the venom amboceptors from binding the serum complement. The reaction of venom and antivenin is certainly a chemical one, being likened by Kyes[89] to that of strong acids upon strong bases. The serum of animals immunized to venoms contains precipitins for the proteins of these venoms, and, to some extent, for the serum proteins of the same species of snakes. These precipitins are highly but not absolutely specific, and they bear no exact quantitative relation to the other antibodies present in the same sera.[90]

As is well known, snakes are nearly or quite insusceptible to snake venom. Cunningham[91] found that serum of cobras was devoid of antitoxic property, so the immunity of snakes must be ascribed to an absence of cell receptors in their tissues, with which their venom amboceptor can combine. The reputed immunity of the mongoose and hedgehog depends partly on a relatively low susceptibility, but probably more on the agility of the mongoose and the defensive spines of the hedgehog. However, Kraus[36] found considerable antivenomous power in the blood of a South American skunk which is immune to snake venom.

Platypus Venom.—The only mammal with a venomous secretion is that strange freak, the duck-billed platypus (*Ornithorhynchus paradoxus*). The males have a hollow movable spur on each hind foot, communicating like a fang with the venom gland, which secretes a venom with properties resembling the venoms of the Australian snakes, but much weaker.

Scorpion Poison[92]

This poison is secreted by a pair of specialized glands in the posterior segment of the elongated abdomen, surrounded by a firm capsule with a sharp apex through which the poison is discharged. Its effect on man is usually confined to local pain, swelling, and occasionally phlegmonous inflammation with constitutional symptoms after bites from the largest species. In Africa a large scorpion (*Androctonus*) exists, that is reputed frequently to cause fatal poisoning, especially in children. Manchurian scorpions (*Buthus martensi, Karchi*) seem to be less toxic than this or

[87] Gunn and Heathcote, Proc. Roy. Soc. London, B., 1921 (92), 81.
[88] Mazza, Compt. Rend. Soc. Biol., 1924 (90), 669.
[89] Berl. klin. Woch., 1904 (41), 494.
[90] See Houssay and Negrete, Rev. inst. bact. Buenos Aires, 1918 (1), 15.
[91] Nature, 1896 (55), 139.
[92] A complete discussion of the literature on poisonous invertebrates, etc., is given by v. Fürth, "Vergleichende chemische Physiologie der niederen Tiere," Jena, 1903; and by Faust, "Die tierischen Gifte," Braunschweig, 1906. Concerning scorpions see Kubota, Jour. Pharmacol., 1918 (11), 447.

Mexican scorpions (*Centrurus exlicauda, Wood*). In Korea, however, of 81 cases collected by Mori,[93] four were fatal. The majority of serious results following scorpion bites, as well as bites of poisonous insects to be considered later, are due to infection of the wound, which occurs readily because of local necrosis and hemorrhages, and also because of the unfavorable conditions existing in tropical climates. Apparently these bites favor local infection much as do those of vipers.

When general symptoms do occur, they are described as resembling strychnine poisoning, with trismus, stiffness of the neck and eventually of the respiratory muscles, which seems to be the chief cause of death (Cavorez). Thompson,[94] however, observed only occasionally severe symptoms, consisting of general paralysis that passed off in a few hours. Most experimenters with scorpion poison describe it as chiefly a nerve-tissue poison, and it also seems to act as a hemolysin and agglutinin (Bellesme and Sanarelli), but Todd[95] found it without action on corpuscles and not capable of combining with nervous tissues. Houssay[96] states that scorpion poison (*B. quinquestriatus*) is above all a muscular poison of the veratrine type, and a powerful peripheral excitant of the salivary and lachrymal secretions. The Algerian scorpion venom is said to have a cardiovascular effect similar to that of adrenaline.[97]

Calmette[98] gives the lethal dose for a guinea-pig as 0.5 milligram, while Phisalix and Varigny put it at 0.1 milligram and state that scorpion blood is also poisonous. Wilson[99] found the toxicity of the venom equal to 0.1 gram per million, that is, one gram of poison will kill 10,000,000 grams of guinea-pig,[1] hence it is much stronger than cobra venom. It is quite stable, and keeps many months in an ice chest; is not affected by heating to 100° for a brief period but is destroyed after 12 or 13 minutes' heating. The active constituents are precipitated by saturating with ammonium sulphate, or by an excess of alcohol, hence they are apparently protein, possibly histones.[2] They are destroyed by either pepsin or trypsin (Kubota).[92] The average amount of toxin in an Egyptian scorpion (*Buthus quinquestriatus*) is sufficient to kill about 35 kilos, which agrees with the fact that fatal poisoning by this scorpion is rare in adults, but reaches 60 per cent. in children. The venom is harmless when taken into the stomach, and is said to be made inactive by ammonia, calcium hypochlorite, and peroxide of hydrogen. Calmette claims that antivenin for cobra in part neutralizes scorpion poison, a statement which could not be corroborated by Todd, who succeeded, however, in preparing an

[93] Korean Med. Soc. Jour. (Chosen Igakukai-Zasshi), 1917, p. 47.
[94] Proc. Acad. Nat. Sci. of Philadelphia, 1886, p. 299.
[95] Jour. of Hygiene, 1909 (9), 69.
[96] Jour. physiol. path. gén., 1919 (18), 305.
[97] Bardier and Stillmunkes, Compt. Rend. Soc. Biol., 1923 (88), 559.
[98] Ann. Inst. Pasteur, 1895 (9), 232.
[99] Records of Egyptian Gov't., School of Med., 1904; abst. in Jour. of Physiol., 1904 (31), p. xlviii.
[1] Exactly the same toxicity is shown by Korean scorpions (Mori).[93]
[2] Wilson, Bull. inst. Egypte, 1921 (3), 67.

efficient antiserum by immunizing horses with scorpion venom.[3] Kraus[4] found that snake venom antitoxin has no curative action on scorpion stings, although it slowly neutralizes scorpion poison in the test tube. Houssay[69] also describes the antiscorpion serum as strictly specific. A large number of naturalists and raconteurs have furnished interesting tales of suicide by scorpions, which are more than improbable in the light of our present knowledge concerning natural immunity. Many animals seem to possess more or less immunity to scorpions (Wilson), especially such wild animals as are much exposed to them.

Spider Poison

The poison apparatus of the spiders consists of two long pouches lying in the thorax and extending into the jaws, at the apex of which the poison is discharged. Some of the larger members of the family are very poisonous, *e. g.*, the Malmignatte (*Lathrodectes tredecimguttatas*), of the vicinity of the lower Volga in southern Russia, is said to have destroyed 70,000 cattle in one year, the bite being fatal in 12 per cent. of all cases, although rarely killing man. Other members of this species in Chili, Madagascar, and other countries are not much less venomous. Kobert[5] has studied the poison of Malmignatte and found it distributed throughout the body of the spider, even in the eggs, and resembling in nature the snake venoms. It is destroyed by heating, and seems to be of protein nature; the chief effect is upon the nervous system and heart.[6]

A number of common spiders investigated by Kobert were apparently not poisonous for mammals, except the "cross spider" (*Epeira diadema*,) which has since been thoroughly studied by him and by Sachs.[7] Walbum[8] states that the chief poison of these spiders is found in the ovaries, the salivary poison being much weaker, and the hemolysin is found chiefly in the albumin fraction. Epeiratoxin resembles the snake venoms strikingly, according to Sachs, for it contains a powerful hemolysin which he calls "arachnolysin," acting very differently with different sorts of blood, and destroyed by heating at 70°–72° for forty minutes, and it behaves with lecithin and cholesterol like cobra venom.[9] By immunizing a guinea-pig Sachs succeeded in securing an antitoxin of some strength. The agglutinin is quite distinct from the hemolysin.[10] Only such blood

[3] A successful serum has also been prepared in Brazil (see Brazil-Medico, 1918 (32), 161).

[4] Brazil Medico, 1923 (1), 120; see also Münch. med. Woch., 1923 (70), 695.

[5] "Beiträge zur Kentnisse der Giftspinnen," Stuttgart, 1901.

[6] In western America and South America is found a spider (*Latrodectes mactans*) the bite of which is capable of causing severe spasm of the abdominal muscles, according to Atwood (Southern Californ. Pract. Vols., 10, 12 and 16). Kellogg and Coleman (Jour. of Parasitol., 1915 (1), 107), found extracts of the poison glands of this spider to be highly toxic. Watson observed severe symptoms in four men bitten by *L. mactans* (Science, 1922 (55), 539). Houssay found that the bites of this spider kill guinea pigs, apparently with bronchospasm (Rivista inst. bacteriol, Buenos Aires, 1919 (2), 189).

[7] Hofmeister's Beitr., 1902 (2), 125.

[8] Zeit. Immunität., 1915 (23), 623.

[9] Pini, Il Policlinico (Sez. Med.), 1909 (16), 208.

[10] v. Szily, Zeit. Immunität., 1910 (5), 280.

is hemolyzed as is able to bind the poison in the stroma of the red corpuscles. The discovery of this hemolysin explains Kobert's observation of hemoglobin, methemoglobin, etc., in the urine of persons bitten by spiders. Spider hemolysins have been studied extensively by Houssay,[11] who finds that spiders without hemolysins poison flies exactly as those with hemolysins.

Von Fürth considers that the bite of the historically famous Italian tarantula is able to cause no more than local inflammation, and Kobert found that the entire extract of six Russian tarantulas (which are supposed to be more poisonous than the Italian) caused no symptoms when injected into a cat. An antitoxin is said to have been secured against the Russian tarantula.[12] Baerg[13] found the bite of the American tarantula, *Eurypelma steindachneri*, to have only slight effects.

In all probability the other poisonous spiders possess toxic substances allied to those of the venoms, with hemolytic, agglutinative, and neurotoxic products, Sachs' studies indicating the general similarity of all the zootoxins.

Centipedes

Undoubtedly the severity of centipede poisoning has been greatly exaggerated, the results being usually limited to local inflammation, frequently spreading some distance in an erysipelas-like manner. An authentic case of fatal poisoning of a child four years old by a centipede (*Scolopendra heros*) has been reported from Texas by G. Linceicum,[14] death resulting five to six hours after the bite was received. Besides the local pain and inflammation, vomiting was marked, occurring also in five other non-fatal cases.

Centipedes secrete their poison in relatively large glands, which discharge at the apices of a pair of specialized claws that take the place of the first pair of legs. The nature of this poison seems not to have been much investigated, but Bekal and Phisalix[15] have found quinone to be the active principle of *Inlus terrestris*, a single insect secreting about 0.22 mg. at a time. Numerous chemical substances are described as secreted by other glands of these animals, including prussic acid and a camphor-like matter (see v. Fürth).

Bee Poison[16]

Bee poison has been better studied than most insect poisons, beginning with the work of Paul Bert (1865). It is secreted by the glands into a small poison sac, and stored up until ejected. Cloez found that bee poison was precipitated by ammonia, tannin, and platinic chloride, and Langer believed it to be a non-volatile organic base. As excreted, it is

[11] Comp. Rend. Soc. Biol., 1916 (79), 658.
[12] Konstanzoff, Russky Wratsch., 1907, No. 17.
[13] Jour. parasitol., 1921 (8), 90.
[14] Amer. Jour. Med. Sci., 1866 (52), 575.
[15] Compt. Rend. Acad. Sci. Paris, 1900 (131), 1004.
[16] An extensive review is given by Nast and Boyd, Jour. Amer. Inst. Homeopathy, 1923 (16), 123; also see Flury, Naturwissenschaften, 1923 (11), 341.

acid, contains 30 per cent. of solids, and one honey-bee secretes 0.0003–0.0004 gm. It has long been considered to contain formic acid[17] and much protein, but it has been stated that the poison is protein-free, and is not destroyed by heat (100°), weak acids, or alkalies. On the other hand, it is said to be destroyed by proteolytic enzymes, which would indicate that it is of protein nature. Arthus[18] believes the evidence indicates that the bee venom is a proteotoxin. However, there are many points of resemblance between the effects of insect stings and the local effects of histamine injection.[19]

Flury[20] believes bee poison to be a complex, from which he has split off choline, glycerol, P_2O_5, palmitic and other fatty acids, and possibly tryptophan, as well as a non-nitrogenous compound which behaves like the hemolytic saponin. These findings suggest that bee poison is a compound of lecithin with basic radicals, resembling in some respects sapotoxins and cantharidin. Hemolysis of all sorts of blood is produced both *in vitro* and *in vivo*, but to very different degrees, thus resembling spider toxin. The hemolytic action is greatly increased by the presence of lecithin, forming a toxolecithid like "cobra lecithid."[21] Locally, bee poison causes necrosis, with marked hyperemia and edema. A 4,500 gm. dog was killed by intravenous injection of 6 c.c. of a 1.5 per cent. solution of pure poison (Langer).[22] Injection of the poison in rabbits causes a slowing of blood coagulation, fall in blood pressure, increased respiration and peristalsis, but in dogs arterial hypertension is produced (Lyssy).[23]

Immunity is undoubtedly possible, for bee-keepers frequently show a great decrease in susceptibility. On the other hand, abnormally great susceptibility is frequently seen, some cases of fatal poisoning having been observed.[24] Dold[25] was unable to secure experimental immunity to bee poison. Acute fatal myelosis of leukemic character has been observed in two persons shortly after they had been severely stung by bees.[26]

Wasps and hornets presumably produce poisons similar to those of the bee. A study by Bertarelli and Tedeschi[27] establishes this for a species of wasp (*Vespa crabro L.*).

Ants also produce formic acid,[28] a fact so well known that it has come to be considered that this is the source of their toxicity.[29] Von Fürth, however, suggests the probability that ant poison, like that of the bees, owes its chief effects to other more

[17] This has been questioned by later observers; see Flury, Klin. Woch., 1923 (2), 2157.

[18] Jour. Pharm. Chim., 1919 (20), 41.

[19] See Eppinger, Wien. med. Woch., 1913 (23), 1414; Sollmann and Pilcher, Jour. Pharm., 1917 (9), 309.

[20] Arch. exp. Path. u. Pharm., 1920 (85), 319.

[21] Morgenroth and Carpi, Berl. klin. Woch., 1906 (43), 1424.

[22] Arch. exp. Path. u. Pharm., 1896 (38), 381; Arch. internat. Pharmac. et Ther., 1899 (6), 181.

[23] Arch. intern. physiol., 1921 (16), 272.

[24] Hospitalstidende, 1905, No. 27.

[25] Zeit. Immunität., 1917 (26), 284.

[26] Parrisius and Heimberger, Deut. Arch. klin. Med., 1924 (143), 335.

[27] Cent. f. Bakt., 1913 (68), 309.

[28] See Stumper, Compt. Rend. Acad. Sci., 1922 (174), 413; 1923 (176), 330.

[29] The sting of nettles is said to be due in part to formic acid. (See Dobbin, Nature, Sept. 18, 1919.)

complex, unknown poisons,[30] and not all ants seem to contain formic acid. Flury[31] believes that in general the effects of formic acid in stings are but slight and transitory, and not responsible for the chief toxic effects in any species, although formic acid is of widespread occurrence in insect poisons.

Lice.—Persons bitten by large numbers of lice may exhibit a distinct intoxication, accompanied by an eruption resembling measles.[32] The nature of the poison is not known, but it does not produce a severe local urticaria like the sting of bees and wasps.

Poisons of Dermal Glands of Toads and Salamanders

It has been known for centuries that toads produce poisonous substances, Paré in 1575 having discoursed interestingly, if inaccurately, on this topic. Numerous studies have been made of these poisons, which are secreted by the dermal glands and therefore cannot be used for poisoning either prey or enemies (except those that feed upon them), the most extensive study being that of Faust.[33] He isolated two constituents, apparently the same in different species of toads; one, which he called *bufotalin*, is very active, resembling in physiological effects the digitalis group; the other, *bufonin*, is much less active. Bufonin is neutral in reaction, soluble in warm alcohol, but slightly in cold. Analysis indicates an empirical formula of $C_{34}H_{54}O_2$. It probably is the cause of the milky appearance of the dermal secretion. Bufotalin seems to be $C_{34}H_{46}O_{10}$, is acid in reaction, soluble in chloroform and alcohol, but not in petroleum ether. Subcutaneous injection of 2.6 mg. bufotalin killed a dog (weighing 4 kg.) in four to five hours; given by mouth it causes much vomiting and diarrhea, so that large doses are not fatal. It causes much local irritation when applied to mucous membranes, but produces no marked change at the site of injection. Bufotalin seems to be derived from bufonin by oxidation, and the latter is quite similar to cholesterol, apparently having the following formula: $HO–H_{26}C_{17}–C_{17}H_{26}–OH$. Handovsky[34] has obtained an alkaloidal poison from the skin glands of *Bufo vulgaris*, which is C_6H_9NO, and called by him bufotamine; it seems to be a pyrrol derivative. Flury says that the skin glands of a single frog may contain enough poison to kill dozens of rabbits. An important consideration is that Faust has also isolated from the venom of cobra and crotalus, poisons which seem related to these toad poisons, the cobra poison being assigned an empirical formula of $C_{17}H_{26}O_{10}$, and the crotalus poison $C_{34}H_{54}O_{21}$. Fühner[35] considers bufotalin to be more closely related to the saponins.

Phisalix and Betrand[36] have found poison in the blood of toads similar to that of the glands. The hemolytic property observed by Pugliese[37]

[30] An attempt by Barratt (Ann. Trop. Med. and Parasitol., 1910 (4), 177) to obtain a poison from culex mosquitos was unsuccessful. The bodies of "black flies" contain an active poison that could not be identified by Stokes (Jour. Cut. Dis., 1914 (32), 830), beyond that it is insoluble in alcohol which does not inactivate it, and that it is destroyed by trypsin.

[31] Ber. pharm. Ges., 1919 (29), h. 9.

[32] Hirschfelder and Moore, Arch. Int. Med., 1919 (23), 419.

[33] Arch. f. exp. Path. u. Pharm., 1902 (47), 279; bibliography and review.

[34] Arch. exp. Path. Pharm., 1920 (86), 138.

[35] Arch. exp. Path. u. Pharm., 1910 (63), 374.

[36] Arch. d. physiol. norm. et path., 1893 (5), 511.

[37] Archivo di farm. e. terap., 1894 (2), 321; Arch. ital de Biol., 1895 (22), 79.

may be due to the acidity of the dermal secretion. The poisons of different species seem to be quite the same in all (Faust). From the dermal secretion of the large tropical toad, *Bufo agua*, Abel and Macht[38] have isolated two distinct active substances; one identical with epinephrine, which constitutes nearly seven per cent. of the crude venom; the other, which makes up 36 per cent., is called *bufagin*, has a composition idicated by the formula $C_{18}H_{24}O_2$, and therefore is presumably related to the rest of this group which arise from cholesterol. In physiological action bufagin resembles digitalis, and it is extremely active. The toad is relatively immune to bufagin,[39] but not at all to the epinephrine. A Chinese drug derived from toad skins has been found to contain similar ingredients (Shimizu[40]), as well as a substance resembling picrotoxin in action.

Salamanders also produce poisonous secretions in their dermal glands, which have been studied especially by Faust,[41] and earlier by Zalesky,[42] who isolated an organic base which he named *samandarin*. Faust describes samandarin as first stimulating and then paralyzing the automatic centers in the medulla. The poison resembles the alkaloids, having the formula $C_{26}H_{40}N_2O$, and produces death in doses of 0.7–0.9 mg. per kilo (dogs) with respiratory failure. Immunization of rabbits was practically impossible. A second alkaloid, *samandaridin* ($C_{20}H_{31}NO$) is also present in even greater quantities than the samandarin, and differs only in being weaker.[43]

Frogs have similar poisons in their skins, extracts of *Rana esculenta* skin being highly toxic.[44] The dermal secretions of most of the amphibians are poisonous, not only for mammals, but also for reptiles, and in large doses for the animals producing them (Phisalix).[45] Bert[46] and also Dutartre[47] have described a digitalis-like poison in the secretion of the dermal glands of frogs.

It is evident that all these poisons are quite distinct from the venoms, and from the true toxins, apparently being simple chemical compounds not related to the proteins and not capable of causing immunization.

Poisonous Fish[48]

There are numerous fish, especially in tropical waters, which defend themselves by injecting poisons into their enemies. This is accomplished by spines, to which are attached poison glands.[49] Dunbar-Brunton[50] has described two such fish (*Trachinis draco* and *Scorpæna scorpha*) of Mediterranean waters. Wounds by these spines cause in animals intense local irritation and edema and paralysis of the part, followed by gangrene about the site of the wound; in fatal poisoning death

[38] Jour. Amer. Med. Assoc., 1911 (56), 1531; Jour. of Pharm., 1912 (3), 319.
[39] See also Novaro, Compt. Rend. Soc. Biol., 1922 (87), 824; 1923 (88), 371.
[40] Jour. Pharmacol., 1916 (8), 347.
[41] Arch. exper. Path. u. Pharm. 1898 (41), 229 (literature); 1900 (43), 84
[42] Hoppe-Seyler's Med. Chem. Untersuch., 1866, p. 85.
[43] A Japanese triton (*T. pyrrhogaster*) also has hemolytic and neurotoxic poisons in its skin and other tissues (Matsusaki and Kabeda, Tokyo Med. News, 1918, pp. 455 and 815).
[44] Caspari and Loewy, Med. Klinik, 1911 (7), 1204.
[45] Jour. Phys. et Path. gen., 1910 (12), 325.
[46] Compt. Rend. Soc. Biol., 1885, p. 524.
[47] *Ibid.*, 1890, p. 199.
[48] Full discussion and literature given by Faust, "Tierische Gifte," p. 134.
[49] For a list of fish with poison glands see Pawlowsky, Zool. Jahrb., 1912 (31), 529.
[50] Lancet, 1896 (ii), 600.

occurs in from one to sixteen hours, with general paralysis. The sufferings of persons so poisoned are said to be extreme, and death may occur either directly from the poison or later from sepsis following the local gangrene. Presumably this poison is not dissimilar to that of the snakes; it probably is not an alkaloid, as Dunbar-Brunton suggests. It affects chiefly the heart, according to Pohl,[51] and contains a hemolytic principle which behaves like the venom hemolysins in that it is activated by serum (Evan).[52] The poison obtained from the spine glands of a Japanese fish (*Plotosus anguillaris*) is said to produce antitoxins in immunized animals.[53] Several other fish such as the sting ray, spiny dog fish and weever fish,[54] secrete poison in glands attached to long spines, one of the most poisonous being *Synanceia brachio*, which is known to have caused fatal intoxication in several instances. Only the *Muræniдæ* seem capable of poisoning by biting; they have a well-developed poison apparatus on the gums, but nothing is known concerning the poisons they produce.

Many fish develop poisonous decomposition products remarkably soon after death, especially in tropical climates, so that a fish that is perfectly wholesome if eaten immediately after being caught may be very poisonous if kept but a few hours.[55] There is a decided difference in fish of different varieties in this respect, so that some cannot be safely marketed. Some of the poisonous products of the decomposition of fish seem to be early products of protein cleavage of high molecular complexity, for they are digested by pepsin and trypsin, but not by erepsin.[56]

There are also other fish whose bodies, even when perfectly fresh, contain very powerful poisons. Savtschenko,[57] in his elaborate atlas of the poisonous fish, describes a number of cases of poisoning by the famous "parrot fish" of Japan (*Tertrodon* or *Fugu*), in which the poison seems to be developed and contained in the ovaries and eggs, and therefore the degree of toxicity varies with the season of the year in which the fish is taken.[58] Poisoning by these fish is very violent, the symptoms appearing quickly, and the cases are divided into two groups by Savtschenko, the algid, or choleriform, and the gastro-intestinal type. The symptoms of the algid form appear almost immediately after eating the fish, and consist of pain in the stomach, with great fear and distress; soon diarrhea and vomiting set in, with cramps in the arms and legs; this terminates in collapse, coma, and death from either respiratory or cardiac paralysis. The entire course of the process may be but ten to twenty

[51] Prager med. Woch., 1893 (18), 31.

[52] British Med. Jour. 1907 (i), 73.

[53] Kabeshima, Nippon Biseibutsugakki Zasshi, 1918 (6), 45; abst. in Japanese Med. Lit., 1920 (5), 23.

[54] See Evans, Brit. Med. Jour. Oct. 29, 1921, p. 690.

[55] Polarized light is said to increase the rate of putrefaction (Bryant, Chemistry and Industry, 1923 (42), 681).

[56] Konstanzoff and Manoiloff, Wien. klin. Woch., 1914 (27), 883.

[57] "Atlas des Poissons Veneneux," St. Petersburg, 1886 (literature).

[58] A Brazilian fish, *Spheroides testudineus*, has extremely toxic tissues, extract of 0.01–0.02 gm. of liver killing a guinea pig in a few minutes (Fonseca, Brazil-Medico, 1917 (31), 97).

minutes, or it may be as many hours. On account of the localization of the poison in the eggs and ovaries, not all persons who eat the fish are poisoned, and not all who are poisoned receive a fatal dose. In the gastro-intestinal form the symptoms appear later, consist chiefly of gastro-intestinal disturbances resembling more closely ptomain poisoning, and the prognosis is not so bad as in the algid form. The pathological anatomy of this form of poisoning has not been carefully studied, but no characteristic or striking anatomical changes have been noted in the bodies examined.

Tahara[59] has described a toxic body, *tetrodo-toxin*, isolated from the ovaries of *Tetrodon*. The purest preparations had a minimum lethal dose of 0.0025 to 0.004 gm. per kilo, and a provisional formula of $C_{16}H_{31}NO_{16}$ was given to it. Tetrodotoxin is neither protein nor alkaloid, nor yet a protamin. Ishiwara[60] believes it to be a gluco-ester, and prefers the name *fugu-toxin*. It is non-antigenic and not hemolytic or agglutinative, but a slight tolerance can be produced by immunization. It is very soluble in water, and can be obtained free from protein, and apparently contains a glucose radical (Ishihara).[61] It anesthetizes motor nerve endings and the central nervous system, paralyzes both motor and sensory nerves, increases the excitability of muscle (Itakura)[62] but does not paralyze sympathetic nerve endings (Ishihara).[61] It causes paralysis of respiration by both central and peripheral action, fall of blood pressure, and is antagonized by adrenaline.[63]

The roe of other fishes contains poisons, that of the pike (*Esox lucuis*) and the barbel (*Barbus fluviatilis*) having been found by McCrudden[64] to be unlike that of *Tetrodon*, for it seemed to be associated with the proteins; it is toxic only by injection, paralyzes the respiratory center and has no hemolytic action. The ovaries of the American gar are also said to be toxic.[65]

In this connection may be mentioned the peculiar erysipelas-like lesions caused by bites of crabs, which indicates the formation of some toxic product by these crustaceans.[66] Gilchrist,[67] obtained a history of bites or injuries by crabs in 323 of 329 cases of "erysipeloid." Crabs, in turn, may be poisoned by cephalopods which secrete an active poison from their salivary glands.[68] Many coelenterates produce active poisons (most famous of these being the Portuguese-man-o'-war), which have especially a paralyzing and a local irritant effect.[69] Some slight antitoxic

[59] Biochem. Zeit., 1910 (30), 256.
[60] Arch. exp. Path. Pharm., 1924 (103), 209.
[61] See Jap. Med. Lit., 1920 (5), 21.
[62] Mitt. med. Fak. Univ. Tokio, 1917 (17), 455.
[63] Iwakawa and Kimura, Arch. exp. Path. u. pharm., 1922 (93), 305.
[64] Arch. exp. Path. u. Pharm., 1921 (91), 46.
[65] Greene *et al.*, Amer. Jour. Phys., 1918 (45), 558.
[66] The livers of the spiny lobster (genus *Penæus*) have been found to be very toxic (Nakano, Jap. Ztschr. Dermatol., 1917 (17), 1).
[67] Jour. Cutaneous Diseases, November, 1904.
[68] Baglioni, Zeit. f. Biol., 1908 (52), 130.
[69] See von Furth, Vergl. chem. Physiol.; also Lojacono, Jour. d. physiol., 1908. (10), 1001.

effect has been reported in the serum of animals immunized with the poison of a Japanese jelly fish (*Olindioides formosa goto*).[70]

Eel Serum

In 1888 Mosso[71] studied the toxic properties of eel serum, which he found was extremely poisonous for experimental animals, 0.1 to 0.3 c.c. per kilo being fatal for rabbits and dogs in a few minutes if intravenously injected; introduced into the stomach it is not toxic, but it produces a violent conjunctivitis when it enters the eye, the poisonous agent being contained in the albumin fraction.[72] The poisonous principle Mosso called *ichthyotoxin*. Death results from respiratory failure with large doses; small doses lead to cachexia and death after a few days. The coagulability of the blood is greatly reduced. Kossel[73] found histological changes in the central nervous system in such animals, that resembled the lesions of tetanus. He succeeded in securing an active antitoxin which neutralized the strongly hemolytic action of eel serum *in vitro*, and also prevented fatal effects in animals. Camus and Gley[74] have studied the physiological action of eel serum and found it strongly hemolytic, and also apparently neuro-toxic. The toxicity is destroyed by heating to 58° for fifteen minutes. By immunization an antitoxic serum can be obtained which neutralizes the eel toxin completely. Tchistovitch[75] secured antitoxic serum, which acted also as a precipitin for eel serum. De Lisle[76] found that eel serum does not act like an amboceptor, since after heating it cannot be reactivated with fresh mammalian serum, and it seems, therefore, to be different from snake serum in its structure.[77] Lamprey serum is likewise toxic,[78] as is also that of the Rays. Not only the serum, but also the palate glands of the Moray (*Muræna helena*) contain toxic antigenic substances resembling snake venoms (Kopaczewski).[79]

[70] Aoki, Japan Med. World, 1923 (3), 55.
[71] Arch. Ital. de Biol., 1888 (10), 141; 1889 (12), 229.
[72] Pöllot and Rahlson, Graefe's Arch., 1911 (72), 183.
[73] Berl. klin. Woch., 1898 (35), 152.
[74] Arch. internat. d. Pharm., 1899 (5), 247.
[75] Ann. Inst. Pasteur, 1899 (13), 406.
[76] Jour. of Med. Research, 1902 (8), 396.
[77] Corroborated by Sato, Nippon Biseibutsugakkai Zassi, 1917 (5), No. 35.
[78] Gley, Compt. Rend. Soc. Biol., 1915 (78), 116; Camus and Gley, *ibid*,. p. 203.
[79] Compt. Rend. Acad. Sci., 1917 (165), 513; Ann. Inst. Pasteur, 1918 (32), 584.

CHAPTER VII

CHEMISTRY OF THE IMMUNITY REACTIONS (I)[1]—ANTIGENS, SPECIFICITY, AND THE NATURE OF ANTIBODIES

ALTHOUGH immunity was first investigated in relation to bacterial infection, it was soon learned that the reactions by which the animal body defends itself against bacteria have not been developed as specific means of defense against bacteria alone, but are reactions against foreign substances of similar chemical nature, whether bacterial, animal, vegetable, or artificially synthetic in origin. Furthermore, these reactions are chemical reactions, and the problems of immunity are chemical problems, although as yet most of the reacting substances are not accessible to chemical investigation. In this place, where our concern is with the chemical aspects of pathological processes, the subject of immunity will be discussed only from the standpoint of the chemistry of the processes and substances involved, leaving to other works the clinical and bacteriological aspects of the subject.[2]

The reactions of immunity are, we find, reactions to chemical substances entering the body from without, or abnormally developed within the body by invading organisms, or by changes in the chemical processes of the body. Furthermore, there seems to be an essential difference between the reactions incited by simple chemical compounds to which the animal body can develop a certain degree of resistance (such as morphine, alcohol, and arsenic), and the reactions against more complex substances such as bacterial toxins, foreign proteins, venoms, etc. The complex substances of the latter group incite reactions which are to a greater or the less degree specific, and usually very highly augment the defense of the body against the foreign substances; with the simple poisons the reactions are largely or altogether non-specific, and the resulting resistance is relatively slight. Substances of the first class we refer to as antigens.

ANTIGENS[3]

This term includes those substances which, when introduced into the blood or tissues of an animal, in proper amounts and under suitable

[1] A more extended discussion is given in "Chemical Aspects of Immunity," H. G. Wells, Chemical Catalog Co., New York, 1925.

[2] Especially to be recommended for a discussion of the scientific problems of immunology is Zinsser's "Infection and Resistance," Macmillian, New York, and for methods and applications see Kolmer's "Infection, Immunity, and Specific Therapy," W. B. Saunders, Philadelphia. For a more concise presentation see Karsner and Ecker, "The Principles of Immunology," Lippincott. Also see Kolle and Wassermann, "Handbuch der path. Mikroorganismen;" Weichardt "Jahresbericht der Immunitätsforschung."

[3] See the Review on Antigens by E. P. Pick, Kolle and Wassermann's Handbuch d. path. Mikroorganismen. Also Sachs, Handbuch. d. Biochem., 1924 (3), 1.

conditions, cause the generation and appearance in the blood of specific antibodies capable of reacting with the antigen.[4] Concerning the chemistry of antigens we can say that all antigens, as far as now known, are colloids. Furthermore, with one exception, every known soluble, complete protein may act at least to some degree as an antigen, and, as yet, it has not been finally established that any colloids other than proteins can act as antigens. The exception is the racemized protein of Dakin, which seems to be entirely non-antigenic although soluble and possessed of all the amino-acids present in the egg albumin used in preparing it.[5] Presumably the inability of racemized proteins to serve as antigens depends on their indigestibility by proteolytic enzymes or tissues, so that they pass out of the body unaltered.[6] The racemization is supposed to depend on a keto-enol tautomerism of the –CH–CO– groups.

Solubility is esssential for antigenic action, for proteins that have been coagulated by heat or other agents lose their antigenic capacity, while proteins that are not coagulated (*e. g.*, casein, ovomucoid) retain their antigenic properties after boiling.[7] A typical incomplete protein, gelatin, is devoid of antigenic power (Starin).[8] The same is true of the protamines and possibly of the histones.[9] Hemocyanin, however, is antigenic (Schmidt.)[10]

Apparently an antigen must be a colloidal molecule, and Zinsser suggests that only such molecules, which, because of their size, cannot diffuse into the cells to be destroyed, incite the cells to form destructive extracellular antibodies as a protective measure. When the foreign molecules can diffuse into the cells no such extracellular mechanism is necessary for their destruction. In support of this is the fact that the entire mass of cleavage products of a protein are not antigentic, whereas if they are resynthesized into a colloidal molecule (plastein formation) this colloid is antigenic.[11]

It has been shown by Gay and Robertson[12] that if the non-antigenic cleavage products of casein are resynthesized by the reverse action of pepsin, into a protein resembling paranuclein, this synthetic protein is capable of acting as an antigen. Protamins and globin, they found, were not antigenic,[13] although globin when combined with casein forms a compound which engenders an antibody that gives complement fixation

[4] Attempts to influence the capacity to produce antibodies by modifying diet have not produced striking results. (See Zilva, Biochem. Jour., 1919 (13), 172; Werkman, Jour. Infect. Dis., 1924 (34), 447).

[5] Wells, Jour. Infect. Dis., 1909 (6), 506; Ten Broeck, Jour. Biol. Chem., 1914 (17), 369; also Schmidt, Proc. Soc. Exp. Biol. Med. 1917 (14), 104; Kahn McNeil.[8]

[6] Dakin, Jour. Biol. Chem., 1914 (17), 369.

[7] Wells, Jour. Infect. Dis., 1908 (5), 449; Jour. Biol. Chem., 1916 (28), 11.

[8] Jour. Infect. Dis., 1918 (23), 139; corroborated by Kahn and McNeil, Jour. Immunol. 1918 (3), 277.

[9] Wells, Zeit. Immunität., 1913 (19), 599; Schmidt, Jour. Infect. Dis., 1919 (25), 207; Browning and Wilson, Jour. Immunol., 1920 (5), 417.

[10] Proc. Soc. Biol. Chem., 1919 (14), lxix; Jour. Immun., 1920 (5), 259.

[11] Hermann and Chain, Zeit. physiol. Chem., 1912 (77), 289; Landsteiner, Biochem. Zeit., 1919 (93), 106.

[12] Jour. Biol. Chem., 1912 (12), 233.

[13] Jour. Exp. Med., 1912 (16), 479; 1913 (17), 535.

reactions with globin. Schmidt also found that protamin edestinate is antigenic for edestin and for itself, but not for protamins, whereas a compound protein, both elements of which were non-antigenic (globin-albumose), was not antigenic.[14]

Of the cleavage products of proteins it is certain that none of the amino-acids and simple polypeptids can act as antigens, and even such large complexes as the proteoses are antigenic to only a slight degree if at all.[15] Whether the entire protein molecule, or only groups thereof, determine the characteristics of the antigen, is not known, there being evidence which can be interpreted in favor of either view, but Wells and Osborne[16] have submitted evidence which indicates that a single pure protein can act with and engender more than one antibody; this is supported by Klein's demonstration of the production of two distinct antibodies by immunizing with casein,[17] and by numerous other observations.

There seems to be no very definite relation between the amount of antigen injected and the amount of antibody produced,[18] but usually the more soluble a foreign protein in the body fluids the better antigen it is.[19]

Numerous substances have been found which react specifically with antisera although not capable of inciting antibody production; these are not true antigens, and they are called *haptenes* by Landsteiner; *e. g.*, the non-antigenic carbohydrate of pneumococci which is thought to be responsible for the type specificity of these organisms[20] or the carbohydrate "residue antigen" of yeast.[21]

NON-PROTEIN ANTIGENS

Proteins cannot serve as antigens unless they are foreign to the animal used in immunizing, for the animal's own proteins do not incite a protective reaction, unless they are altered by introducing chemical radicals into them, when they may be able to serve as antigens in the same animal, *e. g.*, rabbit serum treated with formaldehyde injected into a rabbit may engender antibodies reacting with formol-rabbit serum.[22] As a rule the more widely two animals are separated biologically the more successfully the proteins of one can be used as antigens in the other, whereas the serum of one of two closely related species (*e. g.*, dog and fox, rabbit and hare) will have little antigenic effect in the other. However, some of the tissue proteins seem to be sufficiently foreign to the blood or to other tissues to serve as antigen when introduced into the blood of the animal furnishing

[14] Univ. of Calif. Publ., Pathol., 1916 (2), 157. Review and bibliography on specificity.
[15] See Fink, Jour. Infect. Dis., 1919 (25), 97; full review on proteoses as antigens.
[16] Jour. Infec. Dis., 1913 (12), 341.
[17] Folia Microbiol., 1912 (1), 101.
[18] See Tsen, Jour. Med. Res., 1918 (37), 381.
[19] Wells and Osborne, Jour. Infect. Dis., 1914 (14), 377.
[20] Heidelberger and Avery, Jour. Exp. Med., 1923 (38), 73; 1924 (40), 301.
[21] Mueller and Tomcsik, Jour. Exp. Med., 1924 (40), 343.
[22] Landsteiner and Jablons, Zeit. f. Immunität., 1914 (20), 618.

the tissue; *e. g.*, crystalline lens protein of guinea pigs is able to sensitize guinea pigs to their own lens proteins.

The presence of added radicals in proteins does not interfere with the antigenic capacity of the proteins if it does not destroy their solubility, although such additions may alter the specificity range (*q. v.*). Numerous compound proteins occurring in nature have been shown to be antigenic, *e. g.*, hemoglobin,[23] mucins and mucoids,[24] casein,[25] beta-nucleoproteins[26] and perhaps alpha nucleoproteins.[27] The chemical nature of the highly antigenic bacterial toxins is unknown, and that they are proteins is far from established, for very active toxin solutions have been obtained that did not contain enough protein to give the ordinary protein tests, although the toxins exhibit distinct colloidal characters and in many respects behave like proteins. That tuberculin (*q. v.*,) is an antigen has never been conclusively established.[28]

Among the many accounts of what the authors interpret as the successful production of specific antibodies as a reaction to non-protein antigens, are the following:

Ford[29] found that rabbits can be immunized to extracts of *Amanita phalloides*, and that the serum of such rabbits will neutralize five to eight times the lethal dose for guinea-pigs, and is anti-hemolytic for the hemolysin of amanita when diluted to 1–1000. As he and Abel[30] had found this hemolytic poison of Amanita to be a glucoside, this observation is to be interpreted as a successful production of an antibody for a non-protein poison, a glucoside. This work was further supported by successfully immunizing rabbits to extracts of *Rhus toxicodendron*, and finding that their serum in doses of 1 cc. will protect guinea-pigs from 5–6 lethal doses of the poison, which was found by Acree and Syme[31] to be a glucoside.[32] Subsequent work by the same author confirms the main point, showing that an active hemolysin can be obtained free from demonstrable protein, and that immunization with this protein-free hemolysin will result in strongly active (1–1,000) antihemolytic serum.[33] The antihemolysin unites with the hemolysin in simple multiple proportions.[34] Another, non-hemolytic poison from Amanita, which Ford designates as *Amanita toxin*, was found to contain neither protein nor glucoside, and no antitoxic serum or definite artificial immunity can be obtained for it.

[23] See Hektoen and Schulhof, Jour. Infect. Dis., 1922 (31), 32; 1923 (33), 224; Heidelberger and Landsteiner, Jour. Exp. Med., 1923 (38), 561.
[24] Elliott, Jour. Infect. Dis., 1914 (15), 501.
[25] Review by Wells and Osborne, Jour. Infect. Dis., 1921 (29), 200.
[26] Wells, Jour. Biol. Chem., 1916 (28), 11.
[27] Review by Wells, Zeit. f. Immunität., 1913 (19), 599.
[28] See Long, "Chemistry of Tuberculosis," Wells, DeWitt, and Long, Baltimore, Williams and Wilkins, 1923, p. 70.
[29] Jour. Infec. Dis., 1907 (4), 541.
[30] Jour. Biol. Chem., 1907 (2), 273.
[31] Jour. Biol. Chem., 1907 (2), 547.
[32] Not confirmed for the closely related *Rhus diversiloba* by McNair (Jour. Amer. Chem. Soc., 1916 (38), 1417), who considers the poison to be an unsaturated polyhydric phenol, lobinol, (Jour. Amer. Chem. Soc., 1921 (43), 159).
[33] Jour. Pharmacol., 1910 (2), 145.
[34] Jour. Pharmacol., 1913 (4), 235.

Jacoby believed that he had obtained the phytotoxin ricin free from protein, in which case the well-known and active antiricin must represent an antibody for a non-protein antigen. However, the work of Osborne, Mendel, and Harris[35] has shown that ricin is, in all probability, an albumin, and this, for the present at least, places ricin with the protein antigens. Nucleic acid and nucleinates have been found to be non-antigenic (Wells,[36] Taylor).

The work of Ford is, in our estimation, the strongest evidence yet presented as to the possibility of non-protein antigens. The newer developments in immunological research, moreover, make it seem entirely plausible that a complex glucoside, which can be hydrolyzed by enzymes, can act as an antigen. If we consider the evidence that immunity consists in the development of a special power to hydrolyze foreign substances, when these substances are of such a nature as to stimulate the cells to activity, and that Abderhalden and others have found evidence that specific enzymatic properties appear in the blood of animals injected with carbohydrates and fats, it seems entirely reasonable that a toxic glucoside can have antigenic properties. A similar line of reasoning will apply to the question of lipoid antigens.

However, the impossibility of excluding the presence of antigenic proteins in any preparations whatever that may be used for immunizing, is clearly shown by the studies of Florence Seibert[37] on distilled water. Observing that a large proportion of distilled waters, especially those kept for a time after distillation, give febrile reactions, the cause of these reactions was investigated and found to depend on the growth of bacteria capable of multiplying in distilled water. Moreover, it was found that injection of 20 cc. of such water freed from the bacteria by filtration served to produce agglutinins for these bacteria demonstrable with serum diluted to 1 to 8,000, although this water contained but 0.000014 mg. nitrogen per cc. or but 0.00028 mg. nitrogen in the entire amount of fluid used for immunizing. If quantities of substance as minute as this can serve so effectively as antigen, it is obviously practically impossible to determine whether the antigenic agent is protein, and certainly it is impossible to prove that it is not a protein.

Lipoids as Antigens.—The evident participation of lipoids[38] in immunity reactions, especially the complement-fixation and allied reactions, has naturally led to investigation of the possibility that lipoids may act as true antigens, a possibility made conspicuous by the fact that lipoids can be substituted for true antigens in the Wassermann reaction (*q. v.*). Bang and Forssmann immunized with ethereal extracts of red corpuscles and obtained hemolysins, so that they concluded that the antigenic constituent of the corpuscles is a lipoid, probably a phosphatid. This work has caused much controversy and many workers have failed to confirm their results.[39] It

[35] Amer. Jour. Physiol., 1905 (14), 259.

[36] Zeit. Immunität., 1913 (19), 599. Lichtenstein (Arch. Physiol., 1915, p. 189) reports the production of agglutinins for spermatozoa and yeasts with sodium nucleinate from sperm and yeasts.

[37] Amer. Jour. Physiol., 1925 (71), 621.

[38] Bibliography on Lipoids and Immunity given by Landsteiner, Kolle and Wassermann's Handbuch, 1913 (2), 1240; Jobling, Jour. Immunol., 1916 (1), 491.

[39] Review of literature by Landsteiner Jahresb. Immunitätsfrsch., 1910 (6), 209. See also Hemolysis, Chap. XI.

is a striking fact that when purified phosphatids, from sources favorable for obtaining pure materials, are used, the results are usually negative, while the positive results are generally reported with lipoids of more or less dubious purity.

"Nastin," the lipoid material from a streptothrix, has been used by Much and others, who state that sera are obtained which give complement fixation reactions with nastin used as the antigen.[40] Similar results are described for the fatty materials from tubercle bacilli ("tuberculonastin"). Warden[41] reports securing positive precipitin and fixation reactions, not only with fatty complexes from bacteria and red cells, but also with artificial mixtures of soaps made up to resemble the cellular lipins; indeed, he states that the lipoidal antigens are more specific than proteins, and infers that the specificity of antibodies is in part or wholly due to the fats of the cells. These observations could not be confirmed by Dernby and Walbum[42] and other observers have not been able to demonstrate antigenic action by bacterial fats.[43]

Meyers[44] has reported the production of specific complement fixation antibodies by immunizing rabbits with acetone-insoluble lipoidal material obtained from tape worms and echinococcus. He has found the acetone-insoluble fraction of tubercle bacilli, presumably phosphatids, to serve as antigen in complement fixation reactions with antibodies for tubercle bacilli,[45] and much more effectively than the protein residue of the bacilli, wherefore he concludes that the reactions obtained with the lipoids certainly cannot be ascribed to adherent traces of protein. Bergel[46] observed after lecithin injections in rabbits, not only an increase in the lipase content of the blood and tissues, but also the presence of complement-binding antibodies, and Jobling and Bull[47] have found an increase in serum lipase after immunizing with red corpuscles.[48]

The number of reputed positive results with lipoids makes it impossible at this time to state dogmatically that lipoids may not possess antigenic properties, but it must be taken into account that the successful use of lipoids as antigens in complement fixation reactions is not proof of their true antigenic nature. MacLean,[49] indeed, found evidence that even in the Wassermann reaction the active substance is not lecithin itself, but some other unknown substance which could be obtained practically lecithin-free. Furthermore, we have the testimony of Fitzgerald and Leathes[50] that a lipoidal material from liver, which was itself capable of serving as antigen in the Wassermann reaction, did not engender complement-fixing antibodies in rabbits immunized with this lipoid. Ritchie and Miller[51] could find no antigenic activity in the lipoids of serum or corpuscles. Also Kleinschmidt,[52] who accepts the antigenic nature of nastin, was unable to secure antibodies by immunizing rabbits with it. Neufeld found that rabbits immunized with lecithin developed no opsonins for lecithin emulsions. A suggestive observation is that of Pick and Schwarz,[53] who found that the presence of lecithin increases the antigenic power of bacteria, which may help to explain the activity of possible traces of proteins in lipoid preparations used as antigens.

Landsteiner and Simms[54] have investigated the so-called "heterogenetic lipoidal antigens" of Forssman which, although present in the tissues of animals of many species, incite the formation of hemolytic antibodies for sheep corpuscles. They

[40] Literature in Beitr. Klinik d. Tuberk., 1911 (20), 341.

[41] Jour. Infect. Dis., 1918 (22) 133; (23), 504; 1919 (24), 285.

[42] Biochem. Zeit., 1923 (138), 505.

[43] Borcic, Biochem. Zeit., 1920 (106), 212; Beumer, Biochem. Zeit., 1921 (121), 127.

[44] Zeit. Immunität., 1910 (7), 732; 1911 (9), 530; 1912 (14), 355.

[45] *Ibid.*, 1912 (14), 359; 1912 (15), 245.

[46] Deut. Arch. klin. Med., 1912 (106), 47.

[47] Jour. Exp. Med., 1912 (16), 483.

[48] Bogomolez suggests that the lipoids themselves may be produced in excess for defense against various poisons, which they serve to inhibit, especially the toxin of *B. botulinus*. (Zeit. Immunität., 1910 (8), 35).

[49] Lecithin and Allied Substances, Biochemical Monographs, 1918, p. 170.

[50] Univ. of Calif. Publ., 1912 (2), 39.

[51] Jour. Path. and Bact., 1913 (17), 429; but Wang. (*Ibid.*, 1919 (22), 224) obtained positive results with ether-chloroform extracts of corpuscles.

[52] Berl. klin. Woch., 1910 (47), 57.

[53] Biochem. Zeit., 1909 (15), 453.

[54] Jour. Exp. Med., 1923 (38), 127.

found that the lipoidal element of this antigen[54a] is not antigenic of itself, but when united to proteins the mixture is antigenic. There is much evidence of the importance of lipoid-protein complexes as antigens, but as yet it cannot be said that the antigenic capacity of lipoids as such has been established. One fundamental difficulty in accepting the idea that lipoids are antigenic lies in the fact that antigens must be *foreign* colloids, whereas the lipoids, of all animal sources at least, seem to be quite the same in all species,[55] and very limited in variety, hence they cannot well act as foreign substances, and, as Thiele[56] says, since lipoids possess no specificity they cannot give rise to antibodies. Of course it is possible that bacteria and lower animals, such as tapeworms, may contain lipoids foreign to mammals, which are capable of serving as antigens.

Simple Chemical Antigens.—Many drugs cause a hypersensitization, and in this respect seem to behave as antigens producing anaphylactic antibodies. It happens that most of these chemicals are of such a nature as to permit their union with proteins, and it seems probable that such protein compounds behave as foreign proteins to the animal in which they are formed, for it has been found that guinea-pig serum treated with iodine can render guinea-pigs sensitive to the same iodized serum.[57] Hence, hypersensitiveness to iodine compounds would be a reaction to iodized proteins,[58] and not to the non-protein iodine compound; the same applies to anaphylactic reactions observed with salvarsan, atoxyl,[59] copper compounds,[60] and perhaps aspirin and antipyrin.[61] Zieler, has questioned the validity of many of the experiments on which these views are based,[62] but they seem to have been placed on a sound footing by the studies of Landsteiner and his colleagues[63] on the antigenic capacity of artificial compounds of proteins with simple chemical radicals, for it has been demonstrated that serum proteins so altered by artificial means are antigenic when injected into the animals of the same species as those from which the serum came. It is also possible that certain chemicals may react in such a way with the tissue or blood proteins as to make them sensitive to the animal's own complement, which then forms anaphylatoxin,[64] and thus causes reactions, but the whole anaphylatoxin question is in so uncertain a state at the time of writing that further speculation in this direction is not justifiable.

The attempts to produce antitoxin against cantharidin have not yielded convincing results,[65] nor against epinephrine.[66] The supposed immunity to bile salts could not be confirmed by Ponder.[67] De Angelis[68] claimed that he had produced specific precipitins for various natural and synthetic dyes, but this work has, as was to be expected, failed of confirmation.[69] Elschnig and Salus[70] state that melanin from the eye is antigenic, producing complement-fixing antibodies specific for melanin but not for the species. Woods[71] has corroborated this and also demonstrated anaphylactic sensitization. We know too little concerning the composition of their melanin preparations to interpret these observations.

[54a] In solubility the heterogenetic antibody resembles the cerebrosides (Sordelli *et al.*, Revista Inst. Bact. Buenos Aires, 1925, (4), No. 1).

[55] Levene, Physiol. Reviews, 1921 (1), 327.

[56] Zeit. Immunität., 1913 (16), 160.

[57] Friedberger and Ito, Zeit. Immunität., 1912 (12), 241.

[58] According to Block (Zeit. exp. Path., 1911 (9), 509), iodoform idiosyncrasy depends upon the CH_3 rather than on the iodin, and is a local cellular reaction rather than a humoral reaction, the protoplasm having an increased affinity for methyl radicals. (See Weil, Zeit. Chemotherapie, 1913 (1), 412.)

[59] Moro and Stheeman, Münch. med. Woch., 1909 (56), 1414.

[60] Hollande, Compt. Rend. Soc. Biol., 1918 (81), 58.

[61] Bruck, Berl. klin. Woch., 1910 (47), 1928; Klausner, Münch. med. Woch., 1911 (58), 138.

[62] Münch. med. Woch., 1912 (59), 401.

[63] Biochem. Zeit., 1919 (93), 106; 1920 (104), 280; Jour. Exp. Med., 1924 (39), 631.

[64] See Manoilov, Wien. klin. Woch., 1912 (25), 1701.

[65] Champy, Compt. Rend. Soc. Biol., 1907 (62), 1128.

[66] Pollak, Zeit. physiol. Chem., 1910 (68), 69.

[67] Quart. Jour. Exp. Physiol., 1924 (14), 25.

[68] Ann. di Ig. Sperim., 1909 (19), 33.

[69] Takemura, Zeit. Immunität., 1910 (5), 697.

[70] Graefe's Arch., 1911 (79), 428.

[71] Jour. Immunol., 1918 (3), 75.

In general terms, therefore, antigens are protein molecules, and the reactions of immunity are reactions against proteins foreign to the body of the host, and manifested by the presence in the blood of the reacting animal of substances which combine with and cause recognizable changes in the foreign protein.[72] These changes are recognized in many ways, such as by precipitation, agglutination, complement-fixation, etc., and the question at once arises as to whether these different manifestations depend each upon a separate antibody, or if several or all of them are not caused by a single antibody, the action of which is indicated by the different reactions which are made manifest by different procedures in each case.[73] Certainly there is a growing tendency to assume that probably all the different reactions of immunology depend upon one, or, at most, two classes of antibodies. This question will be discussed further in later paragraphs.

Knowing that the antigens are merely foreign proteins which have been introduced into the body of an animal, there naturally occurs the thought that the animal body is continually receiving in its food foreign proteins, against which it defends itself in the alimentary canal by enzymatic action which disintegrates these proteins until they have lost their colloidal character. Logically following this comes the idea that perhaps the reactions of immunity are simply the same or similar disintegrative enzymatic actions, carried on within the blood and tissues to protect the body in the same way against foreign proteins which the alimentary digestive apparatus has not had the opportunity to destroy.[74] This conception of the nature of immune reactions to antigens has been especially advanced and investigated by Victor C. Vaughan[75] and his co-workers, and by Emil Abderhalden, who has demonstrated in various ways an increased proteolytic power in the blood of animals which have received parenteral injections of foreign proteins.[76] Thus, if the antiserum reacts on the specific proteins within a dialyzing sac, the products of proteolysis diffuse into the surrounding medium where they can be detected by simple chemical reactions. Also, changes in the specific rotation of the protein or peptid solution can be observed by the polariscopic reading before and after the action of the antiserum. Particularly important support of Vaughan's theory is furnished by the behavior of the racemized proteins. Although soluble, racemized protein cannot be attacked by the digestive proteolytic enzymes, presumably because of

[72] Drew has found no evidence of antibody formation by immunizing molluscs and echinoderms (Jour. of Hyg., 1911 (11), 188), from which he concludes that the reaction to foreign proteins is not a universal property of protoplasm; a sweeping generalization which requires more extensive investigation for its establishment. Cantacuzene (Compt. Rend. Soc. Biol., 1913 (74), 111) obtained precipitins by immunizing *Phallusia mamillata* with mammalian blood, but no hemolysins with this or *Aphrodite aculeata* and *Eledone moschata*.

[73] See Dean, Lancet, Jan. 13, 1917.

[74] Carrel and Ingebrigsten (Jour. Exp. Med., 1912 (15), 287) have found that tissues growing *in vitro* with foreign blood produce hemolytic antibodies for that blood, indicating that isolated cells can react to antigens by antibody production.

[75] See Vaughan, "Protein Split Products," Philadelphia, 1913.

[76] Abderhalden, "Abwehrfermente des tierischen Organismus," Berlin, 1913.

its altered configuration; and it is non-antigenic, presumably because it cannot be attacked by the proteases of the blood and tissues. Likewise it cannot be metabolized, whether fed or injected subcutaneously.[77] Here we have good evidence of the fundamental identity of the three processes, digestion, metabolism, antigenic activity.

As immunity reactions manifest themselves, however, there are steps in the process besides simple hydrolysis of proteins, even if this be the ultimate goal of them all. For example, according to Opie[78] the precipitin reaction serves an important function in holding foreign proteins at the point they enter the tissues, thus preventing their dispersion through the body.

Specificity of Immune Reactions[79]

The many attempts to explain the various reactions of immunity solely on the basis of known physico-chemical properties of colloids all flatten out when the striking, characteristic, and often extreme specificity of these reactions is considered. Chemical explanations are but little more satisfactory. In enzyme action we find many comparable examples of specificity,—but this does not help, as the enzymes are as mysterious as the antibodies. But no proposed explanation of any of the reactions incited by antigens can be of value if it fails to take into account the specificity of the reactions. We lack the space here to consider the many ideas and the items of evidence which have been advanced concerning this all-important chemical problem, but refer the reader to other, more detailed discussions.[80] The main facts at present available are the following: Specificity was at first supposed to depend solely upon biological relationships, for it was found easy to distinguish the serum of animals of unlike nature by means of the precipitin and other reactions, but the more closely related the animals the less sharply these reactions distinguish them, until, with such closely related animals as dog and fox, or man and apes, antisera for the blood of one react nearly as well with the blood of the other, the existing differences being only quantitative. The opinion therefore gained ground that the specificity depends upon some peculiar biological relationship of the antigens, and, as serum proteins which seem to be similar chemically but which are from unrelated species are sharply differentiated by the biological reactions, that the specificity must depend upon something quite distinct from ordinary chemical differences. But even with closely related species, immunological differences can often be brought out by means of the process of saturation. (This consists in treating the antiserum with sufficient quantities of an antigen until it no longer reacts with additional quantities

[77] See Ten Broeck, Jour. Biol. Chem., 1914 (17), 369.
[78] Jour. Exp. Med., 1924 (39), 659.
[79] See Review by Wells, Jour. Immunol., 1924 (9), 291.
[80] E. P. Pick, Kolle and Wassermann's Handbuch d. path. Mikroorganismen, 1912 (1), 685; full bibliography, to that date. For more recent features see Wells, "Chemical Aspects of Immunity," 1925, Chemical Catalog Series.

of this antigen, and then trying its reactive power with the other related antigen which one wishes to test.)

As use began to be made of other materials than serum, and especially when more or less purified proteins were employed, it was found that within the tissues of a single animal or plant there might exist antigens which were quite distinct from one another, and, on the other hand, a common antigen may occur in many different species which are often not at all closely related from a zoological standpoint. Thus, in the hen's egg, by means of the anaphylaxis reaction I have been able to distinguish five distinct antigens, and these correspond to as many different proteins which have been distinguished by chemical means.[81] Also, for example, the crystalline lens proteins have been found to be immunologically the same in different species of animals,[82] even as remote from one another as cattle and fish, although two different proteins present in the same lens (called α and β crystallin) are immunologically distinct from each other[83] and from the serum protein of the same animal. Many other examples of the same principle may be cited; for example, caseins from goat and cow milk are immunologically closely related if not identical, while casein from cow milk is immunologically unrelated to lactalbumin from cow milk, and both are distinct from the globulin of cow milk which is identical with the serum globulin of the same species.[84] On the other hand the several proteins of the blood that have been isolated in pure condition, namely, serum albumin, euglobulin, pseudoglobulin,[85] fibrinogen, and hemoglobin,[86] are all immunologically distinguishable even when from the same species, and they are also immunologically distinct from the corresponding proteins of the blood of different species. A certain but slight distinguishable specificity may be observed between proteins from different organs of the same animal,[87] which differentiation is still sharper between the tissue proteins and serum proteins of the animal.[88] Sex cells, especially, seem to be rather distinct immunologically from the body cells.[89] Numerous instances of two separate proteins from the same plant seeds showing entirely distinct immunological specificities have been described.[90] It must also be taken into consideration that different colo-

[81] Jour. Infect. Dis., 1911 (9), 147.

[82] Krusius, Zeit. Immunität., 1910 (5), 699; Hektoen and Schulhof, Jour. Infect. Dis., 1924 (34), 433; Goto, Japan Microbiol. Soc., 1924 (17), No. 11.

[83] Hektoen and Schulhof, Jour. Infect. Dis., 1924 (34), 433.

[84] Review of milk proteins by Wells and Osborne, Jour. Infect. Dis., 1921 (29), 200.

[85] Dale and Hartley, Biochem. Jour., 1916 (10), 408; Doerr and Berger, Zeit. f. Hyg., 1922 (96), 191.

[86] Hektoen and Schulhof, Jour. Infect. Dis., 1923 (33), 224; Hektoen and Welker, ibid., 1924 (35), 294.

[87] According to Kernbach (Compt. Rend. Soc. Biol., 1924 (91), 213) the albumoid substance from bone has organ specificity but not species specificity. Hektoen and Schulhof (Jour. Amer. Med. Assoc., 1924 (83), 1300) have found that alimentary mucosa contains an antigen distinct from the serum proteins.

[88] See Salus, Biochem. Zeit., 1914 (60), 1.

[89] Graetz, Zeit. Immunität., 1914 (21), 150; Hektoen, Jour. Amer. Med. Assoc., 1922 (78), 704.

[90] Wells and Osborne, Jour. Infect. Dis., 1911 (8), 66 *et seq.*, especially 1916 (19), 183.

nies from a pure culture of a given species of bacteria may show different and distinctive reactions with agglutinating antisera, whereas organisms of distinct species (*e. g.*, gonococcus and meningococcus), may be difficult to differentiate by immune serum (Dean).[91]

All these facts taken together indicate that the chemical character of the proteins is undoubtedly more significant than the biological relations of the organisms from which they have been obtained. These and other observations leave little room for doubt that specificity depends upon chemical composition, and that *the differences in species as exhibited by their biological reactions depend upon distinct differences in the chemistry of their proteins.*[92]

Furthermore, it has been shown by Wells and Osborne[93] that a single pure protein may exhibit multiple antigenic properties, and react or fail to react with antisera against other pure proteins according to whether chemical differences can be demonstrated by recognized analytical methods. There is much evidence that the entire protein molecule is not involved in determining the specific character of the immunological reaction, but that this is developed by certain groups or radicals of the protein molecule and that a single protein molecule may contain two or more such groups.[94] Presumably the intact protein molecule is necessary to function as an antigen, but certain groups may determine the specificity, and consequently a single pure protein may exhibit more than one sort of specificity. For example, purified serum albumin from horse serum is immunologically specific when compared with albumin from swine serum and also with globulin from horse serum. On this basis, "group reactions" may depend on the presence of identical proteins or on the presence of distinct proteins with common reactive groups.

The facts cited above indicate conclusively that the immunological specificity of an antigen is not necessarily related to the biological specificity of the living organism from which it is derived. The logical explanation is that there may exist proteins in different species which have chemical resemblances or identity, and this is scarcely to be doubted. We find identical lipoids, fats, nucleic acids, and carbohydrates in different species; many particular types of proteins show apparent chemical identity in different species (*e. g.* gelatin, keratin, lens protein, casein). Therefore, it is highly probable that many tissue proteins may be identical in different forms of animal cells, and even in animal and plant cells.

Indeed, it is altogether reasonable that lens proteins, keratin, mucin, and other proteins whose function is not metabolism, should be nonspecific. Each of these proteins has quite the same function to perform in every species, and is set off from the active tissues to perform this function. There is no more reason why they should be species specific than any other product of cell activity, *e. g.*, trypsin, epinephrine, thyroxin, insulin. All these are products of cell activity with a definite function,

[91] Brit. Med. Jour., 1923, Dec. 1.
[92] See Wells and Osborne, Jour. Infect. Dis., 1916 (19), 183.
[93] Jour. Infect. Dis., 1913 (12), 341.
[94] Jour. Infect. Dis., 1913 (12), 141.

and apparently alike in all species, just as lens proteins have been found to be. So too, the characteristic protein of the thyroid, thyreoglobulin, seems to be immunologically distinct from the blood proteins of the animal from which it is obtained, although not distinct from thyreoglobulin of other animals.[95] A similar distinction from tissue proteins is often seen in proteins set aside for nourishment of the offspring (*e. g.*, casein, ovalbumin, seed proteins) and hence not participating in the metabolism of the organism from which they come.[96]

NON-SPECIFIC IMMUNITY

Another sort of manifestation of apparently non-specific immunity reactions has been observed especially in therapeutic immunization.[97] Beginning with the classical observation of Matthes that the tuberculin reaction could be produced with deutero-albumose, many similar non-specific reactions have been observed. For example, the sharp reaction that follows intravenous injections of killed typhoid bacilli into typhoid patients occurs equally well if colon bacilli are used, or deutero-albumose. One possible explanation of this type of reaction is that the injected substance acts as a common antigen, which causes the production of common antibodies that react also with the antigens of the cause of the disease. Another possibility is that the foreign protein stimulates the tissues that form antibodies, so that they produce not only antibodies for this antigen, but also for the antigens of the specific etiologic factor of the disease that have been stimulating the antibody formation previously. Hektoen[98] has observed, for example, that if an animal that has previously produced precipitins for one foreign protein is reinjected with a different protein it will then produce precipitins for both these proteins, and possibly for other proteins with which it has not been injected.[99] Moreover, the febrile reaction, the leucocytosis, and other phenomena, such as the antiferment index of the serum (Jobling),[1] that injection of nonspecific protein produces, may be responsible for favorably affecting the disease, rather than actual antibody formation.

An interesting illustration of the fact that whatever stimulates the bone marrow may cause it to form, among other blood elements, specific antibodies, is furnished by the behavior of antitoxin-producing horses. If a horse that has been immunized to diphtheria toxin is bled as much as possible, it will be found to have regenerated the lost antitoxin within 48 hours,[2] even although the last immunizing dose of toxin was received

[95] Hektoen and Schulhof, Jour. Amer. Med. Assoc., 1923 (80), 386.

[96] Bence-Jones proteins show distinct immunological specificity corresponding to specificity of chemical structure (Hektoen and Welker, Jour. Infect. Dis., 1924 (34), 440).

[97] See review by Jobling, Jour. Amer. Med. Assoc., 1916 (66), 1753; Petersen, Wm. F., "Protein Therapy and Non-specific Resistance." MacMillian, 1922.

[98] Jour. Infect. Dis., 1917 (21), 279.

[99] See also Hetmann, *ibid.*, 1918 (23), 457; Tsukahara, Zeit. Immunität., 1921 (32), 410.

[1] See review of this subject, Harvey Lectures, 1917 (12), 181; also Cowie and Calhoun, Arch. Int. Med. 1919 (23), 69.

[2] O'Brien, Jour. Path. and Bact., 1913 (18), 89.

long before. Also, it is stated that persons who have once had typhoid, but whose blood no longer contains much agglutinin, may show a high typhoid agglutinin content when infected by some other organism, or after any sharp febrile attack. It is highly possible that many non-antigenic therapeutic agents may similarly act by stimulating the tissues to increased formation of specific antibodies, *e. g.*, arsenic, mercury and other metals, heliotherapy, hemorrhage[3] or phlebotomy, hot baths.

The opposite type of phenomenon, that is, non-specific interference with immunological reaction, is suggested by the observations of J. H. Lewis.[4] He found that small quantities of one protein injected into a guinea pig together with or shortly after large quantities of another protein (*e. g.*, egg albumen in dog serum) would not sensitize the animal, although a similar amount injected alone would always sensitize. The suggested explanation is that the larger amount of the one foreign protein combines with so many of the available cell receptors that few of the smaller number of the other sensitizing protein molecules are able to be bound to the cells and to stimulate antibody formation; this explanation assumes a certain lack of specificity on the part of the cell receptors.

Heterogenetic Antibodies.—A striking example of the existence of identical antigenic properties in materials of biologically unrelated origins, is furnished by the sheep corpuscle hemolysin discovered by Forssman,[5] who found that many different materials, when injected into rabbits, engender in the rabbits' serum active hemolytic amboceptors for sheep corpuscles. This antigenic property has been demonstrated in the organs of the guinea-pig, horse, cat, dog, mouse, chicken, turtle, and several species of fish,[6] although not exhibited by organs of many closely related species (*e. g.*, pig, ox, rabbit, goose, frog, eel, man, pigeon, rat). It is not present in the red corpuscles of these animals, but is present in the corpuscles of the sheep, whose organs do not have this property. It has also been found in paratyphoid and Gärtner bacilli, mouse tumors and sheep spermatozoa. Not only does the serum of rabbits thus immunized show active hemolysis for sheep corpuscles, but if injected into the vein of an animal whose organs contain this antigen there results a prompt, severe anaphylactic intoxication, presumably through reaction between the antigen present in their tissues and the antibodies of the rabbit serum. Furthermore, the antibody can be specifically removed from the immune rabbit serum by contact with any of the antigen-containing tissues, but not by tissues that do not exhibit this antigenic property. The antigen seems to be a lipoid-protein complex closely related to the acetone-precipitated antigen used in the Wassermann reaction; possibly the protein part of the complex confers the antigenic capacity while the lipoid part confers the heterogenetic specificity.[7]

Immunological Specificity Is Dependent on Chemical Individuality.—So remarkable are the manifestations of specificity, so exquisitely delicate are many of the immunological reactions exhibiting specificity, and so wonderfully minute are the traces of proteins that may suffice to demonstrate it, that for some time it was assumed that specificity must depend on chemical or physical differences far too slight to be detected by other

[3] See Hahn and Langer, Zeit. Immunität., 1917 (26), 199; Trommsdorf, ibid., 1921 (32), 379.

[4] Jour. Infect. Dis., 1915 (17), 241.

[5] Review by Doerr and Pick, Biochem. Zeit., 914 (60), 257; also see Jour. Amer. Med. Assoc., 1924 (82), 1465; Hans Schmidt, "Die Heterogenetischen Hammelblutantikörper und ihre Antigene." Leipsig, 1924.

[6] Tsunecka, Zeit. Immunitat., 1914 (22), 567.

[7] Landsteiner and Simms, Jour. Exp. Med., 1923 (38), 127.

than immunological methods. More extensive acquaintance with the composition of the protein molecule and the application of more refined methods to its study, have suggested that *immunological differences between proteins are usually, and as far as now known always, associated with and presumably dependent upon chemical differences which can be detected by chemical or physical methods.*[7a]

It has been found that the immunological differences that exist between different species of bacteria, between extracts of different plants, or between the blood and tissues of different species of animals are dependent on differences in the composition of their proteins whenever isolated proteins are under observation. As far as investigations have been made, any two proteins that are identical immunologically are indistinguishable chemically; those that are readily distinguished by one of these methods are also readily differentiated by the other. Proteins that are shown to be related but still distinguishable by immunological tests are found to be similar to one another in chemical composition but nevertheless show recognizable chemical differences when suitable methods are used. *Biological specificity depends on chemical individuality of proteins, and biological relationship is equally associated with the presence of chemically similar proteins.*[7b]

For example, the alcohol-soluble protein of wheat, gliadin, shows no recognizable chemical difference from the gliadin of rye, and these two proteins react immunologically as if identical, despite their derivation from plants of different species. Hordein of barley is chemically similar to gliadin but nevertheless definitely of different composition; immunologically, hordein is found to be related to but distinguishable from gliadin. Similarly, another protein of wheat, glutenin, is related to but distinguishable from gliadin, despite the common source of the two proteins in the wheat grain. Again, the several proteins of serum which can be differentiated from one another immunologically can be distinguished also by chemical means, and, furthermore, the protein fractions of the serum of one species show, equally, immunological and chemical[8] differences from the corresponding serum proteins of another species.

A study of the chemistry and immunological behavior of milk proteins has furnished further evidence of the same purport (Wells and Osborne).[9] Cow's milk contains four chemically distinct proteins or protein fractions, namely, casein, lactalbumin, lactoglobulin and an alcohol-soluble protein. By means of the anaphylaxis test it can be shown that these four proteins are immunologically distinct. This fact furnishes another striking illustration of the dependence of immunologic specificity on chemical composition rather than on biologic origin. Of these four proteins only one, the globulin, sensitizes to beef serum or causes reactions in animals sensitized to beef serum. This corresponds to the observation of Crowther and

[7a] Wells, Jour. Immunol., 1924 (9), 291.
[7b] Wells, and Osborne, Journ. Infect Dis., 1913 (12), 341; 1916 (19), 183.
[8] Obermayer and Willheim, Biochem. Zeit., 1912 (38), 331; 1913 (50), 369.
[9] Jour. Infect. Dis., 1921 (29), 200.

Raistrick that milk globulin and serum globulin are chemically indistinguishable; and of Woodman that lactalbumin and serum albumin are two distinct proteins.[10] Biologically, casein is quite as distinct from the whey proteins and the serum proteins as it is chemically, whereas the biological reactions and chemical composition of caseins from different species of animals show close relationships. An immune serum for any one casein will give reactions with any other casein, even of remote species.[11]

A particularly interesting observation bearing on the dependence of immunological differentiation on chemical variations has been furnished by the egg proteins. Dale and Hartley had found, in preliminary experiments, that crystallized albumins from hen eggs and from duck eggs sensitized mutually to one another even with the guinea pig uterus method, but by using quantitative methods and by checking up with the desensitizing test, it was later possible for Dale to detect slight immunological differences between the two.[12] The importance of this observation lies especially in the studies carried out by Dakin on the chemistry of these two albumins. When hydrolyzed, their amino-acid content is found to be similar, but when the proteins are first racemized and then hydrolyzed it is found that there are distinct differences in the degree of racemization of the leucine, aspartic acid, and histidine. There being reasons for believing that the amino acids which escape racemization occupy the terminal positions in the peptid chains of which the protein molecule is built, this result indicates that there are at least structural or spatial differences in these two proteins. In other words, a close relationship by immunological tests is here associated with chemical similarity, and a slight difference in chemical structure is found which presumably accounts for a slight immunological difference that can be detected only by the most sensitive methods.

The Chemical Basis of Specificity.—Only the proteins show sufficient variety to account for the manifestations of specific immunity, since the carbohydrates, lipins, and inorganic elements of the body are few in number and of limited variety, but it is possible that the addition of these simpler radicals to the protein molecule may alter its specificity, just as Landsteiner modified the specificity of proteins by introducing simple chemical radicals into the protein molecule.

Contemplating the possible number of variations in the arrangement of the amino-acids in a protein which the great number of these radicals provides, there is no difficulty in understanding the existence of an almost limitless number of specific distinctions between proteins. Abderhalden, indeed, calculates that the 20 amino-acids we find in proteins could form at least 2,432,902,008, 176,640,000 different compounds, and this without including possible compounds varying in quantitative relations. A contribution to the chemical basis of specificity has been made by Kossel,[13]

[10] Biochem. Jour., 1921 (15), 187.
[11] Fleischer, Russky Wratsch, 1908 (7 pt. 2), 1638.
[12] Biochem. Jour., 1919 (13), 248.
[13] Zeit. physiol. Chem., 1913 (88), 163.

who finds certain relations in the proportions and groupings of the scanty number of amino-acids that make up the protamines and histones of sperm, to be characteristic of the sperm of certain species and families.

A conception of the nature of specificity has been developed by Pick, largely on the basis of his own work. He properly accepts the influence of both the physico-chemical properties and the chemical composition of the colloids concerned in immunity reactions as determining specificity. Both these factors undoubtedly come into play in determining the possibility of interaction of antigen and antibody. The electric charges of the amphoteric colloidal antigen and antibody, and perhaps also their surface configuration and the surface forces, all influence their reaction; these physico-chemical factors greatly complicate the possibility of reaction between two colloids, and to these influences are added the influence of the chemical structure in determining subsequent chemical reactions. It would seem possible that the existence of all these factors may account for specificity, it being necessary for each one of a long series of both physical and chemical adjustments to agree perfectly in order that reaction may take place—just as in a combination lock one lever after another is thrown by the proper manipulation of the dial, and only when all the long series of levers is in just the proper position does the bolt engage and the lock open.[14]

The studies of Pick and his colleagues, amplified somewhat by other investigations, have led to the following view of the chemistry of specificity: There exist two sorts of specificity in each protein molecule; one of these is easily altered by simple physical measures, *e. g.*, heat, cold, partial coagulation etc., without essentially changing the chemical composition of the protein. When so altered the antigenic properties of the protein are likewise altered, in that the antibody it engenders differs somewhat in the scope of its reactivity from the antibody engendered by the original unaltered protein; but the alteration does not affect the species characteristics of the antigen. Thus, a heated antigen may engender precipitins that will react with this heated antigen, but not with similar heated proteins from other species of animals, while the antibodies engendered by the same but unheated antigen will not react with the heated protein.

The other sort of specificity is not so easily affected, only marked chemical alterations of the antigen modifying it, and this concerns the species characteristics of the antigen. Pick believed this fundamental species specificity to be closely related to the aromatic radicals of the protein antigen, for it is affected by introducing into the protein molecules substances which are known to combine with the benzene ring, *e. g.*, iodine, diazo, and nitro groups. Proteins thus chemically altered will act as proteins foreign to animals of the species from which they are derived, and the antigens they develop are devoid of species specificity,

[14] The "*resonance theory*" of Traube assumes that the surface forces of reacting substances must harmonize, just as the vibration of one tuning fork starts vibrations in another fork only when the two are in harmony, or as electromagnetic waves incite resonance phenomena (see Zeit. f. Immunität., 1911 (9), 246 and 779).

although quite specific for proteins like themselves; *e. g.*, a nitro-protein made by treating rabbit serum protein with nitric acid, will, if injected into even the same rabbit, cause the formation of antibodies which will react with this same nitro-protein, and also with nitro-proteins derived from entirely different species or even from plants,—but it reacts only with nitro-proteins. It is also possible to cause chemical modifications analogous to the physical modifications previously mentioned, which change only the scope of specificity of the antigen without altering its specificity for species. Appreciating that the number of different aromatic radicals in the protein molecule is not sufficient to account for the innumerable manifestations of specificity, Pick interprets the significance of these aromatic radicals as that of a central complex about which are the groupings which determine species specificity. It is not merely the number and proportion of amino-acid radicals in the protein molecule which determine its specificity, but, more important because presenting greater possibilities for variations, the arrangement of these radicals in the molecule.

Landsteiner and Prasek,[15] however, state that alteration of proteins by simply treating them with acid alcohol also causes them to lose their species specificity, and this without any substitution in the aromatic radicals of the proteins. This observation throws doubt on the hypothesis of Pick that the aromatic radicals are the essential center of species specificity. They found that species specificity may be altered in many other ways than by attacking the aromatic rings of the protein. This they accomplished by esterification with acid alcohol, acetylating with acid anhydride or acid chlorides, or methylation by means of diazomethane. The common feature of these reactions is that the alterations occur in the salt-forming groups of the protein molecules, and they evidently do not depend solely on substitution of H-ions in the aromatic radicals. This artificial, structural specificity is marked, and traverses by far the biological lines; *e. g.*, antiserum for methylated protein of horse serum may react not only with methylated proteins from most diverse animal species, but also with methylated plant proteins.

Landsteiner and Lampl[15a] have also carried on an extensive study of the precipitin reactions of horse serum when combined with azo-compounds, with chlorine and bromine, and with sulfonic and arsenic acids. Their results confirm the observations and conclusions of Obermayer and Pick and of Wells and Osborne, that specificity depends upon certain groups within the protein molecule. They made the interesting observation that if one derivative of a protein reacted with another sort of derivative, the position of the two reactive groups in the molecules of the substituted radicals was identical or closely related. That is, cross reactions depend on chemical relationships, as Wells and Osborne also found by means of the anaphylaxis reactions, and the specificity is determined by relatively small portions of the large antigen molecule. The

[15] Zeit. Immunität., 1913 (20), 211.
[15a] Biochem. Zeit., 1918 (86), 343.

observation that the location in the molecule of definite groups is indicated by their immunological reactions can best be explained as depending on spatial correspondence of antigen and antibody, just as Emil Fischer assumed for the specific action of ferments in his comparison to "lock and key." Here again we get evidence that both chemical composition and spatial relations are concerned in determining specificity. Presumbably there are also innumerable isomeres that cannot be distinguishd by our present methods, which correspond to the racial and individual differences which are so obvious and yet not to be detected by serum reactions.

Reaction of Antibodies with Non-antigenic Compounds.—Landsteiner[16] also found that if he immunized with a protein compounded with a non-protein radical, such as metanilic acid (m-aminobenzol sulfonic acid), para-arsanilic acid (p-amino-phenylarsinic acid) or p-amino benzoic acid, the resulting antiserum would react with compounds of these radicals with many different proteins, no matter how unrelated the proteins are, *e. g.*, rabbit serum, gliadin from wheat and casein from cow's milk. The added groups used in these experiments are unable of themselves to incite antibody formation if injected uncombined with proteins, although they can bind the specific antibodies if they are added to the antisera engendered by proteins to which they had been united before serving as antigens.[17] For example, the specific precipitin reaction between a protein treated with metanilic acid and an immune serum made by injecting this compound protein, is inhibited by metanilic acid and closely related sulfonic acids but not by p-arsanilic acid, and p-amino-benzoic acid, or other unrelated aromatic and aliphatic organic compounds. Apparently these inhibiting radicals can react with the antibodies of the immune serum just as well whether attached to a protein molecule or not, and hence by binding the antibody render it unable to react with the protein antigen containing the radical. That is, they saturate the specific antibody even although unable to produce a precipitation or other observable reaction when not attached to a protein radical.

THE NATURE OF ANTIBODIES

The presence of antibodies may be recognized by several different methods, and according to the procedure employed we designate the antibodies as precipitins, agglutinins, antitoxins, complement fixation antibodies, opsonins, cytolysins, anaphylactins, and so on. These names assume the existence of several different and distinct reactive substances or antibodies, and this assumption has been currently accepted as if it were an established fact, although this is far from the case. Of late there has been a growing tendency to doubt that there is any such considerable variety of antibodies, for we usually find after immunizing even with a single purified protein that we can demonstrate several if not all of these properties in the serum of the immunized animal.

[16] Biochem. Zeit., 1919 (93), 106.
[17] Biochem. Zeit., 1920 (104), 280.

Are There Different Types of Antibodies?—An attractive hypothesis is that there are two fundamental types of immunity reactions.[18] One having to do with substances which are essentially active poisons, neutralizes or inhibits their toxicity by direct chemical action. In this group come the antitoxins for bacterial toxins (diphtheria, tetanus, etc.), and the antibodies for venoms, vegetable toxins (ricin, abrin, etc.), and various bacterial hematotoxins. It is to be noted that these toxic substances, the true toxins, are all similar to one another in being classed as large colloidal aggregates, resembling proteins, but not yet identified as proteins.

The other group of immunity reactions is concerned with defense against foreign proteins, whether toxic or non-toxic, and whether in solution or aggregated into cells (bacteria, corpuscles, tissue cells). In all the reactions of this group we deal with processes that tend to alter the colloidal state of the foreign proteins, by making them larger aggregates (precipitation, agglutination), or smaller aggregates (proteolysis, hemolysis, bacteriolysis, cytolysis), and in each case the process consists of two separable steps, sensitization and reaction. It is tempting to accept the view, championed especially by Friedberger, Zinsser, Dean, and Nicolle, that in this second group of reactions only one and the same antibody is concerned, and that all the reactions are essentially the same, differing merely in the method by which the reaction is demonstrated. There are, indeed, many facts capable of interpretation as supporting this hypothesis, but there are still other items that do not harmonize with it, notably the lack of constant quantitative relations between the different reactions produced by the same immune serum, and as yet it is neither completely established nor disproved.

Evidence Favoring Unity of Antibodies.—Zinsser has christened this the "unitarian" hypothesis, and presented much of the evidence in its favor. As Zinsser[19] says:

> "If the antibody comes in contact with a very finely divided antigen, as in a bacterial extract or in, let us say, horse serum, if electrolytes are present and perhaps other necessary physical factors furnished by the presence of serum, etc., precipitation occurs.
>
> "When we are dealing with whole bacteria of relatively large mass and correspondingly small surface exposure, agglutination is the result, and quantitative parallelism with the precipitin reaction is not to be expected because of the much greater dispersion of the antigen in the latter test.
>
> "When alexin is present, complement fixation or hemolysis or bactericidal effects result, since the changes produced by the sensitization have permitted union with the complement.
>
> "When there are leucocytes present the union makes possible the phagocytosis of the antigen, and when the antibody is absorbed by the cells of an animal, anaphylactic 'sensitization' occurs."

He concludes, "We do not wish by any means to convey the impression that we consider the 'unitarian' view as absolutely and rigidly proved.

[18] Zinsser, Arch. Int. Med. 1915 (16), 223; Nicolle, Jour. State Med. 1920 (28), 293; "Antigens et les Anticorps," Masson e Cie, Paris, 1920; also Nicolle and Cesari, Ann. Inst. Pasteur, 1922 (36), 463; Dean, Lancet, Jan. 13, 1917.

[19] "Infection and Resistance," 1923, p. 319.

We do believe, however, that the denial of such a view necessitates the assumption that the injection of a pure antigen calls forth five or six fundamentally different reactions on the part of the tissue cells, a theory which would be justified only on the basis of incontrovertible proof."

On the other hand, it has been urged against the unitarian hypothesis that there is often no parallelism in the activity of the different reactions with the same immune serum, and indeed, sometimes one reaction may be demonstrable while another type of reaction is entirely negative; for example, Hektoen[20] has observed opsonins in the spinal fluid of dogs when agglutinins could not be demonstrated, although the blood of the animal exhibited both opsonic and agglutinative activity. This finding might suggest that the opsonin had been selectively secreted into the spinal fluid, while the agglutinin had been held back, supporting the view that these are distinct antibodies. However, the failure to secure parallelism in titration of the several antibodies in a given serum lacks force as negative evidence in the face of the known great quantitative inaccuracy of most immunological measurements, and the ready inhibition of the reactions by factors which are often unknown, for the variables in all these tests are far too many and too uncontrollable to permit of exactitude. And furthermore, while it has frequently been seen that agglutinating and precipitating functions are often weaker than other antibody effects, or even absent entirely in such sera, it has rarely been observed that they are powerfully and specifically present when other effects are lacking. Nicolle also holds that there is but one sort of antibody which has the property, as first shown by Bordet, of rendering the antigen susceptible to coagulation by the electrolytes present in the mixture, constituting the agglutinin and precipitin reactions, as well as one phase of the opsonic and anaphylactic sensitizations. If complement is also present in the mixture we get the lytic phenomena of immunity, *i. e.*, hemolysis, bacteriolysis, cytolysis, and possibly the formation of the anaphylactic poison. When the coagulation reaction is especially vigorous the aggregation of the antigen into dense masses seriously interferes with the lytic phenomena, through reducing the surface for attack by the enzymes, which explains why a strong precipitin serum may seem to be weak in lytic activity, rather than because of the existence of separate antibodies. Nicolle would even include the toxin-antitoxin neutralization in the same class.[21]

From the foregoing facts it would seem fair to say that at present it is probable that there are not so many different sorts of specific antibodies as there are means of demonstrating them, and it is certainly possible that there are not more than two types of antibodies, *i. e.*, antitoxins and amboceptors, and there is even room for doubt that these are different, for Ramon has developed a method for testing the antitoxic strength of

[20] Jour. Infect. Dis., 1913 (12), 1.

[21] Interferometric investigations by Bachman (Zeit. Immunität., 1924 (40), 325) gave no evidence that the various immunological reactions are different from one another; all seem to depend on similar mechanisms which are not of a hydrolytic or synthetic character

therapeutic sera which is based on the assumption that precipitins and antitoxins are identical, a view which is not yet fully established.[22]

The Chemical Properties of Antibodies.—In subsequent sections the nature of the several reputed types of antibodies will receive more extended discussion, especially in respect to antitoxins which have been particulary studied because of their practical importance. As for the antibody or antibodies responsible for the other familiar immunological reactions, generally designated as amboceptors, their chemical composition is as unknown as that of the enzymes. As far as chemical properties are concerned, we merely know that they are found in the globulin fraction of the serum, but we do not know whether the amboceptors are serum globulins modified by the process of immunization, or specific globulins formed in and secreted by the cells to unite with the antigen, or specific chemical radicals either attached to or forming part of the protein molecule.[23] Landsteiner and Prasek[24] in 1911 reviewed the evidence advanced up to that time to demonstrate that antibodies are not proteins, concluding that it was not competent to overthrow the weighty evidence establishing their close relation to the proteins, and this conclusion is as justifiable today, although some new evidence against it has been produced.

For example, Huntoon[25] has obtained particularly pure preparations of the agglutinating and protective antibodies present in antipneumococcus serum. Study of such purified preparations indicated that the antibody molecules are evidently colloidal, or attached to colloids, as they do not pass through dialyzing membranes; but apparently they are not ordinary serum proteins since they resist trypsin digestion for long periods of time, do not give the usual protein color reactions, and are not precipitated by 30 per cent. NaCl nor by removal of the electrolytes from their solution; neither are they lipoidal in nature, since they do not dissolve in ether. This probably represents the nearest anyone has yet come to securing antibodies in approximately pure condition, but even here the amount of active substance present in the solutions available for study is so small that it is not possible to secure any satisfactory idea as to its chemical nature.

These results agree with somewhat similar studies made with hemolysins and hemagglutinins, concentrated by fixing the antibodies to red corpuscles, freeing the sensitized corpuscles from the serum, and then separating the antibody from the corpuscles, for the active solution of this antibody has been found to contain at the most but traces of recognizable protein (von Liebermann and Fenyvessy,[26] Kosaki).[27] In some of Huntoon's antibody solutions not only were all the conventional protein tests negative or doubtful, but although material precipitated by such reagents

[22] See Kraus, Loewenstein and Baecher, Wien. klin. Woch., 1924 (37), 561.

[23] Tadokoro states that immune sera show spectroscopic differences from normal sera (Jour. Infect. Dis., 1920 (26), 8).

[24] Zeit. Immunität., 1911 (10), 68.

[25] Huntoon, Masucci and Hannum, Jour. Amer. Chem. Soc., 1920 (42), 2654; Jour. Immunol., 1921 (6), 185.

[26] Cent. f. Bakt., 1908 (47), 274.

[27] Jour. Immunol., 1918 (3), 109.

as picric, phosphotungstic, and phosphomolybdic acid were present, the nitrogen content of the solution was as low as 0.16 mg. per cc. All these facts throw doubt on the protein nature of the antibodies, although they do not disprove it.

There is a striking similarity to be noted between antibodies, toxins, enzymes, and insulin. Each of them acts in quantities that are so infinitesimally small that it is not possible to say whether the proteins which may usually be found in the solutions are themselves the active agent or merely contaminations. Especially similar are the antibodies and the insulin, since each seems not to be the active agent itself, but merely one that accelerates or makes possible the action of other catalysts. Thus, the amboceptor serves to activate the complement; the insulin serves to make possible the burning of sugar by the tissues. Neither of itself accomplishes the final reactions.

The site of antibody formation is not definitely known. Undoubtedly the blood-forming tissues, especially the bone marrow, are active participants, for stimulation of hematogenesis also stimulates antibody formation, whereas serious injury to the bone marrow reduces antibody formation.[28] Carrell[29] found that goat red corpuscles added to an artificial culture of guinea pig bone marrow and lymph gland, stimulated the production of hemolysins in the culture. Przygode[30] also observed the formation of precipitins and agglutinins in cultures of spleen tissue, and Hektoen[28] has found that removal of the spleen often causes a marked reduction in the formation of antibodies. However, there is not a little reason to believe that antibody formation may be a widespread cellular function, perhaps participated in by many or all the different kinds of cells in the body, for Fischer[31] found that even connective tissue cells in artificial culture may become immune to foreign sera. There is also evidence that cells of the fixed tissue phagocytes[32] or reticulo-endothelial system,[33] which are universally distributed, are especially active in the production of antibodies.

[28] Hektoen, Jour. Infect. Dis., 1915 (17), 415; 1918, (22), 28; 1920 (26), 330; Simonds and Jones, Jour. Med. Res., 1915 (33), 183; Hektoen, Jour. Infect. Dis., 1916 (18), 69.

[29] Carrel and Ingebrigtsen, Jour. Exp. Med., 1912 (15), 287.

[30] Wien. klin. Woch., 1913 (26), 841; 1914 (27), 201.

[31] Jour. Exp. Med., 1922 (35), 661.

[32] Kyes, Jour. Infect. Dis., 1916 (18), 277; Motohashi, Jour. Med. Res., 1922 (43), 473, lit.

[33] Billings, Zeit. Immunität., 1923 (38), 193; Maximow, Jour. Infect. Dis., 1924 (34), 549.

CHAPTER VIII

CHEMISTRY OF THE IMMUNITY REACTIONS (Continued II). TOXINS AND ANTITOXINS, AGGLUTININS, PRECIPITINS

TOXINS AND ANTITOXINS[1]

In the discussion of the bacteria and their products (Chapter IV), the nature of the *true toxins* was defined, and attention was called to the fact that one of their most important characteristics is, that immunization of animals against them leads to the accumulation in the blood of substances capable of neutralizing their poisonous action. Such true toxins are produced especially by the diphtheria bacillus and the tetanus bacillus; also, but less strikingly, by *B. pyocyaneus*, *B. botulinus*, pathogenic gas bacilli, dysentery bacilli, and possibly by a few others. In addition to these, numerous bacteria produce *hemolytic poisons* which seem to have properties similar to the toxins; and there are also toxins produced by plants (abrin, ricin, crotin, and mushroom poisons) and by animals (snake venom, scorpion and spider toxin, and eel serum). Against all of these, true antitoxins may be obtained by the immunization of animals.

Ehrlich's Conception of Toxins and Antitoxins.—According to Ehrlich's theory, the action of toxins upon cells is purely chemical. A toxin unites with a cell because some chemical group in the molecule of toxin has a chemical affinity for some particular group in the cell protoplasm. For convenience in description, names have been given to these groups; the group of the toxin that combines with the cell has been called the *haptophorous* group, or haptophore, while the group in the protoplasm that combines with the toxin is known as the *receptor*.[2] Animals that are naturally immune to toxins may owe their immunity to the fact that their vital tissues contain no substances with a chemical affinity for the toxin, and hence the toxin cannot unite with them to cause harm. (In Ehrlich's terminology, the cells contain no receptors for the toxin.) The toxin may not combine with any tissue element at all in such immune animals, and may circulate for some time harmlessly in the blood, or it may combine with some organ where it does little harm, *e. g.*, tetanus

[1] Review by Schlossberger, Handbuch. d. Biochem., 1924 (3), 116.

[2] Ehrlich has used certain diagrams to illustrate these various groups and their relations to the cells and to one another, which are generally used in explaining his theory. From a teaching standpoint they seem to be undesirable, in that the student soon comes to ascribe physical properties and appearances to what should be considered as chemical combinations. The toxophore group becomes "the black fringed end of the toxin," etc. To one accustomed to thinking in chemical terms there is no difficulty in following the literature and understanding the reactions as chemical reactions, which they are.

toxin is said to combine chiefly in the liver of some animals, and therefore it does not harm their nervous system.

According to this theory, *the antitoxin consists of cell receptors that have been produced in excess and secreted by the cells into the blood.* In the blood they combine with any toxin that may have been introduced, and by saturating its affinities render it incapable of uniting with the cells. As the toxin harms cells only after it has been chemically united to them, it is rendered harmless when its affinities for the cell are saturated by cell receptors in the blood stream. The process of immunization consists in injuring the body cells to such a degree that they are stimulated to regenerate the receptor groups with which the toxin combines; these receptor groups are produced in excess, and not only replace those combined by the toxins, but the excessive groups escape free into the blood. Hence the serum of an immunized animal is antitoxic *because it contains free cell receptors* that can unite with the toxin. An important point is that the receptors liberated by all animals which have been immunized with a given toxin seem to be the same—horse serum, sheep serum, or goat serum will neutralize diphtheria toxin if the animals have been made immune to this toxin; and, furthermore, their serum when introduced into the body of an entirely different animal, *e. g.*, a guinea-pig, will neutralize diphtheria toxin within its body. Equally important is the fact that the antitoxin for one toxin will not neutralize any other toxin; *e. g.*, diphtheria antitoxin will not neutralize tetanus toxin, or conversely. This means that diphtheria toxin is attached to chemical groups of the body cells (receptors) which are quite different from the groups to which tetanus toxin unites, and hence different receptors are thrown out in immunizing against each.

It having been observed that a toxin solution rapidly loses its toxicity on storage, without a corresponding loss in its capacity to neutralize antitoxin, Ehrlich concluded that the poisonous element of the toxin must be different from the constituent which unites with the antitoxin, from which arose the conception that the toxin consists of two elements, a toxic or "toxophore" group, and a binding or "haptophore" group. When, through the action of time or certain chemical or physical agents, the toxophore group is thus impaired or removed, the remaining nontoxic group still possesses the power of binding antitoxin or of immunizing animals against the toxin, and Ehrlich called this a "toxoid." Other observations on the behavior of toxin-antitoxin mixtures made it necessary to assume that there are different degrees of affinity for antitoxin in the toxoids present in the toxin solution. Toxoids with a weaker affinity for antitoxin than toxins, were designated as "epitoxoids" and it was also assumed that there exist other substances, "toxons," which are formed as such by the diphtheria bacillus, having the same sort of haptophore group as the toxins, but a different toxophore which gives rise, not to acute symptoms but to slow emaciation and paralysis. Also it was found necessary to assume that there must be other toxoids which have a higher affinity for antitoxin than the toxin itself, and these were chris-

tened "protoxoids." Also there seemed to be toxoids with the same affinity for antitoxin as the toxins, and they were given the name "syntoxoids." In order to visualize the action of such a complex mixture as the toxic filtrate of diphtheria cultures had shown itself to be, Ehrlich described a "spectrum" of reactions occurring between the filtrate and antitoxin.

The toxic filtrate from diphtheria bacillus cultures was supposed to contain the following ingredients reacting with the antitoxin:

A. Toxins, and toxons, differing in the acuteness of their toxic effects, both produced as such by the diphtheria bacillus and both uniting with the antitoxin.

B. Toxoids, derived by disintegration of the toxins and perhaps also the toxons, and of varying degrees of affinity for antitoxin, *e. g.*, protoxoids, syntoxoids.

C. Other possible modifications, for apparently each of these subdivisions of the toxins consists of equal parts of two components which have similar affinities for antitoxin but different capacities to resist deterioration.

These highly complex conditions were assumed by Ehrlich on the basis of the conception that toxin-antitoxin reactions are entirely analogous to those occurring between strong acids and strong bases, in which the reactions are complete in one direction and reversible only to an inappreciable degree—*e. g.*, $H_2SO_4 + 2NaOH = Na_2SO_4 + H_2O$.

Further study of the phenomena of toxin-antitoxin neutralization developed more and more discordant facts, each calling for a new explanation, until the theory as originally advanced by Ehrlich became too complicated to be reasonable. Arrhenius[3] studied the physical chemistry of immunity reactions and came to the conclusion that toxin-antitoxin neutralization was not similar to the nearly irreversible reaction between a strong acid and a strong base (*e. g.*, $H_2SO_4 + 2NaOH = Na_2SO_4 + H_2O$), but more like that between weak acids and bases (*e. g.*, boric acid and ammonia), in which an equilibrium is reached while there are still present appreciable amounts of both the free acid and base, according to the law of mass action. Although many interesting analogies were found by Arrhenius to support this hypothesis, it has not been accepted, for simple dilution of neutral toxin-antitoxin mixtures does not produce the amount of dissociation that this theory calls for.

If toxin is added to antitoxin in several fractions, with some interval of time between each addition, the final mixture is much more toxic than if the same quantities of toxin and antitoxin were put together at one time. This phenomenon is commonly referred to as the *Danysz effect*, and indicates that the toxin-antitoxin union is physical rather than chemical, and a typical colloidal behavior, for it seems to be quite analogous to such a phenomenon as the taking up of more dye by several pieces of blotting paper added in series to a dye solution, than is taken up by the same amount of paper added in one piece. Of similar significance

[3] "Immuno-chemistry," Macmillan, 1907.

is the *zone phenomenon, i. e.*, when antibodies and antigens are brought together an excess of one component may reduce the amount of reaction between the two. This is a phenomenon characteristic of reactions between colloids.

Bordet,[4] who from the first has urged that immunological reactions are fundamentally physico-chemical, explains the spectrum phenomena of Ehrlich on the basis of adsorption. That is, he does not imagine that when one combining unit of antitoxin is added to ten units of toxin, one of the latter unites quantitatively with the antitoxin unit, leaving nine units of completely unaltered toxin still present in the solution as in a simple chemical reaction. Rather, the one unit of antitoxin is distributed upon all the toxin molecules present, modifying somewhat but not completely neutralizing their toxicity.[5] Thus, Ehrlich's "toxons" become merely incompletely neutralized toxin, although the existence of spontaneously detoxicated toxin molecules in the form of toxoids is not denied. For the theory of an exact union of toxin with antitoxin in fixed definite proportions like a simple chemical reaction, Bordet substitutes the hypothesis of union in variable proportions according to concentration and other varying factors obtaining in the solutions in which the reaction takes place. Such a conception is much more closely in harmony with reactions occurring in colloidal systems, which toxins and antitoxins are. Bordet compared such reactions to the phenomenon familiar in dyeing, for the dye colors all parts of the substance, faintly or deeply according to the proportion of dye and absorbent.

When tested in the laboratory, Bordet's "adsorption theory" receives much support. For example, bacteria, corpuscles, or tissue cells in a given suspension with immune serum present, can be shown to be all sensitized to varying degrees according to the amount of available antibody present. The fact that antigen-antibody unions do not undergo any considerable dissociation on dilution, and that after such unions have stood for a few hours dissociation is usually too little to be detected and the union becomes increasingly firmer with longer standing, is not in harmony with the idea of Arrhenius and Madsen that such unions are dissociable compounds analogous to the salts of weak acids and bases. In this respect their behavior is closely analogous to that of colloidal unions, *e. g.*, (1) dyeing with colloidal dyes, which attach themselves progressively more closely to the substrate with a correspondingly decreased tendency to be washed out; (2) the increasing insolubility of proteins precipitated by and standing in alcohol.

On the other hand, the chief shortcoming of the colloidal adsorption theory of immunological reactions is its failure to explain adequately the specificity of the reactions, which seem to depend chiefly on definite chemical differences in the antigens. To account for both the harmonies and short-comings of the purely colloidal explanation of the toxin-anti-

[4] For full review see "Studies in Immunity" by Jules Bordet and F. P. Gay, Wiley and Sons, 1909,

[5] Ann. Inst. Pasteur, 1903 (17), 161.

toxin reaction advanced by Bordet, and supported by Biltz and others, various investigators have suggested its amplification by assuming a primary colloidal adsorption and a secondary specific chemical reaction.

The Chemistry of Toxin-antitoxin Reactions.—The neutralization of toxin by antitoxin was believed by Ehrlich and many other investigators to be a chemical process, which occurs as well in the test-tube as in the body. It seems to occur *according to the laws of definite proportion*, a given amount of antitoxin neutralizing a proportionate amount of toxin under equal conditions (hence the toxin is not destroyed by antitoxin through a ferment action). Neither the toxin nor the antitoxin is destroyed in the process of neutralization, as has been proved by suitable experiments. Pick and Schwarz believe that the union of toxin and antitoxin takes place in two steps—first, colloidal adsorption, and then the specific reaction.[6] There is some question as to whether the union with antitoxin completes the neutralization of the toxin, or whether there is then necessary a further destruction of the toxin in the body. But whether necessary or not, such further destruction does take place. Neutralization occurs more rapidly under the influence of warmth, and more slowly in the cold; and it is more rapid in concentrated than in dilute solutions, just as with ordinary chemical reactions. It is said that it requires two hours for tetanus toxin to be completely combined with the corresponding quantity of antitoxin at 37°.

According to Arrhenius and Madsen, reaction of antitoxin upon toxin is accompanied by the liberation of much heat—6,600 calories per gram molecule, or about half as much as is set free by the action of a strong acid upon a strong base.[7] Union of toxin and antitoxin causes no change in the surface tension of the fluid in which the reaction occurs (Zunz),[8] and the neutral toxin-antitoxin compound (diphtheria) is not adsorbed by animal charcoal, which adsorbs each of the constituents when free. The physico-chemical studies of the reaction between tetanolysin and its antibody, gave results which led Arrhenius to conclude that in the reaction there are formed from one molecule of toxin and one molecule of antitoxin, two molecules of the reaction products (analogous to the reaction between alcohol and acid which yields one molecule of ester and one of water). In general, the union of toxin and antitoxin is dissociated by acids,[9] even after months, showing that neutralization does not cause any marked alterations in either component.

There is apparently no close resemblance between the method of action of antitoxins and enzymes. The antitoxin acts quantitatively,

[6] Also von Krogh (Zeit. f. Hyg., 1911 (68), 251). Bordet, Biltz, and others look upon the neutralization of toxin as an adsorption process entirely.

[7] Literature of chemical and physical reactions of toxin and antitoxin given by Zangger, Cent. f. Bakt. (ref.) 1905 (36), 238; Arrhenius, "Immuno-chemistry," 1907 and "Quantitative Laws in Biological Chemistry," London 1915; also review in Zeit. Chemother., Ref., 1914 (3), 157.

[8] Bull. Acad. Royal Med. Belg., 1911; also Bertolini, Biochem. Zeit., 1910 (28), 60.

[9] Morgenroth and Ascher, Cent. f. Bakt., 1911 (59), 510; de Potter, Bull. Acad. Roy. Med. Belg., 1923 (3), 394.

and produces no detectable alteration in the toxin, or in any other substance, as far as we know. It also has but one functioning group (haptophore), the one with which it combines with the toxin; whereas both toxins and enzymes seem to have two functioning groups, one which unites with the cell or substance that is to be attacked, the other which produces the chemical changes. But there is evidence that union with antitoxin or fixed receptors prepares the toxin for its disintegration, which, presumably, is then accomplished by enzymatic action as with other antigens.

More modern studies of enzyme action, however, indicate that the divergence of enzyme action from the laws of mass action may be more apparent than real, whereas toxin-antitoxin reactions do not follow the law of mass action except under certain conditions, and hence there may be less difference between the behavior of enzymes and of antitoxins than was formerly believed.

Chemical Nature of Antitoxins[10]

This is as entirely unknown as is the nature of the toxins. Investigation of antitoxic serum (principally diphtheria antitoxin) has shown that the antitoxic properties are closely related to the serum globulin, which, however, by no means proves that antitoxin is serum globulin or any other sort of protein. According to Ehrlich's theory, antitoxin consists of free cell receptors, and these receptors are presumably simple chemical groups which may be but a part of a larger molecule, or they may be entire protein molecules. In any event they behave as colloids; moving toward the cathode in an electrical field,[11] diffusing little or not at all, their reaction curve resembling more an absorption curve than the reaction curves of crystalloids, and being influenced by all conditions that influence colloids. Whether the receptor groups are secreted in a free condition in antitoxin formation, or combined with a large molecule, is unknown.

By saturating serum with magnesium sulfate, or half saturation with ammonium sulfate, three chief groups of proteins can be precipitated and isolated.[12] These are *fibrinogen*, *euglobulin* (true globulin), and *pseudo-globulin* (soluble in water). Pick[13] found that the precipitate obtained by 36 per cent. volume saturation with ammonium sulfate contained no antitoxin; the antitoxin came down in the precipitate obtained on raising the strength from above 38 per cent. to 46 per cent. When the globulins are precipitated by electrodialysis the antitoxins are carried with them.[14] According to Pick, in horse serum the antitoxin is

[10] Review and bibliography given by Crawford and Foster, Amer. Jour. Pharmacy, 1918 (90), 765.

[11] According to Field and Teague (Jour. Exper. Med., 1907 (9), 86) both toxin and antitoxin move towards the cathode, which fact is only apparently opposed to the theory that this reaction is simply one of differently charged colloids, as Bechhold, points out (Münch. med. Woch., 1907 (54), 1921.)

[12] See résumé by Gibson, Jour. Biol. Chem., 1905 (1) 161; Gibson and Banzhaf Jour. Exper. Med. 1910 (12), 411.

[13] Hofmeister's Beitr., 1901 (1), 351.

[14] Adolf, Klin. Woch., 1924 (3), 1214.

associated with the pseudo-globulin,[15] and Gibson and Banzhaf[12] found that the blood of horses immunized to either diphtheria or tetanus toxin shows a marked increase (40 to 114 per cent.) in serum globulin, varying somewhat according to the antitoxin content, the more soluble globulins being most increased. At the same time the serum albumin and euglobulin content decreases in proportion, while the fibrinogen shows no characteristic alterations.[16] Meyer[17] and his colleagues, however, find in their study of the blood proteins during immunization, that the proportion of globulins increases according to the severity of the intoxication, and not in any definite relation to the degree of immunity or antitoxin production.[18] The average antitoxic horse serum contains 12 per cent. albumin, 78 per cent. of soluble globulin containing antitoxin, and 10 per cent. euglobulin, whereas in normal horses the proportion is 40 per cent. albumin, 42 per cent. pseudoglobulin and 18 per cent. euglobulin. By heating 12 hours at 57° a considerable part of the soluble globulin becomes insoluble, without a corresponding loss of antitoxin (Banzhaf), and apparently the antitoxic globulins have somewhat smaller or more stable molecules than most of the rest of the serum globulins. Reitstötter[19] found evidence that the globulins of antitoxic sera carry a greater negative charge than the globulins of normal sera.

The relation of antitoxins to proteins has also been investigated by permitting digestive enzymes to act on antitoxic serum. Pick digested the antitoxin-containing globulin of horse serum for several days with trypsin; after five days, when part of the protein was still not digested, the antitoxin was but little impaired in strength; after nine days, when most of the protein was digested, the antitoxin had lost two-thirds of its strength. This indicates a considerable resistance of antitoxin to trypsin, but also shows that it is affected in much the same way as the globulin (which is itself very resistant to trypsin) and therefore is presumably of similar nature. Antitoxin seemed to be much more rapidly destroyed by pepsin-HCl digestion than by trypsin, in which respect it again resembles the serum globulin.[20]

In favor of the view that antitoxin is a definite protein body is also the fact that it is not carried down in indifferent precipitates, as are the enzymes, but comes down always in a certain fraction of the protein precipitates. Another important point has been brought out by Arrhe-

[15] Miss Homer found tetanus and diphtheria antitoxin associated with the pseudo-globulins, but the antibodies in antidysentery and antimeningococcus serum were chiefly in the euglobulin fraction (Jour. Physiol., 1918 (52), xxxiii.)

[16] During immunization the *antitryptic* power of the horse serum increases with the antitoxin increase (Krause and Klug, Berl. klin. Woch., 1908 (45), 1454.)

[17] Jour. Exp. Med., 1916 (24), 515; 1917 (25), 231; Jour. Infect. Dis., 1918 (22), 1.

[18] See also Bächer and Kosian, Biochem. Zeit., 1924 (145), 324.

[19] Kolloid Zeitschr., 1923 (32), 47.

[20] Berg and Kelser (Jour. Agric. Res., 1918 (13), 471; Jour. Amer. Med. Assoc., 1921 (76), 1820), found that trypsin and pepsin destroy the antitoxin and serum proteins at about the same rate, and their failure to observe "significant chemical changes" in the proteins of serum acted upon by weak acid or alkali that slowly inactivates antitoxin, does not seem to warrant their deduction that antitoxin is non-protein. See also Crawford and Andrus, Amer. Jour. Pharm., 1917 (89), 158.

nius and Madsen,[21] who determined approximately the molecular weight of toxin and antitoxin by means of their rate of diffusion, and found that the toxin (diphtheria toxin and tetanolysin) diffused ten or more times as rapidly as the corresponding antitoxin. Gelatin filters also hold back antitoxin and let toxin pass through, and toxins diffuse into cells which seem to be impermeable for the antitoxin. This indicates that the antitoxin molecules are much larger than the toxin molecules, agreeing with the idea that antitoxin is of protein nature and that toxin either is not protein or is smaller than most protein molecules. Taken all together, the evidence indicates a closer resemblance of antitoxins to proteins than has been shown for the toxins, and all attempts to separate antitoxins from proteins have so far failed.[22]

Antitoxins are retained to greater or less extent by porcelain but not by Berkefeld filters,[23] do not pass through dialyzing membranes readily, and are in general easily destroyed by chemical and physical agencies, although much less so than are most toxins. Antitoxins are adsorbed by animal charcoal,[24] and neutral mixtures of toxin and antitoxin when acted upon by precipitins for horse serum are dissociated, the antitoxic protein being attached to the precipitin and the toxin set free. Freezing may also dissociate neutral toxin-antitoxin mixtures, especially if antiseptics are present.[25] Heating to 60°–70° injures, and boiling quickly destroys them, although like the enzymes and the proteins, they resist dry heat to 140°, and also extremely low temperature, without change. Putrefaction of the serum destroys the antitoxins (Brieger).[26] They can be preserved for a very long time when dried completely, but in the serum they gradually disappear, especially if exposed to light and air. Acids and alkalies destroy antitoxins, acids being the more harmful in low concentrations. Like the enzymes, antitoxins are destroyed by ultraviolet rays. They are destroyed in the alimentary tract, without appreciable absorption, except in the case of new-born animals sucking mothers whose blood and milk contain antitoxin.[27] When subcutaneously injected, antitoxin soon disappears from the blood; part may be bound to the tissues, part may be destroyed, since only traces appear in the urine.

AGGLUTININS AND AGGLUTINATION[28]

The relation of agglutination of bacteria by the serum of immunized animals to their immunity is not known, for it is not established that

[21] Festskrift Statens Serum Institut, 1902.

[22] Salkowski (Biochem. Zeit., 1922 (132), 84) reported the preparation of antitoxin practically free from protein, but this experiment has not been repeated.

[23] Berg, Jour. Infect. Dis., 1921 (29), 86.

[24] Eisler, Biochem. Zeit., 1923 (135), 416.

[25] Anderson and Leonard, Jour. Amer. Med. Assoc., 1924 (82), 1679.

[26] Behring states that tetanus antitoxin resists putrefaction.

[27] Römer and Much, Jahrb. f. Kinderheilk., 1906 (63), 684; McClintock and King (Jour. Infect. Dis., 1906 (3), 701) found appreciable absorption of antitoxin when digestion was impaired by drugs. Full review of literature on transmission of antibodies from mother to offspring given by Famulener, Jour. Infect. Dis., 1912 (10), 332; Heurlin, Arch. Mens. Obs. et Gyn., 1912 (1), 497.

[28] Bibliography given by Schiff, Handbuch der Biochemie, 1924 (3), 262.

agglutination helps in the defensive reaction.[29] Agglutinated bacteria seem not to be severely injured by the process, and can grow vigorously in agglutinative serum. Possibly agglutination favors phagocytosis and lessens dissemination of the infecting organisms, but it is not generally considered that the influence on the course of infection is great.[30] Agglutination, therefore, may be looked upon as an incident in the infection, rather than as a definite method of resistance, and it is not essentially a means of defense against bacteria, for agglutinins are equally well produced by immunizing with foreign cells or any foreign protein masses of suitable size which contain soluble antigens.

Normal serum may contain agglutinin;[31] *e. g.*, typhoid bacilli are sometimes agglutinated by normal serum, even when it is diluted thirty times, but by immunization this property can be greatly increased until agglutination may be obtained with dilutions as high as one to a million. Whether normal agglutinins are essentially different from immune agglutinins is not known.[32] Many protein solutions, especially extracts of plant tissues and leguminous seeds, cause marked non-specific hemagglutination.[33] Likewise, bacterial extracts may agglutinate red corpuscles.[34]

For the production of agglutination it is necessary that the cell contain an antigen (*agglutinogen*) which has an affinity for the specific constituent of the serum, *agglutinin*. In immunization the agglutinogen, which is probably an intracellular protein, acts as a stimulator to the formation of the specific agglutinin. Hence, when we inject either extracts of cells or entire cells, we secure agglutinins, for the agglutinogens are liberated from the cells upon their disintegration. In erythrocytes the agglutinogen seems to be in the stroma.[35]

Agglutinins are found in the blood stream in the highest concentrations, but they are also present in the various organs, and to greater or less extent in the other body fluids, excepting usually the spinal fluid (Greer and Becht).[36] The place of their formation is unknown, but they

[29] Bull, however, ascribes much importance to agglutination of bacteria for their removal from the circulation (Jour. Exp. Med., 1915 (22), 484), but it is possible that the removal of bacteria does not depend upon the agglutinative action of the immune serum but upon other effects, such as opsonization (See Bull and McKee, Amer. Jour. Hyg., 1922 (2), 208). Fujimoto (Jour. Immunol., 1919 (4), 67) attributes to agglutinins the power to impair the glucolytic action of *B. coli*, but there is no evidence in his experiments that it is agglutinin rather than some other serum component that is responsible. On the other hand Zironi, (Atti accad. Lincei, 1917 (26), 19) found that agglutination does not modify reproductive or biochemical activities of bacteria.

[30] Blaizot (C. R. Soc. Biol., 1918 (81), 356) states that it is possible to modify typhoid bacilli by treating them with nitric acid or hydroquinone, or by heat, so that they will produce immunity without producing agglutinins.

[31] Even cold blooded animals may have normal agglutinins for bacteria and mammalian corpuscles (see Takenouchi, Jour. Inf. Dis., 1918 (23), 393, 415).

[32] See Andrejew, Arb. kaiserl. Gesundhtsamt., 1910 (33), 84; Heuer, Zeit. f. Hyg., 1922 (95), 100.

[33] Mendel, Arch. Fisiol., 1909 (7), 168.

[34] Fukuhara, Zeit. Immunität., 1909 (2), 313.

[35] Chyosa, Arch. f. Hyg., 1910 (72), 191.

[36] Jour. Infect. Dis., 1910 (7), 127.

can be formed by spleen tissue grown in artificial cultures.[37] Since bacteria contained within a collodion sac implanted in an animal give rise to the production of agglutinins, it is evident that the agglutinogens are diffusible, at least to some extent, through collodion. Old cultures of bacteria contain free agglutinogens, probably liberated from disintegrated cells, and filtrates of such cultures will neutralize agglutinins, showing both that the agglutinogens are filterable, and that the reaction of agglutination is a chemical one and not dependent upon the presence of cells. Agglutinogens are said to pass through dialyzing membranes, while agglutinins do not, so it is evident that the agglutinogen is of smaller molecular dimensions than the agglutinin, just as toxin molecules are smaller than antitoxin molecules. Agglutinogens are not destroyed by formalin, heat, or ultraviolet rays in concentrations sufficient to kill the bacteria containing them.[38] Even heating bacteria to 100° does not reduce their capacity to unite with the specific agglutinins (Lange).[39]

Alterations in the agglutinability of bacteria are marked, *e. g.*, strains of typhoid bacilli freshly cultivated from human infections may be practically inagglutinable even by active serum, but after prolonged cultivation on media they may or may not develop agglutinability. This phenomenon has not yet been satisfactorily explained, but it may depend on an active immunity of the bacteria against the agglutinins. Such bacteria injected into rabbits produce antisera that will agglutinate ordinary agglutinable strains, but not themselves; hence they do not lack agglutinogens. They give normal complement fixation reactions, and hence do not lack receptors, and they agglutinate with acids and chemicals much the same as ordinary agglutinable strains.[40] Moreover, identical strains of bacteria grown on media of different composition may show considerable variations in agglutinability (Dawson).[41]

Just what constituent of the bacteria acts as the stimulus to the production of the agglutinin is unknown.[42] Apparently, there are at least two bacterial substances capable of reacting with antisera, one of which seems not to be a protein, since it is soluble in alcohol, gives no biuret reaction, and resists temperatures up to 165°. The other gives all protein reactions, and is destroyed by heating to 62°. Therefore it has been thought that there are two agglutinogens in the bacterial cell, one, thermostable, the other, thermolabile. The difference in the function of these two "agglutinogens" is still a matter of dispute. Likewise, the question as to whether they occur in the membrane or within the bacterial cell is still open, but Craw found that the insoluble residue of crushed typhoid bacilli, after being washed free of all soluble constituents, was but slightly agglutinated by active serum; therefore, the agglutinogens are probably soluble intracellular substances. Stuber holds that bacterial

[37] Pryzgode, Wien. klin. Woch., 1913 (26), 841.
[38] Stassano and Lematte, Compt. Rend. Acad. Sci., 1911 (152), 623.
[39] Zeit. f. Immunität., 1921 (32), 449.
[40] McIntosh and McQueen, Jour. Hyg., 1914 (12), 409.
[41] Jour. Bact., 1919 (4), 133.
[42] See review by Paltauf, Kolle and Wassermann's Handbuch, 1913 (2), 565.

agglutinogens are lipins;[43] but, as stated elsewhere, it is more probable that these, like other antigens, are closely related to proteins, although they may be combined with lipoids which modify their antigenic activity. Confusion has arisen through the mistaken assumption that substances which react specifically with an immune serum are also antigenic, which is not always true. For example, pneumococci have been found[44] to contain a carbohydrate which, although not antigenic, is responsible for the type-specific character of the reactions with antipneumococcus serum, the antigenic activity of pneumococci being determined by a protein which is specific for the species but not for the type.

Properties of Agglutinins.—Like most of the other immune substances, agglutinins are precipitated out of the serum in the globulin fraction, but Schierge[45] found that sometimes the albumin fraction contains a little agglutinin, probably through adsorption. The isoelectric point of agglutinins (between 2×10^{-6} and 4×10^{-6}) corresponds to that of the serum globulins,[46] but they are not always precipitated out quantitatively with the globulins[47] and their behavior suggests that they are merely adsorbed by the globulins. All attempts to separate them from proteins have been unsuccessful. Stark[48] found that trypsin does not attack the agglutinins readily, corresponding to the resistance of the serum globulins to this enzyme; alkaline papayotin solution destroys them slowly, while pepsin acts more rapidly. Alkalies are destructive even when quite dilute, while acids are much less harmful. The temperature resistance of agglutinins seems to be variable, plague agglutinin being destroyed at 56°, while purified typhoid agglutinin may resist 80°–90°; most agglutinin serums lose their activity at 60°–65°. The rate of reaction of agglutinins increases with the temperature, as long as this is not high enough to injure the reacting substances.[49] They are not precipitated by specific precipitins, but are readily adsorbed by charcoal and other suspensions, especially the hydrosols of the metals[50] but they can be extracted from such adsorbents by the specific bacteria. The observations of Bond[51] suggest that they may become physically bound to other colloids within the body. Ottenberg[52] found that agglutinins, when separated from bacteria to which they have been adsorbed, are precipitated by various metallic salts, from which they can be recovered without loss. This behavior resembles that of the more soluble proteins or of the larger polypeptid molecules.

The structure of the agglutinins (in the Ehrlich theory) is similar to that of the toxin; *i. e.*, there is a haptophore group by which they combine with the agglutinogen, and a toxophore group by which they produce the changes that cause agglutination. The agglutinogen is probably related to the antitoxins in structure having a single haptophore to unite with the agglutinin. By degeneration of the toxophorous group of the agglutinin, *agglutinoids* may be formed. It is believed that agglutinins are cell receptors, which have a group with a chemical affinity for the agglutinogen of the bacterial protoplasm, and also another group which brings about the agglutination. They are, therefore, more complex than the simple receptors that unite with toxins, and are called *receptors of the second order*. According to Ohno[53] the reaction of agglutinin and antigen is in constant proportions, and seems to be a chemical rather than a physical reaction. Coplans[54] finds this reaction associated with an increase in conductivity in the solutions, but whether this depends upon the

[43] Biochem. Zeit., 1916 (77), 388; also Bauer, Biochem. Zeit., 1917 (83), 120.
[44] Dochez and Avery, Jour. Exp. Med., 1917 (26), 447; Heidelberger and Avery, ibid., 1923 (38), 73.
[45] Zeit. f. Immunität., 1920 (29), 527.
[46] Szent-Györgi, Biochem. Zeit., 1921 (113), 36.
[47] Schierge, Zeit. f. Immunität., 1920 (29), 527; Went, ibid., 1923 (35), 503.
[48] Inaug. Dissert., Würzburg, 1905.
[49] Madsen, *et al.*, Jour. Exper. Med., 1906 (8), 337.
[50] Bleyer, Zeit. f. Immunität., 1922 (33), 478.
[51] Brit. Med. Jour., June. 14, 1919.
[52] Proc. Soc. Exp. Biol. Med., 1923 (21), 14.
[53] Philippine Jour. Sci., 1908 (3), 47.
[54] Jour. Path. and Bact., 1912 (17), 130.

agglutinin reaction itself, or upon associated processes, is questionable. The behavior of the agglutinin in reacting with the electronegative bacteria suggests that it possesses basic properties and carries a positive charge (Hirsch),[55] for during the reaction the medium increases in alkalinity as in the dissociation of a basic electrolyte.

Agglutinated bacteria can be again separated from one another by the action of organic and inorganic acids, alkalies, acid salts, and by heating to 70° or 75°, and after once being separated they cannot be reagglutinated by fresh serum.[56]

The Mechanism of Agglutination.—Bacteria and other cells are held in suspension by the Brownian movement which resists the tendency to gravitation, and by the mutually repellant forces of the like electrical charges of the suspended particles, which overcome the surface tension or cohesive force which tends to cause them to agglutinate. Evidently, then, agglutination will occur when the repellant forces are reduced or the cohesive force is raised, and the problem of bacterial agglutination becomes one of physical-chemistry. In the early days of immunology it was believed that the clumping was brought about by loss of motility, until it was found that non-motile bacilli were equally affected. Similarly, the hypothesis of adhesion of the flagellæ was disposed of. Gruber[57] and others supposed that a sticky substance, "*glabrificin*," was absorbed from the serum by the bacilli, which caused them to adhere on contact with one another; but this does not explain the flocking together of non-motile bacilli. Paltauf considered that the specific precipitin (see next section) produced by immunization carried the bacilli down in the precipitate formed, and there is reason to believe that this reaction is of importance. In support of this hypothesis is the observation of Scheller[58] that mixtures of typhoid bacilli and agglutinating serum lose their agglutinability by vigorous shaking, which may be interpreted as the result of disintegration of the agglutinating precipitate. Shaking of either bacteria or serum alone is without effect. Neisser and Friedmann[59] found that if the bacterial cells were saturated with lead acetate, washed in water until all soluble lead was removed, and then treated with H_2S, they were promptly agglutinated and precipitated, supporting other observations that indicate that precipitation within the bacterial cells can lead to agglutination. This sort of agglutination is related to the process of formation of coarse flocculi in solutions, and probably depends upon alterations in surface tension.

Bordet[60] made the important observation that agglutination does not occur if both the bacterial suspension and the agglutinating serum are dialyzed free from salts before mixing; but if, to such mixtures, a small amount of NaCl is added, agglutination and precipitation of the bacteria occur at once. This observation brought the phenomenon of bacterial agglutination into close relation with the precipitation of colloids by electrolytes, Bordet comparing it to the precipitation of particles of inorganic matter suspended in the fresh water of rivers that

[55] E. F. Hirsch, Jour. Infect. Dis., 1922 (30), 651.
[56] Eisenberg and Volk, Zeit. f. Infektionskr., 1902 (40), 192.
[57] For complete bibliography, see Craw, Jour. of Hygiene, 1905 (5), 113.
[58] Cent. f. Bakt., 1910 (54), 150.
[59] Münch. med. Woch., 1904 (51), 465 and 827.
[60] Ann. d. l'Inst. Pasteur, 1899 (13), 225.

occurs when the fresh water meets the salt water of the ocean. He found that the agglutinin combined with the bacteria in the absence of the salts, and the resulting compound was precipitated by the addition of minute amounts of electrolytes,[61] which alone did not precipitate or agglutinate the bacteria or the serum. This indicates that the agglutinins cause a change in the bacteria which brings them under the same physical laws as the inorganic colloidal suspensions, which are characterized by being precipitated by the addition of traces of electrolytes.[62]

In this precipitation by electrolytes the cation is responsible,[63] for the bacteria are electro-negative; and the process follows the valency rule, the more the valency of the cation increases the less becomes the lowest concentration at which agglutination takes place,[64] as in the precipitation of inorganic colloidal suspensions by salts.

The agglutination is undoubtedly due to changes in surface electrical forces and surface tension, and corresponds to the effect of bringing together in a solution two colloids of opposite charges in too small an amount to produce flocculation, for the further addition of even a small amount of electrolytes may then cause flocculation. There is much reason to believe that when agglutinin acts upon cells, the antibody forms a surface film on the particles,[65] for such sensitized antigens have been found to assume the same isoelectric point as that of the euglobulin of the serum, which is the protein with which the antibodies are associated.[66] Before the agglutinin combines with the bacteria they behave like the colloidal solutions of organic colloids, being precipitated only by the salts of heavy metals, alcohol, formalin, etc., or by great concentrations of neutral salts. Field and Teague[67] have found that agglutinins carry positive charges while bacteria are negative, and that by the electric current agglutinins can be separated from bacteria with which they have combined; this shows that the agglutinin is not destroyed in the reaction. Teague and Buxton[68] considered that neutralization of the electric charge of the bacteria is not, however, the only important factor in agglutination, a view now generally accepted. Recent work, nevertheless, emphasizes the importance of the electrical charges in serum agglutination. Thus, Shibley[69] says that immune agglutinating sera exhibit a specific charge-reducing effect, proportional to the agglutinin titer, disappearing when the agglutinin is removed by adsorption by the specific bacteria, and the agglutination

[61] Corroborated for sensitized red corpuscles by Eisner and Friedemann, Zeit. Immunität., 1914 (21), 520.

[62] Arrhenius (Zeit. physikal. Chem., 1903 (46) 415) has attempted to show that the gas laws are applicable to the partition of agglutinin between the bacteria and the medium, which he compares to the partition of iodin between water and carbon disulfid. This idea is not accepted by Craw (*loc. cit.*)[57], nor by Dreyer and Douglas, Proc. Royal Soc., 1910 (82), 185.

[63] Neisser and Friedemann, Münch. med. Woch., 1904 (51), 465 and 827.

[64] Shionyoa, Lancet, 1922, Oct. 28, p. 905.

[65] Dean, Lancet, Jan. 13, 1917.

[66] Coulter, Jour. Gen. Physiol., 1922 (4), 403.

[67] Jour. Exper. Med., 1907 (9), 86.

[68] Zeit. physikal. Chem., 1907 (57), 76.

[69] Jour. Exp. Med., 1924 (40), 453.

occurs only when the charge on the bacteria has been reduced to a certain potential.

According to Bechhold[70] normal bacteria behave like inorganic suspensions that have each particle protected by an albumin-like membrane, which prevents them from being thrown out of suspension by solutions of alkali salts, etc. After being acted on by agglutinin they are so altered that they behave like the unprotected inorganic suspensions, and are precipitated by salts and other electrolytes. Buxton and Shaffer[71] also found that bacteria which have been acted upon by agglutinin behave as if their proteins had been so changed that they are more capable of adsorbing or combining with salts than when in their normal condition. Strong salt solutions inhibit agglutination by preventing the binding of the agglutinin.[72] Tulloch[73] observed that in the presence of salts of mono- and di-valent cations, unsensitized bacteria do not readily precipitate or agglutinate, but sensitized bacteria, as Bordet showed, agglutinate with small quantities of salts. In this respect unsensitized bacteria behave like "non-rigid colloids," such as fresh egg white, while sensitized bacteria resemble "rigid colloids," such as denatured egg white. Hence he advanced the hypothesis that the process of sensitization is akin to that of denaturation of proteins, the specificity perhaps depending on different degrees of denaturation. Mansfield[74] would bring agglutination into line with other serological reactions as a protein digestion process, by his hypothesis that bacteria are held in suspension by protective colloids which are digested by an enzyme, the agglutinin. He finds in favor of this hypothesis that the temperature and reaction curves correspond to enzyme actions, that agglutinating serum contains an enzyme digesting protein extracted from bacteria; during agglutination the agglutinogen is destroyed, and this by removing the source of the mutually repellant charges of the particles, permits them to come close enough together to cohere.[75] The increased conductivity that takes place when agglutinins react on antigens (Coplans)[54] may also be considered as evidence of a digestive process. The presence of serum proteins in excess interferes with agglutination, apparently because of a protective action of the serum albumin (Heuer).[76]

Agglutination obeys the same laws as other similar physical phenomena; the rate of agglutination depends upon the concentration of the suspension and of the electrolytes, and varies with the valence of the cations. In all respects the behavior of bacteria and agglutinin resembles the behavior of suspensions of colloidal mixtures which form an electrically amphoteric colloidal suspension, so that the ions of electrolytes or the electric currents, by discharging them unequally, cause precipitation

[70] Zeit. f. physikal. Chem., 1904 (48), 385.
[71] Zeit. physikal. Chem., 1907 (57), 47.
[72] Landsteiner and St. Welecki, Zeit. Immunität., 1910 (8), 397.
[73] Biochem. Jour., 1914 (8), 293.
[74] Zeit. Immunität., 1918 (27), 197.
[75] Szent-Györgi, Zeit. f. Immunität., 1920 (30), 144.
[76] Zeit. f. Hyg., 1922 (95), 100.

(Neisser and Friedemann).[77] The agglutinin reaction also exhibits well the zone phenomenon which is typical of colloid reactions, *i. e.*, in the presence of an excess of antigen no reaction is observed. Physico-chemical researches, however, have yet failed to explain the specific character of the agglutinins for specific bacteria. Specific reactions will occur between agglutinins and antigens suspended in solutions exhibiting a wide range of H-ion concentration, which would seem to indicate that electrical charges alone cannot be responsible for the specificity of the reactions.[78]

Acid Agglutination.—Michaelis[79] has developed an interesting analogy in the specific agglutination of bacteria by acids. This is based on the fact that the optimum concentration of H-ions which precipitates proteins from solution is characteristic and constant for each protein, and the same is true to some extent for the agglutination of bacteria by acids. Michaelis thought that the agglutination by acids is even more sharply specific in some cases than the agglutination by immune sera; *e. g.* typhoid and paratyphoid bacilli are readily distinguished because the former are agglutinated by a concentration of H-ions from 4 to 8 $\times$ 10^{-5}, while paratyphoids require 16 to 32 $\times$ 10^{-5}, and colon bacilli are not readily agglutinated by acids, but more recent observers have not found acid agglutination to be specific or constant.[80] According to Arkwright,[81] typhoid bacilli contain two extractable proteins that are agglutinated by acids, one at 3.6 $\times$ 10^{-5} and the other at 1.1 $\times$ 10^{-3}; the former seems to be related to, if not identical with, the substance that is precipitated by immune serum. Apparently acid agglutination of bacteria belongs to the same class of reactions as the coagulation by H-ions of amphoteric colloids of preponderatingly acid character. Bacteria which have been sensitized by serum are more sensitive to acid agglutination than are normal bacteria.[82] Because of their electro-negative character bacteria are agglutinated by basic dyes, but without specificity (Brossa).[83]

The Effect of Agglutinins on Electrical Potential and Cohesive Force.—It has been determined for certain bacteria by Northrop and deKruif[84] that whenever the electrokinetic potential difference between the surface of the bacteria and the suspension fluid, which is the force that repels the bacteria, falls below about 15 millivolts, the bacteria will agglutinate even if the cohesive force remains unaltered. If the cohesive force is also reduced sufficiently, of course no agglutination will take place. With bacteria sensitized with immune serum, the agglutination that is produced by addition of electrolytes was found to depend on lowering of the potential by the salts, the cohesive force remaining constant. With large concentrations of salts, however, the cohesive force is so much reduced that no agglutination occurs although the potential is reduced below a measurable amount.

An explanation for the origin of the surface potential of cells is furnished by the Donnan equilibrium, *i. e.*, when a membrane separates two solu-

[77] Münch. med. Woch., 1904 (51), 465 and 827; see also Girard-Mangin and Henri, Compt. Rend. Soc. Biol., 1904, vol. 56; and Zangger, Cent. Bakt. (ref.), 1905 (36), 225.

[78] Michaelis and Davidsohn, Biochem. Zeit., 1912 (47), 59; DeKruif and Northrop, Jour. Gen. Physiol., 1922 (5), 127.

[79] Folia Serologica, 1911 (7), 1010; also Beniasch, Zeit. Immunität., 1912 (12), 268.

[80] Review and bibliography given by Gouwens, Jour. Infect. Dis., 1923 (33), 113.

[81] Zeit. Immunität., 1914 (22), 396; Jour. Hyg., 1914 (14), 261.

[82] Krumwiede and Pratt, Zeit. Immunität., 1913 (16), 517.

[83] Zeit. f. Immunität., 1923 (37), 221.

[84] Jour. Gen. Physiol., 1922 (4), 639, 655.

tions, the membrane being permeable to all the ionic constituents of one solution and to only a part of the ionic and molecular constituents of the other solution, a definite potential difference appears between the solutions when they are at equilibrium depending on the concentrations and the charge of the various constituents (see p. 26). J. Loeb[85] has shown that the quantitative results obtainable with proteins and electrolytes correspond in general to the prediction of the Donnan equilibrium, and undoubtedly the same is true when the proteins are in the form of bacteria and other cells.[86]

Northrop and deKruif found that these principles are applicable to the study of agglutination of bacteria and cells. For example, when immune serum freed from salts by dialysis is added to bacteria in distilled water, their electrokinetic potential is gradually decreased, but not enough to cause agglutination. If salt is now added, as in the Bordet experiment, until the potential is decreased to the critical point (15 millivolts for the bacteria studied), agglutination occurs, indicating that the action of agglutinins is to prevent the reduction of the cohesive force by salts. Hence, when the potential of sensitized bacteria is decreased by salts, the cohesive force remains constant and brings about agglutination as soon as the potential has been reduced beyond the critical point. According to these observations it would seem that the agglutinin unites with the specific antigenic cells to form a surface film which protects them from the action of the electrolytes in lowering the cohesive force, whereas the effect of electrolytes in lowering the potential is unopposed and the mutual repulsion becomes insufficient to keep the cells apart against the action of the cohesive force.

Conglutination.—Under this term Bordet and Gay described the observation that in normal ox serum there is a substance which combines with corpuscles (or bacteria) that have been acted upon by agglutinating sera, and augments the agglutination.[87] Dean finds that, in general, agglutination requires two agents, one being the specific antibody, and the other a precipitable substance, probably a globulin. When cells have combined with the antibody the precipitable substance is aggregated on their surfaces, and, presumably, determines the agglutination. There is reason to believe that this conglutination may depend on the action of traces of fibrinogen present in the serum.[88]

Co-agglutination described by Bordet and Gengou as the agglutination by an antigen and the homologous antibody, of the corpuscles of another animal, is probably closely related to these phenomena (Dean).

PRECIPITINS[89]

If to a solution containing proteins we add in proper proportions the serum of an animal immunized against the same protein, a precipitate will

[85] Jacques Loeb, "Proteins and the Theory of Colloidal Behavior," New York and London, 1922.

[86] See Winslow, Falk and Caulfield, Journal Gen. Physiol., 1923 (6), 177.

[87] Literature given by Dean, Proc. Royal Soc. (B), 1911 (84), 416; Hall, Univ. Calif. Publ., Pathol., 1913 (2), 111.

[88] Maltaner and Johnston, Jour. of Immunol., 1921 (6), 349.

[89] For complete bibliography of the subject of "Precipitins" see the résumé by Michaelis, Oppenheimer's Handb. d. Biochemie, 1909, II (1), 552; Kraus, Kolle and Wassermann's Handb., 1913, II; Uhlenhuth and Steffenhagen *ibid.*, III, 257; Zinsser. "Infection and Resistance."

soon form. In general, what has been said in Chapter VII relative to antigens, antibodies, and specificity applies to the precipitin reaction as to the rest of the immunological reactions. Because of its ready application, however, the precipitin reaction has been of particular importance in medicolegal work and in the study of many problems of biology.

Essentially the precipitin and agglutinin reactions seem to be the same, agglutination being manifested when the particles of antigen are visible and precipitation when the antigen is in solution.[90]

Presumably the precipitin reaction is a means of throwing such foreign proteins out of solution and rendering them harmless. According to Zinsser[91] and others, the function of the precipitin is to sensitize the unformed foreign proteins to the digestive complement, a view in harmony with the prevailing tendency to correlate the immunity reaction with defense through enzymatic hydrolysis. Opie[92] also finds that foreign proteins are retained at the site of injection in immunized animals and thus prevented from entering the circulation.

Precipitin appears in the blood generally about six days after injection of the protein, but disappears after injection of each subsequent dose of protein, to reappear again after a somewhat shorter lapse of time. After injections are stopped, the precipitin disappears rather rapidly, but never appears in the urine, although it may enter the fetal blood from the blood of pregnant female animals. The presence of precipitins in the blood does not seem to prevent the excretion of the foreign protein in the urine, nor are the animals less susceptible to the toxic action of the foreign protein; indeed, the reaction is even stronger in the immunized animals, and sometimes the ordinary dose becomes fatal. Precipitin and antigen have been found to coexist ununited in the circulating blood under certain conditions,[93] but according to Opie[94] this is only the case when the antigen is a complex mixture such as serum; if a pure protein serves as antigen it does not coexist in the blood with the antibody. Certain antibodies are carried down with the precipitates formed when the serum containing them reacts under proper conditions with an antiserum; *e. g.*, diphtheria antitoxin is precipitated when added to the serum of a rabbit immunized to horse serum. This is not true of all antibodies, however.[95] As the precipitates formed in the precipitin reaction, when injected into a guinea-pig make it passively hypersensitive to the protein used as antigen in the precipitin reaction, it would seem that the precipitin and the anaphylactin are identical (Weil),[96] or at least closely associated.

[90] However, Landsteiner and van der Scheer (Jour. Exp. Med., 1924 (40), 91) have found evidence that there may be chemical differences between precipitinogens and agglutinogens.

[91] Jour. Exper. Med., 1912 (15), 529; 1913 (18), 219.

[92] Jour. Exp. Med., 1924 (39), 659.

[93] Bayne-Jones, Jour. Exp. Med., 1917 (25), 837; Foster, Jour. Infect. Dis., 1923 (32), 105, review.

[94] Jour. of Immunol., 1923 (8), 19 and 55.

[95] See Gay and Stone, Jour. Immunol., 1916 (1), 83.

[96] Jour. Immunol., 1916 (1), 1. See also Fujiwara, Mitt. med. Fak. Univ. Kyushu, 1921 (5), 325; Luginbühl, Zeit. Immunität., 1922 (34), 246.

According to the source of the protein used we recognize *bacterial precipitins*, *phyto-precipitins* (for plant proteins),[97] and *zoöprecipitins* (for animal proteins). Although tissue extracts, body fluids, and exudates are generally used in immunizing, purified constitutents of these protein mixtures will also excite precipitin formation, *e. g.*, we may immunize with caseinogen as well as with milk. At present it is not established that precipitins can be secured against lipoids or other non-protein substances. Possibly precipitins can be produced for closely related substances with molecules approximating in size the protein molecule, *e. g.*, certain substances present in supposedly protein-free filtrates of bacterial cultures.

The precipitin reaction exhibits particularly well the zone phenomenon *i. e.*, excess of antigen prevents the formation of precipitate, or redissolves it, but excess of antiserum has less or no effect. Since both reacting substances are colloids they follow the laws governing other mutually precipitating colloids, and precipitation occurs only when they are brought together in concentrations that lie within definite zones of relative proportions. It is, of course, perfectly possible to have a union of precipitin and antigen without any visible precipitate occurring, since the product of the reaction is not necessarily insoluble under all conditions; in this case the occurrence of a reaction must be demonstrated by some other method, *e. g.*, the complement fixation reaction.

As with the agglutinin reaction, electrolytes must be present or precipitation will not occur. Neither the precipitin nor the antigen seems to be altered appreciably by the reaction, since when either is separated from the precipitate it retains its original properties. Precipitin reactions will occur even when the solutions have a range of *p*H as wide as 4.5 to 9.5, and the precipitate is no heavier at the isoelectric point of either the antigen or of the antibody than at other points (Mason).[98] Outside this range a precipitate already formed will be dissolved. Nevertheless, according to Hirsch[99] the reaction of a precipitin serum on the specific antigen is accompanied by an increase in the alkalinity of the solution.

The amount of precipitation obtained is much modified by the amount of inorganic salts present, and, according to Friedemann,[1] there is a general resemblance between the precipitin reactions and the precipitations occurring when colloids precipitate one another; *i. e.*, when an amphoteric colloid reacts with either an acid or a basic colloid.[2] So far, however, attempts to interpret the precipitin reaction, as Arrhenius has tried to do, solely on the basis of the laws of physical chemistry, have not met with much success (Michaelis). We prefer the attitude of Krogh,[3] who

[97] Literature on precipitins for vegetable proteins given by Wells and Osborne, Jour. Infect. Dis., 1911 (8), 66.

[98] Mason, Bull. Johns Hopkins Hosp., 1922 (33), 116.

[99] E. F. Hirsch, Jour. Infect. Dis., 1922 (30), 651.

[1] Arch. f. Hyg., 1906 (55), 361.

[2] See Friedemann and Friedenthal, Zeit. exp. Path. u. Ther., 1906 (3) 73; Iscovesco, Compt. Rend. Soc. Biol., 1906, Vol. 61, and subsequent volumes.

[3] Jour. Infect. Dis., 1916 (19), 452.

states that the colloidal chemical part of immunological reactions is to be looked upon as only a preliminary step to the real chemical process that completes the reaction and gives it the specific characters.

Chemical Properties of Precipitin and Precipitate.—In its *chemical nature* precipitin resembles the "antibodies" generally, being precipitated in the euglobulin fraction of the serum,[4] and slowly destroyed by trypsin, rapidly by pepsin. It cannot be separated from the serum proteins. The precipitation by precipitins is not an enzyme action, for the precipitins are used up in the process. It apparently does not differ from precipitations of colloids by other colloids of opposite electrical charges, except in that the reaction is specific.

As with all the other substances of this class, the precipitins have, in the Ehrlich terminology, a haptophore group by which they unite to the protein molecule, and another group by which they produce the change resulting in precipitation. When the latter group is destroyed by heating to 72°, the precipitin is converted into a *precipitoid*, which possesses the property of preventing the precipitation of unheated precipitin by the specific antigen.[5]

The precipitin is the passive reagent that is thrown down by a trace of the immunizing material (precipitinogen). The resulting precipitate is the insoluble modification of the previously dissolved precipitin, and originates chiefly or entirely in the proteins of the immune serum,[6] according to the work of Welsh and Chapman, especially. But as the precipitate is able to sensitize anaphylactically, both actively and passively, it would seem that it must contain both the antibody (which confers passive sensitization) and antigen, to cause active sensitization (Weil).[7] According to Doerr[8] the precipitate accounts for all the material lost from solution in the antigen-antibody reaction, which is indirect evidence that no cleavage of protein molecules occurs in this reaction.

The precipitate may, when of maximum amount, contain more nitrogen than corresponds to the entire euglobulin of the immune serum, and the euglobulin contains all the precipitin, so it seems probable that the precipitate consists of more than the precipitin alone; however the precipitate is always less in amount than the total globulin of the antiserum.[9] It is always greater when the reaction is between homologous antiserum and antigen, than with even closely related but heterologous antigens,[10] so that the quantitative measurement of the amount of precipitate is of value in applying this reaction to determine the nature of protein solutions. The dilution of the reacting solutions is of influence, however,

[4] Funck (Cent. f. Bakt., (Ref.), 1905 (36), 744) states that if the precipitin serum is very strong, part of the precipitin comes down in the pseudoglobulin.

[5] Bacterial precipitinogens are relatively resistant to moderate heating, and heated extracts of bacteria are used for precipitin tests under the name *thermoprecipitins*. See review by A. Ascoli, Virchow's Arch., 1913 (213), 182.

[6] Moll, Zeit. exp. Path. u. Ther., 1906 (3), 325; Welsh and Chapman, Proc. Royal Soc., B., 1908 (80), 161; Zeit. Immunität., 1911 (9), 517.

[7] Jour. Immunol., 1916 (1), 35.

[8] Kolloid-Zeit., 1920 (27), 277; Doerr and Berger, Biochem. Zeit., 1921 (123), 144.

[9] Franceschelli, Arch. f. Hyg., 1907 (69), 207.

[10] Welsh and Chapman, Jour. Hygiene, 1910 (10), 177.

for if in too dilute solutions weak precipitins may fail to give reactions; with strong precipitins the influence of dilution is much less (Michaelis).

The precipitate itself is very resistant to disintegrative agencies, including putrefaction (Friedberger),[11] and proteolytic enzymes,[12] but is soluble in dilute acids and alkalies. In physiological salt solution its solubility is too small to be demonstrable, but with very strong salt solution it may redissolve.[13] It has the power of binding complement (Gay)[14] and if the complement causes solution of the precipitate, poisonous substances ("anaphylatoxins," *q. v.*) may be formed (Friedberger).

[11] Cent. f. Bakt., 1907 (43), 490.
[12] See Lange, Zeit. f. Immunität., 1921 (32), 449.
[13] See Ottensooser, Zeit. Immunität., 1924 (40), 469.
[14] See Univ. of Calif. Pub. Path., 1911 (2), 1.

CHAPTER IX

CHEMISTRY OF THE IMMUNITY REACTIONS (Continued III). AMBOCEPTOR-COMPLEMENT REACTIONS (BACTERIOLYSIS, OPSONINS, CYTOTOXINS, COMPLEMENT FIXATION, WASSERMANN AND FLOCCULATION REACTIONS, ABDERHALDEN REACTION)

SERUM BACTERIOLYSIS

The bactericidal property of serum may be shown by its destruction of the life manifestations of bacteria without marked alteration in their structure, or it may be accompanied by dissolution of the bacterial cell (*bacteriolysis*). How much of the bacteriolytic process is performed by the serum itself, or how much by the autolytic enzymes of the bacterial cell, is unknown, but the latter is probably also a factor. The bactericidal property of immune serum has been shown to be quite independent of the antitoxic properties and also to have quite a different mechanism. This last is shown in the following manner:

If we heat bactericidal serum made by immunizing an animal against bacteria, say the cholera vibrio, at 55° for fifteen minutes, it will be found to have lost its power of destroying these organisms.[1] Normal serum of non-immunized animals is equally without effect upon the vibrios. If, however, we add to the inactivated heated serum an equal quantity of inactive normal serum, the mixture will be found to be as actively bactericidal as the original unheated immune serum. This phenomenon is interpreted to mean that, by immunization, some new substance has been developed which, although by itself incapable of destroying bacteria, is able, when united with some substance present in normal serum, to destroy bacteria readily. The substance present in normal serum is also incapable of affecting bacteria by itself, but needs the presence of the substance developed by immunizing to render it bactericidal. Hence *the bactericidal property in this case depends on two substances acting together;* one, developed during immunization and therefore called the *immune body*, is specific for the variety of bacteria used in immunization, and is not destroyed by heating at 55°. The other, present in normal serum, is not increased during immunization, is not (altogether) specific in character, and is destroyed by heating at 55°; as its action is comple-

[1] Normal *human* serum often exhibits some power to destroy bacteria, even after heating to 55°. The nature of this thermostable bactericidal agent is unknown. (See Selter, Zeit. Hyg., 1918 (86), 313.)

mentary to that of the specific immune body, it is called the *complement.*[2] The name *alexin* (protective substance) is also used. (See further discussion of complement-amboceptor in hemolysis, Chap. XI.)

It was believed by Ehrlich that the action of these substances is as follows: The immune body is, like antitoxin, a cell receptor which unites the bacteria to the cell. It differs from the antitoxin, however, in that it has two affinities, one for the complement and the other for the bacterial substance. It is, therefore, an *intermediary body,* uniting the complement to the bacterial protoplasm. On account of the existence of the two affinities it was called by Ehrlich an *amboceptor.* Some serums contain such amboceptors for certain bacteria without previous immunization, hence the term *immune amboceptor* is reserved for amboceptors developed by immunization.

Bordet found it unnecessary to accept Ehrlich's view of the amboceptor character of the specific immune body, which assumes the presence of one definite group uniting it to the complement and another to the antigen. He prefers to characterize the action of the immune body as that of a *sensitizer,* which, when combined with the antigen, creates a complex which has an avidity for the complement. Experiments to be cited later have shown that only after the antigen has been acted upon by the immune body can the complement be bound by the latter, for complement and immune body may coexist in the serum without union in the absence of the antigen. Bordet's views now seem to be much more closely related to the facts than the original Ehrlich hypothesis.

Properties of Complement or Alexin.—The complement[3] is the substance that actually destroys the bacteria, in which respect, as well as in its susceptibility to heat, it resembles the enzymes. Complement is present in normal serums, and, as it is not increased in amount during immunization it may not be sufficient to satisfy all the amboceptors, hence it may be impossible to secure marked bactericidal effects even when sufficient amboceptors have been formed. If the complement in an immune serum has been destroyed by heating, it may be replaced by adding normal serum from another animal, even of some other species; indicating either that the complement is not absolutely specific in its nature, or that quite the same complement may be present in the blood of many different animals. Furthermore, the same complement will function with antibodies specific for all possible sorts of antigens.

The origin of the complement is unknown, but it has been urged that the leucocytes are an important source of this substance, if not its chief one;[4] there is evidence, however, that various organs and cells may also produce complement.[5] Its most important characteristics are its extreme

[2] The polynuclear leucocytes also contain bacteriolytic agents, "endolysins," of a similar complex structure, but quite distinct from the serum bacteriolysins (See Kling, Zeit. Immunität., 1910 (7), 1).

[3] Review and bibliography by Noguchi, Biochem. Zeit., 1907 (6), 327.

[4] Cholera antiserum will produce the Pfeiffer phenomenon of lysis of cholera vibrios in animals made leucocyte-free with thorium. (Lippmann, Zeit. Immunität., 1915 (24), 107.)

[5] See Dick, Jour. Infect. Dis., 1913 (12), 111; Lippmann and Plesch, Zeit. Immunität., 1913 (17), 548; Morrison, Jour. Immunol., 1922 (7), 435.

susceptibility to heat, and the resemblance of its action to the action of enzymes.[6] Hektoen[7] found that it could be made to unite with Mg, Ca, Ba, Sr, and SO_4 ions, which rendered the complement (for typhoid bacilli and red corpuscles) inactive. Manwaring[8] found that these ions could be separated again from the complement by simple chemical precipitation. Acids stronger than CO_2 and of the higher saturated or unsaturated fatty acid series, inactivate complement in strengths greater than $n/_{40}$, and alkalies are equally inhibitive.[9] Ultraviolet rays destroy complement.[10] Sherwood[11] has made a study of various substances that may be present in the blood in excessive amounts during pathological conditions, such as CO_2, lactic acid, acetone, etc., and finds that they interfere seriously with the action of complement, which suggests that they may favor infection or interfere with recovery from infection.

Presumably the complement is a protein, for it has antigenic properties, so that immunization with sera containing either complement or complementoid causes anticomplement activity in the blood of the immune animal. Also, it is destroyed by trypsin free from lipase,[12] and, like other colloids, is readily adsorbed by surfaces; like enzymes, complement is destroyed by shaking,[13] and gradually disappears on standing. There are some striking resemblances between the behavior of complement and of certain compounds of protein with soaps and lipoids, as pointed out especially by Noguchi, but that these are identical with true complement is doubtful (see Hemolysis), and there is much reason to believe that complement is not a definite substance but merely the expression of a certain colloidal state or structure.[14]

Its colloid nature is attested by the large loss when complement is filtered through Berkefeld filters.[15] Apparently the complement molecule is larger than that of the immune bodies, for in the early stages of filtration through a Berkefeld filter, complement is held back while the immune bodies pass through. It seems probable that the smallest possible unit which exhibits complement function must consist of multiple protein molecules, which explains the pronounced colloid character and ready inactivation of complement. In support of these views is the production of an artificial complement by Liebermann,[16] consisting of a combination of sodium oleate, proteins (including especially globulins), and calcium salts, forming an emulsion in a colloidal condition. This artificial complement is inactivated by heat like natural complement,

[6] See Walker, Jour. of Physiol., 1906 (33), p. xxi.
[7] Trans. Chicago Path. Soc., 1903 (5), 203.
[8] Jour. Infect. Dis., 1904 (1), 112.
[9] Noguchi, Biochem. Zeit., 1907 (6), 172.
[10] Courmont *et al.*, C. R. Soc. Biol., 1913 (74), 1152
[11] Jour. Infect. Dis., 1917 (20), 185.
[12] Michaelis and Skwirsky, Zeit. Immunität., 1910 (7), 497.
[13] Noguchi and Bronfenbrenner, Jour. Exp. Med., 1910 (13), 229; Ritz, Zeit. Immunität., 1912 (15), 145.
[14] See Klopstock, Deut. med. Woch., 1924 (50), 1790.
[15] See Schmidt, Arch. f. Hyg., 1912 (76), 284; Jour. Hyg., 1914 (14), 437.
[16] Deut. med. Woch., 1921 (47), 1283.

and can be used as complement in the Wassermann reaction. In certain immune reactions, colloids (lecithin, silicic acid)[17] can play the rôle of complement and immune body, but these reactions are probably quite different from those of bacteriolysis by immune serum.

A careful review of the evidence has led Liefmann[18] to the conclusion that the reaction of complement to sensitized corpuscles is more like that of ferment to substrate than of antigen to antibody. In its effect of dissolving bacteria (and also other cells against which animals may have been immunized) *complement resembles the enzymes*, and by many it is looked upon as related to them, but the changes it produces do not resemble those produced by proteolytic enzymes in all details.[19] In particular, complement seems to participate in reactions according to the law of definite proportions, unlike the enzymes;[20] but this distinction may be more apparent than real, for often secondary processes in digestion mixtures lead to inactivation of enzymes so that their reactions come nearer to corresponding to the laws of mass action than the theories of enzyme action predict.[21] However, attempts to find evidences of proteolysis in cytolysis by complement-amboceptor reactions have failed.[22]

Structure of Complement.—According to the Ehrlich theory, complement, like toxins and enzymes, possesses at least two groups: one, the haptophore, by which it unites with the amboceptor; the other, the toxophore (or *zymophore*, because of its enzyme-like action), which attacks the bacterial protoplasm. It may degenerate and lose its toxophore group while retaining the power to combine by means of its haptophore group, thus forming a *complementoid*. Complement and amboceptor exist side by side in the serum, not uniting with one another until the amboceptor has become attached to the bacterial protoplasm.

It is generally stated that if serum containing complement be so treated as to separate the globulins from the albumin, it is found that the complement has been divided into two parts, one present in each of the protein fractions. The globulin fraction of the complement will unite to amboceptor which is fixed to cells, and hence is called the *mid-piece* of the complement, for it will unite also with the *end-piece* of the complement contained in the albumin fraction, and then cytolysis can take place. Without the intervention of the globulin mid-piece the albumin end-piece cannot unite with the amboceptor, while in the absence of end-piece the amboceptor mid-piece complex can cause no cytolysis. Both fractions of the complement are destroyed by heat, but if the mid-piece is bound to the amboceptor it resists heating. The mid-piece corresponds to Ehrlich's haptophore, the end-piece to the toxophore group, and this complex structure is common to both bacteriolytic and hemolytic complement. Bronfenbrenner and Noguchi,[23] however, contend that the supposed cleavage of complement is merely an inactivation by the agencies employed, all the complement being in the albumin fraction in a condition capable of reactivation, not only by globulin but by simple amphoteric substances, a view which has not been generally accepted.[24]

[17] Landsteiner and Jagic, Wien. klin. Woch., 1904 (17), 63; Münch. med. Woch., 1904 (51), 1185.

[18] Zeit. Immunität., 1913 (16), 503.

[19] The curve of complement action resembles that of enzyme action. (Thiele and Embleton, Jour. Path. and Bact., 1915 (19), 372.)

[20] See Liebermann, Deut. med. Woch., 1906 (32), 249.

[21] See Northrop, Jour. Gen. Physiol., 1920 (2), 471.

[22] Jobling and Petersen, Jour. Exp. Med., 1914 (20), 321; Wollman and Graves, Compt. Rend. Acad. Sci., 1923 (117), 1162.

[23] Jour. Exp. Med., 1912 (5), 598; good review of literature.

[24] See Leschley, Zeit. Immunität., 1916 (25), 44; Zinsser and Cary, Jour. Exp. Med., 1914 (19), 345; Schmidt, Zeit. Immunität., 1921 (31), 125.

Properties of Amboceptors or Sensitizers.—There is good reason to believe that these are identical with precipitins, agglutinins and other antibodies, which are discussed elsewhere, and which are either globulins or associated with this fraction of the serum proteins. According to Pfeiffer and Proskauer,[25] digestion of the globulin precipitate, in which amboceptors are carried down, does not destroy their activity completely even when all the proteins are thus removed, as far as ordinary protein tests indicate. Immune serum kept three months in alcohol yielded an extract with distilled water that was rich in immune bodies, but almost free from protein. From these experiments it has been thought by some that the bacteriolytic amboceptor is not itself a protein, although closely associated with the serum globulins. Furthermore, it was a bactericidal amboceptor, the one present in antipneumococcus serum, which Huntoon isolated in a state so free from proteins that no chemical evidence of protein could be found in solutions that were nevertheless strongly bactericidal (see page 177 for details).

The stability of the amboceptors is very considerable: serum prepared in 1895 by Pfeiffer against cholera vibrios was found to have lost almost none of its activity after eight years in an ice-box (Friedberger). Heating twenty hours at 60° scarcely injures them, but 70° for one hour destroys them almost completely, and heating the serum to 100° destroys all the immune bodies. They are quite resistant to putrefaction, and, like the antitoxins, do not dialyze. Strong salt solutions will prevent the union of complement and amboceptor *in vitro*, and probably to greater or less degree in the animal body, but the union of antigen and amboceptor is not prevented by salt.[26] Alkalies may prevent the union of amboceptor with the cells, or extract it from the cell to which it has united; and they may also inhibit the union of amboceptor and complement. Amboceptors are not inactivated by shaking, as is complement, but they are destroyed alike by ultraviolet rays, and both resist *x*-rays.[27] According to Sachs[28] the cataphoresis of bacteria is not altered by their union with the specific amboceptor.

OPSONINS[29]

The correlation of phagocytic and serum immunity was accomplished when A. E. Wright showed that, before any considerable phagocytosis of bacteria can take place, the bacteria must first be acted upon by serum, which in some way prepares them to be ingested by the leucocytes. The hypothetical substances accomplishing this sensitization of the bacteria were called opsonins by Wright and they exist to a certain extent in normal serum, being increased by immunization. Not only bacteria, but cellular elements in general, including especially red corpuscles, and even unorganized particles (such as melanin),[30] are sensitized for phagocytosis by opsonins. Probably phagocytosis by endothelial[31] and other cells also requires sensitization of the bacteria by opsonins. Although there have been many expressions of the opinion that the opsonins are not distinct antibodies, but are identical with agglutinins, bacteriolytic amboceptors, or other antibodies, there is evidence to the contrary.[32] However,

[25] Cent. f. Bakt., 1896 (19), 191.
[26] Angerer, Zeit. Immunität., 1909 (4), 243.
[27] Scaffidi, Biochem. Zeit., 1915 (69), 162.
[28] Zeit. f. Immunität., 1924 (40), 57.
[29] Bibliography given by Neufeld, Kolle and Wassermann's Handbuch, 1913 (2), 440.
[30] Shattock and Dudgeon, Proc. Royal Soc. (B), 1908 (80), 165.
[31] Briscoe, Jour. Path. and Bact., 1907 (12), 66. See also Manwaring and Coe, who found that the Kupffer cells can take up only opsonized pneumococci (Proc. Soc. Exp. Biol., 1916 (13), 171).
[32] See Hektoen, Jour. Infec. Dis., 1909 (6), 78; 1913 (12), 1.

the union of opsonin and bacteria seems to follow the same quantitative laws as other antigen-antibody reactions (Amato).[33]

There are two opsonizing elements in serum, one thermostable and one thermolabile, it being the former which is increased during immunization; the thermostable element unites firmly with the object which is to be opsonized, while the thermolabile element seems to remain free in the fluid (Hektoen),[34] and is perhaps identical with complement.[35]

It would seem that opsonization and phagocytosis constitute but one of a series of similar processes by which foreign proteins are removed from the blood and tissues; *i. e.*, by lysis by extracellular enzymes when this is possible, as it is with simple protein aggregates (albuminolysis) and with some of the more labile cells (hemolysis, bacteriolysis); but in the case of more resistant structures, notably Gram-positive cocci and acid-fast bacilli, extracellular lysis being unsuccessful, these protein structures are taken within the cells where a greater concentration of enzymes may destroy them. *Fundamentally serum bacteriolysis and phagocytosis seem to be the same—in each case specific antibody sensitization prepares the bacterium for lysis by enzymes, either inside or outside the cells that furnish the lytic enzyme.*

As yet nothing is known concerning the change brought about in the bacteria by the opsonin, although it has been established that it is the bacteria that are modified and not the leucocytes. The chemical nature of the opsonins is likewise unknown, except that they may combine with certain inorganic ions and are then inert (Hektoen and Ruediger),[36] since addition of $CaCl_2$, $BaCl_2$, $SrCl_2$, $MgCl_2$, K_2SO_4, $NaHCO_3$, sodium oxalate and potassium ferrocyanide, inhibit the opsonic effect of serum. On the contrary, calcium salts stimulate the phagocytic effect of leucocytes, salts of barium and strontium being inactive.[37] In common with other immune bodies, opsonins are thrown down in the soluble serum globulins.[38] They are very sensitive to acids and alkalies, being destroyed by a concentration of $n/_1$ and their maximum effect is at the neutral point.[39] However, treatment of either the bacteria or the leucocytes with very weak acids or alkalies, increases the rate and amount of phagocytosis (Oker-Blum).[40] Opsonins may be developed by immunizing against substances practically free from protein, *e. g.*, melanin granules.[41] Injection of nuclein preparations may increase the amount of opsonin present in the blood.[42] Cholesterol in excess diminishes phagocytosis, but apparently through its action on the leucocytes.[43] Both the sensitization of bacteria and their ingestion by leucocytes, either with or without sensitization, take place in accordance with the laws regulating an adsorption process according to Ledingham[44] and Schütze,[40] but this is not supported by the later studies of Fenn.[45]

CYTOTOXINS[46]

Just as precipitins can be obtained for proteins derived from other sources than bacterial cells, so also upon immunizing an animal against

[33] Sperimentale, 1918 (71), 459.

[34] Sawtchenko (Arch. Sci. Biol., 1910 (15), 145; 1911 (16), 161) holds that there are two steps in phagocytosis: (1) Fixation of the bacteria to the leucocyte because of modification of surface tension by the fixative substance (opsonin or amboceptor-complement complex); (2) Ameboid motion of the phagocyte; an entirely independent phenomenon. Neither phase of phagocytosis can occur in the absence of electrolytes.

[35] Walravens, Bull. acad. roy. med. Belg., 1920 (30), 204.

[36] Jour. Infect. Dis., 1905 (2), 129.

[37] Hamburger, Biochem. Zeit., 1910 (24), 470; 1910 (26), 66; Höber and Kanai, Arch. ges Physiol., 1923 (198), 401.

[38] See Simon *et al.*, Jour. Exp. Med., 1906 (8), 651; Heinemann and Gatewood, Jour. Infec. Dis., 1912 (10), 416.

[39] Noguchi, Jour. Exp. Med., 1907 (9), 454.

[40] Zeit. Immunität., 1912 (14), 485; Schütze, Jour. Hyg., 1914 (14), 201.

[41] Ledingham, Zeit. Immunität., 1909 (3), 119.

[42] Bedson, Jour. Path. and Bact., 1914 (19), 191.

[43] Dewey and Nuzum, Jour. Infect. Dis., 1914 (15), 472.

[44] Jour. Hyg., 1912 (12), 320.

[45] Jour. Gen. Physiol., 1921 (3), 465.

[46] Literature is given by Fleischmann and Davidsohn, Folia Serologica, 1908 (1), 173; Landsteiner, Handbuch d. Biochem., 1909 (II (1)), 542; Ritchie, Jour. Pathol. and Bact., 1908 (12), 140.

various types of cells other than bacteria, substances appear in its serum that exercise a destructive effect upon the type of cells injected. In other words, the reactions of animals to infection are not specially devised for combating bacteria and their products, but can be equally exerted against non-bacterial cells and their products. In the case of soluble proteins, as before mentioned, the antibodies show their effects by precipitating them, with agglutination of the particles into flocculi and perhaps a subsequent digestion; in the case of cells, whether bacterial or tissue cells, the antibodies cause agglutination and loss or impairment of vitality. This injury may be manifested by loss of motion in the motile cells (bacteria, spermatozoa, ciliated epithelium) or by solution of their contents (bacteriolysis, erythrocytolysis, leucocytolysis, etc.), or by cell death without marked morphological alterations (*B. typhosus*, spermatozoa). If we immunize with red corpuscles, leucocytes, spermatozoa, renal epithelium, or any other foreign cell, the reaction with the immune serum is as specific as it is if we inject bacteria, and of exactly the same nature. Therefore, all that has been said previously concerning bactericidal substances and agglutinins can be transposed to apply to immunity against tissue cells. As a matter of fact, however, the transposition is generally made in the other direction, for red corpuscles are much easier cells to study than bacteria, because their laking gives prompt and readily recognized evidence that the immune serum has brought about changes. Much of our knowledge of bactericidal serum has been obtained through studies of the mechanism of erythrocytolysis, the results of which have then been applied to the subject of bacteriolysis. Both on this account, therefore, and because solution of red corpuscles is of itself an important process in many intoxications and diseases, the subject is of great theoretical and practical importance, and is discussed in Chapter XI.

Not the same degree of success has been obtained in immunizing against other tissue elements. Immune serum can readily be obtained against cells that can be secured quite free from other cells, such as spermatozoa, ciliated epithelium, and leucocytes, but even then the immunity is not specific. Much less is it specific when ground-up organs are used for immunizing, as is the case in the experimental production of *nephrolysins*, *hepatolysins*, etc., for at the same time antibodies are secured for not only the typical parenchyma cells, but also for endothelium, stroma cells, red and white corpuscles, and blood plasma. As a consequence, the early expectations that by this process of immunization against specific cells great progress could be made in our knowledge of physiology, by selectively throwing out of function an organ through the simple process of injecting an antiserum, have been disappointed. Equally little progress has been made in the treatment of malignant growths by the same method. The immune serums usually obtained do, to a certain extent, injure the specific organ, but they also usually injure other organs nearly as much or perhaps more; furthermore they generally contain hemolytic toxins, even if the tissues used in

immunizing are free from blood, and hemolytic poisons may cause serious tissue destruction.[47]

By the absorption of common antibodies it is possible to demonstrate that different organs from the same species, *e. g.*, liver and kidney, do contain distinct antigens as well as common antigens,[48] but this method has not been exhaustively developed.

Beebe[49] reported that he had secured serums by immunizing with tissue nucleoproteins, that were altogether specifically toxic for the type of cells from which the nucleoproteins were obtained; *e. g.*, immunizing with liver nucleoproteins yielded serum destroying liver cells and no others. Other observers have failed to corroborate this work.[50] According to Zinsser[51] the cytolytic antibodies may be quite distinct from the proteolytic amboceptors which are developed against unformed protein antigens. The Abderhalden reaction (q.v.) is based on the principle that when tissues are injured the body reacts by the production of specific defensive enzymes which attack this sort ot tissue, but the status of this reaction as a specific immunological process is still doubtful.

In view of the present uncertain state of the subject, and the very questionable value of much of the work so far done, the consideration of the various cytolysins or cytotoxins may be dismissed by briefly referring to a few of the most important results.

Leucocytolytic Serum.[52]—This may be obtained either by immunizing with leucocytes obtained from exudates or from the blood, or by using emulsions of lymph-nodes. Specific leucocytolytic serum agglutinates leucocytes and produces observable morphologic changes, in the way of solution of the cytoplasm and cessation of ameboid movements; but it may also react with the fixed tissue cells of the same animal.[53] Of the leucocytes, the large granular cells seem most affected, especially those with the most nuclear segments,[54] and the lymphocytes least. When injected into the peritoneal cavity such serum causes an apparent initial leucopenia, and later a decided leucocytosis in the peritoneal fluid. Corresponding with this, if bacteria are injected at the same time as the serum, resistance is found decreased, but later it is much increased. Such serum also contains anticomplement, according to Wassermann, indicating that the injected leucocytes contain complement. Leucocytotoxin obtained by immunizing against lymphatic tissue is very thermolabile, being destroyed by 55° C. for thirty minutes, and the serum can be only partially reactivated by the use of fresh serum. Bacterial filtrates may also contain "leucocidins" analogous to hemolysins. Normal foreign sera are more or less toxic to leucocytes, which can be shown by the reduced capacity of the leucocytes for phagocytosis.[55]

Antiplatelet Serum.—Several experimenters have produced specific antisera for platelets, which indicates that these are chemically distinct from the other blood

[47] See Sata, Ziegler's Beitr., 1906 (39), 1.

[48] Fleisher et al., Jour. Immunol., 1920 (5), 437; 1921 (6), 223; 1922 (7), 51.

[49] Jour. Exp. Med., 1905 (7), 733.

[50] Pearce and Jackson, Jour. Infect. Dis., 1906 (3), 742. See also review by Wells, Zeit. f. Immunität., 1913 (19), 599.

[51] Biochem. Zeit., 1916 (77), 129.

[52] Literature, see Flexner, Univ. of Penn. Med. Bull., 1902 (15), 287; Ricketts, Trans. Chicago Path. Soc., 1902 (5), 178; Christian, Deut. Arch. klin. Med., 1904 (80), 333; Leschke, Zeit. Immunität., 1913 (16), 627; Reeser, Folia Mikrobiol., 1914, H. 3.

[53] Spät, Zeit. Immunität., 1914 (21), 565.

[54] Giuffré, Pediatria, 1924 (32), 1374.

[55] Löhner, Arch. ges. Physiol., 1915 (162), 129.

elements.[56] Lee and Robertson[57] obtained a specific lytic and agglutinative action, requiring complement for its accomplishment. Injected into animals this anti-platelet serum caused a condition resembling exactly *purpura hemorrhagica* in man.

Endotheliolytic Serum.—Every attempt at immunizing an animal with any sort of fixed tissue must of necessity involve the injection of endothelial cells as well as the cells specific to the tissue studied. Therefore, it is possible that cytotoxic serum so obtained will contain endothelial toxins, and so complicate any results of *intra vitam* experiments. There is every reason to believe that endotheliolytic substances are produced in this way. Ricketts[52] found that serum of animals immunized against lymph-glands was toxic to endothelial cells, which was indicated by hemorrhages at the point of injection, and marked desquamation of endothelium when the injection was made into a serous cavity. In snake-venom poisoning the extensive hemorrhages are also due to an endotheliolytic principle, called by Flexner *hemorrhagin*.

Lymphatolytic Serum.—This serum has been studied by Ricketts and by Flexner,[52] who immunized animals with lymph-nodes. As might be expected from the structure of the injected tissue, the resulting serum contained endotheliotoxin, leucocytotoxin, hemolysin, hemagglutinin, leucocyto-agglutinin, and precipitins. When injected into animals, this serum has a marked effect upon the spleen and lymph-nodes, producing great enlargement and congestion of these structures. The bone-marrow is also somewhat affected, and when marrow is used in immunizing, the *myelotoxic* serum produces marked proliferative changes in the lymph-nodes as well as in the marrow. Anti-thymus serum has been described, but Takenouchi[58] was unable to confirm these results.

Nephrolytic Serum.—It has been claimed that if a kidney is destroyed by ligating its vessels or ureter, the remaining kidney develops serious degenerative changes, which are not present if one kidney is entirely removed. This has been attributed to the development of nephrotoxic substances produced in reaction to the absorption of the injured renal tissue that has been left in the body. Other methods of renal injury have been thought to produce similar effects, and serum of animals with kidney disease was said to injure the kidneys of normal animals. Upon this basis it has been thought possible to explain the progressive nature of the chronic nephritides as the result of nephrotoxins produced through the absorption of the injured cells, which nephrotoxins injure still other renal cells.[59] Such a process, however, involves the production of cell toxins in an animal that are toxic for its own cells, that is, *autocytotoxins;* and as it has so far been extremely difficult to produce autolysins of other sorts, it is not altogether probable that the kidney is an exception. Furthermore, Pearce[60] was unable to produce isonephrotoxins, and could not corroborate the statements as to the changes said to have been found in the remaining kidney after ligating the vessels of its mate. He did obtain an active heteronephrolysin, but also found that immunization with liver produced nearly as actively nephrolytic serum as did immunization with kidney. However, Fleisher[43] has demonstrated that antikidney serum does react specifically with at least some of the antigens of kidney tissue.

Neurolytic Serum.—Even as highly specialized cells as those of the nervous tissue seem to produce a reaction with the formation of immune bodies. Perhaps the most positive results are those of Ricketts and Rothstein,[61] who found that serum of rabbits immunized against the brains or cords of guinea-pigs was highly toxic when injected into the vessels of guinea-pigs, causing death with various symptoms only explainable on the assumption of nervous lesions. Microscopically, the ganglion cells showed marked changes in those animals that survived the injection long enough. All the results so far obtained have been with heterogeneous serum.[62] Venoms, particularly that of cobra, possess strong neurolytic substances that are the chief toxic agents in most of the venoms (rattlesnake venom excepted).

Thyrolytic Serum.—There are but few reports on this serum, but that of Portis[63] indicates that after removal of all hemolysis as a factor there do occur changes, in the

[56] Menne, Jour. Infect. Dis., 1922 (31), 455.
[57] Jour. Med. Res., 1916 (33), 323.
[58] Jour. Exp. Zool., 1919 (29), 311.
[59] See Kapsenberg, Zeit. Immunität., 1912 (12), 477
[60] Univ. of Penn. Med. Bull., 1903 (16), 217.
[61] Trans. Chicago Path. Soc., 1903 (5), 207.
[62] An attempt to obtain a specific neurotoxin with corpus striatum was unsuccessful. (Lillian Moore, Jour. Immunol., 1916 (1), 525.)
[63] Jour. Infectious Diseases, 1901 (1), 127.

nature of excessive absorption of colloid, and proliferation after the order of that seen in thyroid regeneration. However, the clinical picture of thyroidectomy was not produced in any case, and the anatomic changes were not great. By immunizing against nucleoproteins derived from thyroid tissue, Beebe[64] has secured an antiserum to which he ascribes some effect upon diseased thyroids (exophthalmic goiter). MacCallum[65] could not get a specific serum for parathyroid tissue.

Numerous reports may be found indicating attempts, with varying success, to obtain serum toxic for other tissues. Among them may be mentioned *epitheliolysin*[66] (for ciliated *epithelium*), *spermat toxin*,[67] *gastro-toxin*[68] *hepatolysin*, *cardiolysin*, *splenolysin*, and *syncytiolysin*.[69] Special attention has been given to the production of specific lysins for cancer cells, without definite success. (See Chapter XXI.) In general it can be said that it has *not* been found possible in this way to throw out of function one particular organ, with or without involvement of other structures.[70] The principles involved in all these experiments are the same, and the results are in no instance altogether satisfactory; therefore no further consideration of these special cytotoxic serums will be made here, the reader being referred to other sources for details.[71] It may be said, however, that recent developments indicate that various tissues not only contain proteins which exhibit the species characteristics of the entire animal, but also other proteins or antigenic radicals which are more or less independent of these and characteristic to a certain degree for the tissue from which the antigen was obtained. For example, Hektoen and Schulhof,[72] have obtained specific precipitins for the proteins of the alimentary mucosa distinguishing them from the serum proteins, and Fleisher's[48] experiments have demonstrated the presence of specific and common antigens in organ extracts. This being the case, we cannot consider the problem of specific cytotoxins a closed chapter; improved methods for separating our antigens may yet enable us to secure antibodies specific for a single tissue or organ. (See Specificity of Antigens, Chap. VII.) However, by the useful method of studying the effect of cytotoxic serum on the growth of tissue cultures *in vitro*, Lambert[73] found no evidence whatever of specificity, although there is a non-specific inhibition of growth by the immune sera.

[64] Jour. Amer. Med., Assoc., 1906 (46), 484. Not corroborated by Portis and Bach, *ibid.*, 1914 (62), 1884.

[65] Med. News, 1903 (83), 820.

[66] See Galli-Valerio, Zeit. Immunität, 1915 (24), 311.

[67] McCartney, Amer. Jour. Physiol., 1923 (63), 207; Kennedy, Quart. Jour. Exp. Physiol., 1924 (14), 279.

Taylor (Jour. Biol. Chem., 1908 (5), 311) found that no spermatolytic serum could be obtained by immunizing with isolated nucleic acid, protamines, or ether extracts of sperm, although immunizing with whole sperm produced active sera.

[68] Miyagawa, Jour. Path. and Bact., 1920 (23), 462; Koopman, ibid., 1924 (27), 323.

[69] Lake, Jour. Infect. Dis., 1914 (14), 385.

[70] An attempt to produce a specific cytolytic serum for the islands of Langerhans by Kamimura was unsuccessful. (Mitt. med. Fak. Univ. Tokio, 1917 (17), 95).

[71] Biochemisches Centralblatt, 1903 (1), 573, *et seq.;* also see Sata, Ziegler's Beitr., 1906 (39), 1; and literature cited previously.

[72] Jour. Amer. Med. Assoc., 1924 (83), 1300.

[73] Jour. Exp. Med., 1914 (19), 277; also Walton, *ibid.*, 1915 (22), 194.

COMPLEMENT FIXATION[74]

The original principle involved in this reaction was first demonstrated by Bordet and Gengou, and is essentially as follows: If a specific antigen and amboceptor unite in the presence of complement, the complement is then united to the amboceptor-antigen compound to complete the reaction. When sufficient amounts of amboceptor and antigen are present the entire quantity of available complement may be thus fixed, and, consequently, the mixture contains no more complement free for further reactions. As complement does not ordinarily unite with amboceptors except when the amboceptors are already united with their specific antigens, the fact that in a given system of

complement + amboceptor + antigen

there is no free complement, is evidence of a reaction between amboceptor and antigen; in consequence of which this reaction can be used to determine the presence of a specific amboceptor in a serum, by using the corresponding antigen; or conversely, with a serum containing a known amboceptor we can detect the presence in a solution of the specific antigen.

The indicator of the presence or absence of complement which is in universal use, is the ability of the mixture to hemolyze erythrocytes in the presence of the specific hemolytic amboceptor. Thus, if typhoid bacilli and a typhoid antiserum which contains both complement and specific amboceptor, are mixed in proper proportions and incubated for a short time, the complement will be bound to the bacilli. If we then add this mixture to sheep corpuscles which have been acted upon and sensitized by an antisheep-corpuscle serum, from which the complement has been previously removed by heating, no hemolysis will occur, for we have added no *free* complement. But if our original mixture had contained dysentery bacilli instead of typhoid bacilli the complement would not have been fixed, and the addition of this mixture, containing free complement, to the sensitized sheep corpuscles would cause prompt hemolysis.

This reaction was at first used for the detection of antibodies in sera, and for the identification of bacteria, and was found to be exquisitely

[74] The reaction of "complement fixation" must not be confused with the entirely distinct reaction of "*complement deviation*," a mistake very likely to happen because of the careless but erroneous use by some writers of the latter term in describing the first-named reaction. Complement deviation (or Neisser-Wechsberg phenomenon) is produced when an excess of amboceptors is present together with antigen and a limited amount of complement, which results in absence of complement activity. The original explanations for this reaction are not now acceptable, and with increasing appreciation of the relation of immunological reactions to colloidal chemistry processes, the tendency has been to put the Neisser-Wechsberg phenomenon into the inhibition-zone reactions discussed in connection with agglutination and precipitation. Pandit (Jour. of Hygiene, 1923 (21), 406), who reviews the earlier work, believes that the complement "deviation" does not depend on special inhibiting antibodies, but is the result of dissociation of the antigen-amboceptor complex which occurs when the amboceptor is in excess. He found that the complement is still free in a reaction mixture exhibiting the Neisser-Wechsberg phenomenon, showing that the "deviation" is not dependent on binding of complement by amboceptors as the foregoing authors believed.

delicate, detecting most minute amounts of antigens with the sharpest specificity limits of any of the immunity reactions. As indicated previously, there is much reason to believe that the antibody (amboceptor) responsible for complement fixation is identical with the precipitins, agglutinins, and other antibodies, and this reaction may often be demonstrable in conditions under which precipitin or agglutinin reactions cannot be detected.

It is not yet known what changes actually take place in the reaction of complement fixation. A favored hypothesis is that the antibody by uniting with the antigen produces large colloidal aggregates, often visible as a precipitate in the precipitin reaction and demonstrable by ultramicroscopic methods when not visible to the naked eye, which absorb the large, labile, colloidal complement aggregates.

Hecht[75] says we should look upon the property of serum which we designate as complement, as merely the property of a mixture of colloids and electrolytes in water, and dependent on such factors as the surface tension, viscosity, conductivity, and dispersion. The complement function is manifested only when the colloid particles are of a certain size and electrolytes are present, and hence it is easily inactivated by changes in either the amount of electrolytes or the degree of dispersion of the colloids. Slight changes therefore produce a reversible inactivation, marked changes inactivate it irreversibly. All procedures that inactivate complement are associated with a reduction in surface tension. Conductivity is also lowered during inactivation, except, of course, when this is accomplished by strong salt concentrations. These changes are not the result of alterations in the complement itself, but merely an expression of the change in the entire colloid complex of the serum.

Kiss[76] believes that the complement can be shown to be a definite body in the serum, consisting of most minute hydrophilic colloidal particles, which cannot be subdivided without loss of their characteristic properties. He found that all forms of complement fixation, whether specific or non-specific, follow the formula of physical adsorption, and hence this must be looked upon as a physico-chemical process, but how the specificity of the reaction is to be explained on a simple physical basis has not been shown. And yet in none of the immunological reactions does the colloidal chemistry aspect stand out more prominently than in that of complement fixation. The ready inactivation of complement through adsorption by colloids is a conspicuous feature of the behavior of this large colloidal complex, for silicic acid, gum mastic suspensions, and many other colloidal aggregates have this effect quite as strikingly as the complex formed by syphilitic serum and the lipoidal suspension which constitutes the complement fixation antibody in the Wassermann reaction. The fact that in this reaction the adsorbent precipitate is formed more rapidly in the ice chest than in the incubator is further evidence that the process is physical rather than chemical.

[75] Zeit. Immunität., 1923 (36), 321.
[76] Biochem. Zeit., 1922 (129), 487.

THE WASSERMANN REACTION[77]

In his original experiments Wassermann carried out the test for syphilis according to the principles of the true complement fixation reaction of Bordet and Gengou. He used extracts of the livers of congenital syphilitic fetuses, which contain great quantities of spirochetes, as an antigen, expecting thereby to be able to determine in a given serum the presence of specific amboceptors for the virus of syphilis, such amboceptors being present in persons infected with syphilis as a result of the reaction to the infection. As originally introduced, then, the Wassermann reaction was supposed to be simply a specific reaction between syphilitic antigen, specific syphilitic amboceptors, and non-specific complement. It was soon learned, however, that the reaction as it occurred in syphilis is decidedly different from the original complement fixation reaction, for it was found possible to substitute in the reaction for extracts of tissues containing syphilitic virus (spirochetes), the most varied sorts of tissue extracts, coming from tissues certainly free from spirochetes (*e. g.*, ox heart). Moreover, when Noguchi succeeded in devising means for cultivating the *Treponema*, it was found that extracts of these organisms were entirely ineffective when used as antigen with the serum of human syphilitics as a source of antibody, although such serum gave strongly positive Wassermann reactions. These and many other observations have shown conclusively that the Wassermann reaction, although almost specific evidence of syphilitic infection, is not a true complement-fixation reaction demonstrating the presence of syphilitic antibodies in the serum of the person infected with syphilis. The actual nature of the reaction is still obscure but the present tendency is to consider it dependent upon physico-chemical and colloidal phenomena.[78]

Extended investigation of these non-specific antigens which give specific complement fixation with syphilitic sera, has shown them to be related to the lipoids, especially the lecithins, as indicated by the fact that the most efficient antigens contain the acetone-insoluble fraction of the tissue lipoids.[79] The antigenic value of this fraction of different liver extracts varies nearly directly with its power to combine with iodin, according to Noguchi and Bronfenbrenner, which indicates that the unsaturated fatty acids are important in the reaction, but this was not corroborated by Browning.[80] Furthermore, saturation of the lipoids with hydrogen, whereby the double bond $-\overset{H}{C}=\overset{H}{C}-$ is reduced to the single

[77] Early literature given by Noguchi, "Serum Diagnosis of Syphilis and Luetin Reaction," Philadelphia, 1912.

[78] See review of physico-chemical theories by Epstein and Paul, Kolloid Zeit., 1921 (29), 310; Arch. f. Hyg., 1921 (90), 98.

[79] This is also supported by the finding of Mizuhara (Zeit. Immunität., 1924 (40), 84) that cobra venom lecithinase destroys the complement activity of heart extracts.

[80] Browning *et al.*, Zeit. Immunität., 1912 (14), 284; Jour. Pathol. and Bact., 1911 (16), 135 and 225. Klein and Fraenkel believe the "antigen" of ox heart extracts to be a combination of lecithin with cholesterol and small amounts of a soap-like substance similar to jecorin (Münch. med. Woch., 1914 (61), 651).

bond form $-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-$ does not remove the activity of the lipoids, indicating that this does not depend on the unsaturated carbon atoms.[81] Crude lecithins from different sources vary in efficiency, heart lecithin being more active than liver lecithin, brain and egg yolk lecithin following. Pure lecithin is not effective, the activity of lipoid solutions depending upon some other substance which is difficult to separate from lecithin (MacLean).[82] Addition of cholesterol to the lipoid solutions increases greatly their activity,[80] although cholesterol itself will not function as antigen.[83] An acetone-precipitated "antigen" of this class is not a true antigen, however, for fixation antibodies are not developed in animals injected with such a lipoid, although this lipoid has been shown to be entirely efficient in the Wassermann reaction.[84]

The antigen is employed as a colloidal suspension in salt solution, and its efficiency depends greatly upon the size of the particles in the suspension, indicating that the function of the antigen depends upon the surface development of the suspension colloid. The efficient lipoid antigen seems to consist of a mixture of particles varying in size from coarse suspensions to true colloidal dispersion, with a relatively large proportion on the borderline between the two stages, which makes for the lack of stability characteristic of and apparently essential for such "antigens." (Epstein and Paul).[78]

The substance in the syphilitic serum which participates in the Wassermann reaction, (the amboceptor) would seem to be related to the globulins, which are decidedly increased in the blood and spinal fluid[85] of syphilitics,[86] especially the euglobulin.[87] Kapsenberg[88] maintains that both the Wassermann and the Sachs-Georgi reactions are given by isolated serum globulin of syphilitic sera, while the serum albumin is inactive. Stern[89] says that the activity lies exclusively in the less labile fraction of the euglobulin, which does not differ in its electrical charge whether active or not. The absence of Wassermann reactions with the blood of syphilitic fetuses may depend on the deficiency of fetal blood in globulins.[90] P. Schmidt[91] ascribes the reaction to the physico-chemical properties of the globulins of the syphilitic serum, which, he believes,

[81] C. L. A. Schmidt and S. E. Coffey, Jour. Infect. Dis., 1923 (32), 119.

[82] Monographs on Biochemistry, "Lecithin and Allied Substances," London, 1918.

[83] Frank, Klin. Woch., 1922 (1), 419.

[84] Fitzgerald and Leathes, Univ. of Calif. Publ., Path., 1912 (2), 39.

[85] Pfeiffer, Kober and Field, Proc. Soc. Exp. Biol., 1915 (12), 153.

[86] See Rowe, Arch. Int. Med., 1916 (18), 455; Schiff and Roser, Monats. f. Kinderheilk., 1920 (19), 15.

[87] Müller and Hough, Wien. klin. Woch., 1911 (24), 167; Harold, Jour. Royal Army Med. Corps, 1922 (39), 83; 1923 (40), 47; Ruppel, Deut. med. Woch., 1923 (49), 46; Herrold, Jour. Amer. Med. Assoc., 1923 (81), 203.

[88] Zeit. Immunität., 1924 (39), 3.

[89] Biochem. Zwit., 1924 (144), 115.

[90] See Levinson, Jour. Immunol., 1922 (7), 497.

[91] Zeit. f. Hyg., 1911 (69), 513. See also Hirschfeld and Klinger. Zeit. Immunität., 1914 (21), 40.

possess a greater affinity for the colloids of the antigen than normal globulins; this affinity is held in check in normal serum by the albumins of the serum, which are relatively or absolutely decreased.

If the globulin and albumin of a positive serum are separated, the isolated globulin contains all the reactive agent, the albumin giving negative reactions (Kapsenberg).[92] During antisyphilitic treatment, furthermore, there is a fall in the globulin content of the blood associated with the disappearance of the Wassermann reaction.[93] Forssmann,[94] however, like many others, believes that the antibody is not globulin, but something intimately associated with the globulin, probably of lipoidal nature. Normal sera can be made Wassermann-positive by such procedures as shaking or treatment with fine suspensions such as kaolin, and lose this artificial reactivity on removal of the globulin. These observations suggest that while alterations in the colloidal state of the globulins may cause a serum to become Wassermann positive, yet the reaction in syphilis depends at least in part on something else besides the globulin. They agree with the view of Sachs[95] who suggests that the reaction involves first the lipoids of the serum and the antigen, and that the specific behavior of the syphilitic serum depends on a primary alteration of the lipoids from which results a secondary alteration of the globulin which modifies the degree of its dispersion.

That physico-chemical factors play a part is evidenced by the common observation that the turbidity of the antigen suspension is closely related to its efficiency, clear solutions being less active.[96] Slight changes in H-ion concentration will change a reaction from negative to positive, or reverse; and neutral salts can change a negative to a positive reaction, but not the reverse (Cumming).[97] Even the mere treatment of a negative serum with ether may cause it to give positive reactions (Forssman),[98] possibly by causing a change in the colloidal state of the lipo-proteins of the serum. Unlike the "antigen," the "amboceptor" is not flocculated by electrolytes in small concentrations, and hence it is apparently an emulsoid or hydrophilic colloid whereas the "antigen" behaves like a suspensoid colloid in which the disperse phase is solid.

The lipoids in syphilitic sera are said by Peritz[99] to be increased, but the lipoid content and the antibody titer do not show any constant relation (Bauer and Skutezky).[1] The cholesterol content of syphilitic blood shows no evidence of a quantitative relation to the Wassermann reaction.[2] Friedemann[3] believes that a globulin soap compound is the active sub-

[92] Ann. Inst. Pasteur, 1921 (35), 648; Zeit. f. Immunität., 1924 (29), 3.
[93] Bircher and McFarland, Arch. Derm. and Syph., 1922 (5), 215.
[94] Biochem. Zeit., 1921 (121), 180.
[95] Kolloid-Zeit., 1919 (24), 123.
[96] According to Zunz (Bull. acad. roy. med. Belg., 1924 (4), 74) the surface tension of syphilitic serum is distinctly lower than normal in syphilis.
[97] Jour. Infect. Dis., 1916 (18), 151.
[98] Biochem. Zeit., 1921 (121), 180.
[99] Zeit. exp. Path., 1910 (8), 255.
[1] Wien. klin. Woch., 1913 (26), 830.
[2] Weston, Jour. Med. Res., 1914 (30), 377; Stein, Zeit. exp. Med., 1914 (3), 309.
[3] Zeit. f. Hyg., 1910 (67), 279.

stance in syphilitic sera. McIntosh[4] says that the active component differs from typical antibodies in not passing through collodion or porcelain filters, and there are many who hold that the reacting substance is a product of tissue disintegration.[5] Wassermann[6] has found evidence that the antibody is derived from the lymphocytes, at least in the spinal fluid of syphilitics.

Whether true antibodies are concerned in the Wassermann reaction is a question. In favor of this view is the fact that the serum of rabbits immunized with congenital syphilis livers contains an antibody giving the Wassermann reaction, exactly like the serum of syphilitics.[7] On the other hand, the actual substance of pure cultures of spirochetes does not ordinarily act as antigen with syphilitic sera in the Wassermann reaction (Noguchi). It is highly probable that when syphilitic liver extracts are used as antigen in the Wassermann reaction, we have a true Bordet-Gengou reaction of complement fixation with the syphilitic substance present in this extract, in addition to the reaction which is accomplished by the lipoids.

Whether the complement is destroyed by enzymes,[8] or is inhibited by anti-complement present in syphilitic serum, or is bound by precipitates formed by the reaction between antigen and amboceptor, or is destroyed by some toxic substance in the serum[9] are matters still under discussion. Bachmann[10] found by interferometer studies no evidence of enzyme action. A favorite interpretation of the Wassermann reaction, which seems to harmonize with the known facts, is that there is a precipitation of serum globulin by the lipoidal colloids of the antigen, and adsorption of the complement by this precipitate.

Apparently the globulins of the serum in syphilis are altered in some specific but as yet unknown way, whereby they acquire in greatly increased degree the capacity to form this adsorbent precipitate,[11] or lose their capacity to act as protective colloids which prevent the aggregation of particles which absorbs the complement.[12] Alterations in the lipoids also seem to exist, for it is known that conditions that modify the serum lipoids also modify the reaction. There seems little doubt that the reaction is not chemical but physical, and the union of complement to antibody follows essentially the laws of absorption. Also its intimate relation to the precipitin reaction seems to be established (Dean).[13] Forssman[14]

[4] Zeit. Immunität., 1910 (5), 76.
[5] Bachmann, Zeit. f. Immunität., 1921 (33), 233; Much and Schmidt, Deut. med. Woch., 1921 (47), 552.
[6] Wassermann and Lange, Berl. klin. Woch., 1914 (51), 527.
[7] Citron and Munk, Deut. med. Woch., 1910 (36), 1560; Eiken, Zeit. Immunität., 1915 (24), 188; Meyer, Zeit. f. Immunität., 1921 (31), 278.
[8] Manwaring, Zeit. f. Immunität., 1909 (3), 309.
[9] Kiss, *ibid.*, 1910 (4), 703.
[10] Zeit. Immunität., 1924 (40), 325.
[11] See Nathan, Zeit. Immunität., 1918 (27), 219; Walker, Jour. Path. Bact., 1917 (21), 184.
[12] Holker, Jour. Path. and Bact., 1923 (25), 281.
[13] Lancet, Jan. 13, 1917.
[14] Biochem. Zeit., 1924 (148), 160.

points out that the Wassermann antibody is inactivated by formalin in quite the same way as other antibodies.

Flocculation Reactions in Syphilis

The changes in character of the blood serum in syphilis are sufficient to give not only immunological but also frank chemical or physico-chemical manifestations. For example, Bruck[15] states that the precipitate obtained when nitric acid is added to syphilitic serum is more abundant and of a characteristic gelatinous appearance. Platinum cloride also produces a heavier precipitate in syphilitic sera (Brown and Iyengar).[16] The globulin responsible for the Wassermann reaction is said to precipitate more readily by ammonium sulfate and other reagents.[17] According to von Dungern[18] the heat coagulation of syphilitic serum is prevented by a smaller relative quantity of an alkaline solution of indigo than is the case with normal serum, a statement disputed by Flood and Fujimoto.[18] Syphilitic serum also flocculates on addition of appropriate colloidal suspensions which will not coagulate normal serum (Vernes),[19] Landau[20] states that syphilitic serum has a heightened power to decolorize and clear up an iodin precipitate produced in the serum.[21]

In general, the serum in syphilis is a less stable colloidal solution than normal serum, and because of this it has been possible to devise numerous more or less specific reactions to demonstrate this property and to determine that the serum is syphilitic. Among these reactions[22] are the following:

Klausner's Serum Reaction.—When distilled water is added in certain proportions to fresh serum, a distinct flocculent precipitate separates out in a few hours, and this property is much more marked in syphilitic than in normal sera. While not specific for syphilis, this reacton is almost invariably present in certain stages of syphilis. This property is not due to the excess of globulin present in syphilitic sera, according to the later studies of Klausner,[23] who believes that the high lipoid content of syphilitic serum is responsible.

Porges-Hermann-Perutz Reaction.—If equal parts of a 2 per cent. solution of sodium glycocholate and an alcoholic cholesterol suspension (0.4 per cent.) are added to inactivated serum from syphilitic patients, a precipitate forms, while with normal serum there occurs no precipitate.[24] Little is known concerning the nature of this reaction.

Coagulation Reaction.—This was described by Hirschfeld and Klinger,[25] and depends on the fact that tissue extracts digested with syphilitic serum lose their ability to coagulate blood. The effect is believed to depend on adsorption of the lipoids of the tissue extract by serum constituents, and hence is fundamentally similar to the Wassermann reaction.

[15] Münch. med. Woch., 1917 (64), 25. See also Smith and Solomon, Boston Med. Surg. Jour., 1917 (177), 321.

[16] Indian Jour. Med. Res., 1915 (3), 95.

[17] See Heller, Biochem. Zeit., 1918 (90), 166; McDonagh, Proc. Royal Soc. Med., 1916 (9), 191, (Derm. Sect).

[18] Münch. med. Woch., 1915 (62), 1212. See also Flood, Jour. Immunol., 1916 (2), 69; Fujimoto, *ibid.*, 1918 (3), 11.

[19] Compt. Rend. Acad. Sci., 1918 (167), 383.

[20] Wien. klin. Woch., 1913 (26), 1702.

[21] Not corroborated by Stillians and Kolmer, Jour. Amer. Med. Assoc., 1915 (64), 1459.

[22] See review on Flocculation Reactions by Levinson, Amer. Jour. Syphilis, 1921 (5), 414.

[23] Biochem. Zeit., 1912 (47), 36.

[24] See Gammeltoft, Deut. med. Woch., 1912 (38), 1934; Ellermann, *ibid.*, 1913 (39), 219.

[25] Deut. med. Woch., 1914 (40), 1607. See also Kolmer and Toyama, Amer. Jour. Syphilis, 1918 (2), 505.

Meinecke Reactions.[26]—These are based on the hypothesis that the colloids of alcoholic extract of tissues disturb the isotonicity of saline solutions, permitting the union of serum globulins and lipoids, the reaction being greater with syphilitic than with non-syphilitic sera. If normal and syphilitic sera are precipitated, respectively, with dilute colloidal solutions of a lipoidal antigen in distilled water, the precipitate which forms in the globulin-rich syphilitic serum is less soluble in salt solution than that from the normal serum.[27]

Bruck's Nitric Acid Reaction.[15]—This is based on the fact that the precipitate formed in syphilitic serum by nitric acid is less soluble in dilute nitric acid than the precipitate from normal serum. It agrees with the Wassermann reaction in about 70 per cent. of positive cases (Kolmer).

Formol Reaction.—Syphilitic sera form a solid gel when treated with formalin in concentrations which do not have this effect on normal sera (Gaté and Papacostas).[28] This reaction is, however, far from constant, and often positive reactions are obtained with normal sera.[29]

Sachs-Georgi Reaction.—A specially prepared cholesterolized lipoid antigen from beef heart gives a much heavier precipitate in diluted inactivated syphilitic than in normal serum. This reaction takes advantage of the precipitation described in syphilitic serum when added to lipoidal "antigen" observed by ultramicroscopic methods by Jacobsthal, which is made visible to the naked eye under the conditions of this test. Active complement is not present in this reaction, which, according to Mackie[30] seems to depend on a different fraction of the serum proteins from the one responsible for the Wassermann reaction. The precipitate consists chiefly of lipoidal matter,[31] but is said to carry down with it the substance of the serum which gives the Wassermann reaction. (Wassermann,[32] Taoka[33]).

The Vernes Reactions.[34]—These are based on the observation that colloidal suspensions of inorganic substances, especially ferric hydrate, produce more flocculation in syphilitic than in normal sera. Colloidal suspensions of organic substances may exhibit the same differences.

As seen in the above reactions the capacity of syphilitic serum (and spinal fluid) to produce flocculation with various colloidal or crystalloid precipitating reagents, is distinctly increased over the normal. The flocculated material is generally found on examination to contain both the serum protein, especially globulin, and the other agent involved in the flocculation. When the latter is a lipoid it seems to be the predominating part of the precipitate.[35] Kolmer[34] believes that the fundamental mechanism of complement fixation in syphilis is identical with these macroscopic colloidal flocculation reactions. The agglutinin and precipitin reactions are undoubtedly colloidal reactions even though the chemical nature of the antibody in the serum is unknown. It is entirely reasonable to asume that in the Bordet-Wassermann reaction the same antibody-like substance is operative as in the various flocculation reactions; that in the former, flocculation occurs, which is invisible to the naked eye, but sometimes visible microscopically by the dark-field illumination method. In a general manner, the tissue extracts yielding best results in the complement-fixation reaction also prove most sensitive in the macroscopic flocculation reaction, *e. g.*, the cholesterolized alcoholic extracts.

The Active Agent in the Syphilis Reactions.—Despite the abundant evidence on the nature of the active substances at play in the complement fixation and specific flocculation tests cited in the foregoing

[26] Berl. klin. Woch., 1917 (54), 613; 1918 (55), 83; Jantzen, Zeit. f. Immunität., 1921 (33), 156.

[27] The principles of the Meinicke reactions are considered in much detail by Epstein and Paul, Arch. f. Hyg., 1921 (90), 98.

[28] Compt. Rend. Soc., Biol., 1920 (83), 1432.

[29] Ecker, Jour. Infect. Dis., 1921 (29), 359.

[30] Jour. Path. Bact., 1923 (26), 120.

[31] Niederhoff, Münch. med. Woch., 1921 (68), 330; Klostermann and Weisbach, Deut. med. Woch., 1921 (47), 1092.

[32] Berl. klin. Woch., 1921 (58), 193.

[33] Kitasato Arch. Exp. Med., 1922 (5), No. 3, 1.

[34] See Kolmer, "Infection, Immunity and Serum Therapy," 1923, p. 523.

[35] Niederhoff, Münch. med. Woch., 1921 (68), 330.

paragraphs, we can say nothing more definite than that *the proportion and character of serum globulins and lipoidal colloidal suspensions are altered in syphilis in some unknown way, so that their stability is greatly reduced and hence they tend to flocculate and to adsorb other colloids, including among them the serum colloids which exhibit the function of "complement."*

THE MEIOSTAGMIN REACTION[36]

Reaction of antigens with their specific antibodies results in lowering the surface tension of the solution in which the reaction occurs, which may be demonstrated by counting the number of drops of fluid per minute, under constant conditions. Ascoli and Izar[37] worked out methods for practical application of this phenomenon, giving it the name of "meiostagmin reaction," from the Greek, meaning "small drop." The number of drops from a stalagmometer is counted, and an increase of two or more per minute is considered a positive reaction, after two hours' incubation of the reacting mixture; the increase is seldom above eight drops. The antigens used are soluble in alcohol but their nature is unknown; the antibody involved in the reaction is referred to as the meiostagmin, but its relation to other antibodies is likewise unknown. There has been an attempt to explain the reported observations as the effect of the action of lipoids, linoleic-ricinoleic acid mixtures having been used by some experimenters.[38] Gouwens[36] and other recent investigators, however, have been unable to demonstrate any such constant change of surface tension to occur, even when antisera of known high antibody content act upon the specific antigen, and hence it seems probable that this reaction is not dependent upon antigen-antibody reaction. Loeb[38] believes that it merely indicates that the serum in some pathological states has less than the normal power to counteract or inhibit the lowering of surface tension by the lipoidal "antigens." Weist-Osborn and Ehrentheil[39] say that a lowered cholesterol content of the serum produces a positive reaction, but that positive reactions may also be obtained with serum with high cholesterol content when this is partly obscured by the formation of globulin-cholesterol compounds.

THE EPIPHANIN REACTION

Besides the supposed reduction in surface tension, other physico-chemical changes result from antigen-antibody reactions, including the rate of diffusion, the osmotic pressure, and, in consequence, according to Weichardt, the neutral point to phenolphthalein of a mixture of barium hydroxide and sulfuric acid is also changed towards the acid side by antigen-antibody reactions taking place in the mixture.[40] This phenomenon has been utilized by Weichardt, under the name of "epiphanin reaction," to determine the occurrence of such interaction of antigen and antibody. The reaction probably depends upon adsorption phenomena, but the exact nature of the change is not yet understood. According to Rosenthal,[41] the epiphanin reaction is especially suitable for demonstrating cancer antibodies and antigens, but Burmeister,[42] Reich[43] and others have not been successful with this procedure.

THE ABDERHALDEN REACTION

This reaction is based upon the hypothesis that the animal body reacts to the presence of foreign proteins by providing specific means of destroying them through proteolysis, and hence is fundamentally the same as the anaphylaxis reaction as

[36] Full review of lit. by Gouwens, Jour. Infect. Dis., 1922 (31), 237.
[37] Münch. med. Woch., 1910 (57), 62, 182 and 403.
[38] N. Blumenthal, Zeit. f. Immunität., 1915 (24), 42; L. F. Loeb, Biochem. Zeit., 1923 (136), 190; Grevé, Münch. med. Woch., 1924 (71), 1466.
[39] Biochem. Zeit., 1924 (148), 308.
[40] See Weichardt, Berl. klin. Woch., 1911 (48), 1935; Rosenthal, Zeit. Immunität., 1912 (13), 383; Angerer and Stötter, Münch. med. Woch., 1912 (59), 2035; Angerer, Arch. f. Hyg., 1914 (83), 77.
[41] Zeit. Chemotherapie, 1912 (1), 156.
[42] Jour. Infect. Dis., 1913 (12), 459.
[43] Zeit. f. Immunität., 1913 (8), 480.

conceived by Vaughan, Friedemann, Friedberger and others. It differs from the other reactions of this class merely in that the methods used for determining the proteolysis are chemical rather than biological. The occurrence of a reaction is indicated by the production of diffusible products of protein hydrolysis, which may be detected by any one of several methods,[44] although most used is "ninhydrin" (triketohydrindene hydrate)[45] which reacts with any alpha-amino acid, the resulting condensation compound being a blue or violet color; or by observing the change in optical rotation that occurs in a solution of peptone under the hydrolytic action of the serum.

It has undergone much the same series of shifting explanations as the other reactions of this class. At first, like the other proteolytic reactions, it was assumed that the antigen was digested; but, as with the precipitin and anaphylaxis reactions, evidence was found by numerous observers that not the antigen but the proteins of the immune serum are the chief or sole source of the cleavage products. For some reason, hard to explain, it has always been referred to as if it were the result of the formation of specific enzymes which attack the antigen, in spite of the repeated demonstration that sera giving positive reactions can be inactivated by heat and reactivated by normal serum,[46] thus throwing it into the class of amboceptor-complement reactions, with which it agrees in principle.

Having been introduced first as a method for diagnosing pregnancy, on the principle that in pregnancy the chorionic cells of the placenta enter the maternal circulation and, as foreign proteins, cause the formation of specific "defensive ferments," it was at once taken up as a clinical procedure, and as a result an enormous literature on this reaction was rapidly produced. Much of this represents highly uncritical work, largely from workers not trained or experienced in immunological principles, and hence it is not profitable to review it *in extenso* here. Abderhalden's own views are given in full in his monographs[47] and there exist numerous critical reviews.[48] The status of the reaction at this writing seems to be as follows:

Animals, or man, after having foreign proteins of any sort enter the blood stream, may, and commonly do show an altered condition of their serum, whereby when their serum is incubated with the antigen under suitable conditions very minute quantities of the products of protein cleavage may be set free, and recognized when dialyzed away from the digesting mixture; or, a measurable change in optical rotation of the digestion mixture occurs. However, perfectly normal sera may at times cause a similar proteolysis, usually but not always less than with the immune serum.

The digestion seems to involve chiefly the serum proteins rather than the antigen, although under certain conditions there may be some digestion of the antigen. Bronfenbrenner holds that the enzymes exhibit no selectivity, digesting both the antigen and the serum impartially.[49]

Apparently the digestion is accomplished by serum complement, or at least normal serum enzymes, rather than by any new-formed specific enzyme, although enzymes set free from the tissues have been held responsible by some, and Abderhalden[50] maintains that the enzyme destroying a specific tissue arises from that sort of tissue, *e. g.*, he says that thyroidectomized animals do not form enzymes attacking thyroid, and castrated animals form no enzymes against testicle substance.

The mechanism of the reaction is not understood. Jobling and Petersen have suggested that the antigen-antibody combination may adsorb or bind the antiproteases of the serum, so that the normal protease digests the serum proteins. Or it may be that union of antigen and antibody activates the complement, or binds it to the antibody so that it digests either the antibody or other proteins of the serum. It also is suggested that enzymes are set free from the tissues injured by the specific protein,

[44] See Abderhalden, Fermentforsch, 1923 (7), 54.

[45] Concerning the mechanism of the ninhydrin reaction see Retinger, Jour. Amer. Chem. Soc., 1917 (39), 1059.

[46] See Stephan, Münch. med. Woch., 1914 (61), 801; Hauptman *ibid.*, p. 1167; Bettencourt and Menezes, Compt. Rend. Soc. Biol., 1916 (77), 162.

[47] Emil Abderhalden, "Schutzfermente des tierischen Organismus."

[48] See Wallis, Quart. Jour. Med., 1916 (9), 138; Bronfenbrenner, Jour. Lab. Clin. Med., 1915 (1), 79; 1916 (1), 573. Hulton, Jour. Biol. Chem., 1916 (25), 163, Oppler, Biochem. Zeit., 1916 (75), 211; Mühlmann, Virchow's Arch., 1921 (230), 448; Wertheimer, Handbuch d. Biochem., 1923 (4), 132.

[49] Supported by Smith and Cook, Jour. Infect. Dis., 1916 (18), 14. De Waele states that it is the serum globulin that is digested (Compt. Rend. Soc. Biol., 1914 (76), 627). See also Abderhalden, Fermentforsch., 1922 (6), 230.

[50] Fermentforsch., 1922 (6), 263.

or by disease, which digest the foreign protein or the cellular proteins that may have escaped from the tissues into the blood stream.

Serum treated with various inert, finely-divided particles, such as kaolin, starch, silicates, etc., may acquire the property of giving positive reactions.[51] This is another point of resemblance to anaphylatoxin formation, and against the specificity of the reaction, indicating that the antigen merely acts as a non-specific adsorbent.

The reaction possesses a certain specificity, but just the degree of this specificity has not been agreed upon. The claim of Abderhalden[52] and his followers, that it is by far the most specific of immunity reactions, whereby disintegration of small amounts of any given organ of an individual can be determined by specific reactions between his serum and that organ, with such refinement that even cerebral localization[53] or prenatal determination of sex[54] is possible, is difficult to accept. There are so many possible sources of error in the original technic that even with great care the charge of incorrect results from incorrect technic cannot be escaped, and therefore, those who do not accept the doctrine of its specificity are always on the defensive. Nevertheless, so many careful and experienced investigators have found the original Abderhalden reaction to give at times absolutely non-specific and hopelessly paradoxical results, that its diagnostic value for either clinical or scientific purposes must be considered at present as unproved,[55] whatever the final decision as to its standing as a specific reaction may be.[56]

By far the most satisfactory results have been recorded in the diagnosis of pregnancy by means of placental antigen. This may be explained by the fact that the protease activity of the serum seems to be increased in pregnancy,[57] and hence the reaction with placenta is more marked than with the serum of non-pregnant individuals. But simply shaking normal serum with kaolin or other foreign substances may cause it to give strong reactions with placenta antigen (Wallis).[48]

[51] Denied by Abderhalden, Fermentforsch., 1921 (4), 338.

[52] A reply to numerous criticisms is given by Abderhalden, Fermentforschung, 1916 (1), 351; this and other numbers of this journal also consist largely of articles on the Abderhalden reaction.

[53] See Retinger, Arch. Int. Med., 1918 (22), 234.

[54] Sellheim, Klin. Woch. 1925 (4), 247, 299.

[55] See Högler and Serio, Wien. Arch. Inn. Med., 1924 (7), 571; Kupelwieser, Biochem. Zeit., 1924 (145), 492; Barikine and Zarodovsky, Ann. Inst. Pasteur, 1924 (38), 909; Wollman *et al.*, ibid., p. 115; Serejski, Biochem. Zeit., 1924 (152), 79.

[56] O. J. Elsesser (Jour. Infect. Dis., 1916 (19), 655), working in my laboratory with the purified vegetable proteins of Osborne, found that at best the specificity of the reaction was less than that of the anaphylaxis reaction, and there were many absolutely non-specific and irrational reactions. As these pure proteins furnish a much more appropriate material for studying specificity than the tissues or sera commonly used, it would seem that the results thus obtained are excellent proof of the uncertainty and unreliability of the reaction. Careful quantitative studies of the setting free of amino-acids by serum incubated with placenta by Van Slyke and his associates, also showed a complete lack of specific proteolysis by pregnancy serum (Arch. Int. Med., 1917 (19), 56; Jour. Biol. Chem., 1915 (23), 377; see also Hulton, *ibid.*, 1916 (25), 163).

[57] See Sloan, Amer. Jour., Physiol., 1915 (39), 9.

CHAPTER X

CHEMISTRY OF THE IMMUNITY REACTIONS (Continued IV). ANAPHYLAXIS, HYPERSENSITIVENESS, ALLERGY

In many instances the injection of a foreign protein into an animal produces severe, perhaps fatal, intoxication. With some proteins this natural toxicity is very marked; thus, eel serum is fatal to rabbits and dogs in doses of 0.1 to 0.3 cc. per kilo, and foreign sera are commonly toxic to other animals; *e. g.*, fresh bovine and human serum are toxic to guinea-pigs. This so-called "primary" toxicity is reduced or destroyed in most cases by heating to 56° for 30 minutes.[1] Almost any non-toxic soluble protein, however, may be made toxic for animals by giving the animal a small dose of this same protein at least eight days previously. This preliminary dose, which may be extremely minute,[2] renders the animal hypersensitive to the same protein, so that a relatively small quantity (a few milligrams in the case of the guinea-pig) of an otherwise entirely harmless protein, such as egg white or milk, produces violent, often fatal, symptoms when introduced into the blood of the animal. We shall not discuss the general features of the reaction, its history and its relation to biology and pathology which are fully covered in many easily accessible reviews,[3] but shall limit our consideration to the more definitely chemical aspects of the reaction.

The Substances Involved (Anaphylactogens).—As far as now known, these are always proteins, and with the exception of gelatin[4] and a few others, practically any soluble protein will produce sensitization and intoxication of susceptible animals, *i. e.*, almost any soluble protein may be an anaphylactogen. As with the other immunity reactions, observations have been made which are interpreted as indicating that non-protein substances can produce this reaction, but these interpreta-

[1] The nature of the toxic agent is unknown, but there is reason to believe that it is formed, at least in part, during the coagulation of the drawn blood.

[2] Julian Lewis has found that if a very small amount of protein is injected at the same time as a large dose of another foreign protein, no sensitization results to the former; presumably the available cell receptors are occupied by the protein given in larger amounts. (Jour. Infect. Dis., 1915 (17), 241.)

[3] Doerr, Ergebn. der Hyg. Bakt. Immun. u. Ther., 1922 (5), 71; Coca, Tice's Practice of Medicine, Prior and Co., New York, 1920, 107; Wells, Physiological Reviews, 1921 (1), 44; Seligmann and Gutfeld, Handbuch d. Biochem., 1924 (3), 152. Concerning anaphylaxis in man, see Longcope, Amer. Jour. Med. Sci., 1916 (152). 625. Concerning cutaneous reactions see Kolmer, Bull. Johns Hopkins Hosp., 1917 (28), 163. Concerning desensitization and antianaphylaxis see Longcope, Physiol. Reviews, 1923 (3), 240.

[4] Wells, Jour. Amer. Med. Assoc., 1908 (50), 527; Jour. Infect. Dis., 1908 (5), 459; Starin, Jour. Infect. Dis., 1918 (23), 139.

tions are not generally accepted. (See Antigens, Chap. VII.) It is possible, however, for non-protein substances to combine with or alter the proteins of an animal so that they become as foreign proteins to that animal, and thus cause sensitization; in this way can be explained apparent anaphylactic reactions to iodin and arsenic compounds and other non-protein substances.[5] As far as my own experiments show, nothing less than an entire protein molecule will suffice, the products of protein cleavage all being inactive.[6] Zunz[7] and Fink,[8] however report some slight positive results with proteoses. Possibly the inefficiency of gelatin as an anaphylactogen depends upon its deficiency in aromatic radicals, since these radicals have been shown (Vaughan, Obermeyer and Pick) to be particularly important in immunological reactions. It is not necessary for a protein to contain all the known amino-acids of proteins to be active, however, for certain vegetable proteins (zein, hordein, gliadin) which lack one or more of such amino-acids as glycine, tryptophan, or lysine, produce typical reactions. Some compound proteins are efficient anaphylactogens (mucin,[9] casein[10]) but with alpha-nucleoproteins which have been thoroughly purified I have obtained only negative results;[11] as also with histone and nucleic acid, the isolated components of nucleins.[12] Bacterial substances,[13] extracts of plant tissues, purified plant proteins, and proteins obtained from invertebrates and cold-blooded vertebrates, have all been found to be anaphylactogens, if they can be introduced by any means into the blood or tissues in a soluble unaltered condition.

If the proteins are rendered insoluble by coagulation they become inert, but proteins which cannot be made insoluble by heating (*e. g.*, casein, ovomucoid) withstand boiling temperatures. Trypsin destroys anaphylactogens in just the same proportion as it splits the protein molecules; thus, globulins resist trypsin longer than albumins, both as regards coagulability and anaphylactic activity. Acids, alkalies, and other chemical agents may modify the reactivity of proteins in proportion to the changes in solubility or constitution which they produce.[14]

[5] Landsteiner, Jour. Exp. Med., 1924 (39), 631.

[6] Abderhalden (Zeit. physiol. Chem,. 1912 (81) 314) states that he has obtained a positive reaction with a synthetic polypeptid containing 14 amino-acid molecules, including only leucine and glycocoll. E. Zunz (Jour. physiol path. gén., 1917 (17), 449) reports obtaining positive results with much simpler polypeptids (3–5 glycylglycine). These reactions consisted in changes in blood pressure and coagulability in rabbits, and it is not probable that typical shock can be obtained in guinea pigs with these peptids.

[7] Zunz (Zeit. Immunität., 1913 (60), 580).

[8] Jour. Infect. Dis., 1919 (25), 97 (Review on proteoses as antigens).

[9] Elliott, Jour. Infect. Dis., 1914 (15), 501.

[10] Wells and Osborne, Jour. Infect. Dis., 1921 (29), 200, review on milk proteins.

[11] See review in Zeit. Immunität., 1913 (19), 599, concerning alpha-nucleoproteins, which is the type usually designated as "nucleoproteins." I have found beta-nucleoproteins to be more effective antigens (Jour. Biol. Chem., 1916 (28), 11).

[12] Zinsser and Mueller (Jour. Exp. Med., 1925 (41), 159), have found that skin reactions of tuberculous animals are produced by the nucleoprotein fraction of tubercle bacilli or tuberculin.

[13] See Zinsser and Mallory, Jour. Immunol., 1924 (9), 75.

[14] See Dold and Aoki, Cent. f. Bakt., Ref. Beilage, 1912 (54), 246.

The amounts of protein necessary to produce reactions in guinea-pigs are very small. With crystallized egg albumin sensitivity has been produced with one twenty-millionth of a gram (0.00000005 gm.) and fatal reactions are obtained after sensitization with one-millionth of a gram. No other animal seems to be so sensitive to this reaction as the guinea-pig, however, and rabbits and dogs require larger, and in many instances, repeated doses to render them anaphylactic. Within certain limits large doses are less effective in sensitizing guinea-pigs than small, *e. g.*, one milligram of most proteins will usually be much more effective than one hundred milligrams. White and Avery[15] found that there is a certain relation between the minimum sensitizing and the minimum intoxicating dose; with extremely minute sensitizing doses a larger intoxicating dose is required to produce fatal reaction than when the sensitizing dose is larger.

The proteins concerned must be foreign to the circulating blood of the injected animal, but they may be tissue proteins of the same animal, (*e. g.*, placenta elements, organ extracts, lens proteins), which are not normally present in its blood. Indeed it has been claimed that by injecting a guinea-pig with the dissolved lens of one eye it will become sensitized so that it will react to a subsequent injection of the lens from the other eye.[16] In general, tissue proteins are less active antigens than the proteins of the blood, lymph, and secretions, probably because less soluble in the tissue fluids, but even keratins may produce anaphylaxis when dissolved[17] and positive results have been obtained with proteins from mummies.[18]

Both the sensitizing and intoxicating agent are (or are derived from) one and the same protein, but the minimum intoxicating dose is always larger than the minimum sensitizing dose; thus, with pure egg albumin the minimum lethal dose for sensitized pigs was one-twentieth to one-tenth milligram by intravascular injection, or about one hundred times more than the minimum fatal sensitizing dose. With less soluble proteins the disparity is even greater, for with such the sensitizing dose is not much changed, but the minimum intoxicating dose is relatively much increased. Apparently an animal may be killed by much less antigen than is required to saturate the antibodies present in its body (Weil). The exact fate of the injected antigens is unknown. Manwaring[19] observed no loss in antigen perfused through organs of sensitized animals, but others have found that antigen injected subcutaneously disappears more rapidly in sensitized or immunized than in normal animals,[20] presumably because fixed at the site of injection by the antibodies,[21] and a violent local inflammatory reaction may result (known as the Arthus phenomenon) which

[15] Jour. Infect. Dis., 1913 (13), 103.
[16] Uhlenhuth and Haendel, Zeit. f. Immunität., 1910 (4), 761.
[17] Krusius, Arch. f. Augenheilk., Supple., 1910 (47), 47; Clough, Arb. kais. Gesundhtsamte, 1911 (31), 431.
[18] Uhlenhuth, Zeit. f. Immunität., 1910 (4), 774.
[19] Jour. Immunol., 1917 (2), 511.
[20] G. H. Smith and Cook, Jour. Immunol., 1917 (2), 269.
[21] Opie, Jour. Exp. Med., 1924 (39), 659.

results in destruction of the foreign protein. Nevertheless, as yet we do not know what part, if any, anaphylaxis plays in protecting the body against bacterial infection.[22]

The Anaphylactic Antibody (Anaphylactin).—That anaphylaxis, like other immunity reactions, depends upon the presence of specific antibodies in the blood of the sensitized animal, is shown by the production of *passive anaphylaxis* in normal animals, by injecting into them a few cubic centimeters of blood or serum from a sensitized animal. Such animals become sensitive in a few hours to the specific antigen, no matter what species of animal furnishes the serum. The potency of a serum in conferring passive sensitization is in direct proportion to its content in precipitins, for which and other reasons it is believed that the antibody responsible for the anaphylaxis reaction is identical with the precipitin and presumably with the complement fixation antibody. (See Antibodies, Chap. VII). An animal sensitized by small doses of antigen may have no free antibodies in its blood and hence be unable to confer passive sensitization, although highly hypersensitive itself by virtue of antibodies present in the tissues.

In active sensitization the anaphylactin appears in the blood in appreciable quantities about eight days after the sensitizing injection, increases to a maximum between the 15th and 30th days, and then very slowly decreases. The reaction of antibody and antigen is strictly quantitative, as with all amboceptor reactions. The amount of antibody developed seems to be limited, for after a sensitized animal is given a sub-lethal intoxicating dose of protein it may be no longer sensitive to this protein, and this refractory or anti-anaphylactic condition persists for three weeks or more. It has been demonstrated especially conclusively by Weil and Coca,[23] that this refractory condition is, as Friedberger suggested, dependent upon saturation or exhaustion of all the anaphylactic antibodies, and hence the amount of these antibodies present free in the blood of a sensitized animal must be relatively small, for a few milligrams of the specific protein is sufficient to saturate them, *e. g.*, the amount of antibody present in 3 cc. of serum from a guinea-pig sensitized with horse serum could be neutralized with from 0.0005 to 0.01 cc. of horse serum.[24] The antibodies are, however, very persistent, remaining in guinea-pigs through the entire life of an animal sensitized when young. They also pass from the mother to the fetus, conferring a passive sensitization which, like passive sensitization from injection of serum from a sensitized animal, is of relatively brief duration, in contrast to the persistence of active sensitization.[25] Anaphylactin, like other amboceptors, resists heating at 56°

[22] See Mackenzie, Jour. Exp. Med., 1925 (41), 53, 65.
[23] Zeit. Immunität., 1913 (17), 141.
[24] Anderson and Frost, Jour. Med. Res., 1910 (23), 31.
[25] The brief duration of passive sensitization presumably depends on the formation of antibodies for the foreign sensitizing serum, constituting the condition of "anti-sensitization" as contrasted with the refractory period which results from the exhaustion of antibodies by antigen. (See Weil, Ziet. Immunität., 1913 (20), 199; 1914 (23), 1).

for one hour, and is salted out from serum in the globulin fraction.[26] It can be formed in animals made leukopenic with thorium-X.[27]

Weil[28] observed certain phenomena which led him to conclude that in anaphylaxis the specific antibody must be largely fixed in the cells, and that it is in the cells that the reaction occurs. Apparently the antibodies present in the blood of the sensitized animal are insufficient in amount to protect its cells from the foreign protein, hence the cellular intoxication. In support of this idea is the observation of Dale[29] that the isolated smooth muscle of sensitized guinea-pigs is specifically sensitized to the foreign protein. Weil states that "all the evidence proves conclusively that anaphylactic shock is induced by reaction between anchored antibody and antigen, and that circulating antibody plays absolutely no rôle in its production." Indeed, if there is an excess of free circulating antibodies in the blood the injected antigen is bound by them and produces no reaction in the tissues. The presence of such excess of free antibodies constitutes the condition of *antianaphylaxis*. Sensitization exists only when there is not enough free antibody in the blood to prevent reaction between antigen and fixed antibodies within the cells (Weil).

The anaphylactin shows quite the same characteristics of specificity as the other immune antibodies,[30] in that proteins of closely related species tend to interact, while proteins of very distinct biological or chemical nature are easily distinguished; thus, guinea-pigs sensitized with chimpanzee serum will react with human serum, but not with serum from dog, ox, or fowl. However, in the final analysis, the specificity depends upon the chemical composition of the antigenic protein, rather than its biological origin, for I have found it possible to distinguish in the hen's egg five distinctly different antigens, and these correspond to five proteins which have been distinguished by chemical measures.[31] Together with Dr. T. B. Osborne, working with purified vegetable proteins, I have found evidence that a single isolated protein (hordein or gliadin) may contain more than one antigenic radical.[32] Whether the chemical differences that determine specificity are of quantitative nature, which can be disclosed by analytic means, or whether they are sometimes dependent upon spatial relationships of the amino-acid radicals, as Pick suggests, remains to be determined. My own experience indicates that usually, at least, proteins distinguishable by anaphylactic reactions also show readily distinguishable chemical differences.[33] (See Specificity, Chap. VII.)

[26] However, Schiff and Moore state that in immune sera the albumin fraction contains both the agent that confers passive sensitization and the constituent that causes "primary toxicity" of foreign sera. (Zeit. Immunität., 1914 (22), 609).

[27] Corper, Jour. Infect. Dis., 1919 (25), 248.

[28] Jour. Med. Res., 1913 (27), 497; 1914 (30), 299–364; 1915 (32), 107.

[29] Jour. Pharm., 1913 (4), 167.

[30] See review in Jour. Infect. Dis., 1911 (8), 73.

[31] The chief proteins obtained by fractionating serum give cross reactions with each other, but strongest with the homologous protein (Kato, Mitt. med. Fak. Univ. Tokio, 1917 (18), 195.

[32] Jour. Infect. Dis., 1913 (12), 341.

[33] Jour. Immunol., 1924 (9), 291.

The Poisonous Agent (Anaphylatoxin).—The symptomatology of the intoxication which follows injection of the protein into an animal sensitized with the same protein, is such as to indicate that a poison is responsible, although as yet the poison has not been isolated. As the symptom complex is practically the same no matter what sort of protein is being used, it would seem that the poison must always be the same or similar—no matter how varied the nature of the proteins capable of inciting anaphylactic intoxication. Possibly the poison is a product of cleavage of protein by tissue or blood enzymes, which act only in the presence of the specific antibodies which unite the protein to the enzyme (or complement), for it is well known that the products of protein digestion exhibit more or less toxicity, with effects often resembling anaphylactic intoxication. Vaughan and his collaborators showed that proteins boiled with an alcoholic NaOH solution might be split into two fractions, one toxic and alcohol-soluble, the other non-toxic and insoluble in alcohol. The toxic fraction gives all the protein reactions (except that of Molisch for carbohydrates) and in doses of 8 to 100 gm. kills guinea-pigs with symptoms practically identical with those of anaphylactic intoxication. The uniformity of the toxic effects with preparations from different sorts of proteins suggests the existence in every protein molecule of some fundamental toxic group, common to all proteins, the specificity residing in other non-toxic attached groups. This and other observations led him to the hypothesis that specific enzymes are developed in response to the presence of foreign proteins in the blood stream, and that upon injection of a second dose of the same protein these enzymes at once disintegrate it, and some of the cleavage products being toxic the anaphylactic intoxication results. Many of the later developments in this field, especially Abderhalden's studies on "protective ferments," added support to this hypothesis, so that in its fundamental conceptions it became for a time the most generally favored explanation of the processes involved in anaphylaxis.[34] Friedberger carried the matter a step farther by showing that if serum from a sensitized animal is incubated for a short time with the antigen, and in the presence of enough complement, a poison is developed which produces the typical symptoms of anaphylactic intoxication when injected intravenously into guinea-pigs. This poison resists heating at 56°, but not at 65°, and is not a true toxin, for it will not produce an antitoxic immunity. In the absence of complement, or when the complement fixation is prevented by strong salt solution,[35] the poison (anaphylatoxin) does not develop; hence the anaphylactic reaction falls into the same class as the lytic reactions, in which the non-specific serum complement is united to a cell by the specific amboceptor, and then causes lysis of the cell. In anaphylaxis not an organized cell but a complex protein molecule is disintegrated by the complement, but in either case a poisonous substance

[34] See Vaughan, Amer. Jour. Med. Sci., 1913 (145), 161; Zeit. Immunität., 1911 (9), 458. Also a full review in his "Protein Split Products," Philadelphia, 1913.

[35] Friedberger's explanation of the inhibiting effect of salt as interference with complement action, has been questioned. (See Zinsser, Arch. Int. Med., 1915 (16), 238).

may be liberated. This agrees with Vaughan's hypothesis in ascribing the poisoning to products of protein disintegration formed by enzyme action, but differs in that specific intermediary substances or amboceptors are supposed to be developed by sensitization, rather than specific enzymes.

There is a growing belief that many or all the different immunity reactions depend upon a single antibody, the different reactions merely being different methods of demonstrating the presence of the antibody in the serum. The precipitin reaction differs from the anaphylactic reaction Friedberger contends, only in that in the latter the specific precipitate is dissolved by complement, yielding the anaphylatoxin. There are some objections to accepting this idea in its entirety which are discussed elsewhere (Chap. VII), but the formation of a poison by a digestive action of complement fixed to the antigen by the antibody, capable on intravenous injection of producing symptoms resembling those of anaphylaxis, seems to be well established as regards *in vitro* reactions.

It would seem probable that proteins may yield a similar poison in whatever way their hydrolysis is brought about, provided the cleavage is not too deep-seated. For example, Rosenow[36] found that pneumococci and other bacteria, permitted to autolyze for a proper length of time, produce poisonous substances with all the toxicologic characters of the anaphylatoxin. Too extensive autolysis again destroys the poison, which is also produced by digestion of pneumococci with serum from normal guinea-pigs, and more rapidly with serum from sensitized animals, which likewise causes a demonstrably more rapid proteolysis. The pneumococcus anaphylatoxic poison is soluble in ether and seems to be a base, containing amino-acids, but Friedberger did not find anaphylatoxin made from serum proteins to be soluble in ether or alcohol, nor was it precipitated with the globulins. The so-called "Abderhalden method" of sero-diagnosis of pregnancy, which depends on the presence of specific proteolytic properties in the blood, is an especially studied instance of these principles, and is discussed later.

Presumably anaphylactic intoxication is but the result of an exaggeration of the normal process of defense of the body against foreign proteins (including bacteria) by means of digestion. Normally this is accomplished in the alimentary tract, and complete disintegration past the toxic stage is made certain by the presence of erepsin in the intestinal wall; but if intact foreign protein molecules reach the blood in any way, this same digestive destruction is performed by the enzymes of the blood or tissues. So abnormal is the "parenteral" introduction of foreign proteins that, once it has happened, the protective mechanism is stimulated to the production of large amounts of proteolytic substances, and on this account if another quantity of the same protein is again parenterally introduced the breaking down of the protein is extremely rapid. Certain of the disintegration products are toxic, but with the normal rate of disintegration the amount present at any one time is inadequate to cause poisoning; when the proteolysis is accelerated, as in the sensitized animal, a poison-

[36] Jour. Infect. Dis., 1912 (11), 94 and 235.

ous dose may be produced, with the resulting anaphylactic intoxication. It has been found that the specific proteolytic power of the blood is increased in sensitized animals, but on the other hand, there is evidence that without the intervention of the liver (at least in dogs) anaphylactic intoxication cannot take place (Manwaring and others).[37] During the reaction, in any event, products of protein hydrolysis appear in the blood (Abderhalden),[38] but there is no demonstrable destruction or binding of the injected foreign protein.[19] Manwaring[39] has found that the blood from the liver of a dog in anaphylactic shock will produce the features of anaphylaxis in a normal dog, and from this and other observations deduces that at least canine anaphylaxis depends in part on toxic substances formed in the liver.

Among possible cleavage products of protein which might be the toxic agent in anaphylaxis, is β-imidazole ethylamine ("histamine *q. v.*"), which is derivable from histidine, and which produces effects resembling acute anaphylactic intoxication.[40] Not only does histamine cause marked fall in blood pressure, bronchial spasm in guinea-pigs and obstruction to the pulmonary circulation in rabbits,[41] but also when applied locally it causes marked urticaria resembling closely that of skin reactions in sensitized persons, and it does all these things in extremely minute dosage, comparable with the dosage of proteins used in anaphylactic reactions. Furthermore, its antecedent amino acid, histidine, is present in every known complete protein. However, the effects of histamine do not in all respects agree with and account for the manifestations of anaphylaxis. Methylguanidine is said to produce somewhat similar but slighter symptoms,[42] and to protect sensitized animals from toxic doses of the antigenic protein.[43] Other amines possibly may be involved. (See Chapter IV, Ptomaines; Chapter XXIII, Pressor Bases.)

The relation of the normal toxicity of certain foreign sera to anaphylactic intoxication has not been determined, but there seem to be both definite similarities and differences,[44] which have been discussed by Loewit;[45] chief of these differences is the absence of the bronchial spasm

[37] Falls found that a larger intoxicating dose must be injected into the portal system than by peripheral injection to produce the same effects. (Jour. Infect. Dis., 1918 (22), 83.)

[38] Zeit. physiol. Chem., 1912 (82), 109.

[39] Jour. Amer. Med. Assoc., 1924 (83), 2092.

[40] See Barger, "The Simpler Natural Bases," London, 1914, p. 30; Hanke and Koessler, Jour. Biol. Chem., 1920 (43), 521–579.

[41] See Dale and Laidlaw, Jour. Physiol., 1919 (52), 355.

[42] Heyde, Cent. f. Physiol., 1911 (25), 441; 1912 (26), 401.

[43] Burns, Jour. Physiol., 1918 (52), xxxix.

[44] It has been found that *organ extracts* are especially toxic to animals, but that this toxicity may be suppressed by a minute dose, for a few minutes later large doses can be injected with impunity, although the blood of the animal is highly toxic during the immune period, which is of brief duration. This condition is called *skepto-phylaxis.* (See Lambert, Ancel and Bouin, Compt. Rend. Acad. Sci., 1911 (154), 21.) Vaughan reports the finding in normal tissues of substances resembling his "protein poisons," which perhaps come from autolysis or tissue metabolism and may be related to the "primary toxicity" of organ extracts.

[45] Arch. exp. Path. u. Pharm., 1913 (73), 1. See also DeKruif and Eggerth, Jour. Infect. Dis., 1919 (24), 505.

with pulmonary emphysema which is characteristic of anaphylaxis in guinea-pigs.

There is, however, much doubt as to the identity of the process of anaphylatoxin formation (as it occurs when antigen, antibody, and complement are incubated *in vitro*) and the process of anaphylactic intoxication. In the first place, a poisonous character, apparently identical with this "anaphylatoxin" may be given to serum without the use of any specific antibody whatever; merely agitating fresh serum with any finely divided foreign material that offers large total surfaces, such as kaolin, agar,[46] or starch, is sufficient, as also is treatment with lipoid solvents, such as chloroform (Jobling).[59] In fact, merely the process of fibrin formation may make the resultant serum highly toxic, even for the very animal from which it came. Furthermore, if anaphylactic shock were the result of anaphylatoxin formation in the sensitized animal through the reaction of antigen with antibody and complement, the intoxication should occur if antibody and antigen are injected simultaneously into an animal; but as a matter of fact the animal receiving antibody in passive sensitization will not react unless the antigen is injected at least three hours after the sensitizing serum is injected.[47] This incubation period is supposed to be required for the anaphylactic antibody to be fixed in the cells where the reaction takes place (Otto), and perhaps in modification of the antibody so that it has a greater affinity for the antigen than it has while free in the serum (Weil);[48] or in acquiring the capacity to affect the cells after union with the specific antigen. Furthermore, it has never yet been proved that complement is a proteolytic ferment, and animals deprived as far as possible of complement will still give anaphylactic reactions. Finally, the isolated nonstriated muscle tissue (uterus) of a sensitized animal gives specific reactions when brought in contact with the specific antigen, no matter how thoroughly the animal's blood has been removed from the tissues; whereas, the uterine muscle of an animal injected with sensitizing immune serum only one hour before killing does not react when in contact with specific antigen. Weil disputes the toxic nature of anaphylaxis, even in the intracellular reaction, which he calls a "cellular discharge." He holds that in the guinea-pig the cellular reaction takes place chiefly in the nonstriated muscles, while in dogs the reaction is essentially hepatic, resulting in a profound congestion of the liver and consequent fall of blood pressure, decreased blood coagulability from hepatic action, and the increase in proteolytic products in the blood characteristic of all acute hepatic injuries.[49] Even in the guinea-pig, however, the liver is affected in anaphylactic shock, and Meinicke[50] adds to the evidence of cellular anaphylaxis by finding that the perfused liver of sensitized guinea-pigs reacts to antigen with a marked inhibition of its capacity to form urea from ammonium lactate, while Löhr[51] found that

[46] See Bordet, Compt. Rend. Acad. Sci., 1924 (179), 243.
[47] See Weil, Jour. Med. Res., 1914 (30), 87; Jour. Immunol., 1916 (1), 109.
[48] Jour. Med. Res., 1915 (32), 107.
[49] Jour. Immunol., 1917 (2), 525.
[50] Zeit. Immunität., 1918 (27), 489.
[51] Zeit. ges. exp. Med., 1923 (37), 442.

the ability of tissues to reduce nitro groups is increased during sensitization and decreased during shock.

Additional items of evidence against the identity of anaphylatoxin intoxication and anaphylactic intoxication are the following:

A sensitized animal may be made highly tolerant to anaphylatoxin by repeated injection without at the same time losing its sensitivity to the specific antigen. On the other hand, its desensitization by injection of the specific antigen does not render it refractory to anaphylatoxin. Therefore, it would seem that poisoning by anaphylatoxin cannot be responsible for the reaction that takes place when antigen comes in contact with sensitized tissues (Dale and Kellaway).[52]

In the anaphylatoxin experiments the existence of capillary embolism or endothelial intoxication has not ordinarily been excluded, and there is reason to believe that most of the observed symptoms are anaphylactoid from pulmonary embolism rather than anaphylactic.[53]

Anaphylatoxins produce symptoms which are not identical with those of true anaphylactic shock, and they do not act on isolated uterine muscle, but only exhibit their action in the presence of circulating blood, apparently through the formation of complexes which keep the colloids finely dispersed.

All attempts to demonstrate that the blood of animals in anaphylactic shock contains a poison responsible for the observed manifestation, have failed (see Weil).[54]

The effect of anaphylatoxin on the *p*H and surface tension of the blood is not the same as that seen in anaphylactic shock.[55]

The fact that, as Dale[56] points out, the specific antigen, when introduced into a bath containing the muscle strip, produces a reaction *immediately it comes in contact with the muscle*, and quite as promptly as a preformed, diffusible stimulant, such as histamine, pilocarpine or pituitary extract, is difficult to reconcile with the theory that the stimulating agent is liberated through proteolysis.

These numerous observations cited above seem to exclude not only the humoral site of the anaphylaxis reaction, but also to rule out the possibility of its dependence on even an intracellular digestion of a sensitized antigen by a ferment, whether serum complement or intracellular proteolytic enzymes.

Nevertheless, the formation of anaphylatoxin is an interesting phenomenon which may well be of importance in human intoxications, even if it is not the essential phenomenon of the anaphylactic intoxication. So readily is blood serum made toxic *in vitro* that it seems highly probable that a similar development of toxicity may take place in the body. It has also been suggested that a process similar to anaphylactic intoxication is responsible for traumatic shock, disintegration of traumatized tissue proteins being the source of the toxic agent.[57] (Quenu and Delbet, Cannon).[58]

Jobling[59] has found that intoxication resembling that from anaphylatoxin formation seems to occur when kaolin is injected intravenously into animals, and hence it is quite possible that the presence in the blood of abnormal, finely divided bodies, such as precipitated proteins, cellular

[52] Trans. Royal Soc. London, 1922 (211B), 273.
[53] See Hanzlik and Karsner.[11]
[54] Jour. Immunol., 1917 (2), 399.
[55] Zunz and La Barre, Compt. Rend. Soc. Biol., 1923 (89), 676.
[56] Jour. Pharm. Exper. Therap., 1913 (4), 167.
[57] Zunz (Soc. belge de biol., July 26, 1919) has suggested that toxic amines (*e. g.*, histamine) may be responsible.
[58] Review by Cannon, Archives Surg., 1922 (4), 1.
[59] Jobling, Petersen and Eggstein, Jour. Exp. Med., 1915 (22), 590.

fragments, even bacteria, may mechanically cause anaphylatoxin formation *in vivo* just as they do *in vitro*. It is necessary to distinguish, however, between the anaphylactoid symptoms that result from capillary embolism and true anaphylaxis, failure to do this undoubtedly having caused many erroneous conclusions.[60] Hanzlik[61] has reviewed the features of anaphylactic and anaphylactoid phenomena and, although recognizing the discrepancies between these conditions, believes that they rest on a common basis, which is the disturbance in the colloidal state of the blood and tissues, although the nature of these changes has not yet been recognized.

The mechanism of anaphylatoxin formation is not yet understood but there is no lack of theories. The original explanation was that anaphylatoxin formation by specific antisera is the result of digestion of antigen *in vitro* by the action of complement united to the antigen by the immune antibody. For the formation of anaphylatoxin by inert finely divided particles the explanation advanced was that the highly developed surfaces of these particles either activated complement, or united it to the serum proteins so that it digested them. Jobling[62] has advanced the hypothesis that normal serum antiferments, which are believed by him to be lipoidal in nature, are bound by the particles or by specific precipitates, so that the complement is free to attack the serum proteins. In any case, it is now generally agreed that *the poisonous substance is derived chiefly, if not entirely from the serum of the intoxicated animal, and not from the antigen*, even in the case of anaphylatoxin formation by specific antigen-antibody complement reactions.[63] This fact would seem to explain why the poison seems to be the same, as far as we can analyze it by pharmacological methods, no matter what protein is used as antigen, or whether produced by immune or by nonspecific reactions, or by chemical means, such as that of Vaughan.

Jobling,[62] who holds to the importance of anaphylatoxin formation as the cause of anaphylactic intoxication, presents the following conception of anaphylaxis: During the course of sensitization there occurs the mobilization of a nonspecific protease, which is greatly increased during acute anaphylactic shock; at this time there is also a decrease in antiferment which permits proteolysis of the animal's own proteins. As a result, there is to be found an increase in noncoagulable nitrogen and amino-acids of the blood, and a decrease in serum proteases. "The acute intoxication is brought about by the cleavage of serum proteins through the peptone stage by a non-specific protease. The specific elements lie in the rapid mobilization of this ferment and the colloidal serum changes which bring about the change in antiferment titer."

As a result of extensive studies, Novy and DeKruif have developed ideas concerning the nature of anaphylaxis quite different from those ordinarily held.[64] Following the observations of Bordet, and others, that incubation of fresh normal serum with agar renders it capable of producing symptoms resembling those of anaphylaxis

[60] See Hanzlik and Karsner, Jour. Pharmacol., 1920 (14), 379; 1924 (23), 173.

[61] Jour. Amer. Med. Assoc., 1924 (82), 2001; bibliography.

[62] Zeit. Immunität., 1914 (23), 71; Jour. Exp. Med., 1915 (22), 401.

[63] That the antigen must be digestible, however, is suggested by the observation of Ten Broeck (Jour. Biol. Chem., 1914 (17), 369) that proteins racemized by Dakin's method, which cannot be digested by proteolytic enzymes, are unable to cause anaphylaxis.

[64] Jour. Infect. Dis., 1917 (20); recapitulation in Jour. Amer. Med. Assoc., 1917 (68), 1524.

they have found reason to believe that the effects of bacteria may also depend on similar phenomena, rather than on hypothetical endotoxins. As little as 9 mg. of agar may produce fatal intoxication if injected in a suitable physical state intravenously into a guinea-pig. Since perfectly dissolved Witte peptone or even distilled water also produce anaphylatoxin when mixed with serum, it seems improbable that the anaphylatoxin formation depends on surface phenomena. Apparently anaphylatoxin formation is closely associated with the coagulation of the blood, which becomes highly toxic in the early stages of clot formation. No evidence could be found of protein cleavage or enzyme action during the formation of anaphylatoxin. It is thought that in specific anaphylaxis the substance that induces the formation of anaphylatoxin is formed by the interaction of the antigen with the antibody. The process of anaphylatoxin formation parallels closely that of fibrin formation, to which it may be related. Anaphylatoxin is believed to be not a proteose but a larger molecular complex than serum albumin, possessing certain globulin characteristics. It is associated with the euglobulin fraction of the serum.[65]

These and many other observations in the literature support the idea that a change in the degree of dispersion of the blood colloids may be the fundamental matter in anaphylaxis.[66] French investigators particularly have attempted to explain the manifestations of anaphylaxis as due to a disturbance in the colloidal equilibrium with partial flocculation of the plasma colloids and possibly of those of the tissues themselves. They refer to this supposed change as "colloidoclasie," and have developed numerous explanations of the phenomena of allergy and anaphylaxis as well as many proposed methods of treatment based upon this hypothesis.[67] However, many of the assumptions of the students of "colloidoclasie" are not in harmony with well attested observations on anaphylaxis, and apparently many of the manifestations discussed are anaphylactoid rather than true anaphylaxis.

The Chemical Changes in Anaphylactic Intoxication.—The anaphylactic poison would seem to be on the order of the alkaloidal poisons, at least from the pharmacological standpoint, since it produces its effects quickly, and these effects, no matter how severe, are strictly transitory, passing off completely in a few hours, which indicates that (like morphine, strychnine, etc.) they do not produce any deep-seated structural alterations in the tissues. According to Schultz[68] the chief effects are directly on the smooth muscles. Such anatomical alterations as are produced, of which hemorrhages and waxy degeneration of the voluntary muscles of respiration[69] are most noticeable, are ascribable to the effect on respiration, which in the guinea-pig often amounts to total asphyxiation through spasm of the musculature of the bronchioles (Auer and Lewis)[70] with profound permanent emphysematous distension of the

[65] DeKruif and Eggerth, Jour. Infect. Dis., 1919 (24), 505. They state also that in primarily toxic sera the toxic element is associated with the pseudoglobulin.

[66] See Kritchewsky, Jour. Infect. Dis., 1918 (22), 101.

[67] See Lumiére, "Theorie Colloidale de la Biologie et de la Pathologie."

[68] Bull. Hyg. Lab. U. S. P. H. and M. H. Service, 1912 (80), 1.

[69] See Cent. f. Pathol., 1912 (23), 945.

[70] In the rabbit the effects seem to be produced chiefly by spasm of the pulmonary arteries (Coca, Jour. Immunol., 1919 (4), 219) while the predominant hepatic and portal effects in dogs are attributed to the highly developed musculature of their hepatic veins by Simonds (Jour. Amer. Med. Assoc., 1919 (73), 1437). Manwaring, (Jour. Med. Assoc., 1923 (80), 303) considers increased capillary permeability to be an important feature of anaphylaxis.

lungs. This effect is peripheral, and is inhibited by atropine.[71] Calcium salts also reduce anaphylactic reactions.[72] The poisonous fraction obtained from proteins by Vaughan's method resembles anaphylatoxin in that it causes a fall in blood pressure by paralyzing the vasomotor endings in the blood vessels (Edmunds[73]). It also produces local urticaria when rubbed into the skin and behaves much like histamine, with which, however, it is not identical. One gram of casein yields enough of Vaughan's poison to kill 800 guinea-pigs, and the poison seems to contain most of the aromatic radicals of the proteins. There is also much other evidence of the importance of the aromatic radicals in anaphylaxis.[74]

Other effects of the anaphylactic intoxication are leucopenia,[75] local and general eosinophilia,[76] acidosis,[77] hyperglucemia with increased lactic acid,[78] an increase in blood chlorides[79] associated with anhydremia[80] but with a decrease in blood calcium, (especially of the diffusible calcium fraction),[81] reduced coagulability of the blood,[82] and a severe fall of temperature unless the dose of antigen is very small when the temperature may rise.[83] The antitrypsin content of the blood is not increased in the anaphylactic animal (Ando[84]). Poisonous substances similar to anaphylatoxin appear in the urine during the anaphylactic intoxication (Pfeiffer).[85] As with other poisons, the anaphylactic poison produces different symptoms in different animals.[70] In dogs the chief effects are a great fall in blood pressure without bronchial spasm,[86] loss of coagulability of the blood, hemorrhagic enteritis, enormous engorgement of the liver with blood (Weil),[87] loss of glycogen from the liver.[88] Manwaring[70] has found that if the lungs of a dog sensitized to horse serum are perfused with a saline solution containing horse serum there occurs a tremendous pulmonary edema, and he believes this increased permeability of the capillaries to be the dominant fundamental change in anaphylaxis reactions. He has attributed the effects of anaphylaxis to some substance liberated from the liver, exhibiting a histamine-like action on

[71] Jour. Exp. Med., 1910 (12), 127.
[72] Kastle, Healy and Buckner, Jour. Infect. Dis., 1913 (12), 127.
[73] Zeit. Immunität., 1913 (17), 105. See also Underhill and Hendrix, Jour. Biol. Chem., 1915 (22), 465.
[74] See Baehr and Pick, Arch. Exp. Path., 1913 (74), 73.
[75] Because of accumulation of leucocytes in the pulmonary circulation (Webb, Jour. Path. and Bact., 1924 (27), 79).
[76] Literature by Moschowitz, New York Med. Jour., Jan. 7, 1911; Schlecht and Schwenker, Arch. exp. Path. u. Pharm., 1912 (68), 163; Deut. Arch. klin. Med., 1912 (108), 405.
[77] See Bigwood, Compt. Rend. Soc. Biol., 1924 (91), 375.
[78] Zunz and La Barre, ibid., 1924 (91), 121.
[79] *Ibid.*, p. 802.
[80] Descamp, *ibid*, p. 894.
[81] Blum *et al.*, Compt. Rend. Acad. Sci., 1924 (179), 1294.
[82] See Bulger, Jour. Infect. Dis., 1918 (23), 522.
[83] See Vaughan, *et al.*, Zeit. Immunität., 1911 (9), 458.
[84] Zeit. Immunität., 1913 (18), 1.
[85] Zeit. f. Immunität., 1911 (10), 550.
[86] Probably from influence upon the nerve endings of the vessels (Pearce and Eisenbrey, Jour. Infect. Dis., 1910 (7), 565).
[87] See Dean and Webb, Jour. Path. and Bact., 1924 (27), 51.
[88] O'Neill, Manwaring and Moy, Jour. Amer. Med., Assoc., 1925 (84), 1102.

the vessels. In rabbits the heart is severely affected, through spasm of the pulmonary arteries,[89] while in guinea-pigs there is a remarkable lack of interference with the heart, so that it beats long after respiration ceases. A pressor substance has been found in the serum of intoxicated guinea-pigs, which is not present in the artificial anaphylatoxin and therefore presumably is produced in the body of the animal,[90] and there is evidence of altered permeability of the endothelium.[91] In man the symptoms are most like those in the guinea-pig. If the antigen is injected into the skin of a sensitized animal there follows a severe local reaction—hyperemia, edema, even necrosis, constituting the Arthus phenomenon[92]—indicating that in this specific proteolysis, poisons are formed which have a profound local effect, especially on the blood vessels. Repeated anaphylactic intoxication may result in structural changes in the kidneys, heart muscle, and liver (Longcope[93]). Metabolism studies may show an increased toxicogenic destruction of protein,[94] but the increase in amino acids, presumably resulting from proteolysis in the sensitized individual, is not large enough, if it does occur, to be significant.[95] However, in anaphylaxis in guinea pigs, as well as after peptone poisoning, there is a considerable increase in noncoagulable and urea nitrogen in the blood, as well as a slight increase in creatinine and amino nitrogen, but it is not known whether this comes from the tissues or from the antigen-antibody reaction in the blood,[96] although the former seems more probable.

The gas metabolism of the animal as a whole, and its tissue respiration, are decreased in true anaphylactic shock.[97] The asphyxia of anaphylaxis is associated with marked acidosis,[98] and when the carbon-dioxide combining capacity of the blood falls below 25 volume per cent. the animal usually dies. With this acidosis is a demonstrable fall in the *p*H of the plasma and a decrease in the surface tension.[99] However, according to Mendeleef[1] the changes in *p*H in anaphylactic shock are not always downward. Numerous other changes have been described in the blood of animals dying in anaphylactic shock,[2] including an increase in the total proteins, with alterations in their colloidal behavior so that they are more precipitable by various means; also marked reduction in the number of

[89] See Drinker and Bronfenbrenner, Jour. Immunol., 1924 (9), 387.
[90] Hirschfeld, Zeit. Immunität., 1912 (14), 466.
[91] See Fleisher and Mayer, Jour. Immunol., 1924 (9), 319.
[92] See Opie, Jour. Immunol., 1924 (9), 231–268.
[93] Jour. Exp. Med., 1913 (18), 678; 1915 (22), 793; also Boughton, Jour. Immunol., 1916 (1), 105; 1919 (4), 213. Not confirmed by Bell and Hartzell, Jour. Infect. Dis., 1919 (24), 618.
[94] See Major, Deut. Arch. klin. Med., 1914 (116), 248.
[95] See Auer and Van Slyke, Jour. Exp. Med., 1913 (18), 210; Barger and Dale, Biochem. Jour., 1914 (8), 670; Major, Bull. Johns Hopkins Hosp., 1923 (34), 104.
[96] See Hisanobu, Amer. Jour. Physiol., 1920 (50), 357.
[97] Abderhalden and Wertheimer, Pflüger's Arch., 1922 (195), 487.
[98] Eggstein, Jour. Lab. and Clin. Med., 1921 (3), 555; Hirsch and Williams, Jour. Infect. Dis., 1922 (30), 259.
[99] Zunz and La Barre, Compt. Rend. Soc. Biol., 1923 (88), 990; Ibid., 1924 (90), 658.
[1] P. Mendeleef, Arch. Internat. physiol., 1923 (21), 15.
[2] Wittkower, Klin. Wochenschr., 1923 (2), 450.

leucocytes and platelets and in the coagulability of the blood, together with some increase in the refractive power, but not in the rotatory power.[3] There has also been described a slight increase in the blood sugar (Hirsch and Williams)[98] and a slight and irregular decrease in sodium, potassium, magnesium, and calcium.[4]

There is evidently a marked change in endothelial permeability in anaphylactic intoxication, as shown by the local edema and an augmented flow of thoracic lymph containing an increased protein content, the globulin and fibrinogen being especially increased when the shock is severe.[5] If the phagocytic endothelial cells of the body as a whole are occupied by previous injections of finely divided suspensions, such as oxide of iron[6] or india ink,[7] the animal is protected from anaphylatic shock, indicating the importance of these cells in anaphylaxis as in other immunity reactions, but as yet it is not clear how this occupation of the reticulo-endothelium by phagocyted particles modifies anaphylactic shock.

HAY-FEVER

In 1873, C. H. Blackley[8] demonstrated conclusively that typical hay-fever, in its several forms, is due to reaction to pollen of various sources, but this important observation was largely neglected, until the work was confirmed by Dunbar in 1902. Since that time the anaphylactic character of the disease has been demonstrated by Koessler, Walker, Cooke, Coca and many others. In Germany the disease seems to come chiefly from pollen of the grasses and grains (rye pollen being most active), whereas in America the most important pollen seems to come from members of the *Ambrosia* (ragweed)[9] and *Graminaceae* (grasses).

Aside from the features of anaphylaxis, the chief chemical interest lies in the chemistry of the pollens. Dunbar found that the toxic constituent could be dissolved from the pollen in salt solution, and seemed to be a protein. The protein constituents of the pollen of rye were studied further by Kämmann,[10] who found three proteins, one of which, an albumin, was found to contain all the active matter. This constitutes about 5.5 per cent. of the entire weight of the pollen, is weakened but little by heating to 80°, and is not destroyed by boiling; it is but partly destroyed by pepsin and trypsin, and resists acids but not alkalies. Analysis of pollen from *Ambrosia* by J. H. Koessler[11] showed that most of the nitrogen present is protein nitrogen, with 14.72 per cent. arginine, 14.05 per cent. histidine and 3.18 per cent. lysine. Heyl[12] obtained from *Ambrosia* pollen a mixture of albumose, peptone, albumin and glutelin, the latter being most abundant. The albumin seems to lack histidine, which is present in the glutelin.[13] A solution containing 0.008 mg. of pollen protein, which amount is contained in two or three pollen grains, produces a reaction in susceptible individuals, but large amounts have no effect on normal persons.

Dunbar manufactured an "antitoxic" serum by immunizing horses against the pollen, believing that he was dealing with a toxin, but its efficacy is more than doubtful.

[3] Abderhalden and Wertheimer, Pflüger's Arch., 1922 (197), 85.

[4] Azzi, Arch. Sci. Med., 1922 (45), 356; La Barre, Compt. Rend. Soc. Biol., 1924 (91), 1293.

[5] Petersen and Levinson, Jour. Immunol., 1923 (8), 323; Petersen and Hughes, Jour. Biol. Chem., 1925 (63), 179.

[6] Petersen *et al.*, Jour. Immunol., 1923 (8), 367.

[7] Moldovan and Zolog, Compt. Rend. Soc. Biol., 1923 (89), 1242.

[8] Full review of subject and literature given by Koessler, Forchheimer's Therapeutics, 1914 (5), 671; Walker, Oxford Medicine, 1920 (2), 243.

[9] See Cooke and Van der Veer, Jour. Immunol., 1916 (1), 201; Goodale, Boston Med. Surg. Jour., 1914 (171), 695.

[10] Hofmeister's Beitr., 1904 (5), 346; Biochem. Zeit., 1912 (46), 151.

[11] Jour. Biol. Chem., 1918 (35), 415.

[12] Jour. Amer. Chem. Soc., 1917 (39), 1470; 1919 (41), 670.

[13] Heyl and Hopkins, Jour. Amer. Chem. Soc., 1920 (42), 1738.

The processes involved in hay fever are characteristically of the nature of an anaphylactic reaction to a foreign protein, in which case we cannot speak of either a toxin or an antitoxic serum. The hypersensitization seems sometimes to be established spontaneously through inheritance, but no antibodies can be demonstrated in the blood of sensitized persons, although the cells of the skin and mucous membranes are reactive,[14] so that the specific protein responsible for the trouble may be determined by skin or conjunctival tests. Essentially, therefore, hay fever is one of the cases of hypersensitivity to foreign protein, of the same class as horse asthma, food urticarias, etc.

[14] See Cooke, Flood and Coca, Jour. Immunol., 1917 (2), 217.

CHAPTER XI

HEMOLYSIS[1] OR ERYTHROCYTOLYSIS

In hemolysis the essential phenomenon consists in the escape of the hemoglobin from the stroma of the corpuscles into the surrounding fluid. As it is not exactly known in what way the stroma holds the hemoglobin normally, whether purely physically[2] or in part chemically, or whether the stroma consists of a spongioplasm, the ultimate processes that permit the escape of the hemoglobin are not finally solved. However, the agents by which the escape is brought about are well known and extensively studied, and they are found to be of extremely various natures. They may be roughly classified as: (1) known physical and chemical agents; (2) unknown constituents of blood-serum; (3) bacterial products; (4) certain vegetable poisons; (5) snake venoms.

Hemolysis by Known Chemical and Physical Agencies

The Mechanism of Hemolysis.—If distilled water is added to corpuscles of any kind, osmotic changes are bound to occur, since within the cells are abundant salts, soluble in water, which will begin to diffuse outward in an attempt to establish osmotic equilibrium between the corpuscles and the surrounding fluid. Conversely, water enters the corpuscles at the same time, and accumulating there leads to swelling until such injury has been produced as permits the hemoglobin to escape and enter the surrounding fluid. Before this occurs the fluid is opaque because of the obstruction to light offered by the red cells, but on the completion of hemolysis the fluid becomes transparent. The stroma now settles to the bottom, while the hemoglobin diffuses into the fluid, making it red, but perfectly transparent. This process has long been known as the "laking" of blood, and is essentially the condition present in all forms of hemolysis. That the hemoglobin escapes only through injury of the stroma and not through simple osmotic diffusion, is shown by the fact that if salt solution of the same concentration as normal serum is used

[1] Through usage this term has been limited to the solution of the red corpuscles, which is more accurately described by the term *erythrocytolysis*. For bibliography see Sachs, Ergebnisse der Pathol., 1902 (7), 714; 1906 (11), 515; Kolle and Wassermann's Handbuch, 1913 (II), 793; Landsteiner, Handbuch d. Biochem., 1909 (II (1),) 395.

[2] Gough (Biochem. Jour., 1924 (18), 202) says that the red corpuscle is a fluid droplet in which the continuous phase is salts and water and the disperse phase is hemoglobin, salts and water, with solid lipoids in the surface film. Rockwood (Jour. Lab. Clin. Med., 1924 (10), 19) believes that the apparent cell surface is unimportant in hemolysis, and that stromatolysis is more or less independent of hemolysis, although the hemoglobin seems to be held to the stroma by adsorption only.

instead of distilled water, no such escape of hemoglobin occurs. As hemoglobin is perfectly soluble in salt solution, it should pass out if it diffuses as do the salts. Since there is no escape of hemoglobin in such a salt solution, it is evident either that the stroma is not permeable to hemoglobin, or else the hemoglobin is in some way attached to or combined with the stroma. Again, if the corpuscles are placed in a solution of salt more concentrated than their own fluids, water escapes and the corpuscles shrink; as no hemoglobin escapes with the water, it is evident that the stroma is not permeable to hemoglobin when intact.

Because of the resemblance of the process of hemolysis to the rupture of plant cells with escape of their contents when they are placed in distilled water, it might be assumed that hemolysis is largely a physical matter, but if a red corpuscle in an isotonic solution is cut into pieces, the hemoglobin does not escape, indicating that its structure is quite dissimilar to that of the simple vegetable cell and that there is some union of stroma and of hemoglobin, either physical or chemical.[3] Physico-chemical studies also indicate that there is no true covering membrane to red corpuscles, for the adsorption of ions by hemoglobin is the same as the adsorption of corpuscles.[4] M. H. Fischer[5] interprets hemolysis as a separation of lipoid-protein stroma and adsorbed hemoglobin, which process can be duplicated experimentally with a combination consisting of a corresponding solid hydrophilic colloid, fibrin, and a hydrophobic colloid dye, carmine; this artificial combination behaves exactly like a corpuscle to simple hemolytic agents.[6]

Bechhold's[7] observations also support the view that the stroma behaves as a protein mesh holding by adsorption a homogeneous mixture of cholesterol and lecithin. Apparently the swelling or precipitation of any of the components of this complex colloidal mixture is capable of loosening the entire structure.[8] According to Bechhold this may be brought about by:

(*a*) Adsorption of the lipoid mixture away from the protein framework (*e. g.*, shaking with inorganic suspensions).

(*b*) Unequal alterations in the state of hydration of the lipoid mixture and the protein framework by physical means (*e. g.*, freezing, heating); or by chemical means (*e. g.*, heavy metals).

[3] Stewart (Jour of Physiol., 1899 (24), 211) found that in hemolysis by physical means or under the influence of serums, there is no marked increase in the electrical conductivity, but hemolysis by saponin and by water causes an increase of conductivity, presumably because of the escape of electrolytes; corroborated by A. Woelfel, Biochem. Jour., 1908 (3), 146; see also Moore and Roaf, *ibid.*, p. 55.

[4] Rohonyi, Kolloid-chem. Beihefte, 1916 (8), 337, 391. Knaffel-Lenz (Arch. ges. Physiol., 1918 (171), 51) also finds evidence that there is no limiting lipoid membrane about red cells. However, Gorter and Grendel (Jour. Exp. Med., 1925 (41), 439) believe that the erythrocytes are covered by a layer of fatty substance that is two molecules thick, much as the molecules of a soap bubble are arranged.

[5] Kolloid Zeit., 1909 (5), 146.

[6] Concerning the influence of H-ion concentration on hemolysis see Walbum, Biochem. Zeit., 1914 (63), 221.

[7] Münch. med. Woch., 1921 (68), 127.

[8] Neuhausen and Breslin, Bull. Johns Hopkins Hosp., 1923 (34), 199.

(*c*) The breaking up of the mixture of lecithin and cholesterol (*e. g.*, water and hypotonic salt solution).

(*d*) Solution of the lipoids (*e. g.*, ether, bile salts).

(*e*) Action of agents which coagulate the lipoids, or dissociate them from their existing unions, or go into combination with them (*e. g.*, saponin, cobra venom, tetanolysin, etc.).

Just where in this scheme specific hemolysis by immune serum belongs has not yet been determined.

The fact that chloroform, ether, bile salts, soaps,[9] and amyl alcohol will cause laking is probably intimately connected with the fact that lecithin and cholesterol, important constituents of the stroma, are both soluble in these substances.[10] In general it can be said that hemolytic agents dissolve lipoids or hydrolyze proteins or lipoids, thus destroying the power of the stroma to retain the hemoglobin.[11]

Nearly all the non-specific hemolytic agents are inhibited to greater or less degree by the serum, in which inhibition both the proteins and cholesterol are concerned.[12] Possibly the calcium of the serum has an important function in preventing the hemolytic action of fatty acids and soaps, although the lipins and proteins probably also play a part in the protective action of serum.[13] In human anemias the serum has a decreased power to protect corpuscles against hemolysis by soaps, but it is not known on what this deficiency depends. The serum proteins seem to have little or no protective effect against saponin hemolysis, but may be of more importance in protection against hemolysis by sodium oleate, a property which is reduced especially in cancer.[14]

The resistance of the corpuscles to hemolysis by various agents differs greatly in disease, although fairly constant in normal blood, the differences being caused in some cases by changes in the permeability of the corpuscles, and sometimes by changes in the environment of the corpuscle or in the quantity of protective substances in either the corpuscles or the plasma. Brinkman[15] finds that the resistance of different corpuscles in a given sample of blood, shows variations normally, about 80 per cent. exhibiting an equal osmotic resistance, 10 per cent. being much less resistant (old cells) and 10 per cent. more resistant (young cells). Washing a suspension of red cells may increase the resistance by removing hemolysing lipoids (probably phosphatids) from the surface of the corpuscles. Increasing the phosphatids of the blood reduces the resistance of the corpuscles,[16] which effect can be neutralized by increasing the cholesterol; in fact, lecithin increases the hemolytic action of many substances, not only venoms, but quinine and many other substances.

[9] See Ponder, Quart. Jour. Exp. Physiol., 1924 (14), 67; Biochem. Jour., 1924 (18), 845.

[10] See Koeppe, Pflüger's Arch., 1903 (99), 33; Peskind, Amer. Jour. Phys., 1904 (12), 184; Moore, Brit. Med. Jour., 1909 (ii), 684.

[11] See Herzfeld and Klinger, Biochem. Zeit., 1918 (87), 36.

[12] See v. Eisler, Zeit. exp. Path., 1906 (3), 296.

[13] Clark et al., Bull. Johns Hopkins Hosp., 1921 (32), 113 and 328.

[14] See Decker, Zeit. ges. exp. Med., 1924 (38), 174.

[15] Arch. néerland. physiol., 1922 (6), 451.

[16] See Frieda, Zeit. Immunität., 1924 (40), 69.

Under certain conditions hemolysis is reversible, *i. e.*, the hemoglobin which has been separated from the stroma may reattach itself, an observation now repeatedly confirmed (see Rockwood[2]). This observation is in full agreement with the hypothesis that the red corpuscle owes its character to a stroma to which hemoglobin is held by adsorption.[17]

Hemolysis by Physical Means.—Repeated alternate freezing and thawing is a simple physical means of bringing on hemolysis. Heating to 62°–64° C. causes hemolysis of mammalian corpuscles; in cold-blooded animals this seems to occur at slightly lower temperatures. Sufficiently hypertonic solutions produce hemolysis, and it may be that freezing and desiccating cause hemolysis through the resulting hypertonicity.[18] Finely dispersed inorganic suspensions are hemolytic only if they have sharp irregular surfaces[19] or are strongly agitated with the corpuscles, and hence their action is probably mechanical. However, contact even with clean glass facilitates hemolysis.[20] In the presence of photosensitive substances, such as eosin, light has a strong hemolytic action,[21] which possibly depends on the oxidation of aromatic radicals in the proteins of the stroma of the corpuscles.[22]

Hemolysis by Chemical Agents.—Some chemical agents are capable of liberating hemoglobin, even when the corpuscles are in isotonic solutions. The ordinary salts of serum, of course, do not have this property, but ammonium salts are strongly hemolytic. The chemical agents that dissolve red corpuscles seem to be those that have the power of penetrating the stroma. Ammonium salts and urea penetrate the corpuscles freely and cause hemolysis. Sugar and NaCl seem not to penetrate the corpuscles, and therefore do not produce hemolysis. Of the permeating substances, there seem to be two types: one, like urea, does not produce hemolysis when in a solution of NaCl isotonic with the serum; the other, like ammonium chloride, is not prevented from producing hemolysis by the presence of NaCl.[23]

All these agents seem to effect hemolysis *by acting on the stroma*, for when the stroma of corpuscles hardened in formalin has its lecithin and cholesterol removed with ether, *saponin*, a powerfully hemolytic substance,

[17] L. E. Bayliss does not accept the occurrence of reversible hemolysis (Jour. Physiol., 1924 (59), 48).

[18] Guthrie, Jour. Lab. Clin. Med., 1917 (3), 87.

[19] Friedberger and Putter, Zeit. Immunität., 1920 (30), 227.

[20] Fenn, Jour. Exp. Med., 1922 (35), 271.

[21] Literature given by Pfeiffer and Bayer, Zeit. exp. Med., 1921 (14), 137.

[22] Schmidt and Normal, Jour. Gen. Physiol., 1922 (4), 681; see also Testoni (Arch. sci. biol., 1923 (4), 123), who does not agree with these authors.

[23] Hamburger, in his book, "Osmotischer Druck und Ionenlehre," reviews exhaustively the physical chemistry of hemolysis. The following is his summary of the permeability of red corpuscles by various substances:

Organic Substances.—(*a*) *Impermeable* for sugars; namely, cane-sugar, dextrose, lactose, also arabit and mannit. (*b*) *Permeable* for alcohols, in inverse proportion to the number of hydroxyl groups that they contain; also for aldehydes (except paraldehyde), ketones, ethers, esters, antipyrin, amides, urea, urethan, bile acids and their salts. (*c*) *Slightly permeable* for neutral amino-acids (glycine, asparagin, etc.).

Inorganic substances, not including the salts of the fixed alkalies. (*a*) *Completely impermeable* for the cations Ca, Sr, Ba, Mg. (*b*) *Permeable* for NH_4 ions, for free acids and alkalies.

seems to have no effect. The action of saponin and of many other hemolytic agents can be prevented by the presence of *cholesterol* in excess, suggesting that it is this constituent of the stroma that is affected.[24] By studying hemolysis under dark field illumination, Dietrich[25] found that in water hemolysis, a diffusion of hemoglobin takes place through the corpuscular substance, which is not visibly altered; in hemolysis by immune serum there is first a precipitate formed in the outer layer, which swells. There is no evidence that the erythrocytes contain proteolytic enzymes of their own that might disintegrate them.[26]

Arseniuretted hydrogen, when inhaled, causes intravascular hemolysis, and there are many other drugs and chemicals with hemolytic properties, among which may be mentioned nitrobenzol, nitroglycerin and the nitrites, guaiacol, pyrogallol, acetanilid, and numerous aniline compounds. Probably the hemolysis produced by autolytic products belongs in his category.[27] Alcoholic extracts of tissues are commonly hemolytic; these extracts when added to serum take on properties which cause them to resemble closely hemolytic complement (Noguchi), and the soaps seem to be the active constituents of the extracts. AsH_3, although strongly hemolytic in the living body, does not hemolyze corpuscles in the test tube (Heffter), and this is true of some other poisons, which probably produce their effects through tissue changes.[28] The bile acids and their salts will also produce hemolysis, as seen in jaundice.[29] Sodium bicarbonate solutions of one or two per cent. are hemolytic for some varieties of corpuscles, but 0.1 per cent. Na_2CO_3 and $NaHCO_3$ do not cause hemolysis. A study of the hemolytic properties of one class of lipolytic hemolytic agents, the terpenes, shows that their hemolytic activity varies much according to their physical properties, generally decreasing directly with increase in the solubility in water (Ishizaka).[30]

Hemolysis by salts of alkalies and alkali earths shows a constant difference in the effects of both the anions and the cations, independent of the osmotic pressure of the solutions, indicating that the ions have specific effects *per se*,[31] which depend largely on the *p*H of the blood and the electrical charge of the corpuscles. Haffner[32] found that electrolytes acting on red corpuscles, in general were lytic in small concentrations and agglutinating in high concentrations, but NaCl is often inhibitory. Agglutination by salts in low concentrations depends on a flocculation of the stroma at the isoelectric point, which is associated with an increase

[24] Ransom, Deut. med. Woch., 1901 (27), 194; Kobert, "Saponinsubstanzen" Stuttgart, 1904; Abderhalden and Le Count, Zeit. exp. Path. u. Ther., 1905 (2), 199. Noguchi (Univ. of Penn. Med. Bull., 1902 (15), 327) found lecithin to be without this property.

[25] Verh. Deut. Path. Gesell., 1908 (12), 202.

[26] Von Roques, Biochem. Zeit., 1914 (64), 1.

[27] Concerning hemolysis by alcohols, ketones, etc., organic acids, and essences, see Vandevelde, Bull. Soc. Chim. de Belgique, 1905 (19), 288.

[28] Friedberger and Brossa, Zeit. Immunität., 1912 (15), 506.

[29] See Ponder, Proc. Roy. Soc. London, 1922 (93*B*), 86.

[30] Arch. exp. Path., 1914 (75), 195.

[31] Beckmann, Biochem. Zeit., 1923 (135), 317.

[32] Arch. ges. Physiol., 1922 (196), 15.

in resistance to hemolysis. Evidently, then, the stroma behaves like a hydrophile colloid, similar to globulin. The lysis by electrolytes seems to depend on adsorption of ions which increase the hydration of the hemoglobin.

Leucocytes are also dissolved by some of these hemolytic agents, particularly the bile salts, although they are affected by no means so rapidly or so much as are the erythrocytes. There seems to be no relation between the erythrolytic and leucolytic powers of these substances. Water causes swelling, with solution of the granules in time, and the same is true of ammonium-chloride solutions.

Various chemicals cause morphological alterations in the leucocytes, and of bacterial products the toxins of pyocyaneus, pyogenic staphylococci,[33] and diphtheria seem to be particularly leucocidal, causing a marked karyorrhexis (Schürmann).[34]

Hemolysis by Immune Serum

Normal blood-serum of many animals causes hemolysis to greater or less degree when mixed with red corpuscles of another species of animal,[35] and this property can be greatly increased by immunizing the animal with red corpuscles in the usual way. This hemolysis by serum occurs both in the test-tube and in the body, in the latter case causing severe anatomical changes or even death. In all respects *the mechanism of hemolysis by immune serum seems to be identical with that of bacteriolysis.* Two substances are concerned, one the *amboceptor*, which resists heat and which is increased by immunizing,[36] the other, *complement*, which is destroyed at 55° and which is present in normal serum. In this case the substances may be referred to as hemolytic amboceptors and hemolytic complements. (See discussion of amboceptor-complement reactions in Chap. IX.)

In spite of the availability of these particular cytolytic substances for study, very little has been learned of their exact nature and properties. It is known that amboceptor is combined with the red cells in a certain sense quantitatively, a definite amount being required to saturate a given amount of corpuscles so that they will all be hemolyzed when complement is added; and that this reaction is complete in less than fifteen minutes at 45°. What change this addition of amboceptor brings about in the corpuscles is unknown. It has also been shown that at 0° the affinity between the amboceptor and the corpuscle is greater than it is between amboceptor and complement, so that it is possible at this temperature to remove all the amboceptor from a serum by treating it with red corpuscles, and thus we can obtain complement free from amboceptor. This experiment also shows that the two bodies exist side by side in

[33] Tobler, Zeit. Kinderheilk., 1924 (37), 354.

[34] Cent. f. Pathol., 1910 (21), 337.

[35] The normal hemolytic activity of serum may depend on the fatty acids and soaps (Brinkman and Szent-Györgyi, Proc. Acad. Sci. Amsterdam, 1923 (26), 470).

[36] In an extensive study of the hemolytic antibody, Thiele and Embleton (Zeit. Immunität., 1913 (20), 1) describe its formation as in several steps, at first being thermolabile and uniting with the corpuscle only when warmed. They also find complement to have several components. This is not confirmed by Sherman, Jour. Infect. Dis., 1918 (22), 534.

the serum without combining, and that combination occurs only after the amboceptor has become united to the erythrocyte. Moreover, the hemolytic amboceptor can be separated from the antigen to which it has been combined.[37] Hemolysis by immune sera takes place best in a medium with a reaction corresponding to that of the blood, acids being more harmful than alkalies; with unfavorable reaction the complement does not unite with the amboceptor, although the latter unites with the corpuscle.[38] As with other immunological reactions, electrolytes must be present to permit of the hemolysis of sensitized corpuscles by complement.[39] Gouwens[40] has found evidence that the hemolytic process is influenced by heat in the same way as a true enzyme action, but the absorption of amboceptor by red cells does not behave in this way but like a simple physical process; the complement is apparently not firmly bound to the amboceptor-corpuscle complex, and seems to act only through surface forces.[41]

The Amboceptor.—Amboceptor is, as a rule, destroyed by heating to 70° or higher.[42] Its place of origin is unknown. Metchnikoff holds that it is derived chiefly from the leucocytes, in support of which view is the fact that leucocytes dissolve red corpuscles after ingesting them; however, other phagocytic cells have the same power, particularly endothelial cells, and it is an open question whether the intracellular digestion of engulfed cells is the same process as extracellular hemolysis; probably it is not, for there seem to be more disintegrative changes in intracellular digestion than in hemolysis. Quinan[43] found that the diffusible constituents of hemolytic serum play no rôle beyond that of maintaining osmotic pressure. He was unable, however, to localize the immune body in any of the protein constituents, and Liebermann and Fenyvessy[44] believe that they obtained the amboceptor in a protein-free condition, in which it behaves like a weak acid. Amboceptors are insoluble in lipoids or lipoid solvents (Meyer),[45] and they move towards the cathode in an electric field, as do other antibodies.[46] The amboceptor complement reaction resembles a bimolecular reaction which is accelerated by its end products (v. Krogh).[47] Many of the effects of hemolytic amboceptors can be duplicated with silicic acid;[48] likewise, other colloidal suspensions may enhance the hemolytic activity of normal serum,[49] and a dye, brilliant green, may in minute quantities sensitize corpuscles so that they are hemolyzed by very small amounts of normal serum, or by lecithin.[50]

The amboceptors of normally hemolytic serum seem to be no different from those in immune serum, and amboceptors of one animal can combine with complement furnished by the serum of an entirely different animal. It is the amboceptor alone that gives the specific nature to the reaction, and, as is the case with all other immunizations, it is very difficult to secure antibodies by immunizing an animal with blood from another animal of its own species, *isohemolysins*. The place of origin of hemolysins is unknown, but that it is not in the blood seems to have been established

[37] Kosakai, Jour. Immunol., 1918 (3), 109.
[38] Michaelis and Skwirsky, Zeit. Immunität., 1909 (4), 357.
[39] Georgi, Zeit. Immunität., 1920 (29), 406.
[40] Jour. Infect. Dis., 1923 (32), 421.
[41] On the basis of mathematical considerations, Hill and Parker (Jour. Path. and Bact., 1925 (28), 1) have reached the conclusion that complement acts within the cell.
[42] Ultraviolet light destroys immune hemolysin (Stines and Abelin, Zeit. Immunität 1914 (20), 598).
[43] Hofmeister's Beitr., 1904 (5), 95.
[44] Jahresber. d. Immunität., 1911 (7), 2.
[45] *Ibid.*, 1909, Vol. 3.
[46] Teague and Buxton, Jour. Exper. Med., 1907 (9), 254.
[47] Biochem. Zeit., 1909 (22), 132.
[48] Landsteiner and Rock, Zeit. Immunität., 1912 (14), 14.
[49] Sachs and Stilling, Zeit. Immunität., 1917 (26), 530; Kondo, Zeit. Immunität., 1923 (36), 76.
[50] Browning and Mackie, Zeit. Immunität., 1914 (21), 422.

conclusively by Hektoen and Carlson,[51] and there is much reason to believe that hemolysins are produced in the reticulo-endothelial elements.[52] Immune hemolysins cannot pass from the mother to the fetus before birth[53] but they can be transmitted through the colostrum (Famulener).[54]

Although Ehrlich held that the union between cell and amboceptor is purely chemical and follows ordinary chemical laws, especially the law of multiple proportions, Bordet and other French observers have claimed that the union between amboceptor and corpuscle is physical and not chemical.[55] Probably the union is with the stroma rather than with the hemoglobin, and the result of the union is to render the stroma permeable to the hemoglobin, or to separate the bonds that unite the hemoglobin to the stroma.[56] There are grounds for believing that the amboceptor not only binds the complement, but that it also produces changes in the corpuscles (Muir). Mathes[57] contends that red corpuscles cannot be dissolved by hemolytic serum or by pancreatic juice until after they have been killed; as heated serum does not kill them, this is presumably done by the complement. Corpuscles that have been killed can then be dissolved in their own serum. Levene[58] tried to produce hemolytic serums by immunizing with different constituents of corpuscles, using—(1) pure crystalline hemoglobin; (2) proteins of the stroma soluble in salt solutions; (3) an extract with alcohol-ether; and (4) an extract in 1.5 per cent. sodium bicarbonate. Only the last gave positive results, and the serum was almost devoid of agglutinative properties. Injection with corpuscles that had been digested with trypsin gave about the same results as alkaline extracts; corpuscles digested by pepsin gave a much weaker serum; in neither was agglutination obtained. According to Bang and Forssmann[59] and others ethereal extracts of red corpuscles give rise to production of hemolysins on immunization, and this "lysinogen" substance can be precipitated with acetone, is insoluble in alcohol, is not destroyed by boiling, and gives rise to no agglutinin. Numerous other observers, however, have failed to confirm these findings. Ford and Halsey[60] obtained serum with both lytic and agglutinative powers by injecting either the stroma or the laked blood free of stroma. Stewart[61] obtained similar results by immunizing with corpuscles laked by physical means, by serums, or by saponin. Others have found that stroma as antigen produces serum rich in hemolysins but poor in hemagglutinins.[62] Pure hemoglobin itself is antigenic, and specific[63] for the species furnishing it.

According to Guerrini,[64] nucleoprotein obtained from dog's blood engenders specific hemolysins, and Beebe states that nucleoproteins from visceral organs do not have this

[51] Jour. Infect. Dis., 1910 (7), 319.

[52] See Portis, Jour. Infect. Dis., 1924 (34), 159; Bieling and Isaac, Zeit. ges. exp. Med., 1922 (28), 154.

[53] See Sherman concerning normal antibodies in the fetus, Jour. Infect. Dis., 1918 (22), 534).

[54] Jour. Infect. Dis., 1912 (10), 332.

[55] Bang and Forssmann (Hofmeister's Beitr., 1906 (8), 238) suggest that the amboceptor merely renders the corpuscle permeable for the complement, perhaps through action on the lipoid membrane; the complement then acts directly upon some constituent of the corpuscle, without the amboceptor acting as a combining substance in any way. They found that the substance in blood which stimulates the antibody formation in the case of hemolysin formation, is chemically separable from the substance in blood which unites with these antibodies; therefore, they conclude, the "receptors" of cells are *not* identical with the antibodies. (See controversy with Ehrlich in Münch. med. Woch., Vols. 56 and 57.)

[56] Corpuscles treated with osmic acid will unite with hemolysins of diverse origin, but when used for immunizing they engender no hemolysins (Coca; also v. Szily, Zeit. Immunität., 1909 (3), 451). Heating corpuscle stroma alters greatly the reactivity (Landsteiner and Prasek, *ibid.*, 1912 (13), 403).

[57] Münch. med. Woch., 1902 (49), 8.

[58] Jour. Med. Research, 1904 (12), 191.

[59] Hofmeister's Beitr., 1906 (8) 238.

[60] Jour. Med. Research., 1904 (11), 403.

[61] Amer. Jour. of Physiol., 1904 (11), 250.

[62] Hadjopoulos, Arch. Int. Med., 1921 (27), 441.

[63] Hektoen and Schulhof, Jour. Infect. Dis., 1922 (31), 32; 1923 (33), 224; Heidelberger and Landsteiner, Jour. Exp. Med., 1923 (38), 561.

[64] Riv. crit. di clin. med., 1903 (4), 561.

effect. Levene's alkaline erythrocyte extracts probably also contained nucleoproteins. Vedder[65] was unable to produce hemolysins when he used ether extracts of corpuscles as antigen, or with globulin from stroma, but the protein extract left after removing the globulin, presumably albumin, as well as lipoid-free stroma, produced hemolysin. On the other hand, Bennett and Schmidt[66] obtained hemolysin by immunizing with the globulin precipitated from hemolyzed erythrocytes by CO_2.

Immunization with extracts of tissues and cells of various sorts, even when entirely free from blood (*e. g.*, spermatozoa), may produce hemolytic sera. The fact that various tissues from many different species of animals, when used as antigen, may give rise to hemolysin for sheep corpuscles, is an interesting but so far unexplained phenomenon, which is discussed under "Specificity" (Chapter VII).

The Complement.—Hemolytic complement possesses the same properties as bacteriolytic complement, with which it is presumably identical resembling enzymes to the extent that it is susceptible to heat, causes a disintegration of cells, and is largely retained by Berkefeld filters.[67] The joint action of amboceptor and complement is strikingly like the activation of trypsinogen by kinase. On the other hand, hemolysis by serum is quite different from the effect of trypsin on corpuscles, as trypsin completely disorganizes the hemoglobin and destroys the stroma, while in hemolysis the stroma and hemoglobin seem to be merely separated from one another but not chemically altered. Again, *hemolysin acts quantitatively*, although this may be due to a difference in the way the binding to the cell occurs, rather than in the method of action of the complement. Landsteiner and others have suggested that a lipoidal complement dissolves the corpuscle lipoids, liberating the hemoglobin, while Neuberg and others have supported the hypothesis that complement is virtually a lipase which splits the lipoids out of the corpuscles. Bordet believes that the hemolysin causes a lesion of the stroma which changes the resistance to osmotic influences. Complement is present in the plasma in about the same amounts as in the corresponding sera, so it is not a substance set free only by coagulation of the blood (Watanabe).[68] Dick[69] has found evidence that the complement is a ferment formed in the liver, and that it causes actual proteolytic changes. Jobling[70] associates the serum lipase with end piece of hemolytic complement.[71] Ohta[72] observed no increase in non-coagulable nitrogen during hemolysis, Dick[69] found an increase in the free amino acids, but others have failed to demonstrate the presence of products of protein disintegration in hemolyzed corpuscles;[73] therefore, as yet agreement has not been reached as to whether hemolysis depends in any way upon proteolysis or lipolysis in the corpuscle stroma.

Since the serum of one animal may complement the immune bodies in serum of several other varieties, and also produce lysis of many sorts of cells, it is probable that there is but one sort of complement. Noguchi,[74] Liebermann and Fenyvessy, and others have pointed out the striking resemblance between hemolytic complement and certain compounds of soaps or lipoids with serum proteins, and it is possible that such compounds are of importance in serum hemolysis; but there seems also to be evidence of the existence of distinct protein complements, entirely different from these,[75] and

[65] Jour. Immunol., 1919 (4), 141.
[66] Jour. Immunol., 1919 (4),29.
[67] Muir and Browning, Jour. Path. and Bact., 1909 (13), 232.
[68] Jour. Immunol., 1919 (4), 77.
[69] Jour. Infect. Dis., 1913 (12), 111.
[70] Jobling and Bull, Jour. Exper. Med., 1913 (17), 61; also Bergel, Deut. Arch. klin. Med., 1912 (106), 47; Olsen and Goette, Biochem. Zeit., 1920 (112), 188.
[71] Thiele and Embleton, however, state that hemolysin is not a lipase, and that the hemolytic power of serum has no relation to its lipolytic power (Jour. Path. and Bact., 1914 (19), 349).
[72] Biochem. Zeit., 1912 (46), 247; see also McNeil and Kahn, Jour. Immunol., 1918 (3), 295.
[73] Wollman and Graves, Compt. Rend. Acad. Sci., 1923 (177), 1162; Browning, Brit. Med. Jour., 1915, Feb. 6, p. 242.
[74] Biochem. Zeit., 1907 (6), 172 and 327; Jour. Exper. Med., 1907 (9), 436.
[75] See Liefmann, *et al.*, Zeit. Immunität., 1912 (13), 150; Hans Schmidt, Zeit. Immunität., 1924 (40), 368.

it is possible that the protein complements are the important agents in specific hemolysis by immune sera.[76]

Antibodies can be obtained for both complement and hemolytic amboceptor by immunizing against serum containing them, and in many serums *antihemolysins* exist normally. Against certain vegetable hemolysins this antihemolytic action is very strong (Kobert). Antihemolysins are generally anticomplements, but in a number of instances anti-amboceptors have been obtained. The existence of immune bodies specific for hemolytic amboceptor and complement, supports the view that both of these agents are proteins.

In hemolysis as in bacteriolysis the complement exhibits two functions, corresponding to the "end-piece" and "mid-piece" fractions. Herzfeld and Klinger[77] consider the mid-piece to be a globulin which renders the surface of the corpuscles more capable of taking up certain disintegration products contained in the serum (persensitization) which constitute the so-called end-piece, and which produce hemolysis by direct hydrolysis or solution of the stroma elements.

Hemagglutination.—Agglutination of red corpuscles occurs under the influence of immune serum as well as under the influence of some normal serums. In all respects the principles seem to be the same as those described for bacterial agglutination. The hemagglutinating antibody behaves like the other antibodies and proteins under the influence of chemical and physical agencies, but Landsteiner and Jagic have obtained strong agglutinating solutions containing very little protein. According to Schütz and Wöhlisch,[78] serum isoagglutinin is precipitated with the globulins, is resistant to alcohol precipitation, and does not pass through ultrafilters; the receptor or "agglutinable substance" is loosely bound to the surface of the corpuscles, and agglutination results from lowering of the surface charge by a precipitate which is easily removed by washing.[79] Holt and Reynolds[80] found the hemagglutinating properties of normal human isoagglutinating serum to be restricted to the pseudoglobulin fraction.

Agglutination occurs at much lower temperatures than hemolysis, and also is not checked by heating the serum to 55°; hence it is possible to observe hemagglutination independent of hemolysis. Serums may contain hemagglutinins and not be hemolytic; the reverse is also true. As agglutination occurs in corpuscles that have been fixed in formalin or

[76] Liebermann and Fenyvessy (*loc. cit.*)[44] believe that serum hemolysis takes place as follows: First, the amboceptor acts on the corpuscle, injuring it so that it becomes less resistant; second, this combination acts upon the complement (a soap compound) and frees the soap so that it can unite with the amboceptor-corpuscle system; third, the soap causes hemolysis; fourth (as a separate step), the escape of the hemoglobin from the corpuscles. Tissot ascribes importance to the fatty acids of the plasma (Compt. Rend. Acad. Sci., 1919 (168), 1283). Bergel (Zeit Immunität., 1918 (27), 441) supports the hypothesis that immune hemolysis and agglutination depend on a solution of the lipoids of the cells. In this reaction the lipoids act as antigen, the newformed amboceptor is formed by the lipoids of the lymphocytes as a zymogen which is activated by serum complement, and is specifically bound by the lipoid antigens of the corpuscles. That is, the lipoids are the haptophore groups of the antigen; they bind the receptor of the thermostabile lipase zymogen, which is activated by the nonspecific complement.

[77] Biochem. Zeit., 1918 (87), 36.

[78] Klin. Woch., 1924 (3), 1614.

[79] Bergel (Zeit. Immunität., 1912 (14), 255; 1913 (17), 169) contends that hemagglutination is produced by lipase from the lymphocytes, which alters the lipoid membranes of the erythrocytes.

[80] Jour. Amer. Med. Assoc., 1922 (79), 1684. .

sublimate, it is probably not the proteins that are affected, but some other of the ingredients of the stroma, of which lecithin and cholesterol seem to be the chief.

Corpuscles that have been sensitized by hemolytic amboceptors are much more readily agglutinated by salts of heavy metals, especially copper and zinc, presumably because of quantitative alterations in the electrical charge of the corpuscles induced by the antibody;[81] but these effects are not constant.[82] When red cells are agglutinated in an isotonic saccharose solution the optimum *p*H is 4.75, but with cells sensitized with agglutinins the optimum is 5.3. As the latter figure corresponds with the isoelectric point of euglobulin it seems probable that the agglutination depends on precipitation of the antibody which is bound to the antigenic cells (Coulter).[83] Northrop and DeKruif[84] found that hemagglutination exhibited the same physicochemical phenomena as bacterial agglutination (see p. 190), the agglutinin raising the critical potential; *e. g.*, unsensitized red cells did not agglutinate until the potential had been depressed below 4 millivolts, whereas cells sensitized by agglutinin were agglutinated when the potential was reduced to about 12 millivolts.

The *conglutinin* effect of beef serum (Bordet and Gay) is also observed with corpuscles as with bacteria, and may be due to the presence in the serum of unclotted fibrinogen, for if this residual fibrinogen is removed by treating the serum with platelets it no longer exhibits the conglutinin effect.[85]

Agglutination of the corpuscles during life may be of great pathological importance, for such masses of agglutinated corpuscles may readily produce capillary thrombi and emboli, which, if wide-spread, may create much disturbance. Sometimes the serum of one individual of a species agglutinates the corpuscles of another individual of the same species (*isoagglutination*), a fact which must be taken into account in performing transfusion of blood, lest dangerous agglutination take place. Agglutination of an individual's corpuscles by his own serum (*autoagglutination*), may also be observed under experimental and perhaps under pathological conditions (Landsteiner),[86] this pathological autoagglutination probably occurring especially at temperatures below 37°. (See Paroxysmal Hemoglobinuria.) Many bacteria produce substances that are agglutinative for human red corpuscles, among them being *B. typhosus*, *pyocyaneus*, and staphylococcus. Flexner[87] has found, in typhoid fever, thrombi that seemed to be composed of agglutinated red corpuscles, almost free from fibrin and leucocytes. Probably many of the so-called "hyaline thrombi" found frequently in infectious diseases are really com-

[81] Eisner and Friedemann, Zeit. Immunität., 1914 (21), 520.
[82] Schürmann and Baumgärtel, Zeit. f. Immunität., 1921 (31), 151.
[83] Jour. Gen. Physiol., 1922 (4), 403.
[84] Jour. Gen. Physiol., 1922 (4), 639, 635.
[85] Maltaner and Johnston, Jour. of Immunol., 1921 (6), 349.
[86] See also Clough and Richter, Bull. Johns Hop. Hosp., 1918 (29), 86; Rous and Robertson, Jour. Exp. Med., 1917 (27), 509.
[87] Univ. of Penn. Med. Bull., 1902 (15), 324; Amer. Jour. Med. Sci., 1903 (126), 202.

posed of agglutinated, partly hemolyzed red corpuscles (see "Thrombosis," Chap. XV).

Certain *vegetable poisons* produce agglutination of red corpuscles, especially ricin, abrin, and crotin, and the fact that ricin has little or no hemolytic action shows the independence of the processes. Antisera for these vegetable poisons are also antiagglutinative, acting, as Ehrlich showed, on the poison and not on the corpuscles. The seeds of many non-poisonous leguminous plants, and also of *Solanaceæ*, yield extracts that are strongly agglutinative for red corpuscles; in *Phaseolus multiflorus* the active substance is found in the proteose of the seed, and seems to be a part of the stored food (Schneider).[88] It is not present in other parts of the plant. *Snake venoms* contain agglutinins, destroyed by heating to 75°; their agglutinating power being in inverse ratio to their hemolytic power. Corpuscles agglutinated by venoms may be again separated by potassium permanganate solutions.[89] Silicic acid and certain other *colloids* may act as agglutinins, their effects bearing a relation to the effects of electrical charges upon agglutination of bacteria or of colloids (*q. v.*).[90]

Hemolysis by Bacteria[91]

Both pathogenic and non-pathogenic bacteria produce hemolytic substances that are excreted into the fluids in which they grow. During many infectious diseases marked hemolysis occurs, especially in those diseases accompanied by septicemia, and in gas bacillus infections.[92] After death the hemoglobin of the blood goes into solution, and the resulting staining of the walls of the blood-vessels, and later of the tissues everywhere, is generally familiar. In the *post-mortem hemolysis* probably the putrefactive organisms are chiefly concerned, although it is marked a very short time after death in many cases of septicemia, particularly when the infecting organism is the streptococcus, and here probably the pathogenic organism is the chief cause of the hemolysis. The hemolytic action of bacteria can be studied both *in vitro* and *in vivo*. Among the best known hemolytic bacterial toxins are *tetanolysin*, *pyocyanolysin*, *typholysin*, *staphylolysin*,[93] and *streptocolysin*, as they have been termed, and the hemotoxin of the gas bacilli. Of these, the pyocyanolysin is questionable, because it has been described as resisting heat above the boiling-point, and Jordan[94] seems to have proved that the hemolysis is

[88] Jour. Biol. Chem., 1912 (11), 47; bibliography.
[89] See Flexner, Univ. of Penn. Med. Bull., 1902 (15), 324 and 361.
[90] See Landsteiner and Jagic, Münch. med. Woch., 1904 (51), 1185.
[91] See Pribram, Kolle and Wassermann's Handbuch., 1913 (II), 1328.
[92] See Henry, Jour. Path. and Bact., 1922 (25), 1.
[93] Analysis of staphylolysin by Burkhardt (Arch. exp. Path. und Pharm., 1910 (63), 107), showed it to be dialyzable, protein- and biuret-free, thermolabile and soluble in ether, and hence probably not a true toxin, but Julia Parker (Jour. Exp. Med., 1924 (40), 761) has obtained from *S. aureus* a true toxin which produces characteristic skin lesions and engenders a potent antitoxin. From *B. putidum* Burkhardt isolated a hemolytic substance which seems to be a derivative by oxidation of erucacic acid (oxydimethylthiolerucacic acid).
[94] Jour. Medical Research, 1903 (10), 31.

ascribable to the alkalinity that this organism produces in culture-media. Other bacterial hemolysins are, however, destroyed by heat at 70° or less for two hours; but they are altogether different from ordinary serum hemolysins. Apparently streptolysin is simply a toxin for red cells,[95] and unites directly to the cell receptors without the intervention of any intermediary body. As a similar structure has been shown for staphylolysin and tetanolysin, it is probable that the *bacterial "hemolysins" are all merely toxins with a particular affinity for red cells*, and against some of these bacterial hematoxins antitoxic sera are obtainable, although there is usually some question as to how much of the antagonistic effect depends on true antitoxins and how much upon the cholesterol in the serum. However, a strong antiserum has been obtained against the hemotoxin of gas gangrene bacilli.[96] Of course bacteria may also form many nonspecific hemolytic substances as products of their metabolism, such as acids and bases; (*e. g.*, staphylococci may produce hemolytic fatty acids by splitting fats),[97] and there is usually much doubt as to how much of the hemolytic activity of bacterial filtrates depends on true antigenic toxins and how much on nonantigenic hemolytic substances.[98] The possible rôle of hemolysins from intestinal bacteria in the production of pernicious anemia, is still undetermined.[99]

Secondary anemia occurring in the infectious diseases is probably to be explained largely by this hemolytic property of bacterial toxins. Hemoglobinuria may also be produced in the same way in some instances. Intravenous injections of filtrates of the saprophyte, *B. megatherium*, will produce hemoglobinuria in guinea-pigs, hence hemolysis is not an exclusive property of pathogenic bacteria, and with streptococci, Lyall[1] found that the hemolysin titer did not afford a criterion of virulence. No immunity to streptococci is produced in animals immunized with streptococcus hemolysin.[2] Pneumococci produce an intracellular hemolytic toxin which is very labile and antigenic; living pneumococci convert hemoglobin into methemoglobin, but this the hemolytic extracts of pneumococci cannot do (Cole).[3] Streptococcus viridans has the same property,[4] which may play a part in the effects of infections with these organisms. Streptocolysin is a labile substance, apparently with colloidal properties, which is produced only in cultures containing certain unknown substances derived from tissue infusions.[5] Kämmerer[6] found that of a large series of organisms, only pneumococcus and *Strep. hemolyticus* produce methemoglobin. Clearing of zones about cultures in blood-agar

[95] Jour. Amer. Med. Assoc., 1903 (41), 962; Jour. Infect. Dis., 1907 (4), 277.
[96] Ford and Williams, Jour. Immunol., 1919 (4), 385; Henry, loc. cit.[92]; Wuth, Biochem. Zeit., 1923 (142), 19.
[97] Orcutt and Howe, Jour. Exp. Med., 1922 (35), 409.
[98] See Julianelle, Jour. Infect. Dis., 1922 (31), 256.
[99] See Busson and Kosian, Zeit. ges. exp. Med., 1921 (25), 199.
[1] Jour. Med. Res., 1914 (30), 515.
[2] McLeod and McNee, Jour. Path. and Bact., 1913 (17), 524.
[3] Jour. Exper. Med., 1914 (20), 347, 363.
[4] Blake, Jour. Exper. Med., 1916 (24), 315.
[5] Meader and Robinson, Jour. Exp. Med., 1920 (32), 639.
[6] Arch. exp. Path. Pharm., 1920 (88), 247.

plates depends on tryptic activity, while hemolysis in fluid media seems to be accomplished by lipase (Kämmerer), and is not accompanied by chemical evidence of proteolysis (Wollman and Graves).[7] Some of the products of hemoglobin disintegration seem to inhibit or destroy Gram-positive bacteria.

Hemolysis by Vegetable Poisons

A number of plant poisons are strongly hemolytic, and some of them owe much of their toxicity to their effect on the erythrocytes. One group consists of the bodies often called "vegetable toxalbumins," because they seem to be proteins, and includes ricin, abrin, crotin, curcin and robin.[8] Of these, crotin and curcin are particularly actively hemolytic, while ricin, abrin, and robin are more marked by their agglutinating action, hemolysis being produced only by relatively large doses. Their effects vary greatly, however, according to the species of animals whose blood is used. They resemble the bacterial toxins, in that immunity can be secured against them, and the immune serum will prevent their hemolytic action. Heating the toxalbumins to 65° or 70° does not destroy the hemolytic or agglutinating action except with phallin, but 100° does. The action of these substances is not like that of the enzymes, in that it is quantitative, a given amount acting on a given amount of corpuscles, to which it is bound. Madsen and Walbum[9] observed that red corpuscles had the power of dissociating neutral mixtures of ricin and antiricin, the ricin entering the corpuscles from which it could be recovered.[10] Ford and Abel believe the hemolytic agent of amanita to be a glucoside. (The general nature and other properties of these substances are considered under the heading of "Phytotoxins," in Chap. VI.)

Saponin Group.—Another quite distinct group of vegetable hemolyzing agents consists of the "*saponin substances.*[11] These are a closely related group of *glucosides*, found in at least 46 different families of plants, and they are strong protoplasmic as well as hemolytic poisons.

The best-known of these are the following:

Sapotoxin, one of the most actively toxic and hemolytic products of *quillaja.*

Cyclamin, also a member of this group (derived from *Cyclamen*), and is said to be the most active of all as a hemolytic agent (Tufanow).

Solanin[12] is obtained from all parts of the potato plant, combined with malic acid; it is found particularly in young sprouts, but not in any considerable amounts in normal potatoes.[13] Its formula is unknown, but as it splits up into an alkaloid (*solanidin*) and sugar it is called a *glyco-alkaloid.* In its action it resembles the

[7] Compt. Rend. Soc. Biol., 1924 (91), 50.

[8] The sap of *Cotyledon Scheideckeri* contains hemolytic substances of peculiar character. (See Kritchewski, Jour. Exp. Med., 1917 (26), 669.)

[9] Cent. f. Bakt., 1904 (36), 242.

[10] According to Pascucci (Hofmeister's Beitr., 1905 (7), 457), ricin combines directly with lecithin, the compound being strongly hemolytic.

[11] Complete literature on saponin given by Kobert, "Die Saponinsubstanzen," Stuttgart, 1904; also Kunkel, "Handbuch der Toxokologie," Jena.

[12] Literature, see Meyer and Schmiedeberg, Arch. f. exp. Path. u. Pharm., 1895 (36), 361; Perles, *ibid.*, 1890 (26), 88.

[13] See Kunkel, "Handbuch der Toxokologie," p. 873.

saponins, being a powerful protoplasmic poison, killing bacteria, and hemolyzing blood in very great dilutions.[14]

They differ altogether from the true toxins, being heat resistant, having no resemblance to proteins, and not giving rise to antibodies on immunization of animals.[15] The degree of their toxicity is not directly proportional to their hemolytic activity; they seem to injure chiefly the nerve-cells. Apparently hemolysis is brought about by action upon the lipoids of the red corpuscles, for addition of cholesterol to saponin prevents its hemolytic effect;[16] lecithin does not have the same property.[17] Both cholesterol and lecithin combine with saponin, the cholesterol compound being quite inert, whereas the lecithin compound is both hemolytic and toxic. The compound formed between a typical saponin, *digitonin*, and cholesterol, is so insoluble that it has been found useful in the quantitative analysis of cholesterol.[18] Careful immunization leads to a slight increase in the antihemolytic action of the serum, possibly due to an increased formation of cholesterol (Kobert). The resistance of corpuscles to saponin hemolysis varies in disease, being especially low in jaundice (M'Neil).[19] In general, the resistance of the erythrocytes to saponin hemolysis in disease varies directly with the cholesterol content of the blood,[20] being decreased by phosphatids and little altered by the proteins of the plasma.[21] Nevertheless, saponin injures the bone marrow even when its hemolytic action is prevented by cholesterol,[22] and it is highly toxic to platelets.[23]

A study of the toxicity of the members of this group by Kobert[24] shows that in general they have similar properties, but that minor differences exist between them. All cause hemolysis, some in dilution as great as 1:100,000. Some produce hemoglobinuria when injected intravenously, others do not. All paralyze the heart, but the injuries to the central nervous system are the chief cause of death. Marked local changes are produced at the site of injection, but the leucocytes are apparently not injured, although sterile suppuration is produced. There is a period of latency after intravenous injection of small doses—twenty-four hours or more—before the appearance of symptoms.

[14] Concerning human solanin poisoning see Rothe, Zeit. f. Hyg., 1919 (88), 1.

[15] Saponins are characterized by their ready solubility in water and the foaming, soapy character possessed by the solution; hence their technical applications as soap bark, etc. Heated with dilute acids they split off sugar; also when acted on by glucoside-splitting enzymes (from spiders), according to Kobert. Saponin from *Quillaja* (soap-bark) has the formula $C_{19}H_{30}O_{10}$ (Stütz). Most are colloids, but some crystallize.

[16] Ransom, Deut. med. Woch., 1901 (27), 194; Madsen and Noguchi, Cent. f. Bakt., 1905 (37), 367; Pascucci, Hofmeister's Beitr., 1905 (6), 543.

[17] Noguchi, Univ. of Penn. Med. Bull., 1902 (15), 327; Meyer, Hofmeister's Beitr., 1908 (11), 357.

[18] Windaus, Chem. Berichte, 1909 (42), 238.

[19] Jour. Path. and Bact., 1910 (15), 56.

[20] See Neilson and Wheelon, Jour. Lab. Clin. Med., 1921 (6), 487, 568; bibliography.

[21] Ehrentheil and Weis-Ostborn, 1923 (36), 356.

[22] Gaisböck and Bayer, Wien. klin. Woch., 1924 (37), 952.

[23] Firket and Campos, Bull. Johns Hopkins Hosp., 1922 (33), 271.

[24] Arch. exp. Path. u. Pharm., 1887 (23), 233.

Several hemolytic poisons have been obtained from poisonous mushrooms. Best known of these is:

HELVELLIC ACID, from *Helvella esculenta*, which has the empiric formula $C_{12}H_{20}O_7$.[25] Intravenously injected it produces hemoglobinuria and icterus, with hemoglobin infarcts in the kidneys (Bostroem).[26]

PHALLIN, or *Amanita hemolysin*, described by Kobert as a toxalbumin, has been found by Abel and Ford to be a glucoside, and thus belongs to the saponin group. (See Chap. VI for further discussion.) In the leaves of the ivy, *Hedera helix*, a hemolytic glucoside has been found by Moore.[27] It is of interest that Faust believes the poisonous agent of cobra venom to be a glucoside, closely resembling sapotoxin.

As will be seen, all these last-mentioned vegetable hemolytic agents are essentially different from either the bacterial or serum hemolysins, or from the abrin, ricin, crotin, or robin group, in that they are of relatively simple chemical composition, and quite unlike proteins, enzymes, or toxins. The manner in which they cause hemolysis is unknown,[28] but from their relation to saponin it is probable that, like it, they cause injury by combining with or dissolving the lipoids of the stroma of the corpuscles. Extracts of *Morchella esculenta* do not hemolyze corpuscles *in vitro*, although powerfully hemolytic when injected into animals, and causing severe hemoglobinuria; so that it is probable that they cause their hemolytic effects indirectly through the changes which they produce in the tissues of the poisoned animal.[29]

HEMOLYSIS BY VENOMS[30]

The laking of blood-corpuscles by venoms is of peculiar interest from the standpoint of immunity phenomena, since it was demonstrated by Flexner and Noguchi that the hemolytic principle of the venoms resembles an amboceptor, in that some substance behaving like complement has to be furnished by the blood. Kyes found that this complementing agent is lecithin,[31] and was able to produce what he considers to be compounds of the hemolysin with lecithin, called "lecithids." The hemolytic activity of these lecithids is very great, and they seem to be free from the neurotoxic principle of the venoms. Whether they represent true compounds of a hemolytic amboceptor with lecithin, or are simply actively hemolytic products of the cleavage of lecithin by an enzymatic activity of the venom, is at present unsettled;[32] it seems probable, however, that the hemolysin of cobra venom is a lipase that splits lecithin into two hemolytic components, oleic acid and "desoleolecithin" (Coca).[32]

[25] Boehm and Külz, Arch. exp. Path. u. Pharm., 1885 (19), 403.
[26] Deut. Arch. klin. Med., 1883 (32), 209.
[27] Jour. Pharmacol., 1913 (4), 263.
[28] See Pickering and Taylor, Biochem. Jour., 1923 (17), 916.
[29] Friedberger and Brossa, Zeit. Immunität., 1912 (15), 506.
[30] General review of literature on the hemolytic properties of animal poisons given by Sachs, Biochem. Centralblatt, 1906 (5), 257; Noguchi, Jour. Exp. Med., 1907 (9), 436.
[31] Cruickshank also found that other lipoids than lecithin may activate cobra venom (Jour. Path. and Bact., 1913 (17), 619).
[32] See Kyes, Jour. Infect. Dis., 1910 (7), 181; v. Dungern and Coca, *ibid.*, 1912 (10), 57; Manwaring, Zeit. Immunität., 1910 (6), 513; Bang, *ibid*,. 1910 (8), 202; Cocoa, Jour. Infect. Dis., 1915 (17), 351.

Noguchi suggests that not only lecithin, but also soaps, especially of unsaturated fatty acids, and probably protein compounds of soaps and lecithin, may act as the hemolytic "complement" which activates venoms. However, lecithin has the capacity to increase the hemolytic power, not only of venoms but also of simpler chemical substances, such as quinine and many others (Frieda).[33]

The hemolytic agents of venom seem to be secreted by the salivary glands of the reptiles from their blood, which contains almost identical amboceptors, differing chiefly in that they can be activated only by agents contained in snake blood, while the amboceptors of venom can be activated by nearly all sorts of blood. Venoms from cobra, rattlesnake, moccasin, and copperhead possess in each a variety of intermediary bodies (amboceptors) that seem to be at least partly identical in nature, although they may vary in quantity. In order of decreasing hemolytic power for mammalian corpuscles come venoms from cobra, water moccasin, copperhead, and rattlesnake. These venoms are also agglutinative for all corpuscles tried, and agglutination will occur at 0° C. Exposure for thirty minutes at 75°–80° C. destroys the agglutinating property. The hemolytic intermediary bodies are resistant to heat, suffering but slight loss of power at 100° C. By adsorption with animal charcoal the hemolytic element may be separated from the element which unites with antihemolysin (deHoussay).[34] In general, the hemolytic power of the venoms for different sorts of corpuscles varies in inverse proportion to their agglutinative power. Red corpuscles of the frog are not hemolyzed by venom, and those of *Necturus* (mud puppy) but slightly, agreeing with the known resistance of cold-blooded animals to snake-bites. (The general properties of venom are discussed in Chap. VI.)

The erythrocytes of different individuals show considerable variations in their resistance to hemolytic agents, perhaps depending upon the amount or upon the manner of fixation of the lipoids in the corpuscles; thus the corpuscles of syphilitics show a heightened resistance to hemolysis by cobra venom (Weil)[35] except in the earliest stages, when they are hypersensitive. Also, the serum of persons suffering from various diseases, especially mental diseases, inhibits the hemolysis of human corpuscles by cobra venom.[36] After splenectomy there is an increased resistance to venom hemolysis.[37]

Eel serum is remarkably hemolytic, so much so that a quantity of 0.1 c.c. per kilogram of body weight will kill a rabbit or guinea-pig in three minutes when injected intravenously. Heating at 54° C. for fifteen minutes destroys the hemolytic action, and, unlike ordinary serum hemolysins the addition of complement does not restore its activity. Animals can be immunized against this serum. Introduced into the stomach in ordinary quantities eel serum is not toxic. It can be dried and redissolved without losing its activity, but acids and alkalies readily destroy it. Mosso, who first

[33] Zeit. Immunität., 1924 (40), 69.
[34] Revista Inst. Bact. Buenos Aires, 1921 (2), 197.
[35] Jour. Infect. Dis., 1909 (6), 688; Stone and Schottstaedt, Arch. Int. Med., 1912 (10), 8.
[36] See articles on this subject in the Münch. med. Woch., 1909, Vol. 56.
[37] Kolmer, Jour. Exp. Med. 1917 (25), 195.

discovered the toxicity of eel serum, called the unknown active principle *ichthyotoxin*. It is found chiefly in the albumin fraction of the eel serum.[38] Many other animals produce hemolytic poisons (*e. g.*, spiders, bees) which are discussed under Zootoxins, Chapter VI.

Hemolysis in Disease

During health there is always going on a certain amount of destruction of red corpuscles that have outlived their usefulness; hence in disease we may have to deal with either an alteration in the normal processes of blood destruction or the introduction of entirely new processes.[39] Although the place and manner of normal red corpuscle destruction is not completely known, yet it seems probable that there is relatively little hemolysis within the circulating blood. When a red corpuscle becomes damaged, it seems to become more susceptible to phagocytosis, and it is then picked out of the blood, chiefly by the endothelial cells of the sinuses of the liver, spleen, hemolymph glands, and bone-marrow. Within these cells it apparently undergoes hemolysis. Eventually, the resulting pigment is split up by the liver, the non-ferruginous portion forming the bile-pigments, while the iron seems to be mostly withheld to be worked over into new hemoglobin.[40] (See "Pigmentation," Chap. XX.) Whenever during disease red corpuscles are more rapidly injured than they are under normal conditions, these processes of normal hemolysis are exaggerated and we not only find the phagocytic cells of the spleen and lymph nodes packed with corpuscles, but endothelial cells elsewhere, and also leucocytes, take on the hemolytic function. At the same time there results an excessive production of bile-pigment from the destroyed red corpuscles, which if sufficient in amount leads to jaundice. If hemolysis is excessive, the blood pigment accumulates in other organs than the liver and spleen.

According to Pearce[41] and his associates, when the blood contains at one time more than 0.06 gm. of free hemoglobin per kilo of body weight, it begins to be excreted by the kidneys; smaller amounts are cared for chiefly by the liver, and even when much larger amounts of hemoglobin are present in the blood the liver takes care of most of it, only a relatively small proportion, 17 to 36 per cent., being excreted in the urine. Hence it is possible to have hemolytic jaundice without hemoglobinuria. Part of the pigment is converted into urobilin, and the amount of this pigment in the stool is an index of the amount of hemolysis.[42] In persons with hemolytic hemoglobinemia, intravenous injection of hemoglobin will produce hemoglobinuria with smaller dosage than in normal persons, who require at least 17 c.c. of laked corpuscles to produce hemoglobinuria.[43]

[38] Sato, Kyoto Jour. Med. Sci., 1917 (14), 36.

[39] See review on Destruction of Red Corpuscles in Health and Disease by Peyton Rous, Physiol. Reviews, 1923 (3), 75.

[40] Muir and Dunn (Jour. Path. and Bact., 1915 (20), 41), find that after acute hemolytic anemia in rabbits the excess iron stored in the organs has been nearly all absorbed by the time regeneration of the blood is complete.

[41] Jour. Exp. Med., 1912 (16), several articles.

[42] See Robertson, Arch. Int. Med., 1915 (15), 1072.

[43] Sellards and Minot, Jour. Med. Res., 1916 (34), 469.

The hemoglobin seems to be excreted by both the glomerules and the tubules,[44] and Haessler[45] suggests that probably hemoglobin at times passes into the tubules but is reabsorbed and does not appear in the urine.

It is possible that the globin, which is quite toxic when free,[46] may play a part in the symptomatology of hemolytic poisons. The stroma of the erythrocytes also seems to be toxic,[47] but Bayliss[48] could find no evidence that hemoglobin or other products of hemolysis are toxic, at least in cats and dogs.

The hemolysis of the *acute febrile diseases* is readily explained by the demonstrable hemolytic property of the products of the organisms that cause them, such as streptocolysin, staphylolysin, etc. Infection with organisms of the gas bacillus group, which produce strong hemotoxins, also results in rapidly progressing anemia (Henry).[92] Perhaps at the same time products of altered metabolism may also play a part in the hemolysis but it does not seem probable from experimental results that the thermic condition *per se* has much effect. In malaria, although the parasites enter and destroy the corpuscles in which they live, yet this alone does not account for all the blood destruction of the disease, for the amount of anemia is quite without relation to the number of parasites to be found. There is good reason to believe that the plasmodia produce hemolytic substances that are discharged into the serum.

In the *primary anemias* hemolysis seems to be the essential process, although the agents involved are at present unknown. Absorption of hemolytic products of intestinal putrefaction or infection has always come in for much suspicion, without ever becoming completely established. Here also the hemolysis seems to take place in the endothelial cells rather than in the vessels. In such a disease as pernicious anemia there is much reason to assume that defective or abnormal hematogenesis is an important factor, but hemolytic products of intestinal bacteria have naturally been suspected, especially since it is known that infection with gas bacilli results in a marked hemolytic anemia. The normal power of the serum to protect the red cells against hemolytic agents is decreased in pernicious anemia and in the hemolytic anemias, which deficiency may be an important factor in the production of the anemia, although as yet we do not know what changes in the serum are responsible.[49] Hemolytic lipoids produced by intestinal parasites have been found to be capable of producing a profound anemia experimentally, and are believed to explain the anemia observed in persons infested with the fish tape worm (See Pernicious anemia).

In some diseases associated with anemia it has been found that the blood-serum of the patient is distinctly *isohemolytic*, although *isoagglutination* seems to be more frequent. Persons repeatedly given intravenous

[44] Fukuda and Oliver, Jour. Exp. Med., 1923 (37), 83.
[45] Jour. Exp. Med., 1922 (35), 515.
[46] Schittenhelm and Weichardt, Münch. med. Woch., 1912 (59), 1089.
[47] Barratt and Yorke, Brit. Med. Jour., Jan. 31, 1914.
[48] Brit. Jour. Exp. Path., 1920 (1), 1.
[49] Clark and Evans, Bull. Johns Hopkins Hosp., 1920 (31), 354; 1921 (32), 113.

injections even of compatible blood may become sensitized to this foreign blood so that subsequent injections may result in serious accidents.[50]

The fluids that can be obtained from cancers have been found to be hemolytic, while antihemolysin has been found in ascitic and pleural effusions. Autolytic disintegration of liver, and presumably other tissues, may also lead to the presence of hemolytic substances in the blood.[51] Arseniuretted hydrogen may produce hemolysis in some such indirect way, since it causes no hemolysis in the test tube (Heffter). Probably the anemia of nephritis is at least partly the result of hemolytic action of the retained products of metabolism, in which connection the hemolytic properties of ammonium compounds may be recalled. The very great hemolytic action of soaps and free fatty acids, which varies directly with the number of unsaturated carbon atoms they contain (Moore[52]), makes it possible that these substances play a part in the hemolysis of disease, especially since the fatty acids of the liver are characterized by their high content of free fatty acids. Bile is strongly hemolytic, and in icterus this is an important consideration.[53]

In many forms of poisoning hemolysis is a prominent feature; in some it seems to be the chief effect of the poison, *e. g.*, potassium chlorate and arseniuretted hydrogen. In severe extensive burns there may occur hemolysis, and hemoglobinuria may also result. The hemoglobinuria of "blackwater fever" has been the cause of much discussion as to whether the malarial parasite or the quinine is the cause, with a divided opinion resulting, although, undoubtedly, cases do occur in malaria without administration of quinine. The studies of Brem[54] indicate that the hemolysis is produced by a hemolysin coming from the plasmodium, and that the quinine influences the condition by preventing the action of an antihemolytic substance present in the blood.

After removal of the spleen, hemolysis by the hemolymph nodes exceeds that of the primitive spleen, causing an excessive destruction of red corpuscles (Warthin[55]). This suggests that the spleen may normally dispose of some hemolytic agent which acts either by stimulating phagocytosis or by so altering the red cells that they are particularly susceptible to phagocytosis. This idea is not substantiated by the work of Pearce,[56] who found the anemia of splenectomy accompanied by an increased resistance of the corpuscles to hemolysis, and no hemolytic

[50] See Bowcock, Bull. Johns Hopkins Hosp., 1921 (32), 83.

[51] Maidorn, Biochem. Zeit., 1912 (45), 328. Hemolytic lipoids are believed by some to be liberated from injured tissues (see Kirsche, Biochem. Zeit., 1913 (55), 169), but McPhedran (Jour. Exp. Med., 1913 (18), 527) could find no evidence that any particularly hemolytic fatty acid, more active than oleic acid, can be isolated from either normal or diseased tissues.

[52] Brit. Med. Jour., 1909 (ii), 684; see also Lamar, Jour. Exper. Med., 1911 (13), 380.

[53] Urine contains hemolytic substances, possibly bile salts (Ponder, Brit. Jour. Exp. Path., 1921 (2), 34).

[54] Arch. Int. Med., 1913 (9), 129.

[55] Jour. Med. Research, 1902 (7), 435.

[56] Pearce *et al.*, Jour. Exp. Med., 1912, Vol. 16. See also Roccavilla, Arch. Med. Exp., 1915 (26), 508.

agent was present in the blood. There also occurs the group of anemias associated with great enlargement of the spleen, and in which removal of the spleen may result in a return to normal blood conditions; a fact suggesting, among other possibilities, that there may be poisons which stimulate directly the hemolytic action of the spleen, independent of the natural stimulation of splenic hemolysis which comes from the presence in the splenic blood of injured red corpuscles.[57] Hereditary hemolytic jaundice, (which is discussed in Chapter XX), also exhibits marked improvement, together with a decrease in the abnormal fragility of the red, cells after splenectomy. Numerous other conditions leading to pathological hemolysis are discussed elsewhere, especially under Anemia (*q. v.*). In closing, however, I would emphasize the comment of Rous[39], that an injury insufficient to produce hemolysis under test-tube conditions may bring it about in the animal body. Furthermore, an amount of serum hemolysin, which suffices to lake but a few cubic centimeters of blood *in vitro* may, when introduced into the organism, give rise to great destruction. This comes about in part through a transfer of the hemolysin from cell to cell, and partly through an increased fragmentation and phagocytosis of corpuscles to which they become liable, without any liberation of pigment having taken place. Also, some agents give rise to hemolysis only secondarily through changes they produce in the tissues.

Resistance to hemolysis varies greatly in disease conditions[58] and often specifically,—*i. e.*, resistance may be increased to one agent, decreased for another, and normal with a third. Attempts have been made to use this resistance as a diagnostic or prognostic index, but not with great success in most cases. Apparently changes in the plasma lead to alterations in the permeability of the corpuscles, which determines their behavior with hemolytic agents; also changes in the proportion of lipoids and hemoglobin may modify hemolysis. As an example of this condition may be cited observations on hemolysis by cobra venom, the corpuscles having been found less resistant to venom hemolysis in dementia precox, more resistant in carcinoma and syphilis. Butler[59] states that fragility of the corpuscles is abnormally high in exophthalmic goiter, cancer, syphilis, tabes, anemia, and malaria. In obstructive jaundice the corpuscles show an increased resistance to hemolysis by hypotonic salt solution, but in congenital hemolytic jaundice the resistance is decreased.[60] Resistance to hypotonicity is greatest at *p*H 9.5,[61] which corresponds with the point of minimum swelling of the cell colloids when in alkaline solutions. Bile salts increase the resistance of the corpuscles to osmotic hemolysis, decreasing at the same time the resistance to other hemolytic agents, and when in any disease the osmotic resistance is found increased without any jaundice, it may be concluded that the increased resistance depends on the presence of young corpuscles.[62]

Using saponin hemolysis, Bigland[63] found the resistance greatly decreased in icterus, although the serum had an increased protective action because of antagonism between the saponin and the bile salts; in all anemias resistance was found increased, except pernicious anemia, which showed normal or slightly subnormal resistance; high temperature decreases resistance. As will be seen from the few examples cited, the resistance to different hemolytic agents may vary with the same corpuscles.[64] Fur-

[57] See Banti, Semain Méd., 1913 (33), 313.
[58] Review by Paltauf, Kr ehl and Marchand's Handb. allg. Pathol., 1912 (II (1)), 83.
[59] Quarterly Jour. Med., 1913 (6), 145.
[60] See Richards and Johnson, Jour. Amer. Med. Assoc., 1913 (51), 1586. Giffin and Sanford, Jour. Lab. Clin. Med., 1919 (4), 465.
[61] Haffner, Arch. ges. Physiol., 1920 (179), 144.
[62] Rusznyak and Barat, Wien. Arch. inn. Med., 1922 (3), 429.
[63] Quart. Jour. Med., 1914 (7), 370.
[64] Bibliography by Krasny, Folia Hematol., 1913 (16), 353.

thermore, as pointed out by Brinkman,[15] the old corpuscles of the blood are less resistant, the young corpuscles more resistant. Undoubtedly the presence of a high cholesterol content of the blood increases resistance, while the phosphatids decrease it,[65] although the protective power of serum does not seem to be directly proportional to or wholly dependent on the serum lipoids.[66]

Paroxysmal Hemoglobinuria.[67]—This condition seems to depend upon the presence in the serum of a hemolytic amboceptor (an *autohemolysin*), which will combine with the corpuscles of the same individual and sensitize them for his own complement (Donath and Landsteiner, Eason). This autohemolysin can react with the corpuscles only at low temperature, such as may be furnished in the peripheral vessels by exposure to cold, and the complement unites with the sensitized corpuscles when the temperature again reaches 37° in other parts of the body. In susceptible persons attacks of hemoglobinuria may be brought on merely by holding the hands in cold water, and their blood serum will sensitize to hemolysis human corpuscles (even of normal individuals[68]) *in vitro* at low temperatures. Certain infections, especially syphilis,[69] predispose to paroxysmal hemoglobinuria. It is said that the hemolysis resulting from chilling in these cases may cause the blood to give a positive Wassermann test even in the absence of syphilis.[70] The autohemolysin of Donath and Landsteiner, however, is said not to be identical with the agent responsible for the Wassermann reaction,[71] and its effect is inhibited by cholesterol.[72] Not only the hemolytic amboceptors, but also an autoopsonin is present (Eason) and the resistance of the red corpuscles is decreased to various harmful agencies, including CO_2 and other weak acids.[73] The corpuscles of three cases studied by Moss[74] showed an increased resistance to hypotonic NaCl solutions. Just before the rigor, hemolysins may be found in the blood, disappearing after the hemoglobinuria (Roberts).[75] In a case studied by Cennie and Robertson,[76] hematuria resulted from destruction of only 6.3 c.c. of the patient's blood, and 90 per cent. of the liberated hemoglobin was excreted within two hours. There also are conditions in which *autoagglutination* without hemolysis occurs when the blood is cooled.[77]

Pathological Anatomy in Hemolysis.—The lesions produced in the organs of animals poisoned with hemolytic agents are usually pronounced and quite characteristic.

[65] Ehrentheil and Weis-Ostborn, Zeit. Immunität., 1923 (36), 356.
[66] Clark and Evans, Bull. Johns Hopk. Hosp., 1921 (32), 113.
[67] Landsteiner, Handbuch d. Biochem., Vol. 2 (1), p. 492; Meyer and Emmerich Deut. Arch. klin. Med., 1909 (96), 287.
[68] See Lorant, Deut. Arch. klin. Med., 1918 (126), 148.
[69] Matsuo, Arch. f. klin. Med., 1912 (107) 335; Lüdke, Deut. med. Woch., 1924 (50), 103.
[70] Burmeister, Zeit. klin. Med., 1921 (92), 19.
[71] MacKenzie, Proc. Soc. Exp. Biol., 1923 (20), 276.
[72] See Weis-Ostborn, Zeit. Immunität., 1923 (37), 165.
[73] Berghausen, Arch. Int. Med., 1912 (9), 137.
[74] Folia Serologica, 1911 (7), 1117.
[75] Brit. Med. Jour., 1915 (2), 398.
[76] Arch. Int. Med., 1915 (16), 205.
[77] See Rous and Robertson, Jour. Exp. Med., 1918 (27), 563; Clough and Richter, Bull. Johns Hop. Hosp., 1918 (29), 86.

There is often a subcutaneous edema, which is usually blood-stained, and similar fluid may be present in the serous cavities. The fat is yellowish, and the muscles are darker in color than normal. The spleen is usually much swollen, soft, friable, and very dark in color. The liver is usually swollen and mottled with red areas in a yellow background. The renal cortex is dark in color, even chocolate-colored, and the pyramids are comparatively light; hemoglobin is frequently present in the urine. In the lungs are often found hemorrhages or areas resembling small infarcts. The blood may be thin and even distinctly transparent. Microscopically the red corpuscles are found in all conditions of degeneration, and often fused together. In the liver, besides patches of congestion, fatty changes are present if the animal lives long enough. Large phagocytic cells packed with red corpuscles are abundant in the spleen and lymph nodes, as well as diffuse accumulations of the blood-cells, which are often fused; and much pigment is also present, both free and in the cells. Pigment also accumulates in the renal epithelium, which often shows much disintegration; congestion is prominent, and hemorrhages into both interstitial tissue and glomerules are frequent. Some of the lesions are due to the hemolysis, and some to the associated agglutination of corpuscles, which form hyaline thrombi. Pearce[78] has found that agglutinative serum when injected into dogs causes widespread necrosis in the liver, which is followed by proliferation of connective tissue and the production of changes resembling cirrhosis. There is a marked decrease in the glycogen content of the liver, and of its lipolytic activity (Andrea).[79]

[78] Jour. Exp. Med., 1906 (8), 64, Jour. Med. Research, 1906 (14), 541.
[79] Arch. internat. pharmacodyn., 1905 (14), 177.

CHAPTER XII

CHEMICAL MEANS OF DEFENSE AGAINST NON-ANTIGENIC POISONS[1]

ALTHOUGH the examples of acquired immunity against poisons of known chemical composition are few indeed, nevertheless the body possesses means of defense against many such poisons, which decrease to greater or less degree their harmful effects. It is to be noted, however, that the increased tolerance to such poisons is far less than the degree of tolerance characteristic of immunity to true toxins; thus, in arsenic eaters the maximum observed tolerance is but three or four times the minimum, and less than the certainly fatal dose (Hausmann); dogs can be made tolerant to only about three times the fatal dose of morphine (Faust). Furthermore, with many poisons of this class the tolerance is largely fictitious, since in spite of the absence of acute symptoms chronic poisoning is taking place; and, of course, with many poisons no distinct increase of tolerance can be produced. True immunity, associated with the production of neutralizing substances in the blood, has as yet been obtained only against substances of protein nature or substances very closely resembling the proteins. Ehrlich[2] believed that simple toxic chemicals are, like toxins, bound to the cells by special receptors, *chemoreceptors*, which, in view of their simpler function he assumed to be simpler than the receptors for toxins. He thought them to be more firmly fixed to the cells, and being, therefore, less easily discharged than bacterial receptors no free antibodies are produced by immunization.

Colloidal character apparently is essential for antigenic action of any substance, for, as Zinsser suggests, the more readily diffusible foreign substances can enter the cells and be destroyed or neutralized there, extracellular protective agents being needed only in defense against substances which cannot enter the cells. To be sure, there have been observations interpreted as evidence of immunity to large molecular non-protein complexes, especially such as lipoids and glucosides, but as yet the positive establishment of the formation of antibodies by reaction to non-protein antigens has not been accomplished. It must be taken into consideration, however, that various chemical substances introduced into the blood or tissues of an animal, may form compounds with the animal's proteins which then behave like foreign proteins, to which the animal reacts by becoming hypersensitive; in this way are explained the instances of idiosyncrasy, with reactions of anaphylactic character, which

[1] Bibliography by Hausmann, Ergebnisse Physiol., 1907 (6), 58.
[2] Beiträge z. exp. Path. u. Chem., Leipzig, 1909, p. 189.

are sometimes shown with iodoform, antipyrine, salvarsan, and other substances. (See Antigens, Chapter VII).

Studies on bacterial immunity and allied topics have as yet shown nothing to explain the acquirement of tolerance to morphine, alcohol, arsenic, and other similar poisons. A few observers have claimed that the serum of animals immunized to morphine will neutralize to some degree the toxic effects of morphine, but these results have not been substantiated. Others have claimed that increased oxidative powers are developed under the stimulation of the poison, which permits of its more rapid destruction, especially in the liver, but the experimental support of this hypothesis is slight. Still another idea is that, at least in the case of morphine,[3] decomposition products are produced, and accumulate in the body, that neutralize physiologically to some extent the morphine itself;[4] this hypothesis can scarcely be applied to arsenic and alcohol tolerance, nor has it been substantiated for morphine habituation.[5] It has been found that in animals habituated to morphine there is an increased power to destroy morphine; nevertheless, the blood of such animals still contains quantities of morphine toxic for normal animals, so there must be a certain refractoriness or cellular immunity in addition (Rübsamen).[6] Caffein tolerance also seems to depend on a refractory condition of the tissues,[7] but the local reaction which results from intradermal injection of morphine is said to be just as marked in morphine addicts as in normal individuals.[8] Schoen[9] found that the acidosis and depression in basal metabolism which ordinarily follow morphine administration is not observed in the habituated.

According to Schübel,[10] morphine tolerance in dogs is associated with hypochlorhydria, lipuria, and increased nuclein metabolism, the withdrawal symptoms being largely due to hyperchlorhydria. In man, most extended morphine addiction produces no noticeable change in protein metabolism.[11]

[3] Concerning immunity against morphine see Du Mez, Jour. Amer. Med. Assoc., 1919 (72), 1069; full bibliography. He summarizes the evidence as follows: "The only knowledge of a positive nature that we have at present concerning these problems is that the different organs and centers of the body acquire tolerance to morphine and heroin to a different degree and with varied degrees of readiness; that these drugs as such are excreted in the feces in diminishing amounts during the period of acquiring tolerance; and that there is evidently present in the blood serum of tolerant animals (dogs) during periods of abstinence a substance or substances which, when injected into normal animals of the same species, causes the appearance of symptoms identical with the so-called withdrawal phenomena. Whether or not the disappearance of these drugs from the feces is due to their increased destruction in the organism is still an unsettled question. It has not been proved that the destruction of morphine in the organism, if it does take place to an increased degree, is a causative factor in the production of tolerance. It may be only a concomitant phenomenon."

[4] Valenti, Arch. exp. Path. u. Pharm., 1914 (75), 437.

[5] Pellini and Greenfield, Arch. Int. Med., 1920 (26), 279; 1924 (33), 547.

[6] Also Takayanagi, Arch. exp. Path. u. Pharm., 1924 (102), 176.

[7] See Myers, Jour. Pharmacol., 1924 (23), 465.

[8] Pilcher and Sollman, Arch. Int. Med., 1924 (33), 516. However, reduced cutaneous sensitivity was found in addicts by Hecht, Zeit. ges. exp. Med., 1923 (38), 123.

[9] Arch. exp. Path. u. Pharm., 1924 (102), 205.

[10] Arch. exp. Path. u. Pharm., 1920 (88), 1.

[11] Glaubitz, Zeit. ges. exp. Med., 1921 (25), 230.

Schweisheimer[12] has shown that when chronic alcoholics and total abstainers are given equal quantities of alcohol, the alcohol content of the blood reaches a higher level, and persists for a longer time at a high level, in the abstainers. Apparently the alcohol-habituated organism can destroy alcohol more readily, presumably through more rapid oxidation.[13] Other factors are, however, involved in alcohol tolerance, for with equal quantities of alcohol in the blood the abstainers show a more marked intoxication than the habitual drinker. So, too, in morphine tolerance any general resistance through augmented oxidation seems inadequate in view of the specific increase in the tolerance of the respiratory center observed in this condition.[14] We also find that tolerance to one drug may be accompanied by tolerance to other drugs exerting similar physiological action.[15]

A consideration of all the evidence on acquired tolerance to hypnotics led Gunn[16] to the conclusion that, at present, it is permissible to make the following deductions:

"(1) Among drugs generally, it is chiefly to depressants of the central nervous system that tolerance can be acquired,—to alcohol, chloral, morphine, etc., rather than to, *e. g.*, codeine, strychnine or other alkaloids. (2) When a substance combines a depressant action on certain parts of the nervous system with a stimulant action on other parts of the nervous system or with other physiological actions, it is to the former chiefly or only that tolerance is established. The experiments of Van Egmond, Van Dongen and Tamura with morphine, Langer with heroin, and of Biberfeld with paracodeine, are all in agreement on this point. (3) In regard to these hypnotics, therefore, acquired tolerance is largely if not entirely due to the fact that certain parts of the central nervous system can acquire an increased resistance to the action of depressants. (4) It may not be accidental to this that tolerance can be acquired the more easily in different animals in proportion to the higher development of the brain, *e. g.*, in the order man, dog, rabbit, frog. (5) The fact that some degree of tolerance can be acquired to alcohol, chloral, veronal, morphine, cannabis indica, hyoscine, substances with little or no chemical similarity but which agree in being cerebral depressants, suggests strongly that tolerance is more intimately connected with their actions on the nervous system, in which they agree, than with increased destruction of them, in the mechanism of which they must necessarily disagree."

Defensive Mechanisms.—It is possible that the cell constituents with which some poisons ordinarily combine are produced in increased amounts under the stimulus of the poison, just as they are in the case of immunization with toxins, with the difference that the combining substances are

[12] Deut. Arch. klin. Med., 1913 (109), 271.

[13] See also Völtz and Dietrich, Biochem. Zeit, 1915 (68), 118. J. Hirsch, *ibid.*, 1916 (77), 129.

[14] Van Dongen, Arch. ges. Physiol., 1915 (162), 54.

[15] See Myers, Jour. Pharmacol., 1916 (8), 417. However, Biberfeld finds morphine tolerance to be specific (Biochem. Zeit., 1916 (77), 283).

[16] Gunn, Physiol. Reviews, 1923 (3), 41.

not thrown off into the blood. For example, it has been claimed that arsenic is ordinarily combined and held in the liver by a nucleoprotein, and the suggestion has been made that in arsenic habitués this nucleoprotein is increased in amount. Again, saponin seems to act upon the cholesterol of the red corpuscles, and Kobert observed increased resistance to the action of saponin exhibited by the serum of immunized animals, which he attributes to an increased amount of cholesterol, perhaps liberated by the corpuscles decomposed by the injected poison, or perhaps produced in excess by the tissues. Wohlgemuth[17] has also suggested that in the case of poisoning with large amounts of substances which combine with glycuronic acid (*e. g.*, lysol), excessive quantities of this substance are formed by the cells and excreted into the blood, where they neutralize the poisons in much the same manner as the antitoxins neutralize toxins.

Sherwin believes that under necessity the animal body may build up out of waste nitrogenous products (urea or its antecedents) the necessary amino acid for detoxication of certain aromatic fatty acids.[18] His results indicate that glycine and glutamine may thus be built up from urea by mammalian organisms, while fowls form ornithine for the same purpose. Nevertheless, if such adaptations do exist they are probably of no great range or persistence, for it is apparent that the amount of combination of simple chemical poisons which may be thus detoxicated is very limited, and that it is usually easily possible to introduce an excess which goes uncombined.

There exists also a natural tolerance to many poisons, which may differ strikingly in different species, and is, therefore, of hereditary origin, just as some types of hypersensitivity are hereditary. Gunn[16] has reviewed the literature of this subject and calls attention to the fact that with a great many drugs, *e. g.*, most heavy metals, quinine, chloral, phenol, etc., the minimum lethal dose per kilogram is very nearly the same for all species of warm-blooded animals; on the other hand with some drugs the difference may be as much as 100 or more to 1. When one species shows a high resistance as compared with the majority, it is regarded as congenital tolerance in the former; if a low resistance, congenital intolerance or hypersusceptibility. Among the examples of congenital tolerance may be mentioned that of the toad to the digitalis-like poison of the toad's dermal glands as well as to digitalis itself, and other poisons of the digitalis group; also the resistance of rats and grass snakes to these same poisons. This tolerance depends upon a specific lack of susceptibility of the heart muscle of these animals, not accompanied by increased tolerance to many other poisons. Even more striking in degree is the resistance of the hedgehog to cantharidin, for Ellinger[19] calculated that one gram of cantharidin is a fatal dose for 20,000 kgm. of man, 500 kgm. of rabbit and 7 kgm. of hedgehog. He found that this tolerance is not

[17] Biochem. Zeitschr., 1906 (1), 134.
[18] Shiple and Sherwin, Jour. Amer. Chem. Soc., 1922 (44), 618.
[19] Arch. exper. Path. u. Pharm., 1900 (45), 89.

due to defective absorption, for it is true also of intravenous injection. It is not due to chemical change or neutralization in the body because cantharidin could be recovered unaltered in the urine. His experiments showed clearly that the tolerance is due, partly at least, to an insusceptibility of the kidney cells to the action of cantharidin. Also to be mentioned in this list is the congenital tolerance of the rabbit and frog to atropine, which seems to depend on a particular capacity of the liver of these animals to destroy this alkaloid.

But besides these scanty examples of tolerance to poisons, the body possesses a number of methods for opposing many other poisons with more or less success; and, poisons invariably acting chemically, the defenses are in turn largely chemical. We have elsewhere referred to the destructive action of the enzymes of the digestive tract upon bacterial and similar poisons; this means of defense cannot apply to non-protein chemical substances except possibly glucosides and toxic lipoids. But the acidity of the gastric juice, the alkalinity of the bile and pancreatic juice, and the precipitating effect of the hydrogen sulfide formed in intestinal putrefaction, are all factors that help to neutralize or prevent the absorption of certain poisons, their total efficiency, however, being on the whole very slight. The epithelium of the alimentary canal seems to have some remarkable and as yet unexplained capacity to protect the blood from the entrance of poisons. It is easy to understand that poisonous colloids such as snake venom, are held back or destroyed by the epithelium, but apparently crystalloidal poisons are also often controlled by the intestinal wall. For example, Koessler and Hanke[20] found that histamine, which is extremely toxic when given beneath the skin, may be introduced into the stomach in enormous amounts without harm, although fatal results may follow absorption from the mouth.

It is also of interest to consider that *unicellular organisms* may show a marked capacity to increase their resistance to poisons, as shown especially by Ehrlich's studies on trypanosomes, which readily become immune to various trypanocidal drugs, including arsenic compounds, and which transmit this acquired immunity through succeeding generations.[21] Yeasts and bacteria can also exhibit increased tolerance to antiseptics, and Effront[22] found that yeasts owe their augmented tolerance to fluorides to an increased content of calcium, which precipitates the fluoride which enters the cells; this tolerance is also transmitted to new generations of yeasts. He found that the tolerance of yeast to arsenic depends on the production of H_2S or some other compound, according to the strain of yeast, while protection against formaldehyde he ascribes to an increased capacity to oxidize it to formic acid. The mechanism of this acquired tolerance exhibited by yeasts he attributes to selection rather than true immunization. Perhaps the best studied example of the development of

[20] Jour. Biol., Chem., 1924 (59), 889.
[21] See Voegtlin *et al.*, Jour. Pharm., 1924 (23), 55.
[22] Réunion soc. belg. biol., 1920, p. 99.

tolerance by bacteria to chemicals is that of the resistance of the pneumococcus to optochin,[23] but there are other examples.[24]

The acquired tolerance is specific in all these cases, and may, indeed, be accompanied by a decreased resistance to other poisons; thus, protozoa accustomed to alcohol may be more susceptible to other chemicals.[25] *Paramecia* made immune to antimony or arsenic transmit this specific immunity to succeeding generations (Neuhaus);[26] they seem to protect themselves by converting the trivalent As and Sb to the less toxic pentavalent form (Neuschloss).[27] Malarial parasites notoriously develop a resistance to quinine used therapeutically, and Neuschloss[27] found that *Paramecia* rendered quinine-resistant owe this to their increased capacity to destroy quinine. However, persons habituated to quinine show no evident increased capacity to destroy it.[28]

With isolated mammalian cells in tissue cultures some increased tolerance to copper and arsenic may be obtained by habituation,[29] suggesting that such habituation depends on substances within the cells.

After absorption of a poison a large series of chemical reactions and physiological processes is brought into play, and there are few poisons indeed whose harmful influence is not more or less decreased by these means. Kobert[30] classifies these protective processes as follows:

1. Rapid elimination, either before absorption by means of diarrhea and vomiting or by the same means after absorption in case the poisons are excreted into the digestive tract (*e. g.*, morphine, venoms, antimony, and many other metals). Many poisons are very rapidly eliminated by other routes (*e. g.*, anesthetics, curare), in some instances causing harm, particularly to the eliminating organ (*e. g.*, kidneys in phenol poisoning, intestines in ricin poisoning). The routes and conditions of elimination of poisons have been fully discussed by Lewin.[31]

2. Deposition and Fixation in Single Organs or Tissues.—In this respect the liver is especially important, probably because of its location and function as a filter for all the blood coming fresh from the alimentary tract.[32] The manner and means by which this fixation is brought about are unknown. It is possible that the power of the tissues to bind poisons may become increased by repeated doses, leading to "specific acquired tolerance."[33] According to Slowtzoff[34] arsenic is fixed by the nucleus in a very firm combination;[35] mercury by globulins in a less stable combination; copper by the nucleins, but less firmly than the arsenic. Other poisons, chiefly alkaloids are probably combined with bile acids. Possibly some poisons combine with *glycogen*.

[23] Morgenroth and Kauffmann, Zeit. f. Immunität., 1912 (15), 610; Schnabel, Klin. Woch., 1924 (3), 566.

[24] See Dale, Physiol. Reviews, 1923 (3), 366; Richet *et al.*, Compt. Rend. Acad. Sci., 1922 (174), 345.

[25] Daniel, Jour. Exper. Zool. 1909 (6), 571.

[26] Arch. Internat. Pharmacodyn., 1910 (20), 393.

[27] Arch. ges. Physiol., 1919 (176), 223; 1920 (178), 61 and 69.

[28] Giemsa and Halberkann, Deut. med. Woch., 1917 (43), 501.

[29] Wilson, Bull. Johns Hopkins Hosp., 1922 (33), 375.

[30] "Lehrbuch der Intoxikationen," Stuttgart.

[31] Deut. med. Woch., 1906 (32), 169; see also Mendel *et al.*, Amer. Jour. Physiol., 1904 (11), 5; 1906 (16), 147 and 152.

[32] Concerning the detoxicating function of the liver see Woronzow, Dissertation, Dorpat, 1910; Rothberger and Winterberg, Arch. internat. Pharmacodyn., 1905 (15), 339.

[33] Santesson, Skand. Arch. Physiol., 1911 (25), 28.

[34] Hofmeister's Beitr., 1901 (1), 281; 1902 (2), 307.

[35] Denied by Heffter (Arch. internat. de Pharmacodyn., 1905 (15), 399), who considers it more a physico-chemical process.

These compounds are but slowly broken up, and thus the poison reaches the more susceptible and more important tissues in a relatively diluted condition. The bones seem to hold in harmless form poisonous fluorides, and to less extent arsenic, barium, and tungsten, which persist in the bones for a great length of time. Leucocytes are possibly important binders of poisons, perhaps through combination with their nucleins,[36] but storage in these labile cells is necessarily of relatively brief duration.

3. Combination with substances formed or contained in the tissues; the resulting substance being less toxic than the poison alone. Under this heading may be included both chemical combination and physical absorption or solution, such as the deviation of the lipoid-soluble narcotics from the central nervous system by excessive tissue fats, or by fats therapeutically introduced.[37] Many poisons combine with the inorganic constituents of the tissues; *e. g.*, barium and various aromatic substances with SO_4, silver with Cl, etc.

4. Chemical alteration, with or without subsequent combination with other substances, by such means as oxidation, reduction, hydrolysis, and neutralization.

5. Impaired absorption should also be considered as a means of defense against poisons. This may depend upon the injury to the gastro-intestinal tract produced either by the poison itself or by some independent pathological condition. Cloetta considers impaired absorption important in acquired immunity to arsenic (see below) and it may also modify the effects of other poisons.[38]

The chemical reactions employed in defense against simple chemical poisons have been particularly considered by E. Fromm,[39] whose outline is here partially followed, and to which the reader is referred for early bibliography.

INORGANIC POISONS

Metallic poisons, such as lead, silver, mercury, and arsenic, are made insoluble, particularly by forming compounds with proteins in the alimentary tract, intestinal walls, blood, or internal organs; also by forming sulfides with the H_2S of the intestinal contents.

According to Cloetta[40] immunization against arsenic depends entirely upon a reduction of absorption in the intestine, for the longer arsenic is taken, the less appears in the urine and the more appears in the feces.[41] At the same time the resistance to arsenic injected subcutaneously is not increased at all, and no increase in resistance can be obtained by repeated subcutaneous injections of sublethal doses. There is, however, reason to question the authenticity of the reputed tolerance of habitués to arsenic (Joachimoglu),[41] and Schwartze[42] points out that much of the supposed tolerance to arsenic may depend on the insolubility of arsenious oxide when administered in the usual coarse crystalline form, for no tolerance can be obtained against soluble arsenic compounds.

Antimony does not produce tolerance in experimental animals (Cloetta).[43] The manner in which various inorganic ions antagonize the

[36] Stessano, Compt. Rend. Acad. Sci., 1900 (131), 72.
[37] See Graham, Jour. Inf. Dis., 1911 (8), 147.
[38] v. Lhota, Arch. internat. pharmacodyn., 1912 (22), 61.
[39] "Die chemischen Schützmittel des Tierkörpers bei Vergiftungen," Strassburg, Karl Trübner, 1903. See also résumé by Ellinger, Deut. med. Woch., 1900 (26) 580.
[40] Arch. exp. Path. u. Pharm., 1906 (54), 196; Corresponbl. Schweizer Aerzte, 1911 (41), 737.
[41] Not accepted by Hausmann, Ergebnisse Physiol., 1907 (6), 58; or Joachimoglu, Arch. exp. Path., 1916 (79), 419.
[42] Jour. of Pharm., 1922 (20), 181.
[43] Arch. exp. Path. u. Pharm., 1911 (64), 352.

physiological action of one another (*e. g.*, sodium and potassium, calcium and magnesium) is still an unsettled problem.[44]

Free acids and alkalies are partly neutralized by the alkaline and acid contents of the gastro-intestinal tract, partly by forming compounds with the proteins, and partly by the alkalies and carbonic acid of the blood stream. (See "Acid Intoxication," Chap. XXII.) Phosphorus[45] and sulfides are oxidized after absorption into phosphoric and sulfuric acid, which are in turn neutralized by the alkalinity of the blood and tissues. Lillie[46] has called attention to the close, palisade arrangement of the nuclei of the epithelium lining the alimentary tract, which makes it necessary for all substances absorbed to pass through the zone of their active oxidative influence, a fact undoubtedly of great importance in the defense of the body.

Reduction of iodic acid, chloric acid, hypochlorous acid, and their salts occurs in the body, resulting in their conversion into the much less toxic iodides and chlorides. Tellurium compounds are also reduced and rendered insoluble. This reaction occurs to some extent in the intestines; how much in other organs is unknown.

Methylation, the addition of CH_3 groups, is observed in poisoning by tellurium, which is eliminated in the breath as methyl telluride, and also in the sweat and feces.[47] Selenium, pyridine, and some other substances also combine with methane. The source of the methane is possibly in the xanthine molecule, and Tomita[48] found evidence that the chief site of methylation is in the liver.

Summary.—There are, therefore, three chief reactions used against inorganic poisons in the body, *oxidation*, *reduction*, and *splitting off of water;* neutralization of acids or alkalies and the formation of albuminates and sulfides being included under the last heading, since in these reactions the splitting off of water is an essential step.

ORGANIC POISONS[49]

In the case of organic poisons an equally small number of primary reactions is employed in their detoxication, but in more complicated manners and combinations corresponding with the complexity of organic compounds. As Sherwin has pointed out, animals of different species may employ different means of defense, *e. g.*, in man, phenyl-acetic acid combines with glycine, in birds with ornithine.[50]

[44] See Osterhout, Proc. Phil. Soc., 1916 (55), 533.

[45] Increased tolerance to phosphorus may be obtained by repeated small doses, but it lasts only while the poison is being given continuously (Oppel, Ziegler's Beitr., 1910 (49), 543). Accompanying the tolerance are structural changes in the liver cells to which are ascribed some significance by Oppel.

[46] Amer. Jour. Physiol., 1902 (7), 412.

[47] See Mead and Gies, Amer. Jour. Physiol., 1901 (5), 105. Caffein may be demethylated in the liver, Kotake, Zeit. physiol. Chem., 1908 (57), 378.

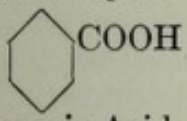
[48] Biochem. Zeit., 1921 (116), 48, 55.

[49] Review by Sherwin, Physiol. Reviews, 1922 (2), 238.

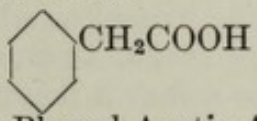
[50] Crowdle and Sherwin, Jour. Biol. Chem., 1923 (55), 13.

Oxidation, which has already been mentioned as a means of destruction of bacterial toxins, is naturally one of the most effective agents in the destruction of simpler organic substances, since the ordinary decomposition of all organic food-stuffs is through oxidation. There are numbers of specific examples of the conversion of a poisonous into a less poisonous or non-poisonous substance by oxidation. All acids of the fatty acid series are oxidized vigorously in the body, eventually into CO_2 and H_2O; and pathologically produced acetic and lactic acids are destroyed in this way. The liver contains an oxidase that destroys alcohol, and it is not increased in the livers of animals made tolerant to alcohol (J. Hirsch).[51] Uric acid is oxidized vigorously by many organs (except in man), as are other members of the purine series, such as caffeine and theobromine. Presumably oxidation of organic poisons as well as of food-stuffs is brought about by the oxidizing enzymes of the cells, as shown by Ehrlich's *indophenol reaction* (see p. 57), which consists of the oxidation of paraphenylene diamine and α-naphthol, with a resulting synthesis. This reaction is said by Lillie[46] to occur principally in and about the cell nuclei or cell membranes.

In general, aliphatic compounds are oxidized completely, with few exceptions, whereas most aromatic compounds resist oxidation of the benzene ring, although an aliphatic side chain will be partially oxidized. If the side chain contains an odd number of carbon atoms the final product is benzoic acid, whereas with even numbers of carbon atoms in the side chain the end product is phenyl-acetic acid.



Benzoic Acid



Phenyl Acetic Acid

From observing this law, Knoop developed the principle of beta oxidation, that is, that these compounds are broken down two carbon atoms at a time because the oxidation attacks the β-carbon atom. On the other hand, alpha-amino acids are attacked at the α position in the process of deamination, but after the α carbon is oxidized the resulting fatty acid is then subject to oxidation at the β position only. If a benzene ring is attached to an amino acid side chain it may then be readily oxidized, *e. g.*, tyrosine, phenylalanine.

Reduction is less common than oxidation, but numerous examples are known, *e. g.*, quinone to hydroquinone, chloral to trichlorethyl alcohol. Nitro compounds especially are likely to undergo reduction to amines, which then are often acetylated. Sometimes oxidation and reduction occur simultaneously and as complementary processes.

Deamination is an important process in the metabolism of amino acids, and may assume importance as a protective mechanism in the destruction of poisonous amines formed by decarboxylation of amino acids.

Combination, with or without Preliminary Oxidation.—Oxidation is also an essential preliminary step to many of the protecting

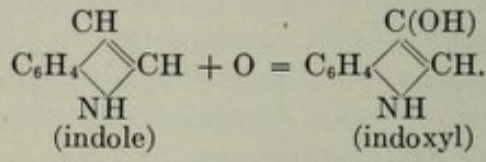
[51] Biochem. Zeit., 1916 (77), 129.

combinations, in which a cell constituent is united to an organic poison. The most important of these combining substances are:

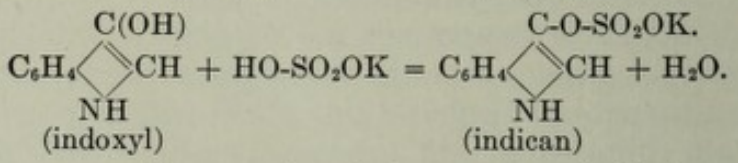
1. Sulfuric Acid.—One of the earliest and most important observations on the protective action of sulfuric acid was made by Baumann and Herter,[52] who showed that phenol is eliminated as a potassium salt of the sulfuric acid derivative, as follows:

$$C_6H_5OH + HO\text{-}SO_3K = C_6H_5O\text{-}SO_3K + H_2O,$$

a reaction that has been put to practical use in treating phenol poisoning. As phenol and cresols are produced constantly in intestinal decomposition, this reaction is undoubtedly of great service, since the salt formed is relatively harmless. *Indole* and *skatole* are similarly detoxicated by being converted into corresponding salts, but only after a preliminary oxidation into *indoxyl* and *skatoxyl*, according to the following reaction:



(indole) (indoxyl)



(indoxyl) (indican)

A host of other aromatic organic substances are similarly combined with sulfuric acid, with or without preliminary oxidation, including all substances resembling phenol or which through oxidation are changed into phenols, such as cresol, thymol, aniline, naphthaline, pyrogallol, and tannin. By this means a poisonous substance is converted into a relatively harmless one, which is readily soluble and rapidly eliminated.

2. Glucuronic acid occupies the same position as sulfuric acid, combining particularly with naphthol, thymol, camphor, chloral hydrate, and butyl chloral. Sometimes a substance may appear in the urine combined in part with sulfuric, in part with glucuronic acid, showing the similarity of their function. Apparently when there is not sufficient sulfuric acid in the body to combine with all the poison, the excess unites with glucuronic acid,[53] although combination between glucuronic acid and the aromatic substance begins to occur before all the sulfuric acid is exhausted.[54] Glucuronic acid represents merely a first step in the oxidation of glucose, as follows:

$$\underset{\text{(glucose)}}{OHC\text{-}(CHOH)_4\text{-}CH_2OH} + O_2 = \underset{\text{(glucuronic acid)}}{OHC\text{-}(CHOH)_4\text{-}COOH} + H_2O.$$

This oxidation occurs after the aldehyde group of the glucose has been combined by some other substance; hence the aldehyde group escapes oxidation, although ordinarily more easily oxidized than the alcohol group.

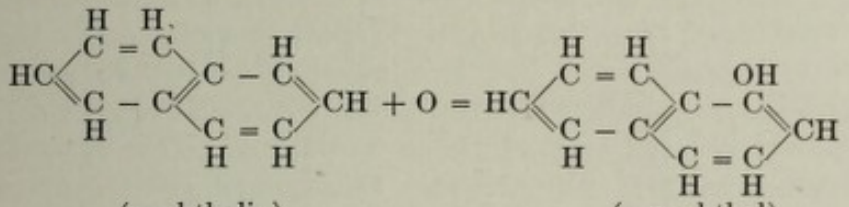
[52] Zeit. physiol. Chem., 1877 (1), 247.

[53] See Austin and Barron, Boston Med. and Surg. Jour., 1905 (152), 269. Wohlgemuth has observed a case in which all the sulfuric acid of the urine was in organic combination (Berl. klin. Woch., 1906 (43), 508).

[54] See Salkowski, Zeit. physiol. Chem., 1904 (42), 230.

Sherwin says that, in general, an organic compound incapable of complete oxidation is usually converted into an alcohol or an acid, the former usually forming for excretion an ethereal sulfate or a glucuronate, the acids combining with an amino acid.

Just as with the addition of sulfuric acid, oxidation may be a preliminary step to the addition of glucuronic acid; *e. g.*, naphthalin is oxidized into α-naphthol, before uniting to glucuronic acid, as follows:



(naphthalin) (α-naphthol)

The same is the case with many camphors and terpenes. Reduction may be the preliminary step as with chloral hydrate, which is first reduced to trichlor-ethyl-alcohol. In still other cases splitting off of water is the chief preliminary step.

3. Glycine is one of the longest-known combining substances, the observation of the combination of glycine with benzoic acid to form *hippuric acid* being the first proof of synthesis in the animal body, discovered by Wöhler (1824). The reaction is as follows:

$$\underset{\text{(benzoic acid)}}{C_6H_5COOH} + \underset{\text{(glycine)}}{H_2N\text{-}CH_2\text{-}COOH} = \underset{\text{(hippuric acid)}}{C_6H_5CO\text{-}HN\text{-}CH_2\text{-}COOH}.$$

A special enzyme has been found in kidney substance which can bring about this reaction outside the body. Normally this enzyme occurs chiefly in the kidney but may also occur in other organs. Many other aromatic compounds also combine with glycine before elimination, *e. g.*, salicylic acid. Some are first altered to a suitable form by oxidation; *e. g.*, toluene is oxidized to benzoic acid, xylene to toluic acid, nitrobenzaldehyde to nitrobenzoic acid. Many of the substances that can be made to combine with glycine in the body are of such a foreign nature that they never could need neutralization under any other than experimental conditions, but here, as with the sulfuric and glycuronic acid reactions, combination occurs whenever a suitable substance is present in the blood, glycine always being abundant as a cleavage product of the proteins. Shiple and Sherwin[55] found evidence that the animal body may also form additional glycine from the urea fraction of urinary nitrogen when needed for purposes of neutralizing poisons. In fowls instead of glycine, *ornithine*, a constituent of arginine, serves to neutralize benzoic acid.

4. Glutamine.—This may combine with phenyl acetic acid, just as glycine combines with benzoic acid, and when large amounts are needed it may be built up at the expense of urea.[55]

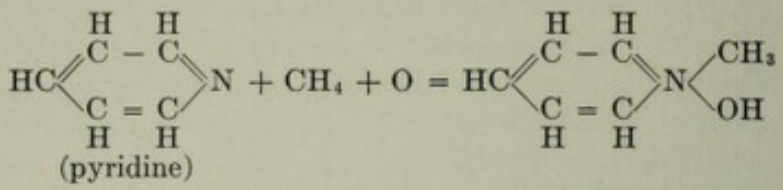
5. Cysteine.—This amino acid has been found to combine with certain halogenized benzene compounds, *e. g.*, brombenzol. It is not known

[55] Jour. Amer. Chem. Soc., 1922 (44), 618.

to unite with any other toxic substances, although Hopkins has given evidence that it may be of importance in vital oxidation.

6. Urea may also be a means of defense, forming salts with organic acids which are rapidly eliminated; *e. g.*, amido-benzoic acid and nitro-hippuric acid.

7. Methane.—Methylation, which occurs also with tellurium, is observed on administration of pyridine, as shown by the following equation:



This reaction is of special importance, because many alkaloids contain a pyridine group; and the resulting methyl compound may be less toxic than the original alkaloid—*e. g.*, methyl morphine.

8. Sulfur split off from proteins may combine with CNH and CNK, converting them into the much less toxic sulfocyanides.[56]

9. Bile Acids.—All the above mentioned reactions are protective largely because the substances formed are soluble and rapidly eliminated, as well as being less toxic than the original poison. Compounds of many poisons are formed with bile acids which are insoluble, and therefore only slowly dissolve or decompose, thus protecting the body from overwhelming doses of the poison. Such compounds are formed, not only with inorganic poisons, but also with alkaloids, especially strychnine, brucine, and quinine. They are then deposited in the liver, to be slowly dissolved and eliminated.

10. Acetic Acid.—Acetylation is observed chiefly in the formation of acetylamino compounds, but furfural unites directly with acetic acid to form furfuracrylic acid.

11. Calcium may be considered a defensive agent against certain poisons [oxalic and citric acids] with which it forms insoluble compounds, and it is probable that the toxicity of oxalates depends largely upon their robbing the cells of calcium.[57]

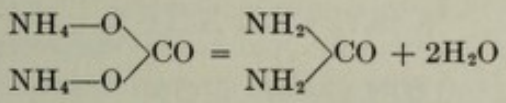
Neutralization of organic acids entering the body or formed in metabolism is accomplished by the sodium carbonate of the blood when in small amounts; if excessive in quantity (*e. g.*, diabetic coma), a portion is combined with ammonia and appears as an ammonium salt in the urine. As Shiple and Sherwin point out, inorganic acids are commonly neutralized by ammonia, whereas aromatic organic acids are united with amino acids even at the expense of urea formation. Magnesium and calcium salts may also help in the neutralization, probably at the expense of the bone tissue.[58] (See Acid "Intoxication," Chap. XXII.)

[56] See Meurice, Arch. int. Pharmacodyn., 1900 (7), 11.

[57] See Robertson and Burnett, Jour. Pharmacol., 1912 (3), 635.

[58] In this connection it may be mentioned that the bactericidal power of the blood is increased if the blood is more alkaline, decreased if it is less alkaline, than usual.

Dehydration, which plays a prominent part in a number of the above-mentioned syntheses, is particularly important in the change of ammonium carbonate into urea:



As ammonium salts of all sorts are very toxic, especially hemolytic, while urea is not, this process is probably one of the most important detoxicating reactions of the body because of the great amount of ammonium compounds that is constantly being formed in nitrogenous metabolism.

Summary.—The variety of reactions and the variety of defensive substances are both remarkably small in number. The reactions are: Oxidation and reduction, hydration and dehydration, and perhaps simple addition (methylation). The chief known protective substances are the alkalies of the blood, proteins, hydrogen sulfide, sulfuric acid, glycine, urea, cysteine, glutamine, bile acids, glucuronic acid, and acetic acid. *All these substances are normally present in the body, and none of them is specific against any one poison,* but each combines with several poisons. This last fact is interesting in comparison with the highly specific nature of the immune substances against bacteria and their products.

As far as we know, no particular increase in the neutralizing substances results from the administration of inorganic or organic poisons. The body does not appear to produce any excessive amounts of sulfuric acid in carbolic-acid poisoning, or of glycine when benzoic acid is administered. Both substances are present in the body normally, and as much as is available combines with the poison; if there is not enough, the remaining poison combines with something else, or goes uncombined. In other words, the neutralizing substances described above do not appear to be the result of any special adaptation to meet a pathological condition. They are present in the body as a result of normal metabolism; they have an affinity for various chemical substances, some of which happen to be poisons; if these poisons happen to enter the body, they may be combined and neutralized to some extent, but, as a rule, very incompletely. There appears to be no elaborate process of defense against the chemically simple poisons, such as seems to be called into action by bacterial infection, and hence a degree of resistance or immunity similar to that present after an attack of scarlet fever or smallpox does not exist for strychnine or phosphorus.

CHAPTER XIII

INFLAMMATION[1]

ALTHOUGH morphological alterations are prominent features of the reaction of the tissues to local injury and infection, yet at the bottom the processes of inflammation are brought about by and result in chemical alterations. The causes of inflammation are in nearly all cases chemically active substances, but for the most part their nature is too little known to permit of speculation as to what chemical characteristic or characteristics a substance must possess to exhibit the power of causing an inflammatory reaction. Even in the case of inflammation due to mechanical, thermal, and electrical injuries, it seems probable that most of the features of the inflammatory reaction are brought about by the action of chemical substances produced by alterations in the tissue constituents at the point of injury,[2] for tissue proteins that have been altered in necrosis are chemotactic,[3] as also are extracts of tissues.

The essential features of inflammation, namely, local hyperemia and related vascular disturbances, exudation of plasma, migration of leucocytes and their phagocytic action, all may be caused by the action of chemical substances upon the vessels and leucocytes. *Active hyperemia* in the case of inflammation is due to stimulation of the vasodilator nerves or paralysis of the vaso-constrictors, or direct paralysis of the muscular fibers of the arterioles; these may result from mechanical, thermal, or electrical stimuli, but in local infection the cause is usually chemical products of bacterial growth or of tissue disintegration; *e. g.*, amino acids have a vasodilator effect.[4] On the other hand, anything reducing vasodilatation reduces the inflammatory reaction.[5]

The *escape of blood plasma* (inflammatory edema) appears to depend upon a number of factors (discussed more fully under "Edema," Chap.

[1] For early reviews and bibliography see Adami, in Allbutt's System of Medicine; reprinted also as a monograph, "Inflammation," 1909; also Opie, Arch. Int. Med., 1910 (5), 541.

[2] Schlaepfer (Zeit. exp. Path., 1910 (8), 181) finds that the reduction of methylene blue is decreased in inflammatory areas, and advances the hypothesis that inflammatory stimulants are oxidation stimulants, inflammation occurring only when the amount of oxidation aroused by the stimulant is insufficient. In accord with this is the observation of Amberg (Zeit. exp. Med., 1913 (2), 19) that substances facilitating oxidation reduce inflammatory reactions. (See also Woolley, Jour. Amer. Med. Assoc., 1914 (63), 2279.) The O consumption of inflamed skin is greater than normal. (Gessler, Arch. exp. Path. u. Pharm., 1921 (91), 366.) Another observation of similar significance is that phagocytosis is stimulated by H_2O_2, and that phagocytes react to HNC in the same way as the respiratory center (Hamburger, Internat. Zeit. phys.-chem. Biol., 1915 (2), 245–264).

[3] Bürger and Dold, Zeit. Immunität., 1914 (21), 378.

[4] Brouha and Fredericq, Compt. Rend. Soc. Biol., 1924 (91), 1169.

[5] See Hirschfelder, Amer. Jour. Physiol., 1924 (70), 507.

XVI) of which the most important seem to be: (1) Injury to the capillary walls, produced largely by the chemical causes or products of the inflammation; (2) increased osmotic pressure in the tissues, due to increased or abnormal formation of crystalloidal substances with high osmotic pressure from large molecular compounds, many of which are colloids (proteins) without appreciable osmotic pressure; (3) alterations in the hydration capacity of the colloids, whereby, through decrease in salts or increase in acidity, they come to possess a greater affinity for water (M. H. Fischer). There are abundant changes in the tissues to account for the escape of fluid on these physico-chemical grounds, for Schade[6] has found that in an area of inflammation the osmotic pressure may be raised to as high as eleven atmospheres and the H-ion content may be increased 50 times the normal. The tissues in the vicinity of inflamed areas also exhibit an excessive demand for oxygen (Gessler).[2]

By far the most characteristic feature of acute inflammation, however, is the *behavior of the leucocytes*—their increase in number in the blood, their migration from the vessels and accumulation about the point of injury, and their engulfing and destroying various solid particles, such as bacteria and degenerating tissue elements. These processes, which seem to indicate something approaching independent volition on the part of the leucocytes may, however, be well explained by application of known laws of chemistry and physics, without passing into the realms of the metaphysical. This will be attempted under the heading of:

AMEBOID MOTION AND PHAGOCYTOSIS

The accumulation of leucocytes at a given point in the body indicates that some means of communication must exist between this point and the leucocytes in the circulating blood. No direct communication by the nervous system or other structural method existing, the only possible explanation is that the communication is through the fluids of the body, and depends upon changes in their chemical composition or physical condition. As the latter generally depends upon the former, the communication is considered to be accomplished by chemical agencies, and called *chemotaxis*.

CHEMOTAXIS

Changes in the chemical composition of a fluid have been shown frequently to affect the motion of living organisms suspended in it. One of the earliest observations was that of Engelmann,[7] who noticed that *Bacterium termo* suspended in water tended to accumulate about a bubble of oxygen in the water. Pfeffer[8] discovered that the spermatozoids of certain ferns were attracted powerfully by very dilute solutions of malic acid, which is contained in the female sperm cell, indicating that the migration of the sperm cells in the proper direction depends on a

[6] Münch. med. Woch., 1924 (71), 1.
[7] Botanische Zeitung, 1881 (39), 441.
[8] Untersuch. aus dem Bot. Institute in Tübingen, 1881-1888, Bd. 1 und 2.

chemical communication, and he proposed the term chemotaxis for this phenomenon. Strong solutions of malic acid, on the other hand, repelled spermatozoids. Cane-sugar was found to attract the spermatozoids of a certain foliaceous moss. In the case of the malic acid, it seems to be the anion that produces the effect, since salts of malic acid have exactly the same property.

Stahl's[9] experiment with a large jelly-like plasmodium (*Aethalium septicum*) growing on bark in wet places, has become classical. He found that if the plasmodium was placed on a moist surface, and nearby was placed a drop of an infusion of oak bark, the organism moved by the process of protoplasmic streaming toward and into the infusion. If a piece of oak bark was placed in the water, plasmodial arms were stretched out to it and the piece of bark was soon completely surrounded by the organism. These movements were found to occur in any direction, even exactly against the force of gravity. Other substances, as acids or strong solutions of salt or sugar, were found to cause the plasmodium to move away from them, although when sufficiently dilute they exerted an attraction. A plasmodium might, however, move into a strong sugar solution if kept with a scanty supply of moisture for some time, and after it had lived for a while in such a strong solution (2 per cent.), a weaker solution or pure water was as injurious as the concentrated sugar solution previously had been. Temperature was also found to exert a marked *thermotactic* effect. If a plasmodium was placed on a filter-paper, one end of which was in water at 7°, and the other in water at 30°, it would move toward the warmer end.

The Theory of Tropisms.—Ciliated protozoa, which can move about freely in water, show very distinct reactions to stimuli of all sorts. The first step in their change of direction of movement is considered by many observers to be an orientation of the organism until it is headed in the axis along which it is to move. This is ascribed by J. Loeb[10] to the existence of a certain degree of equality of irritability of symmetrical parts of the body. The stimulant, whether it be rays of light, or diffusing chemicals, or heat-waves, moves along definite lines, and the organism receives at first unequal stimuli on symmetrical parts of the body, unless the axis of the organism is parallel to the lines of motion of the stimulant. As long as the stimulant acts on symmetrical parts of the body unequally, these parts will react unequally until at length the body is swung into a position where the stimulation is equal, when it will stay in this position and move along a line parallel to the line taken by the stimulant. Not only protozoa, but much higher forms, including some vertebrates, are believed to react in this way to stimuli—*e. g.*, the maintenance by fish of a position heading up stream. The above constitutes the so-called "*theory of tropisms*," and we have such reactions to stimuli of all sorts, not only *chemotropism* and *thermotropism*, but also *heliotropism* (reaction to light); *geotropism* (to gravity), *electropism* (to electricity), *thigmotropism* (reaction to contact), etc.

The work done upon tropisms applies particularly to ciliated, freely motile organisms, and interests us less in connection with leucocytes than do the observations on such forms as *Amœba*.[11] In passing may be mentioned the *thigmotaxis* or *thigmotropism* (reaction to mechanical stimuli) shown by spermatozoa, which explains their apparently difficult feat of advancing in opposition to the cilia of the epithelial lining of the female generative tract. It may also be noted that the nature of reactions

[9] Botanische Zeitung, 1884 (42), 145 and 161.

[10] Comparative Physiology of the Brain, New York, 1900, p. 7.

[11] For full details see Jennings (Publication No. 16, Carnegie Institute, Washington, 1904, also J. Loeb, "Studies in General Physiology."

of organisms to various stimuli is not constant for even the same organism. Copepods (minute crustaceæ) may be negatively heliotropic in the day and go away from the bright surface of the water, whereas at night the same animals are positively heliotropic and swarm to the surface illuminated brightly by a lantern. Variations in heliotropism may, in some cases, be explained as due to chemical changes that occur in the organism, which explanation is made more probable by J. Loeb's experiments, which show that change in composition in the fluid in which animals are suspended may cause a complete reversal in their reaction to a constant stimulus. Motile bacteria seem to behave much like ciliated protozoa in their reaction to stimuli.[12]

Chemotaxis of Leucocytes[13]

That leucocytes come to the site of an infection because of chemical substances produced by bacteria at this point, that is to say, through chemotaxis, was first clearly pointed out by Leber[14] in 1879, who likened the attraction of such substances for leucocytes to the effect of malic acid upon spermatozoids as shown by Pfeffer. He found that in keratitis, leucocytes invaded the avascular cornea from the distant vessels, not in an irregular manner, but all moved directly toward the point of infection, where they collected. As dead cultures of staphylococci produced a similar, although less marked, accumulation of leucocytes, he sought the chemotactic substance in their bodies, and isolated a crystalline, heat-resisting substance, *phlogosin*, which attracted leucocytes in animal tissues. He also observed that capillary tubes filled with phlogosin or with staphylococci were soon invaded by masses of leucocytes.

Since Leber's experiments, many other investigations have been made showing that chemical substances of many different origins other than bacterial exert a chemotactic influence on leucocytes. Some substances are indifferent in effect, most are positive, while some are believed to repel leucocytes; *i. e.*, are negatively chemotactic.

Negative Chemotaxis.—Probably the substances that repel leucocytes are few in number; Kanthack, indeed, doubted the existence of really negative chemotactic action upon leucocytes. Verigo[15] also considers that no actual negative chemotaxis has been satisfactorily demonstrated. But, by analogy with the effects of chemicals on amebæ, ciliata, and plasmodial forms, which all show a decided negative chemotaxis under certain influences, it would seem most probable that leucocytes also should be repelled as well as attracted by chemicals.

Non-bacterial Chemotactic Substances.—One of the earliest significant studies of the effects of non-bacterial substances upon chemotaxis was made by Massart and Bordet,[16] who showed that products of the disintegration of leucocytes and other cells had a strong positive chemotactic influence. They also corroborated the statement of Vail-

[12] The method by which bacteria may invade the tissues is indicated by the studies of Stuart and Emily Mudd (Jour. Exp. Med., 1924 (40), 633) who have observed the bacteria to move along phase boundary lines by virtue of forces resulting from local inequalities in interfacial tension.

[13] Review of literature on leucocytes by Helly, Ergeb. allg. Pathol., 1914 ($17_{(1)}$), 1; Bunting, Physiol. Reviews, 1922 (2), 505.

[14] Fortschr. der Med., 1888 (6), 460.

[15] Arch. d. Méd. exper., 1901 (13), 585.

[16] Ann. d. l'Inst. Pasteur, 1891 (5), 417.

lard and Vincent that *lactic acid* is an actively repellant substance,[17] for they found that tubes containing a pyocyaneus culture, which ordinarily became filled with leucocytes rapidly, did not become invaded at all if lactic acid was also added in a strength of 1:500, although leucocytes did enter when the dilution was 1:1000. In general, however, with the relatively low concentration of H-ions possible within the tissues, leucocytes tend to migrate towards the point of greater H-ion concentration.[18]

Gabritchevsky[19] studied the chemical influence of a large number of substances on leucocytes and divided them into three groups: I. Substances exerting "negative chemotaxis," including those that attracted only a few leucocytes.[20] II. Substances with "indifferent chemotaxis" which attracted moderate numbers of leucocytes. III. Substances with positive chemotaxis. If we correct the groupings made by Gabritchevsky we have the following classification:

I. Substances negatively chemotactic or indifferent:
(*a*) Concentrated solutions of sodium and potassium salts; (*b*) lactic acid in all concentrations;[21] (*c*) quinine (0.5 per cent.); (*d*) alcohol (10 per cent.); (*e*) chloroform in water solution; (*f*) jequirity (2 per cent., passed through Chamberland filter); (*g*) glycerol (10 per cent. to 1 per cent.); (*h*) bile; (*i*) *B. choleræ gallinarum.*

II. Substances with feeble chemotaxis:
(*a*) Distilled water; (*b*) dilute solutions of sodium and potassium salts (1–0.1 per cent.); (*c*) phenol; (*d*) antipyrin; (*e*) phloridzin; (*f*) papayotin (in frog); (*g*) glycogen; (*h*) peptone; (*i*) bouillon; (*j*) blood and aqueous humor; (*k*) carmine.

III. Substances with strong positive chemotaxis:
(*a*) Papayotin (in rabbits); (*b*) sterilized and living cultures of bacteria, whether pathogenic or non-pathogenic.

These results can only be considered as suggestive and not as accurate findings, in view of other contradictory results. Buchner[22] obtained from the *pneumobacillus* of Friedlander, a protein which exerted a strong chemotactic influence, thus showing the chemical nature of the attraction of leucocytes by bacteria, and he isolated other similar proteins from other bacteria. He also obtained a "glutincasein" from grain, which was related chemically to the bacterial proteins, and which was equally chemotactic. The metabolic products of bacteria, however, he found to be negatively chemotactic. Alkali albuminate and hemi-albumose were strongly positive, but peptone was not. Glycine and leucine were found to be chemotactic, but urea, ammonium urate, skatole, tyrosine, and trimethylamine were not. It was also observed that if the positively

[17] Leucocytes are very sensitive to acids *in vitro* (Alice Evans, Jour. Immunol., 1922 (7), 271).

[18] See Gräff, Münch. med. Woch., 1922 (69), 1721.

[19] Ann. d. l'Inst. Pasteur, 1890 (4), 346.

[20] Evidently these substances were not all negatively chemotactic, but were relatively slightly chemotactic or indifferent; yet in the literature generally these experiments have been cited as indicating a negative chemotactic influence of the substances studied.

[21] The supposed exaltation of virulence of bacteria by lactic acid, described by Much (Deut. med. Woch., 1921 (47), 621) seems not to have been substantiated by others.

[22] Berl. klin. Wochenschr., 1890 (27), 1084

chemotactic substances were injected subcutaneously, they produced general as well as local leucocytosis. The products of the action of serum on bacteria, "anaphylatoxins," produce inflammatory reactions, and probably are important factors in pathology; the products of tissue disintegration have similar effects.[23] Certain drugs (notably quinine, morphine and chloral) when injected subcutaneously seem to reduce the amount of leucocytic emigration at a point of local injury (Ikeda).[24] In gas gangrene negative chemotaxis is striking, possibly depending on the abundant organic acids produced by gas bacilli.[25]

v. Sicherer[26] found that chemotaxis of leucocytes may be observed outside the body. If a tube containing positively chemotactic substances (dead beer-yeast cells and dead staphylococci were the strongest) is placed with one end in a leucocyte-containing exudate, the leucocytes pass up into it against gravity.

Bloch[27] demonstrated that carbol-glycerol extracts made from each of the different viscera and tissues exerted a positive chemotaxis, discrediting the statements of Goldscheider and Jacob that only extracts of hematogenetic tissues showed positive chemotaxis. Egg-albumen, gelatin, albumen-peptone, and alkali albuminate were also positive, carbohydrates feebly so, and fat not at all. Metallic copper, iron, mercury, and their salts have also been found to be chemotactic substances, but it is very probable that they act in part through destroying the tissues in their vicinity, which give rise to decomposition-products having a positive effect. Adler,[28] however, found that bichloride of mercury as dilute as 1:3000 caused more leucocytic invasion of a piece of saturated elder pith than did even cultures of pyogenic bacteria.[29]

Metchnikoff observed that leucocytes might, after a time, be attracted toward substances that at first seemed to repel them. If the blood is full of toxins, the subcutaneous introduction of bacteria does not lead to a local accumulation of leucocytes, presumably because the difference in chemotaxis between the blood and the tissue fluids is not great enough to cause emigration; in this connection should be mentioned Pfeffer's observation that *B. termo* in a peptone solution will not migrate toward another stronger peptone solution, unless the latter is at least five times as strong as the former. Leucocytes will migrate freely toward substances that kill them; of the bacterial products the toxins of pyocyaneus and diphtheria bacilli being especially destructive and causing typical karyorrhexis.[30] Substances soluble in lipoids are said by Hamburger[31] to increase phagocytic activity when in extreme dilutions, although stronger concen-

[23] See Dold, Arb. Path. Inst. Tübingen, 1914 (9), 30.
[24] Jour. Pharmacol., 1916 (8), 137.
[25] See Emrys-Roberts and Cowell, 1917 (21), 473.
[26] Cent. f. Bakt., 1899 (26), 360.
[27] Cent. f. allg. Path., 1896 (7), 785.
[28] Festschr. for A. Jacobi, 1900, New York.
[29] Concerning the effects of iodin and iodides upon the leucocytes, See Heinz, Virchow's Arch., 1899 (155), 44.
[30] Schürmann, Cent. f. Pathol., 1910 (21), 337.
[31] Arch. Néerland, 1912 (III, B), 134; Brit. Med. Jour. ,1916 (1), 37.

trations are highly toxic for leucocytes. If an electric current is passed through two fingers there will be found more leucocytes in the tissues of the cathode finger than in the anode finger, presumably because the OH-ions increase ameboid movement.[32]

Feringa[33] observed that widely varying substances injected into the abdominal cavity of animals led to the exudation of many leucocytes, independent of the nature of the injected substance. He found that the *p*H of the abdominal exudate invariably fell to 7.2 from the normal of 7.6, without any relation to the initial reaction of the injected fluid, and if by injection of alkalies this development of acidity was prevented, the leucocytic accumulation was prevented. This suggests that positive chemotaxis depends on the effect of H-ions, but the results of *in vitro* experiments on chemotaxis do not support this view, and conditions known to be associated with relatively high H-ion concentrations in the tissues, such as violent muscular activity[34] or local asphyxia, do not lead to noticeable leucocytic accumulation.

Miss E. P. Wolf[35] found that positive chemotaxis is exerted *in vitro* by certain substances and not by others, entirely independent of the *p*H or the osmotic pressure of the solutions. For example, the calcium ion was the only inorganic ion found to be positively chemotactic *per se*, but when in the form of calcium citrate the negative chemotaxis of the citrate ion neutralizes the positive effect of the calcium. Phosphates are positively chemotactic, except when combined with the negatively chemotactic potassium ion. It was also found that substances which produce a very acute inflammation, such as cantharidin, histamine, or turpentine, are positively chemotactic by this method, but substances, such as mustard gas, which produce a marked necrotizing effect are found to be negatively chemotactic, or neutral, though physiologically they would appear to be positively chemotactic. All amino acids and amines are positively chemotactic to a certain extent. It seems that the longer the carbon chain, the greater the degree of chemotaxis, though this is not absolute. These studies of Miss Wolf's were made in the attempt to learn what chemical, physical or physico-chemical properties a substance must have in order to incite inflammation, or that it may be unable to incite inflammation, in the hope that it might be found possible to predict from the known chemical properties of a substance its capacity to cause inflammation. It seemed, from these and subsequent studies, [6] that *a substance capable of inciting inflammation must be both fat-soluble and positively chemotactic*, but not all substances that are positively chemotactic *in vitro* will produce inflammation *in vivo*. No laws could be established to account for the presence or absence of chemotactic influence in a

[32] Schwyzer, Biochem. Zeit., 1914 (60), 454; see also Sliasoph, Zeit. ges. exp. Med., 1924 (41), 317.

[33] K. J. Feringa, Proc. Acad. Sci. Amsterdam, 1922 (25), 36. Dissertation, Groningin, 1922, Abst. in Ber. ges. Physiol. u. exp. Pharm., 1922 (14), 93; Arch. ges. Physiol., 1924 (203), 672.

[34] Wells, Jour. Exp. Med., 1909 (11), 1.

[35] Jour. Exp. Med., 1921 (34), 375.

[36] Jour. Exp. Med., 1923 (37), 511.

given substance, closely related substances being sometimes contradictory in chemotactic effect, while altogether dissimilar substances may have similar properties in respect to chemotaxis.

Many substances have been used to increase the number of leucocytes in the circulating blood in the hope of increasing resistance to infections, a result that does not seem to follow artificial leucocytosis with any recognizable uniformity. A compilation of the literature on this subject by Gehrig[37] shows such contradictory findings as to indicate that most of the recorded work is of little value. He was unable to corroborate the current statement that antipyretic drugs increase the number of leucocytes in the blood. Nucleinic acid and tissue extracts seem to increase circulating leucocytes with considerable regularity, while with thorium-*X* and benzol they can be reduced to almost complete extinction. The behavior of inflammatory processes in animals thus deprived of available leucocytes has considerable experimental interest.[38] If less than 1,000 leucocytes per cubic mm. are present in the blood, no leucocytic exudate can be produced,[39] although the other features of inflammation occur as usual.

Relation of Cell Types to Migration.—Of the leucocytes, the most actively affected by chemotaxis is the polymorphonuclear variety, but not all substances affect each variety of leucocyte in the same way; for example, infections with most animal parasites result in both local and general increase in the eosinophilous forms, and similar effects have been obtained by the injection of extracts of animal parasites. *Lymphocytes* are supposed to be much less active, presumably because they contain less of the mobile cytoplasm and consist chiefly of the structurally fixed nuclear substance. However, McCutcheon[40] has found evidence that their power of locomotion is much greater than had been supposed. Undoubtedly many of the cells in so-called lymphocytic accumulations seen in certain conditions, such as tuberculosis, are not lymphocytes from the blood, but are newly divided cells of the tissue.[41] The experimental evidence concerning lymphocytic emigration is very contradictory. Fauconnet[42] has found that tuberculin injections cause in man general increase in leucocytes, but only of the polymorphonuclear form; long-continued intoxication of animals, however, may result in lymphocytic increase. Wolff[43] claims that tetanus and diphtheria toxins produce lymphocytosis in experimental animals. Wlassow and Sepp[44] state that lymphocytes are not capable of ameboid movement or phagocytosis in the body, although after heating to 44° they may become motile for a short time. Particularly significant is the experiment of Reckzeh[45] who found that in lympha-

[37] Zeit. exp. Path., 1915 (17), 161.
[38] See G. Rosenow, Zeit. exp. Med., 1914 (3), 42.
[39] Camp and Baumgartner, Jour. Exp. Med., 1915 (22), 174.
[40] Amer. Jour. Physiol., 1924 (69), 279.
[41] See résumé by Maximow, Physiol. Reviews, 1924 (4), 533.
[42] Deut. Arch. klin. Med., 1904 (82), 167.
[43] Berl. klin. Woch., 1904 (41), 1273.
[44] Virchow's Arch., 1904 (176), 185.
[45] Zeit. f. klin. Med., 1903 (50) 51.

tic leukemia with the lymphocytes greatly exceeding the polymorphonuclear forms in the blood, the pus from an acne pustule or from cantharides blisters contains practically no lymphocytes, but is composed of the usual polynuclear forms.

Experiments on the nature of the leucocytes attracted by different chemotactic agents have been made by Borissow[46] and Adler.[47] Both agree in stating that none of the substances tested shows any special affinity for any single type of leucocytes. However, Zieler[48] observed that in the skin of rabbits exposed to the Finsen light, active migration of lymphocytes takes a prominent part in the reaction. General lymphocytosis may be produced by certain substances (pilocarpine, muscarine, $BaCl_2$) which cause contraction of the smooth muscles and force these cells out of the spleen (Harvey),[49] but such a process has no relation to chemotaxis. It is notorious that infections with animal parasites cause both local and general increase in eosinophiles, and we may even have local mast-cell leucocytosis.[50]

Tissue cells were found by Adler to migrate far into blocks of elder pith, apparently rather later than the leucocytes. As they showed changes of form indicating ameboid motion he considers their migration to be an active process. These cells were probably chiefly of the clasmatocyte type. The existence of the polymorphonuclear forms in the pith seems to be very transient.

The position taken by the young blood-vessels and cells in granulation tissue, at right angles to the surface, possibly also depends on chemotaxis determining the direction in which the new cells shall proliferate.

Giant-cell formation may also be considered as the result of chemotaxis, the cells, especially those derived from the reticulo-endothelium, moving toward the attracting particle, and when the particle is larger than the cells they spread out upon its surface, their cytoplasm flowing together because of altered surface tension. The peripheral disposition of the nuclei probably depends on the fact that in ameboid motion the nucleus of the cell plays an entirely passive rôle, being dragged along by the cytoplasm, and hence it is located most remotely from the attracting particle. Digestion of materials taken into a giant-cell seems to go on as in the individual cells that compose it.[51]

Thermotaxis of Leucocytes.—Heat seems to affect leucocytes much as it does amebæ, moderate temperatures being positively thermotactic. Mendelssohn[52] states that the thermotaxis is most pronounced at a temperature of 36°–39° C. (97°–102° F.), but is still marked as low as 20° C. Temperatures higher than 39° C.

[46] Ziegler's Beiträge, 1894 (16), 432.
[47] Festschrift f. A. Jacobi, New York, 1900.
[48] Cent. f. Pathol., 1907 (18), 289.
[49] Jour. of Physiol., 1906 (35), 115; see also Rous, Jour. Exper. Med., 1908 (10), 238.
[50] See Milchener, Zeit. klin. Med., 1899 (37), 194; Massaglia, Cent. f. Path., 1910 (21), 534.
[51] See Faber, Jour. of Path. and Bact., 1893 (1), 349.
[52] Roussky Vratch, 1903.

(102° F.) do not seem to attract them. Wlassow and Sepp[53] found that motility of leucocytes is increased by warming to 40° C., and that a temperature of 42°–46° C. causes the movements to become very irregular, with feeble power of contraction. Lymphocytes are not motile at ordinary temperatures, but at 44° they begin to move, and once motile, they continue their motion when cooled as low as 35°; this motility is considered to be entirely abnormal and only the result of degenerative changes. Murphy[54] and his colleagues have found that exposure of animals to suitable degrees of overheating, leads to marked lymphocytosis.

If mixtures of leucocytes and bacteria sensitized with opsonins are kept at low temperature, the bacteria become attached to the surface of the leucocytes, not being ingested until the mixture is warmed.[55] This indicates that two separate processes are involved in phagocytosis.

Temperature probably plays but a minor part in attracting leucocytes in pathological processes, however. The local heat of an inflamed area is due chiefly to the accumulation of blood in the part, and would not influence the leucocytes to migrate from the still warmer blood into the tissues. Segale,[56] however, has demonstrated that there is some actual heat production through increased metabolism in inflamed tissues, which may have a slight effect. By increasing motility the temperature of fever may favor migration and phagocytosis, and local application of heat to inflamed areas may induce local leucocytic accumulation. In burns the duration of the period of excessive temperature is usually too brief to account for the attraction of leucocytes that results; this accumulation is undoubtedly due to the products of the resulting cell degenerations.

The influence of light,[57] mechanical stimulation, and gravity upon leucocytes seems not to have been studied.[58] The phagocytosis of insoluble non-nutritive particles has been ascribed to *tactile stimulation* but the details of the operation of such stimuli are unknown, and the entire question of tactile stimulation is unsettled. In experiments with elder pith it has been observed that leucocytes penetrate to the center, even when the pith contains only physiological salt solution. As Adler remarks, it is difficult to explain such migration as due to tactile stimuli; but on the other hand, no other explanation has been offered.

Phagocytosis[59]

The engulfing of bacteria, cells, tissue products, etc., by leucocytes seems to be but an extension of the phenomenon of chemotaxis. When the substance toward which the leucocyte is drawn is small enough, the leucocyte simply continues its motion until it has flowed entirely about the particle, or else the leucocyte surface merely flows about particles of proper qualities with which it chances to come in contact, for when phago-

[53] Virchow's Archiv., 1904 (176), 185.

[54] Jour. Exp. Med., 1919 (29), 1.

[55] Ledingham, Proc. Royal Soc., 1908 (80), 188; Sawtchenko, Arch. Sci. Biol., 1910 (15), 145.

[56] Jour. Exp. Med., 1919 (29), 235.

[57] Ultraviolet rays in not too heavy doses are said to promote phagocytosis (Irala, Annale d'igiene, 1920 (30), 28).

[58] Abramson (Amer. Jour. Med. Sci., 1924 (167), 702) has suggested that the current of injury may be of importance in determining migration, especially of lymphocytes.

[59] See review by Metschnikoff, Kolle and Wassermann's Handb. d. Path. Mikroorganismen, 1913 (II), 655; also H. J. Hamburger. "Physikalisch-chemische Untersuchungen über Phagocyten," Bergmann, Wiesbaden, 1912, where is given a full account of the author's important researches on the principles of phagocytic behavior.

cytosis is observed in tissue cultures no active motion towards the engulfed substance can be seen[60] deHaan[61] also holds that ameboid and phagocytic activity are independent.

After phagocytosis the particle becomes, as a rule, more or less altered within the cell, unless it is a perfectly insoluble substance, such as a bit of coal-dust. This action upon the engulfed object is undoubtedly due to the action of intracellular enzymes.[62] Protozoa take their food into a specialized digesting vacuole, which has been shown by Le Dantec[63] (in *Stentor*, *Paramœcium*, and some other varieties) to contain a strongly acid fluid. Miss Greenwood[64] also demonstrated acid in several forms of protozoa, which is formed under stimulation of injected particles, whether nutritious or not. Mouton[65] has been able to extract from the bodies of protozoa (rhizopods) a feebly proteolytic enzyme. This "*amibodiastase*," as he calls it, is active in alkaline and faintly acid media, and digests colon bacilli that have been killed by heat, but not living bacilli. This last fact is highly suggestive in connection with the important question of whether leucocytes engulf and destroy virulent bacteria or only those that have been previously injured by the tissue fluid. Whether bacteria are digested in leucocytes by the same enzymes that digest the leucocytes themselves after they are killed (*i. e.*, the autolytic ferments), or by some specialized enzyme, is not known. Metchnikoff, however, has noted the localized production of acid in the cytoplasm of leucocytes of the larva of *Triton tæniatus*. The eventual excretion of the remains of the bacteria or other foreign bodies by the phagocytes is ascribed by Rhumbler to changes in the composition of the particles through digestion, so that they have a greater surface affinity for the surrounding fluids than for the protoplasm of the cell.

According to some observers, calcium and magnesium salts increase phagocytosis and leucocytic migration,[66] while changes in osmotic pressure decrease these activities, as also does quinine even in dilutions of 0.001 per cent. Otsubo,[67] however, found phagocytosis inhibited within the peritoneal cavity by injection of M/8 solutions of $MgSO_4$, $MgCl_2$, SrC_2,Na_2CO_3 and $CaCl_2$.

Phagocytosis cannot take place in the absence of electrolytes, according to Sawtchenko.[68] Fat-soluble substances in general increase phagocytosis (Hamburger),[69] but cholesterol inhibits phagocytosis,[70] (its effects

[60] Smith, Willis and Lewis, Amer. Rev. Tuberc., 1922 (6), 21.
[61] Arch. néerl. physiol., 1922 (6), 388.
[62] See Opie, Jour. Exp. Med., 1906 (8), 410.
[63] Ann. d. l'Inst. Pasteur, 1890 (4), 776.
[64] Jour. of Physiol., 1894 (16), 441, see also Scarth, Science, 1924 (60), 431.
[65] C. R. Acad. des Sciences, 1901 (133), 244.
[66] Hamburger, Biochem. Zeit., 1910 (26), 66; Eggers, Jour. Infect. Diseases, 1909 (6), 662. According to Radsma (Arch. neerl. physiol., 1918 (2), 301) calcium salts only favor phagocytosis in leucocytes that have previously had their calcium bound by citrate or oxalate in the process of isolation.
[67] Jour. Infect. Dis., 1921 (28), 18.
[68] Arch. sci. biol. St. Petersburg, 1911 (16), 161; 1912 (17), 128.
[69] Hamburger and de Haan, Arch. Anat. und Physiol., 1913, Phys. Abt., p. 77.
[70] Dewey and Nuzum, Jour. Infect. Dis., 1914 (15), 72

being suppressed by lecithin)[71] acting apparently by virtue of its OH group. Agents facilitating oxidation favor phagocytosis (Arkin).[72] Wadsworth and Hoppe[73] found that cultures of many sorts of bacteria contain substances of unknown nature which inhibit phagocytosis, these substances being absorbed by the leucocytes from which they can be removed by washing, with restoration of the phagocytic activity; they are distinct from true toxins if such are present in the cultures, are thermostable and not neutralized by immune sera.

Maximum phagocytosis occurs at the normal body temperature of the animal furnishing the leucocytes (Madsen and Wulf).[74] *In vitro*, at least, the optimum phagocytosis is at about *p*H 7.0 (Fenn).[75] Cross[76] has found that leucocytes may retain their phagocytic activity within the body for some days after death.

Phagocytosis cannot be readily ascribed to chemotaxis in the case of phagocytosis of perfectly insoluble, chemically inert particles, such as coal-dust. The leucocytes seem to take up foreign bodies without reference to their nutritive value, absorbing India-ink granules and bacteria impartially when they are injected together, and loading themselves so full of carmine granules that they cannot take up bacteria subsequently injected. Carbon and many other inert particles are rapidly removed from the blood stream by the tissue phagocytes.[77]

It is possible that foreign bodies first become coated with a layer of altered protein which then leads to phagocytosis, but there is no convincing evidence for this surmise. Kite and Wherry[78] state that leucocytes take up carbon particles and similar substances because the leucocytes are "sticky," which presumably is correct, but what constitutes the "stickiness" and why it varies under the influence of serum is not indicated. Presumably it represents an altered viscosity, which is known to be increased by increased acid content such as might be produced by local asphyxia.[79] The nature of mechanical stimulation of cells is explained by Osterhout[80] as a chemical reaction to rupture of semipermeable cellular surfaces, and there is evidence from plant cells supporting this hypothesis, but its applicability to animal cells apparently has not been investigated. The experiments of Schaeffer[81] seem to show that amebæ exhibit positive chemotaxis towards such insoluble substances as carbon particles and glass fragments, even at a distance, although the mechanism is unexplained. Similar investigations have not been made with leucocytes.

[71] Stuber, Biochem. Zeit., 1913 (51), 211; 1914 (53), 493.
[72] Jour. Infect. Dis., 1913 (13), 418.
[73] Jour. of Immunol., 1921 (6), 399.
[74] Ann. Inst. Pasteur, 1919 (33), 437.
[75] Jour. Gen. Physiol., 1922 (5), 169.
[76] Bull. Johns Hop. Hosp., 1921 (22), 238.
[77] See Wislocki, Amer. Jour. Anat., 1924 (32), 423; Ledofsky, Wien. klin. Woch., 1924 (37), 694.
[78] Jour. Infect. Dis., 1915 (16), 109.
[79] See Woolley, Jour. Amer, Med. Assoc., 1914 (63), 2279.
[80] Proc. Natl. Acad. Sci., 1916 (2), 237.
[81] Biol. Bull., 1916 (31), 303.

Not only leucocytes but tissue cells are capable of moving and performing phagocytosis when properly stimulated, and apparently all or nearly all fixed cells may act as phagocytes under some conditions. The so-called reticulo-endothelial cells, resting wandering cells, histiocytes, or clasmatocytes, as they are variously called,[82] seem to be particularly active as phagocytes, and the evidence is growing to indicate that they may be quite as important protective elements as the leucocytes of the blood. Their injury during disease may play an important role in lowering resistance.[83] Apparently they obey the same laws as the leucocytes, and not only take up bacteria, but also fragments of cells and tissues, red corpuscles, and even intact leucocytes and other cells. Brodie[84] considers that phagocytosis by endothelial cells in lymph nodes is the natural end of the leucocytes, and red corpuscles seem to have a similar fate in the spleen.

Phagocytosis is usually accomplished solely by the cytoplasm of the cells, the nuclei maintaining a passive rôle; but, according to Detre and Selli,[85] the phagocytosis of particles of lecithin is accomplished by the nuclei, which seem to have a specific affinity for this substance.

Influence of the Serum on Phagocytosis (Opsonins).—Phagocytosis of bacteria by leucocytes seems not to be merely a reaction between the leucocytes and the bacteria. Wright and Douglas have demonstrated that certain substances in the blood-serum are necessary to prepare the bacteria for phagocytosis, these substances being termed by them "*opsonins*," and by other authors, "bacteriotropins." If leucocytes are washed free from serum with salt solution and let stand in a test-tube with such bacteria as *Streptococcus pyogenes*, *Staphylococcus pyogenes*, *B. typhosus*, *B. coli*, *B. tuberculosis*, and various other organisms, no phagocytosis occurs. If, however, some serum from a normal or an immunized animal is added to the mixture, active phagocytosis soon takes place. The action of opsonins is also involved in phagocytosis by macrophages.[86] The character and properties of the opsonins are further considered among the reactions of immunity (Chapter IX).

Results of Phagocytosis.—After phagocytosis has been accomplished, the fate of the engulfed object depends upon its nature. If digestible by the intracellular enzymes it is soon destroyed, but in the case of engulfed living cells, it seems probable that they must be first killed—they form no exception to the rule that living protoplasm cannot be digested. This brings forward the question of so much importance in the problems of immunity: Do living bacteria enter phagocytes, or are they first killed by extracellular agencies before they can be taken up? At the present time it seems to be positively established that leucocytes do take up bacteria which are still viable, and which may either grow inside the leucocytes or may be destroyed by intracellular processes.[87] Even such resistant organisms as tubercle bacilli may be seen to be digested within intracellular vacuoles in cells in tissue cultures.[61,82] On the other hand, leucocytes are said not to take up extremely virulent bacteria, and hence

[82] See Maximow, Jour. Infect. Dis., 1924 (34), 549; Physiol. Reviews, 1924 (4), 533.

[83] See Ledofsky, Wien. klin. Woch., 1924 (37), 694.

[84] Jour. of Anat. and Physiol., 1901 (35), 142.

[85] Berl. klin. Woch., 1905 (42), 940.

[86] Briscoe, Jour. Path. and Bact., 1907 (12), 66.

[87] See Ruediger, Jour. Amer. Med. Assoc., 1905 (44), 198.

the question as to the relative importance of the part placed by the leucocytes and by the body fluids is still undetermined. Ruediger's experiments showed that emulsions of organs, with the exception of bone-marrow, do not destroy streptococci which are readily destroyed by leucocytes. However, the phagocytic activity of the tissue macrophages or clasmatocytes, especially in exudates, lymph sinuses and the Kupffer cells of the liver, is so great that these cells may equal or surpass the leucocytes in bactericidal power.[88] Leucocytes do not seem to bind bacterial toxins.[89]

Indigestible substances may remain in cells, particularly in fixed tissue cells, for very long periods, if the substances are chemically inert. The leucocytes seem to transfer the indigestible particles which they have engulfed to other tissues, particularly to the lymph nodes; this is probably accomplished by phagocytosis of the laden leucocytes by the macrophages of the lymph sinuses, but how the insoluble particles are later transferred to the stroma or perilymphangial tissues, where they are chiefly found in such conditions as anthracosis, etc., is unknown.

Leucocytes contain substances which are strongly bactericidal, independent of the action of the blood serum, and which have been called *endolysins;*[90] they are resistant to 65° or even higher, and seem to be bound rather firmly to the protoplasm of the leucocytes, for they resist extraction except by vigorous methods; they have a complex structure like the amboceptor-complement bacteriolysins of the serum, and are not specific (Weil).[91] They do not pass through porcelain filters readily, are precipitated by saturation with ammonium sulphate, and resemble the enzymes in many respects.[92] It is probable that the endolysins act upon bacteria that have been phagocytized and perhaps also upon free bacteria when liberated in suppuration through disintegration of the leucocytes. Lymphocytes and macrophages seem to be devoid of this endolysin.[93]

Fundamentally, serum bacteriolysis and bacterial destruction by phagocytosis seem to be the same—in each case specific antibody sensitization prepares the bacterium for lysis by enzymes, either inside or outside the cells that furnish the lytic enzyme. It would seem that opsonization and phagocytosis constitute but one of a series of similar processes by which foreign proteins are removed from the blood and tissue; *i. e.*, by lysis by extracellular enzymes when this is possible, as it is with simple protein aggregates (albuminolysis) and with some of the more labile cells (hemolysis, bacteriolysis); but in the case of more resistant structures, notably Gram-positive cocci and acid-fast bacilli, extracellular lysis being unsuccessful, these protein structures are taken within the cells where a greater concentration of enzymes may destroy them.

[88] See Gay and Morrison, Jour. Infect. Dis., 1923 (33), 338.

[89] Pettersson, Zeit. Immunität., 1911 (8), 498. Kobzarenko, however, states that horse leucocytes neutralize diphtheria but not tetanus toxin. (Ann. Inst. Pasteur, 1915 (29), 190.)

[90] For general review see Kling, Zeit. Immunität., 1910 (7), 1

[91] Arch. f. Hyg., 1912 (74), 289.

[92] Manwaring, Jour. Exp. Med., 1912 (16), 250.

[93] See Schneider, Arch. f. Hyg., 1909 (70), 40.

Phagocytosis of living virulent bacteria may not always be an unmixed benefit. Besides the obvious possibility of transporting the bacteria and spreading infection, we have also evidence that living bacteria may be protected through phagocytosis, against the action of bactericidal substances in the blood and tissues (Rous and Jones).[94]

THEORIES OF CHEMOTAXIS AND PHAGOCYTOSIS

On the assumption that leucocytes obey the same laws in their motions as do the amebæ, studies of the latter and of other forms of protozoa have furnished most of the ideas, hypotheses, and theories of the forces involved in leucocytic activities. The structural relation of the leucocyte to the ameba is striking, although by no means complete; the relation of their activities is even closer. Each is a microscopic, independent, unicellular organism moving freely in all directions by means of psuedopodia and protoplasmic streaming, taking other smaller bodies into its substance and digesting them, reacting similarly to like stimuli, and containing similarly a nucleus and many granules. The differentiation of the protoplasm of the ameba into a clear outer ectosarc and an inner granular endosarc is perhaps an important difference, but as far as the two forms of cells have been studied, the effect of this difference in structure does not seem to have been considered. That the unicellular protozoa, devoid of any central nervous system, and without any apparent co-ordinating mechanism, seem able to move about in a purposeful way, going toward food supplies and away from injurious agencies, toward or away from light, heat, and chemicals, has long attracted the interest of physiologists, particularly as in these single-celled organisms we may look for the simplest conditions of existence and the most elementary life processes. It seems absurd to imagine that a *paramœcium* goes toward a dilute acid because it "likes it," that an ameba rejects a piece of glass because it "does not taste good," as we explain similar manifestations in higher forms; furthermore, it has been shown by Verworn that minute enucleated fragments of protozoan cells react to stimuli just as does the entire cell, and, therefore, it seems that the only possible explanation of movements in protozoa must be a direct reaction of the stimulated part to the stimulus. The nature of the stimulus and the nature of the stimulated substance must determine the nature of the resulting reaction, and most of the observations so far made suggest that these reactions can be explained according to the known laws of the physics of fluids. An ameba, or a leucocyte, may be looked upon as a drop of a colloidal solution, surrounded by a delicate surface layer which is more or less readily permeable to solvents and to substances in solution, and suspended in a fluid of quite different composition.

Surface Tension.—Such a drop of fluid suspended in another different fluid obeys well-known laws of physics. The particles of each fluid are all under the influence of a very considerable force, called the cohesion pressure, which tends to

[94] Jour Exper. Med., 1916 (23), 601.

draw them together closely. Within the drop each particle is subjected to this force alike from all sides, so that the forces neutralize one another, and each particle is as free as if there were no cohesion pressure. But the particles on the surface are subjected to unequal pressure, for that of the fluid outside the drop is different from that inside, and so the pressure on the surface particles is equal to the difference of the cohesion pressure of the two fluids; this constitutes the surface tension. It is this tension that pulls in upon the surface continually, causing it to tend always to reduce the free surface to a minimum, which condition exists perfectly in the sphere. The amount of cohesion affinity is very different in different fluids, and therefore some have a high surface tension and some a low. When one substance dissolves in another the surface tension is a resultant of the surface tension of the two substances, and hence the surface tension of a liquid may be raised or lowered by dissolving various substances in it.

Artificial Imitations of Ameboid Movement[95]

Imagine a drop of fluid suspended in water—let it be a drop of protoplasm, or oil, or mercury; the drop owes its tendency to assume a spherical shape to the surface tension, which is pulling the free surface toward the center and acting with the same force on all sides. The result is that the drop is surrounded by what amounts to an elastic, well-stretched membrane, similar to the condition of a thin rubber bag distended with fluid. If at any point in the surface the tension is lessened, while elsewhere it remains the same, of necessity the wall will bulge at this point, the contents will flow into the new space so offered, and the rest of the wall will contract; hence the drop moves toward the point of lowered surface tension. Conversely, if the tension is increased in one place, the wall at this point will contract with greater force than elsewhere, driving the contents toward the less resistant part of the surface, and the drop will move away from the point of increased tension. The resemblance of these changes of form and the type of motion produced, to ameboid movement is apparent, and much experimenting has been done to determine how far the processes of motion as shown by amebæ and leucocytes can be reproduced by fluid drops under various conditions of experiment, and to ascertain if such ameboid movement of living cells can be entirely explained by the laws of surface tension.

Gad,[96] in 1878, pointed out the resemblance to ameboid motion of the changes in shape observed in drops of rancid oils in weak alkaline solution. These changes in shape are due to the formation of soaps which lower the surface tension of the drop in places, so that the fluid flows toward these places and produces pseudopodium-like projections. G. Quincke[97] later ascribed the contractions and other movements of amebæ to alterations of the surface tension of the living substance in relation to that of the surrounding medium, believing the substances responsible for the alterations to be albuminous soaps.

Bütschli[98] found that drops of "foam structure" made by mixing rancid oil and potassium carbonate solution show "*protoplasmic streaming*" when placed in glycerol, and that they exhibit positive chemotaxis

[95] Full review by Rhumbler, Abderhalden's Handbuch d. Arbeitsmethoden, 1921, Teil 3, heft 2.

[96] DuBois Reymond's Arch. f. Physiol., 1878, p. 181.

[97] Wiedmann's Annalen, 1888 (35), 580.

[98] "Protoplasm," translation by Minchin, London, 1894.

toward soap solution and other chemicals, the motion being accompanied by current formation in the drops. The "pseudopodia" formed by the drops also show currents rushing along their axes and returning by way of the surface. Heat leads to increased activity of motion. The motions were ascribed by Bütschli to the bursting of some of the superficial globules of the foam, which then spread over the surface of the drops, lowering its surface tension at the point of contact. He believed that ameboid motion, likewise, depended upon rupture of surface globules of protoplasm, for the "foam structure" of which he has been the leading advocate.

Bernstein,[99] basing his work on some observations of Paalzow, observed that a completely inorganic substance, a drop of quicksilver, could be made to imitate ameboid motion under the influence of chemical changes. If a crystal of potassium dichromate is placed near a drop of quicksilver in a nitric acid solution, as soon as the yellow color made by diffusion of the dichromate reaches the drop the quicksilver begins to show motion and advances toward the crystal. This movement is due to local oxidation of the surface mercury, which lowers the tension on that side of the drop, toward which the mercury then flows. If the crystal is removed, the drop follows, often flowing about it as if to take it in, but soon again withdrawing when the acid dissolves away the oxide formed on the surface, only to return again later. All these movements, which may be very life-like, are readily explained by changes in surface tension that take place under the influence of the dichromate and the acid, and are unquestionably referable to surface phenomena.

Artificial Amebæ.—By far the most suggestive experiments on the simulation of ameboid activity by non-living substances are those of Rhumbler (1898) in his great work, "Physikalische Analyse von Lebenserscheinungen der Zelle."[1] On the assumption that the living protoplasm was but a more or less tenacious fluid, following the simple physical laws of fluids, especially in relation to its surface tension, he devised a number of experiments to determine the correctness of these views. An ameba may be regarded as such a mass of viscid fluid, in a medium in which it is nearly or quite insoluble; it is also constantly undergoing chemical changes within itself, and taking substances from or secreting them into the surrounding water. To reproduce partly these conditions a drop of clove oil is placed in a mixture of glycerol and alcohol; the alcohol and clove oil are miscible, the glycerol merely retarding the diffusion.[2] Such a drop of oil will move about, changing its form and sending out pseudopodia much as an ameba does. These movements are undoubtedly due to changes in the surface tension brought about by the irregular mixing of the alcohol and the clove oil. The effect of chemotaxis upon an ameba can likewise be imitated with such an "artificial ameba." If some stronger alcohol is carefully introduced into the fluid near the drop, the surface tension on that side will be lowered, and the drop will flow in that direction. The effect of chemical changes within the drop upon its motion may be demonstrated similarly by injecting a little alcohol into the substance of the drop near one edge—the drop will send out a pseudopodium on that side, and perhaps flow along in the direction of the pseudopodium. We can imagine that metabolic changes in the body of an ameba may account for many of its seemingly purposeless movements by altering surface tension in some part of its circumference. Thermotaxis, the effect of heat in modifying or impelling ameboid motion, may be equally well demonstrated in such an "artificial ameba," the drop being "positively thermotactic," and flowing rapidly toward a heating point in the solution, because heat lowers the surface tension.

[99] Pflüger's Arch., 1900 (80), 628.

[1] Arch. f. Entwicklungsmechanik, 1898 (7), 103.

[2] The details of these experiments are given briefly by Jennings, Jour. of Applied Microscopy, 1902 (5), 1597.

Even as highly specialized a process as the taking of food may be closely simulated experimentally. Amebæ seem to possess the faculty of selecting substances that are suitable for their food, crawling over particles of sand, wood, etc., and rejecting them when they are pushed against or into the surface of the ameba, which, however, readily takes up bacteria, diatoms, algæ, etc., digests them, and later throws out the undigested particles. If there is any property of the ameba that suggests voluntary action, it seems to be exhibited in the choice of its food, although this is not so well developed a selective process as might be expected, for amebæ will take up many harmful objects, and they may be made to fill themselves so full of useless substances that they cannot take up food. However, a drop of chloroform in water, which makes a good artificial ameba, if "fed" with various substances, will refuse some and take in others in a surprisingly life-like manner. Pieces of glass or of wood placed in contact with the drop, exert no influence; if pushed into the substance of the drop, they carry the surface ahead, and on being released they are thrown out with some force. If a piece of shellac, paraffin, styrax, or Canada balsam be brought in contact with the surface of the drop, however, the drop flows around it immediately, and takes it within its substance, where it is soon dissolved. Even more strikingly like phagocytosis and intracellular digestion, however, is the result of a similar experiment with a piece of glass covered with shellac; the chloroform "ameba" takes it up as readily as it does the shellac alone, but after all the coating is dissolved away the piece of glass is then cast out of the drop. The resemblance to the engulfing, digestion, and excreting of indigestible particles of bacteria, etc., by amebæ, is so striking that it seems impossible that there can be any fundamental differences in the two processes. It will also be noticed that the drop takes in only what it can dissolve and rejects what it cannot.

One of the most remarkable actions of the amebæ, which seems almost certainly the result of voluntary action, is this: Oftentimes in feeding, an ameba gets hold of a suitable material which is in the form of a long thread, much too long for the ameba to surround. It then proceeds to coil up the thread within its body, by stretching a slight distance along the thread, bending over, and forming a bend in the thread, and by repeating the process it crowds the thread into a neat coil within its body, where it can be digested. The process is done so systematically and with such evident adoption of the means at hand to the desired end, that it seems as if it must be an adaptation of the ameba to circumstances, the result of long experience or of heredity. That an artificial ameba can perform the same manouvers seems hardly credible, but it is readily done with almost no difference in detail. If the chloroform drop is given a long fine thread of shellac, it proceeds to bend the thread in the middle, and to send pseudopodia out along the thread to pull it into the drop, coiling it up inside as the chloroform softens the substance of the thread, until it is all contained within the drop, provided, of course, that it is not too long (a thread six times as long as the chloroform drop may be taken in completely). The bending and coiling of a thread in this experiment is entirely in accord with the known laws and phenomena of surface tension.

Fully as striking an ameboid action as the coiling up of a thread too long to be taken in, is the building, by some of the protozoa closely related to the ameba (*Difflugia*) of a shell which the animal seems to form by cementing together grains of sand, or diatom shells, or other suitable particles. The particles are united so closely and fitted together so well that they are almost perfectly free from crevices. Even this process is accurately imitated in Rhumbler's experiments. If a drop of oil is mixed with fine grains of quartz sand, and dropped into 70 per cent. alcohol, the grains are thrown out to the surface, where they adhere to the surface of the drop and to one another exactly as do the particles in a difflugia shell. So well fitted are the particles that the artificial shell may remain intact for months, and resemble the natural shell indistinguishably.

Furthermore, the phenomenon of cell division can be imitated to some extent by oil droplets. Bütschli considered that the cleavage furrow of dividing cells represented an area of greater surface tension, and McClendon imitated cell division as follows: He suspended a drop of rancid oil and chloroform between water and salt solution, and allowed sodium hydrate to flow from pipettes against two opposite points in the droplet, whereon the surface tension was lowered and the drop bulged at these points, the band of higher surface tension constricting the drop between these two points. Burrows states that the changes seen in cells dividing beneath the microscope correspond well to these experimental observations.[3]

[3] See Trans. Congress Amer. Phys., 1913 (9), 77.

Relation of the Above Experiments to the Phenomena Exhibited by Leucocytes in Inflammation

The experiments cited indicate strongly, to say the least, that amebæ, and presumably leucocytes, react to stimuli of various kinds, chiefly through the effect of these stimuli upon surface tension. The stimuli may come from within the cell, being in this case the result of changes in composition brought about by metabolic processes; such chemical products alter the tension of the surface nearest their point of origin, causing what appears to be spontaneous motion. Stimuli acting from without may be chemical, thermal, electrical, or mechanical, but in any event they act as stimuli to motion through their effect upon surface tension; if they decrease the surface tension the cell goes toward them; if they increase the tension, the cell moves away.[4] The behavior of leucocytes in inflammation may be explained on these purely physical grounds very satisfactorily, as follows:

At the point of cell injury or of infection, substances are produced that exert positive chemotaxis, as can be shown by experiments both outside and inside the body; these substances are chemotactic because they influence the surface tension of the leucocytes, and since with most if not all the products of cell disintegration the effect is to lower surface tension, the chemotactic effect is positive. As the chemotactic substances are produced, they diffuse through the tissues until they reach the walls of a capillary, through which they begin to pass, presumably most rapidly through the thinnest parts of the wall, the "stomata" and intercellular substance. The leucocytes passing along in the bore of the capillary will be touched most by the chemotactic substances on the side from which the substances diffuse; the surface tension will be lowered on this side, causing the formation of pseudopodia and motion in this direction. When the leucocytes come in contact with the wall, their surfaces, because saturated with the chemotactic substances, will have a tension much the same as that of the cells of the capillary wall, which are likewise saturated with the same substances, and the two surfaces will tend to cling to one another; explaining the phenomenon of adhesion of leucocytes to the capillary wall, when, according to the usual description, "the leucocytes behave as if either they or the capillary wall had become sticky."[5] Surface tension of the leucocytes will be least nearest the points where the most chemotactic substances are entering the capillary, namely, the stomata or intercellular junctions; hence the pseudopodia will form in this direction and flow through the openings, the rest of the cytoplasm flowing after and dragging the nucleus along in an apparently passive manner. Since it is the cytoplasm that seems to be chiefly affected in these processes, the nucleus appearing to be rendered inert by its relatively dense and fixed

[4] OH-ions decrease, H-ions increase the surface tension of leucocytes (Schwyzer, Biochem. Zeit., 1914 (60), 306, 447, 454), which may explain the fact that lactic and other acids exhibit negative chemotaxis.

[5] Kreibich (Arch. f. Dermatol., 1912 (114), 585) describes in the vessel walls during the early stages of inflammation, a diffuse sudanophile change throughout the endothelial cells, in the form of fine, dust-like particles. Probably this depends simply on an aggregation of the intracellular lipoids.

structure, the leucocytes with most cytoplasm are most active in migration, while those with the least, the lymphocytes, are affected relatively little or not at all.

Once through the vessel wall, the motion continues in the same manner, toward the side from which the chemotactic matter comes, just as the mercury drop flows toward the crystal of potassium dichromate, or the drop of oil flows toward the alcohol. If the leucocyte meets a substance that lowers its surface tension sufficiently, it will flow around the object and enclose it, just as the chloroform drop flows about the piece of shellac or balsam; this constitutes phagocytosis. The motion of the leucocyte will continue in a forward direction until one of several possible things happens: (*a*) The leucocyte may reach a point where the chemotactic substances are so thoroughly diffused that the effects on its surface are the same on all sides; there will then be no tendency to move in any direction. (*b*) It may reach a material that exerts a marked positive influence upon it, causing much lowering of the surface tension, but which is so large that the cytoplasm flowing along its surface cannot surround it; other leucocytes will experience the same change, their cytoplasm will fuse together because of the equal lowering of their surface tension; and soon we get a mass of leucocytes with fused cytoplasm surrounding the object, forming a "foreign body giant-cell." (*c*) The leucocyte may reach a place where the concentration of the chemicals is so great that chemical changes are produced in its cytoplasm. If these changes are of a coagulative nature, the surface of the cell will be stiffened so that it cannot migrate farther; if of a solvent nature, the leucocyte is destroyed. (*d*) It may reach the margin of an area where the preceding leucocytes have become coagulated or otherwise rendered immobile, so that they block its path, while it is held fixed by the attraction on this side. (*c* and *d* explain the formation of solid leucocytic walls about areas of inflammation, and the frequent absence of leucocytes within the central necrotic areas.) (*e*) The formation of chemotactic substances may cease because the substance causing the inflammation has been used up, or because the bacteria have been destroyed, or from any of the causes that terminate inflammation. Those leucocytes still advancing will reach a point where there is as much chemotactic substance behind as in front—they will then stop advancing.[6] As the fluids exuded in the central portion continue to dilute the chemotactic substances and wash them out, there will eventually be less chemotactic substance in the center of the inflamed area than there is farther out, hence the leucocytes will move away from the center toward the periphery, following the chemotactic substances back into the blood-vessel and the lymph-stream. These are the conditions that exist at the close of the inflammatory process, which results in the dispersion of the leucocytes.

[6] The phagocytic action of leucocytes *in vitro* is decreased by substances that lower the surface tension, *e. g.* chloroform (Hamburger, K. Akad. Wetensch., 1911 (XIII (2)), 892) Ether-soluble substances from bacteria have no effect on phagocytosis (Müller, Zeit. Immunität., 1908 (1), 61).

General leucocytosis can be explained equally well on the same grounds. Chemotactic substances from the area of inflammation enter the blood-stream, and so, in a very dilute form, pass through the bone-marrow. The chemotaxis in the blood will be greater than that of the marrow, and the leucocytes will move toward and into the blood. As long as the blood contains more chemotactic substances than the marrow, leucocytosis will increase, to stop when the amount in blood and marrow is alike or when there is less in the blood than in the marrow.

Behavior of Tissue-cells and Formation of Giant-cells.—The free cells of the tissues involved in inflammation can, of course, obey the same influences as the leucocytes, and apparently do so in so far as they are not checked by structural impediments to flowing motion; *i. e.*, the more closely a cell is related to a single drop of fluid protoplasm, the more closely does it resemble in the simplicity of its reactions the "artificial ameba." An illustration of the chemotaxis of epithelial cells is furnished by B. Fischer,[7] who found that stained fats cause growth and migration of epithelial cells in the direction of the fat. Cells with much cytoplasm are best fitted to move freely, as a rule, and hence we see chiefly the large reticulo-endothelial cells and the large hyaline and granular cells of the blood acting as phagocytes, for phagocytosis is no different from ameboid motion which continues about a particle until it is surrounded; likewise we see the "epitheloid" cells with their abundant cytoplasm fusing together to form giant-cells.[8] (Note that such giant-cells are formed particularly in conditions in which the epithelioid cell is more abundant than is the leucocyte, *e. g.*, tuberculosis and other chronic inflammations. The cells that fuse about an infected catgut ligature are often the leucocytes, for they are most abundant in such a place.) A good illustration, also, is the giant-cell formed by fusing of leucocytes about blastomycetes in minute abscesses in the epithelium in blastomycetic dermatitis; the epithelial cells cannot flow or coalesce well because of their abundance of stiff keratin and their specialized cell-wall, and hence do not participate; perhaps as the leucocytes are individually too small to surround the fungus cells they flow about them in the abscess exactly as they will do experimentally in a test-tube or in a guinea-pig's abdomen (Hektoen). The method of growing tissues *in vitro* permits of observation of the process of giant-cell formation, and establishes that, for foreign body giant-cells at least, they are formed by fusion of wandering cells (Lambert),[9] and in cultures of blood cells the mononuclear leucocytes form giant cells.[10] The formation of giant-cells is, on this ground, but an amplification of ameboid movement and phagocytosis. The fusing of the individual cells is due to the lowering of their surface-tension by the materials diffusing from the body which is to be absorbed, until parts of the surface of each cell become alike, when the surface tension at the point where the cells are in contact becomes zero and the cytoplasm runs together.

[7] Münch. med. Woch., 1906 (53), 2041.
[8] See Maximow,[82].
[9] Anatomical Record, 1912 (6), 91.
[10] M. R. Lewis, Amer. Jour. Path., 1925 (1), 91.

Objections to the Above Hypothesis.—Physical explanations of ameboid movement seem to fit very closely the known facts concerning the actions of leucocytes. There arise but a few difficulties in applying these laws to leucocytic action; one is the phagocytosis of chemically inert bodies, such as coal particles, tattooing materials, stone dust, etc. We know that amebæ also may take up such inert materials, although they generally refuse them, and it is believed that the particles exert some local injury to the cell wall that leads to an alteration in its tension. Amebæ seem also sometimes to excrete a sticky substance over their surfaces or over the foreign matter that is to be engulfed, which excretion seems to be the result of surface stimulation. Possibly leucocytes do the same. We must bear in mind, however, that the protoplasmic cells have much greater possibilities for action than the "artificial ameba," since within the protoplasm countless chemical changes are going on which must cause continual alteration in surface tension; it is quite possible that mere mechanical action may alter chemical action at the point of contact, so that the injuring particle may become surrounded through local liquefaction of the protoplasm.

A series of studies by Fenn[11] on the phagocytosis of simple inert particles, such as carbon and quartz, by rat leucocytes, led to results that seem to him not to be in harmony with the theory that phagocytosis is determined by surface tension alone, although they do not offer any other explanation. For example, he found that smaller particles were ingested less rapidly than particles of a certain optimum size, although from the principles of surface tension the reverse should be the case. Furthermore, it was found that leucocytes ingest quartz particles more rapidly than carbon particles in acid solutions, whereas the carbon is taken up better in faintly alkaline solutions, but in the presence of acacia the carbon is taken up better than quartz even in acid solutions, although theoretically in the presence of such a protective colloid as acacia the surface charges of different suspended particles should be the same as that of the protective colloid. Therefore, it becomes difficult to explain the differences in behavior of carbon and quartz particles in phagocytosis on the basis of endosmotic charges. It is evident that the complete explanation of chemotaxis and phagocytosis still awaits further investigation, although it is safe to predict that the explanation will be of a physico-chemical nature in view of the evident close relation of the processes of chemotaxis and phagocytosis to surface tension phenomena.

With the ameba, unfortunately, the explanation of all its activities by purely physical analogies is apparently not so successful. Although simple pseudopodia may be produced experimentally, and their formation explained readily on the surface tension basis, yet we find many forms of pseudopodia in the great family of amebæ. Some of them are branching, some are fixed in extension, some have a stiff elastic axis. It would also be difficult to explain cilia as produced by changes in surface tension, yet we find in some protozoa that pseudopodia may take on the persistence and action of cilia, and the cilia may seem to change into pseudopodia. Jennings has made a most extended study of the relations of the "Behavior of Lower Organisms"[12] to the physical theories of ameboid motion, and is unable to corroborate the claim that the processes that go on in "artificial amebæ" exactly reproduce those of living amebæ, or to accept the statement that living protoplasm behaves exactly as any similar drop of fluid would under the same conditions. He states that the currents set up in artificial amebæ by changes in surface tension are not the same as those in living amebæ, contrary to Rhumbler and to Bütschli. The movement of ameba, he maintains, is not due to the flowing of the contents of the cell in a central, axial current out into the pseudopodium and back on the sides, as occurs in the artificial ameba; but rather to a rolling forward of the upper surface over the anterior edge to the lower surface, where it becomes fixed to the surface on which the ameba is crawling. The part played by surface tension, he claims, is in the case of amebæ a very subordinate one, and not sufficient to explain the movements of the living cell.

However the discussion concerning the amebæ may turn, it must be appreciated that there are some important differences between even the ameba and the leucocyte. The latter has by far the simpler organization, and approaches in structure, and presumably, therefore also in

[11] W. O. Fenn, Jour. Gen. Physiol., 1921 (3), 439, 575; 1922 (4), 331, 373; 1923 (5), 143, 169.

[12] Publication No. 16, Carnegie Institute, Washington, 1904; also see American Naturalist, 1904 (38), 625

response to stimuli, more closely to the simple drop of colloid matter. It has no pulsating vacuoles, no specialized pseudopodia, never forms shells or coverings, and does not conjugate as do the amebæ. The external surface of the leucocyte is much simpler,[13] an important fact in connection with surface tension effects, for in the leucocyte the surface seems to be practically undifferentiated, naked protoplasm; whereas in amebæ it is formed of a well-differentiated "ectosarc," which has marked motile powers, being able to contract sufficiently to cut an injured ameba completely in two. At the very least *the surface tension explanation of leucocytic action agrees perfectly with most of the observed actions of leucocytes*, and seems to be the most reasonable theory offered.[14] There seems to be no middle ground between such a physical theory and a metaphysical theory which would endow a single cell, without organs or nervous system, with the reasoning powers of highly developed animals, a position incompatible with the entire evidence of experience.

SUPPURATION[15]

For the formation of pus two conditions are necessary; (1) the accumulation of leucocytes, and (2) necrosis and liquefaction of cells and tissue elements. Many leucocytes may be present in a tissue without suppuration; *e. g.*, erysipelas. Necrosis of cells with their gradual liquefaction and absorption may also occur without suppuration; *e. g.*, infarcts, aseptic liquefaction necrosis, etc. Hence for suppuration to occur there must be produced substances with positive chemotaxis, to cause accumulation of leucocytes, for if a necrotic area is devoid of leucocytes, it does not suppurate; *e. g.*, caseous tubercles. Secondly, necrosis must occur, for digestion and liquefaction of *living* cells and tissues does not take place. Only substances meeting these requirements—*i. e.*, causing positive chemotaxis and cell necrosis—will cause suppuration. Therefore,

[13] See Edwards, Jour. Exp. Zool., 1923 (38), 1; Mast, Proc. Nat'l. Acad. Sci., 1923 (9), 258.

[14] The hypothesis that chemotaxis depends on osmotic forces alone has been advanced by Maltaner and Hoppe, (Jour. Hyg., 1920 (19), 309) who found that leucocytes migrate into capillary tubes containing various substances only when these are in solutions of greater density than the leucocyte suspension. Since in doing this the leucocytes move in a direction with the water current and opposite to the diffusing substances, the deduction is made that their motion depends on osmotic forces; the leucocyte being relatively impermeable to the crystalloids in solution and relatively rich in water it moves with the water and against the crystalloids. In support of this they cite experiments with artificial leucocytes made by filling a small celloidin sac with water; such a sac moves towards strong solutions of crystalloids diffusing into water. Also, they found that if a piece of agar jelly containing 3 per cent. NaCl, or a casein-trypsin digestion mixture, is suspended in an emulsion of leucocytes these remain in contact with the agar from which the crystalloids are diffusing, whereas if the agar contains no crystalloids the leucocytes gravitate to the bottom of the tube. Furthermore, they were unable to confirm Buchner's experiments which he advanced as showing that bacterial proteins are positively chemotactic, nor did they find other reputedly positive substances to have this effect when in hypotonic solutions. But it would seem that if positive chemotaxis depends solely on osmotic pressure, any hypertonic solution should be positively chemotactic, which is certainly not the case, whereas some of the substances most actively chemotactic *in vivo*, *e. g.*, aleuronat, turpentine, are active in solutions that are probably of relatively low osmotic pressure.

[15] Inflammatory Exudates, their formation and composition, are considered in Chapter XVI.

although bacterial infection is the usual cause of suppuration, it may be produced by many other substances, among which the following are the best known: Bacterial proteins, even from non-pathogenic bacteria; oil of turpentine, mercury, croton oil, silver nitrate solutions (5 to 10 per cent.), and certain vegetable proteins (vegetable "caseins").

An excellent example of the importance of leucocytes for suppurative softening is the caseous tubercle, which is usually free from leucocytes and does not undergo suppuration. If for any cause leucocytes are attracted into the caseous area, softening and pus formation promptly occur. Hence Heile[16] found that while pus from a "cold" tuberculous abscess will not digest fibrin and does not give the biuret reaction, both reactions appear after a leucocytosis has been brought about by injection of iodoform. It was formerly considered that the softening was due to the digestive action of the enzymes of the infecting bacteria, many of which were known to produce digestive enzymes dissolving protein culture-media; *e. g.*, *Staphylococcus pyogenes*. Although to some extent these enzymes may be a factor in causing the softening of the fixed tissues and of the killed leucocytes, their effect is probably insignificant as compared with the enzymes liberated by the leucocytes, as shown by the production of active experimental suppuration under aseptic conditions with turpentine, croton oil, etc. Suppuration is, therefore, the result of three processes: (1) Necrosis of cells; (2) local accumulation of leucocytes; (3) digestion of the necrotic cells, fibrin, and tissue elements by enzymes which are derived from three sources, as follows: (*a*) The leucocytes; (*b*) the infecting bacteria (if such are present); (*c*) the fixed tissue-cells. Possibly small quantities of enzymes are also introduced in the blood plasma, but these are probably very inconsiderable.[17] Normal serum, and probably also normal cells, contain antibodies for the proteolytic enzymes of the leucocytes, and hence neutralization or destruction of these antibodies must be an important factor in determining the rate and amount of suppuration.[18]

The influence of the antienzymes is well shown by the rabbit, with serum rich in antienzymes and leucocytes poor in protease, so that infections with pus cocci do not usually lead to the formation of liquid pus (Opie). In man we see a similar relation, in that exudates rich in serum do not suppurate because the enzymes are inhibited by the serum; but if the excess of serum is removed suppuration may then occur. With an excess of enzyme (*i. e.*, leucocytes) the inhibiting effect may also be overcome, and suppuration then begins. Variations in the proportion of leucoprotease and serum antiprotease determine, therefore, the occurrence of suppuration, and the inflammatory reaction is seen to be fundamentally the same as the humoral reactions of immunity, in that in each case the

[16] Zeit. klin. Med., 1904 (55), 508.

[17] Grawitz (Virchow's Arch., 1921 (232), 35) maintains that the leucocytes have little part in tissue disintegration, which he believes is accomplished by lymph free from cells.

[18] See Opie, Jour. Exper. Med., 1905 (7), 316; 1907 (9), 207; Arch. Int. Med., 1910 (5), 541.

essential process is the provision of proteolytic enzymes to remove foreign or abnormal protein substances. In inflammation the proteolytic enzymes are brought in the leucocytes, in humoral reactions the enzymes are present free in the plasma. The antiproteases may be of the nature of lipoids, probably with unsaturated fatty acids (Jobling).

The proteolytic enzymes of the leucocytes and tissue-cells have been previously considered in connection with the subject of autolysis (Chap. III), and it is necessary here only to call attention to the fact that these enzymes are of at least two varieties: (1) Proteolytic enzymes of the polymorphonuclear leucocytes, which act best in alkaline medium (Opie[19]); (2) autolytic enzymes of the tissue-cells, which act best in an acid medium or after a preliminary acidification (Hedin, *et al.*). The mononuclear leucocytes contain, like the tissue-cells, enzymes acting in an acid medium. The antienzymatic action of serum is favored by an alkaline reaction, but is altogether lost in an acid medium (Opie).

Composition of Pus

Because of its method of production, pus consists of the following substances: (1) The constituents of the exuded blood plasma; (2) the constituents of the leucocytes (and tissue-cells) that exist free in the pus; (3) the products of digestion of the proteins of the leucocytes and necrosed tissues. All analyses of pus that are recorded in the literature are in harmony with the above statements. In general the analyses consider pus as composed of two chief portions, the pus corpuscles and the pus serum. As is to be expected, the composition of pus-corpuscles is simply that of a large mass of leucocytes, which contain minute quantities of substances taken up from the pus serum by absorption and phagocytosis.[20] The old analyses of pus-corpuscles by Hoppe-Seyler[21] are given in the following table:

Table I

Quantitative Composition of Pus-cells (in 1,000 *parts of the dried substance)*

	I		II
Proteins	137.62		
Nuclein	342.57	685.85	673.69
Insoluble bodies	205.66		
Lecithin		143.83	75.64
Fat			75.00
Cholesterol	74.00	.....	72.83
Cerebrin	51.99	.....	102.84
Extractive bodies	44.33		

Mineral Substances in 1,000 *Parts of the Dried Substance*

NaCl	4.35
$Ca_3(PO_4)_2$	2.05
$Mg_3(PO_4)_2$	1.13
$FePO_4$	1.06
PO_4	9.16
Na	0.68
K	trace

[19] Jour. Exper. Med., 1906 (8), 410.

[20] The *electrical conductivity* of whole pus is somewhat greater than that of blood, and pus plasma conducts much more than whole pus, because of the resistance of the leucocytes (Tangl and Bodon, Biochem. Zeit., 1917 (84), 183).

[21] Med.-Chem. Untersuchungen.

As abnormal constituents of the leucocytes contained in abscesses may be mentioned glycogen, fat (from phagocytosis and from "fatty degeneration" of the leucocytes), and "peptone" (Hofmeister).[22]

Pus serum[23] differs from blood-serum chiefly in the substances added to it through the proteolytic changes that occur in the pus, and also in that it has lost its antiproteolytic property, containing instead free leucoprotease. The fibrinogen that escapes from the vessels into suppurating areas becomes so altered that pus will not coagulate, even upon addition of fibrin ferment (defibrinated blood). The reaction of the serum is usually slightly alkaline, becoming strongly alkaline if much ammonia is produced, which occurs especially if there is secondary infection with the organisms of putrefaction. Sometimes, however, lipase derived either from bacteria or from the cells causes the splitting of sufficient amounts of fatty acids from the fats to make the reaction acid; lactic and other fatty acids are also sometimes formed.

For example, Schade[24] found the pus from acute abscesses to have a *p*H of 5.95–6.50, as compared with pus from chronic purulent exudation, *p*H = 6.55–7.0, the *p*H of normal tissue fluids being about 7.10–7.40. Presumably the nature of the infecting organism will modify the reaction, for some (*e. g., staphylococcus*) cause an acid formation in media, while others (*e. g., pyocyaneus*) cause an alkaline reaction. Pneumococcus pus is said to become markedly acid.[25]

Hoppe-Seyler's analysis of pus serum gave the following results, which show no considerable deviation from the composition of blood plasma, except in an increased proportion of fatty matter and extractive substances.

TABLE II

	Quantitative Composition of Pus Serum		Plasma Normal
	I	II	III
Water	913.7	905.65	908.4
Solids	86.3	94.35	91.6
Proteins	63.23	77.21	77.6
Lecithin	1.50	0.56	
Fat	0.26	0.29	1.2
Cholesterol	0.53	0.87	
Alcohol extractives	1.52	0.73	4.0
Water extractives	11.53	6.92	
Inorganic salts	7.73	7.77	8.1

Quantitatively the chief abnormal constituent of pus serum is the so-called "*pyin*" of the older writers, which is nucleoprotein derived from the decomposing leucocytes, and hence increasing in amount progressively with the age of the pus;[26] it is characterized by its insolubility in acetic acid. The same substance is found more abundantly in the entire pus, on account of the presence of the cells, and when treated with 10 per cent.

[22] Zeit. physiol. Chem., 1880 (4), 268.

[23] Administration of NaCl to patients with suppurating wounds increases the amount of pus serum secreted (Zuntz, Biochem. Zeit., 1920 (107), 106).

[24] Zeit. ges. exper. Med., 1921 (24), 11.

[25] Netter, Bougault and Salanier, Compt. Rend. Soc. Biol., 1917 (80), 97.

[26] Strada, Biochem. Zeit., 1909 (16), 193.

NaCl solution it forms a stringy mass which was formerly called "*Rovida's hyalin substance.*" Glucothionic acid, derived from the leucocytes, is also present in pus.[27] In the pus serum are found all the other constituents of the leucocytes, including particularly lecithin, cholesterol, fats (and soaps), cerebrin, "jecorin," and glycogen; and also the usual components of the blood-serum as well as some small quantities of pigment derived from decomposed red corpuscles.

The *products of autolysis* are of particular interest, and they are found in varying amount, but usually less abundantly than might be expected, probably because of their solubility and consequent rapid absorption. Albumoses and peptones seem to be constantly present (Shattock).[28] The common occurrence of albumosuria during suppuration presumably depends on the absorption of digestion products from the pus,[29] but true peptone has not been satisfactorily identified in the urine. Leucine and tyrosine have also frequently been found in pus,[30] but Taylor[31] could find no workable traces of either monoamino- or polyamino-acids in a liter of pus, which may depend on their having been either absorbed or transformed into ammonium compounds. Presumably this is in part the explanation of the large urea excretion in persons with extensive suppuration, as observed by Ameuille.[32] Weiss[33] found pus, and also the product of digestion of leucocytes with trypsin, to be free from tryptophan.

From the nucleoproteins *purine bodies* are formed and may be found in the pus. The relation of the purine bases to local leucocytosis is shown by Heile,[16] who found in cold tuberculous abscesses a proportion of purine bases equal to 0.5 per cent., in similar abscesses after injection of iodoform, 1.57, and in acute suppuration, 10.7. *Spermin* crystals are also occasionally found in old pus collections.[34] *Free fatty acids* and volatile fatty acids, such as butyric, lactic,[35] valerianic, and formic, have been found. Products of bacterial activity, such as bacterial proteins and pigments (*e. g.*, pyocyanin), may also be present. It is probable that in many instances these autolytic products are bactericidal, and thus help to terminate the infection. Direct tests have shown that the autolysate of fibrin is bactericidal for staphylococci and streptococci.[36] (See also discussion of "Autolysis of Exudates" Chap. III.)

[27] Mandel and Levene, Biochem. Zeit., 1907 (4), 78.

[28] Trans. London Path. Soc., 1892 (43), 225.

[29] Literature on albumosuria, see Yarrow, Amer. Med., 1903 (5), 452; Elmer, *ibid.*, 1906 (11), 169; Senator, International Clinics, 1905 (IV), series 14, p. 85. See also "Albumosuria," Chap. XXIII.

[30] Müller (Cent. inn. Med., 1907 (28), 297) recommends the tyrosine reaction with Millon's reagent as a means of differentiating tuberculous from ordinary pus, the former not giving the reaction because of lack of leucocytic enzymes; but there is disagreement as to the constancy of this reaction in pus (Dold, Deut. med. Woch., 1908 (34), 869).

[31] Univ. of California Publications (Pathol.), 1904 (1), 46.

[32] Bull. Acad. Méd. Paris, 1917 (78), 8.

[33] Biochem. Zeit., 1919 (98), 116.

[34] See Williams, Boston Med. and Surg. Jour., 1901 (145), 355.

[35] d-lactic acid is a constant constituent of pus from the pleura (Ito, Jour. Biol. Chem., 1916 (26), 173).

[36] Bilancioni, Arch. di Farmacol., 1911 (11), 491.

All the numerous *enzymes* of the blood plasma, the leucocytes and the tissue-cells, are present in pus. Thus Achalme[37] found evidence of the presence of the following enzymes in pus: Proteolytic enzymes,[38] lipase (splitting monobutyrin), diastase, rennin, gelatinase, catalase, and oxidase, the last being very abundant. These seem to exist chiefly in the leucocytes, the pus serum being free from them. No evidence could be found of enzymes acting on amygdalin, saccharose, inulin, or lactose. Fibrin ferment is said to be absent from pus. Presumably the bacteriolytic "endolysins" of the leucocytes are also present in pus.

There is little difference in the effect on metabolism produced by a sterile suppuration and one due to *localized* bacterial infection (Cooke and Whipple),[39] one of the chemical features in each being a precipitous and sustained rise in the urinary N excretion. Presumably the reaction in both cases results from toxic products of protein cleavage, rather than from bacterial secretions in the case of septic inflammation. Probably only part of the excessive urinary N comes from the local injury, the greater part being derived from toxicogenic destruction of tissue proteins.

SPUTUM[40]

The chemistry of sputum may be properly considered in this connection. In reaction, sputum is ordinarily alkaline, but in case of marked bacterial decomposition in bronchiectatic cavities the reaction may become acid. The inorganic constituents usually correspond closely to those of the blood. Its specific gravity is from 1.008 to 1.026, usually varying directly with the number of leucocytes; the average specific gravity is about 1.013. The greenish color frequently observed depends generally upon blood-pigment (except in case of icterus), although in some instances the pigment is of bacterial origin. Hemosiderin is often present, especially with passive congestion. Renk[41] has studied the proteins of sputum with special reference to the loss of protein to the body and its relation to cachexia. In three patients (consumptives) studied, the daily amount of sputum of two averaged 145 grams for each; for the third it was 82 grams. This contained (average) 5 to 6 per cent. of solids; including mucin, 2–3 per cent.; protein, 0.1–0.5 per cent.; fat, 0.3–0.5 per cent.; ash, 0.8–0.9 per cent. The daily loss of nitrogen was 0.75 gram, which equals about 6 per cent. of the total daily nitrogen output of persons under condition of starvation.[42]

[37] Compt. Rend. Soc. Biol., 1899 (51), 568.

[38] Concerning proteolytic enzymes of pus see Opie, Jour. Exper. Med., 1906 (8), 410.

[39] Jour. Exp. Med., 1918 (28), 222.

[40] Complete bibliography given by von Hoesslin, "Das Sputum," Berlin, 1921, J. Springer publ.; and Plesch, Handbuch d. Biochem., 1924 (5), 418. A full discussion of the chemistry of sputum is given in "Chemistry of Tuberculosis," Wells, DeWitt, and Long, Baltimore, Williams and Wilkins, 1923.

[41] Zeit. f. Biol., 1875 (11), 102.

[42] Plesch (Zeit. exp. Path. u. Ther., 1906, Bd. iii, July) found that 4.8 per cent. of all the absorbed calories were lost in the sputum in an advanced case of phthisis. Under similar conditions the amount of salts excreted by the sputum may equal or exceed that in the urine.[9]

Wanner[43] found characteristic variations in the amount of coagulable protein in sputum from different conditions, as follows: In bronchitis the amount of protein is very small; in bronchiectasis protein is present, but the amount of uncoagulable nitrogen from autolysis is relatively large; in phthisis as well as in bronchiectasis the amount of protein does not exceed 1 per cent.; in pneumonia it may reach 3 per cent., but it is highest in pulmonary gangrene. Any protein content that causes more than a slight turbidity on boiling indicates an inflammation; *e. g.*, in case of doubt between a diagnosis of pneumonia and infarct a high protein content speaks for the former. Rogers[44] stated that the sputum in every case of tuberculosis shows albumin,[45] but this has been questioned, especially as to chronic or quiescent cases.[46] Albumin, or better, coagulable protein is also present in the sputum of patients with pulmonary edema and pleurisy. According to Works[47] in active tuberculosis there is usually 0.2 per cent. or more of coagulable protein in the sputum. The mucin of sputum yields 33.6 per cent. of *glucosamin* when split with HCl, which gives an index of the quantity of mucin; this is highest in chronic bronchitis and lowest in pneumonia and phthisis. Kossel found 0.1–0.33 gm. of nucleins in the sputum daily.

The following table by Bokay (taken from Ott) gives the proportion of the organic constituents of sputum in parts per thousand:

TABLE III

	Bronchitis in typhoid	Fibroid phthisis	Phthisis, early in apex	Phthisis, cavities	Phthisis, advanced	Phthisis, advanced
Fatty acids as fat..	0.224	0.845	0.462	2.468	3.468	9.725
Free fatty acids....	trace	0.184	0.521	0.370	0.307	0.902
Soaps.....	traces	0.380	0.430	0.537	0.516	3.973
Cholesterol.....	traces	0.4	1.617	0.172	1.160	0.141
Lecithin.....	traces	traces	1.543	.....	1.165	1.245
Nuclein.....	traces	0.102	.....	....	0.260	0.489
Protein.....	0.898	2.040	.....	4.430	3.455	5.115

On account of the digestion of the exudates by the leucocytes, sputum contains proteoses, peptones, and amino-acids, generally in proportion to the richness of the exudate in leucocytes; they are, therefore, most abundant in pneumonia.

It has been said that tuberculous sputum is less likely to contain free tyrosine than sputum from diseases with more leucocytes, but this seems

[43] Deut. Arch. klin. Med., 1903 (75), 347.

[44] Presse Méd., 1910 (18), 289; 1911 (19), 409; also Ganz and Hertz, *ibid.*, 1911 (19), 41; Kaufmann, Beitr. Klin. d. Tuberk, 1913 (26), 269; Hempel-Jörgensen, *ibid.*, p. 392.

[45] Review by Cocke, Amer. Jour. Med. Sci., 1914 (148), 724.

[46] Fischberg and Felberbaum, Medical Record, Oct. 21, 1911; Acs-Nagy, Wien. klin. Woch., 1912 (25), 1904.

[47] Jour. Amer. Med. Assoc., 1912 (59), 1537.

not to be sufficiently established. Using a special method, Pissavy and Monceaux[48] report that in tuberculosis a positive tyrosine reaction indicates an active lesion. Leucine and tyrosine have also been found in the sputum with pulmonary cancer.[48a] Simon[49] found considerable albumose in phthisical sputum, but no nucleohistone or free histone. In febrile cases of tuberculosis the amount of albumose may exceed the coagulable protein, which rarely exceeds one per cent. of the moist weight.[50] Staffregen, however, could find no true peptone in phthisical sputum, but Stadelmann[51] found that such sputum contained enzymes hydrolyzing fibrin, and attributed this largely to bacteria. Probably most of the enzymes present in sputum come from the leucocytes. In the early stage of pneumonia the sputum has no proteolytic action, presumably because inhibited by the large amount of serum present; but with resolution active proteolytic properties appear (Bittorf).[52] In tuberculosis sputum the tryptic and antitryptic properties fluctuate, and lipase is absent (Eiselt).[53] Pneumonic sputum before the crisis has but slight action on peptids, but acquires marked peptolytic activity thereafter.[54] Most sputa contain enzymes splitting casein and polypeptids.[55] Sputum may contain indole, derived either from the putrefying proteins or excreted from the blood.[56] Positive tuberculin reactions may be obtained with extracts of tuberculous sputum.[57]

The amount of *fats* seems to depend directly upon the number of pus-corpuscles and the age of the pus (*i. e.*, the amount of fatty degeneration). Jacobson found from 0.08 to 1.6 grams of fatty matter per day, containing on an average 14.76 per cent. of soaps, 15.79 per cent. of higher fatty acids, 0–10 per cent. of water-soluble fatty acids, 13.58 per cent. lecithin, and 10.49 per cent. cholesterol.

As to the *inorganic substances*, Bamberger found two types of sputum, catarrhal and inflammatory. In the inflammatory there is a deficiency in alkali phosphate, SO_3 constitutes more than 8 per cent. of the salts, and the ratio, $\frac{Na_2O}{K_2O}$ equals $\frac{15}{41}$. In catarrhal sputum the alkali phosphates constitute 10–14 per cent., $\frac{Na_2O}{K_2O} = \frac{31}{20}$, and the SO_3 is from 0.6–1.2 per cent. Chlorine is about the same in both forms. These differences are, however, not as constant as Bamberger believes, according to

[48] Bull. Soc. Méd. Hôp., 1922 (46), 376; see also Fiessinger and Blum, *ibid.*, 1923 (47), 274.
[48a] Tellgmann, Deut. med. Woch., 1923 (49), 1521
[49] Arch. exp. Path. u. Pharm., 1903 (49), 449.
[50] Prorok, Münch. med. Woch., 1909 (56), 2053.
[51] Zeit. klin. Med., 1889 (16), 128.
[52] Deut. Arch. klin. Med., 1907 (91), 212; see also Abraham, Zeit. klin. Med., 1923 (96), 245.
[53] Zeit. klin. Med., 1912 (75), 91.
[54] Abderhalden, Zeit. physiol. Chem., 1912 (78), 344.
[55] Maliwa, Deut. Arch. klin. Med., 1914 (115), 407.
[56] Binda and Cassarini, Gaz. Med. Ital., 1913 (64), 461.
[57] Maniscaeco, Tuberculosi, 1922 (14), 1.

several later investigations. The results of his analyses are shown in the following table:

TABLE IV

	Chronic phthisis	Acute phthisis
Water	94.55	92.38
Organic matter	4.67	6.88
Inorganic salts	0.78	0.74
One hundred parts of the salts contain:		
Chlorine	35.78	33.40
SO_3	0.70	0.80
P_2O_5	13.05	14.15
K_2O	24.07	19.99
Na_2O	27.90	31.69
Calcium phosphate	1.63	4.32[58]
Iron phosphate	0.09	0.14
Magnesium phosphate	1.20	....
Ca and Mg carbonate and sulfate	1.74	0.22
Silicic acid	0.9	0.3

[58] Including magnesium.

CHAPTER XIV

THE CHEMISTRY OF GROWTH AND REPAIR (INCLUDING VITAMINS AND THE DEFICIENCY DISEASES)

PROLIFERATION AND REGENERATION

The factors that incite cells to proliferate, as well as those that cause the cessation of proliferation after it has once started, are too entirely unknown to permit of speculation as to their exact nature. It seems probable, however, that they are, as Ziegler says, "identical with the stimuli which excite or increase functional and nutritive activity," and these are certainly in many instances of chemical nature. Thus the application of various irritating substances in not too concentrated a form (*e. g.*, painting the skin with iodine) may lead to proliferation without causing discernible degeneration of the cells. Mallory's[1] observations on the phenomena of proliferation and phagocytosis show that the same bacterial products which destroy the cells when concentrated, when sufficiently dilute cause proliferation of similar cells.[2] Numerous dyes are known to stimulate cell growth greatly, (*e. g.*, the growth of epithelium into oils containing sudan III, etc.) and sometimes seem to lead by virtue of this fact to cancer growth (*e. g.*, cancer of the bladder in dye workers). Chemical products from decomposition of vegetable matter have a particularly active stimulating effect, so that cancers have been experimentally produced by painting the ears of rabbits with tar (Yamagiwa). Many other instances of proliferation in response to chemical stimuli might be cited, but in nearly all cases it is extremely difficult to determine that the proliferation is not, after all, reparative in compensation for degenerative changes, and, therefore, possibly obeying some other biological law than that of a simple reaction to a chemical stimulus.

Perhaps the most striking example we have of growth stimulation by chemical agencies is furnished by the proliferation and hypertrophy which take place in the uterus[3] and mammary gland during pregnancy.[4] The same phenomena can be produced by injecting the lipoid fraction of extract of placenta and corpus luteum (Frank).[5] Even transplanted bits of uterine tissue are stimulated to grow by these substances, thus

[1] Jour. Exp. Med., 1900 (5), 15.

[2] Carnot and Lavlièvre (Arch. Méd. Exper., 1907 (19), 388) have obtained evidence that actively growing kidney tissue, whether fetal or adult regenerating kidney, contains something which is capable of stimulating growth of renal epithelium when injected into other animals.

[3] See Leo Loeb, Jour. Amer. Med. Assoc., 1908 (50), 1897; 1915 (64), 726.

[4] Review on internal secretions of reproductive organs by Marshall, Physiol. Reviews, 1923 (3), 335.

[5] Jour. Amer. Med. Assoc., 1920 (74), 47.

excluding possible nervous control of growth.[6] Dried placenta fed to mothers also increases the rate of growth of the suckling infant (Hammett).[7] The nature of the growth-stimulating agency in placenta and corpus luteum is unknown but it strongly resists chemical agents. However, it may be pointed out that *tethelin*, described by Robertson[8] as the growth-promoting substance of the hypophysis, is soluble in lipoid solvents. Acromegaly and gigantism give evidence that even far more than normal growth may be produced, presumably through the agency of an internal secretion of the hypophysis, but whether tethelin actually is the substance responsible is at present unknown. This substance is obtained from the anterior lobe, about 10 mg. for each gland, and contains 1.4 per cent. of phosphorous. It is said to retard growth of animals before adolescence and to increase post-adolescent growth;[9] also it has been reported that wound repair is stimulated by tethelin.[10] At this time, however, the status of tethelin is not fully determined. The influence of the other ductless glands on growth is discussed further in Chapter XXIV.

The studies by Whipple and his colleagues on the repair of the liver after extensive chloroform necrosis, indicate that a mixed diet rich in carbohydrate is more effective in facilitating this repair than meat or fat, and that thyroid extract does not stimulate repair.[11] Also the healing of wounds is more rapid in meat-fed than in fat-fed dogs (Clark).[12] Attempts to find specific substances that will cause increased rate of wound healing have so far been unsuccessful,[13] except for the stimulating influence of leucocytes pointed out by Carrel.[14] Regeneration of blood protein after hemorrhage is said to be most rapid on a protein rich diet,[15] especially one containing red meat, liver, hemoglobin, butter fat, and spinach,[16] vitamins being of accessory value.[17] When an incomplete protein, gliadin, is the sole protein of the diet the hemoglobin is not regenerated, but degenerated liver cells seem to be repaired normally and more rapidly than in animals fasting or dependent on gelatin as a sole source of protein.[18]

Although proper nutrition is necessary for cell proliferation, yet it does not seem that excessive nourishment can lead to excessive cell multiplication, or by itself cause cell proliferation to take place.

Oyxgen and certain inorganic salts are essential for cell division even in the lowest forms, and among such simple organisms as sea-urchins and certain other marine forms segmentation of the unfertilized ova may be incited by changes in osmotic concentration, leading eventually to

[6] Frank, Surg. Gyn. Obst., 1917 (25), 329.
[7] Jour. Biol. Chem., 1918 (36), 569; Endocrinology, 1919 (3), 307.
[8] See Robertson, "The Chemical Basis of Growth and Senescence," 1923.
[9] Robertson and Ray, Jour. Biol. Chem., 1920 (42), 71.
[10] Review by Barney, Jour. Lab. Clin. Med., 1918 (3), 480.
[11] See Arch. Int. Med., 1919 (23), 689.
[12] Bull. Johns Hopkins Hosp., 1919 (30), 117.
[13] See DuNoüy, Amer. Jour. Physiol., 1919 (49), 121.
[14] Jour. Exp. Med., 1922 (36), 385.
[15] Kerr *et al.*, Amer. Jour. Physiol., 1918 (47), 456.
[16] Whipple, Arch. Int. Med., 1922 (29), 711.
[17] Jencks, Amer. Jour. Physiol 1922 (59), 240.
[18] Smith and Moise, Jour. Exp. Med., 1924 (40), 209.

formation of perfect larvæ (J. Loeb, *et. al.*).[19] In lower animals very dilute solutions of alkalies stimulate the rate of cell growth and somewhat higher concentrations cause extremely irregular cell division; in mammals the feeding of alkalies causes great wasting as if through cell stimulation.[20] In tissue cultures it has been found that fibroblasts exhibit an optimum growth at *p*H 7.4–7.8 (A. Fischer)[21] which is slightly more alkaline than tissue fluids usually are, and they are more susceptible to injury by increased acidity than by a corresponding increased alkalinity. It is of interest in this connection that old animals with uranium nephritis, seem less able to maintain a normal acid-base balance than young animals (MacNider).[22]

The products of nuclein hydrolysis are said to stimulate cell growth.[23] Potassium salts seem to be particularly important for proliferating cells, and Beebe and also Clowes and Frisbie[24] have found that actively growing malignant tumors are rich in potassium and poor in calcium, whereas in slow-growing tumors the reverse is the case. Dennstedt and Rumpf[25] also found that in hypertrophy of the heart the amount of potassium is increased, while in chronic degeneration of the myocardium the calcium and magnesium are usually increased. The proportion of nitrogen in the different parts of the heart is not changed during hypertrophy (Bence),[26] but the amount of NaCl is much increased in hypertrophy,[27] as is the coagulable protein of the heart.[28]

Chemical studies of proliferation are lacking,[29] except in regard to the development of the embryo, etc.[30] New tissues differ from adult tissues in having a larger proportion of water,[31] and in having a larger proportion of the "primary" cell constituents and a smaller proportion of the various secondary constituents, since these last are largely products of the activity of the adult cell.[32] Of the primary constituents, the proportion

[19] See J. Loeb, Studies in General Physiology, 1905.
[20] Moore *et al.*, Biochem. Jour., 1906 (1), 294; 1912 (6), 162.
[21] Jour. Exp. Med., 1921 (34), 447.
[22] Science, 1917 (46), 643.
[23] Calkins *et al.*, Jour. Infect. Dis., 1912 (10), 421.
[24] See "Tumors," Chap. XXI.
[25] Zeit. klin. Med., 1905 (58), 84.
[26] Zeit. klin. Med., 1908 (66), 441.
[27] Rzentkowski, *ibid.*, 1910 (70), 337.
[28] Domagk, Zeit. klin. Med., 1924 (98), 171.
[29] The composition of *granulation tissue* has been determined by Hirsch (Amer. Jour. Med. Sci., 1920 (159), 356), who analyzed the "castration granulomas" of swine. These are large inflammatory tumors, probably resulting from subacute infection of the operation wound, and consist of dense fibrous tissue with few cells and a scanty blood supply, but sometimes more or less edematous. His figures are as follows: Water 81.9 per cent.; solids, 18.1; lipins, 2.3; proteins, 14.4. The protein contained sulfur, 0.34 per cent.; phosphorus, 0.32; purine N, 0.08.
[30] Literature on the chemistry of growth given by Aron, Handbuch d. Biochem., Ergänzungsband, 1913. Full review on the metabolism of the developing egg by Needham, Physiol. Reviews, 1925 (5), 1.
[31] In fact, both in plants and animals, increased hydration is associated with and perhaps in some way responsible for growth (See MacDougal, Proc. Am. Phil. Soc., 1919 (58), 346).
[32] Preliminary studies of the placenta at different periods suggest the occurrence of a regular change in the direction of decrease in arginine and increase in monoamino acids (Ehrenberg and Liebenow. Arch. ges. Physiol.. 1923 (201). 387).

of the nucleoproteins is particularly high, and a number of interesting facts concerning the nucleoproteins in cell division have been determined. Most important, perhaps, is the classical observation of Miescher, who found that during the migration of salmon up stream to the spawning grounds, during which time no food is taken, the proteins of the muscular tissue become largely transformed into the protamine type of protein (characterized by containing large proportions of the polyamino-acids, such as arginine, histidine, and lysine),[33] which unite with nucleic acids to form the abundant nucleoprotein of the spermatozoa and ova.[34] Whether such a transformation of proteins occurs in mammalian cells during cell multiplication cannot be stated, but certainly from some source an additional supply of nucleoprotein is derived. Developing sea urchin eggs synthesize great quantities of nucleoprotein,[35] even when in a solution free from phosphates, and here the only available source for the phosphoric acid of the nucleins would seem to be the phospholipins of the egg (J. Loeb). The nucleoproteins during karyokinesis undergo a chemical change in that they become of a more acid type (presumably through splitting off of part of the proteins from the nucleic acid), which results in the characteristic increase in affinity for basic dyes, and the increased negative charge which is easily demonstrated.[36] This suggests the participation of an enzyme in the process of karyokinesis, just as in the production of pycnosis in degenerating cells, but there seems to be no conclusive evidence on this point. Gies[37] could find no enzyme in spermatozoa that incites cell division in the ova of sea-urchins (*Arbacia*). The fertilization of eggs makes them more permeable to ions,[38] which possibly determines many of the subsequent changes.

In *metaplasia* we have what may be interpreted as a chemical alteration due to mechanical stimuli, *e. g.*, the formation of keratin by cells that ordinarily do not do so; the deposition of calcium salts and osteoid transformation of connective tissues in rider's bone, etc. That such is the case, however, cannot be positively stated from the evidence at hand.

As to the nature of the stimuli which start and control tissue growth, our chief information comes from the work by Carrel and his associates, on the so-called

Trephones.[39]—These authors, from whom I quote below, have made studies on the food requirements of cells in pure cultures, which indicate that epithelial and connective tissues do not obtain from blood serum the material necessary for the synthesis of protoplasm, but demand for that purpose certain substances contained especially in embryonic tissues[40] and secreted by lymphocytes and possibly by other cells. In fact, blood plasma has an inhibiting effect on tissue growth. The principles manufactured by certain cells and used by others for the building up of protoplasm are

[33] Concerning protamines, see résumé by Kossel, Biochem. Centr., 1906 (5), 1 and 33.

[34] See also Greene, Jour. Biol. Chem., 1919 (39), 435.

[35] Not accepted by Masing, Zeit. physiol. Chem., 1910 (67), 161.

[36] See Gallardo, Arch. Entwickl. Organ., 1909 (28), 125; Pentimalli, *ibid.*, 1912 (34), 444.

[37] Amer. Jour. Physiol., 1901 (6), 54.

[38] See McClendon, Carnegie Inst. Publ., 1914, No. 183.

[39] Review with bibliography by Carrel, Jour. Amer. Med. Assoc., 1924 (82), 255.

[40] See also Drew, Brit. Jour. Exp. Path., 1923 (4), 46.

called trephones, meaning "I feed." They differ from the hormones, which stimulate the cells to activity without possessing any nutrient property, but both trephones and hormones may be required for the growth of tissues.

These growth-promoting substances are found also in extracts of adult tissues, such as muscle, thyroid and renal tissues, in extracts of leucocytes, and also in heterologous tissues. However, it is probable that adult tissues, as well as serum, contain substances toxic for the homologous cells, and progressively overcome the effect of the growth-promoting constituents, but leucocytes seem to contain a store of the growth-promoting substance even in the adult. Although fibroblasts and epithelial cells remain in a condition of cultivation only in embryonic tissue juices, lymphocytes and large mononuclears behave differently, for they can build up protoplasm from some constituents of serum while neither fibroblasts nor epithelial cells possess this property.

Apparently, leucocytes secrete their growth-promoting substances, not only *in vitro*, but also *in vivo*, for aqueous extracts of inflamed connective tissue were found to increase the rate of fibroblast proliferation. Lymphocytes and macrophages can be considered as mobile unicellular glands which transform some of the serum constituents into trephones and set them free in blood plasma or interstitial lymph as a food for fixed cells. They play the role of trephocytes, or nursing cells, for epithelium and connective tissue.

Carrel believes that the assumption of cell activity in wound healing is due to the trephones set free by the lymphocytes, and possibly from the injured tissue cells,[41] which supply the food material required for the production of new cells and which is not obtainable from the blood plasma of an adult animal. Thus, the irritant does not act by itself but causes the embryonic reserves of the organism to be made available to the wound. Serum and interstitial lymph do not allow fibroblast proliferation in the adult animal. But when cell multiplication is needed, the inhibition due to serum is overcome by an excess supply of trephones. These observations seem to explain why a perfectly clean wound, free from debris of tissue and blood clots, and absolutely protected from outside irritation, does not heal, for under such conditions the inhibiting effect of the plasma is unopposed. But if a mild tissue injury is produced, or leucocytes are attracted, then trephones are set free which overcome the inhibition, and reparative proliferation begins.

Although serum inhibits the multiplication of fibroblasts, it contains a small amount of growth-promoting substances, the effect of which is shielded by more powerful inhibiting substances. The growth-promoting substances have been isolated from serum by precipitation with carbon dioxid, and hence are probably associated with the globulins. They are much less abundant in the serum of an old animal than in that of a young one, and the increased inhibiting effect of serum in old age is partly due to their disappearance. It has also been found that there is a definite relation between the age of the animal and the amount of new tissue produced in cultures in its plasma in a given time. The duration of life in the fibroblasts in cultures also decreases in a similar way with the age of the animal furnishing the plasma. A mathematical study of these and other data on senescence by Brody,[42] led him to the conclusion that the course of decline of vitality with age due to the process of senescence, when not complicated by the process of growth, follows a simple exponential law; that is, the degree of vitality or of senescence (defining vitality

[41] See also Gaza, Arch. klin. Chir., 1922 (121), 378.

[42] Jour. Gen. Physiol., 1924 (6), 245.

as the reciprocal of senescence) at any moment is, regardless of age, a constant percentage of the degree of vitality or senescence of the preceding moment. This exponential law is the same as the law of monomolecular change in chemistry.

CHEMICAL BASIS OF GROWTH AND REPAIR[43]

We do not know just what substances are necessary to maintain individual cells in normal condition, what are needed to stimulate them to multiplication, or what elements they require to permit them to multiply, but it has been learned that certain definite materials are required by the organism as a whole. It is not sufficient that a given number of calories with a certain quantity of proteins, carbohydrates, fats, and salts be supplied; it is essential that certain specific constituents be provided among these foodstuffs. This fact was first clearly pointed out by Gowland Hopkins in 1906, although in 1795 Sir Gilbert Blaine introduced lemon juice into the rations of the British navy to prevent scurvy, in 1897 Eijkman discovered that beriberi and experimental neuritis might result from a one-sided diet of polished rice, and in 1902 Röhmann reported that purified food stuffs do not suffice to maintain and rear mice.

The proteins[44] must not only provide a sufficient amount of nitrogen, but they must also provide certain specific amino-acids, as has been especially demonstrated by the investigations of Willcock and Hopkins[45] and Osborne and Mendel.[46] Apparently the presence of some of the simple straight-chain amino-acids can be dispensed with (*e. g.*, glycine), and the animal will grow and thrive if other nutritive supplies are adequate, but certain, at least, of the more complex cyclic amino-acids must be provided. Furthermore, the requirements for growth (quantitatively speaking at least), seem to be something more than the requirements for mere preservation of health and equilibrium, for it was found that animals could live and preserve nitrogen equilibrium when the protein of the diet furnished at most small quantities of lysine, but young animals were unable to grow with such a restricted supply of this amino-acid. If lysine was added to the defective protein (gliadin from wheat) the animal would then be able to grow at a normal rate. Of particular importance is the fact that animals can be kept in a stunted condition on such a deficient diet until they have reached an age at which normally all growth would have long since ceased, and then when supplied with sufficient lysine they will begin to grow and continue until full size is reached.[47] This last observation proves that growth is not conditioned by age, and that

[43] See Mendel, "Nutrition and Growth," Harvey Society Lectures, 1914–15; Amer. Jour. Med. Sci., 1917 (153), 1; Lusk, "Science of Nutrition," Saunders, Phila., 1917.

[44] Review on nutritive value of proteins by Mitchell, Physiol. Reviews, 1924 (4), 424.

[45] Jour. Physiol., 1906 (35), 88.

[46] Series of papers in Jour. Biol. Chem., 1912, *et seq.*

[47] Jour. Biol. Chem., 1915 (23), 439.

we do not stop growing because a certain age is reached; the capacity for growth may remain latent and capable of exhibiting itself when proper conditions are furnished. But no amount of any amino-acid will cause a fully grown animal to grow any more, so it would seem that the capacity for growth becomes extinguished when it has been utilized to a certain fixed extent, and remains potent until it has been completely utilized.

If the only protein furnished contains no tryptophan the animal cannot maintain itself and slowly loses weight until it dies, unless tryptophan is supplied. If zein from corn, which yields neither lysine nor tryptophan, is the sole protein, then the animal cannot grow unless both lysine and tryptophan are added to the diet. So too, pure casein is not adequate to maintain growth because of its low content in cystine, but if cystine is added the nutritive value is much increased.[48] Histidine also seems to be essential,[49] but this amino acid is present in all proteins. That the pure isolated amino-acids can meet the deficiencies when added to the imperfect protein ration, demonstrates that proteins serve for food as amino-acids, and not as larger complexes.

On the other hand, carbohydrates do not seem to be necessary if proteins and other necessary substances are supplied in abundance (Osborne, Mendel) and apparently fats are not essential (Drummond).

VITAMINS OR FOOD HORMONES AND DEFICIENCY DISEASES[50]

Not only must the proteins present certain essential chemical compounds to the living and growing organism, but also an adequate supply of the essential inorganic salts and certain other, as yet unidentified, substances are necessary to permit of maintenance, growth, and repair. It has long been recognized clinically that certain diseases, notably scurvy, may result from the absence of some essential in the food supply. More recently other diseases have been proved to have a similar cause, and the study of one of these, beriberi, has led to a better appreciation of the nature of the food essentials concerned. This disease usually follows the use of polished rice as the chief constituent of the diet, and can be checked by feeding unpolished rice, or rice polishings, or even extracts of rice polishings, as first demonstrated by Eijkman. A somewhat similar condition may be produced readily in birds by feeding them only polished rice, the chief feature being a severe neuritis, which is relieved with remarkable rapidity by remedying the food deficiency. This experimental neuritis of fowls (*polyneuritis gallinarum*) has served as a valuable means of study of diseases of this class, and led to the demonstration that not only extracts of rice polishings, but also many other food materials, contain the essential materials without which health cannot be maintained.

[48] See Lewis, Jour. Biol. Chem., 1920 (42), 289.

[49] Rose and Cox, Jour. Biol. Chem., 1924 (61), 747.

[50] Full reviews are given in the following publications: Sherman, Physiol. Reviews, 1921 (1), 598; Eddy, "The Vitamine Manual," Williams and Wilkins, 1921; Sherman and Smith, "The Vitamins," Chemical Catalog Co., 1922; Ragnar Berg, "Die Vitamine," S. Herzel, Leipzig, 1922; McCollum, "The Newer Knowledge of Nutrition," Macmillan, 1923; Aron and Gralka, Handbuch d. Biochem., 1924 (6), 345.

One of the early investigators of this subject, Casimir Funk, gave to "the hitherto unrecognized essential dietary factors" the name "vitamines," which, in spite of certain logical objections, has been widely adopted.[51] Although so essential for life the amount required is very small, for whole rice is said to contain not over 0.1 gm. per kilo, and probably much less, of the active substance.

As there exists a large literature on these important agents, which is growing daily and which is well summarized in the accessible reviews cited previously,[50] it is not necessary to discuss here more than the essential facts concerning the vitamins and the pathological conditions which may result when they are inadequately supplied in the diet.[52] In brief, the chief effects and properties of each of the vitamins now generally recognized, are as follows:

Vitamin A (Fat-soluble Vitamine A).—Found especially in cod liver oil,[53] milk and butter, fresh green plants, egg yolk, animal viscera such as heart, liver, and kidney, but it is scanty in muscle. Animals deprived of adequate supplies of this vitamin show a decreased capacity for growth, increased susceptibility to infections,[54] alterations in the œstrus cycle, and the development of a characteristic eye disease designated as keratomalacia or xerophthalmia (*q. v.*, p. 316). A marked decrease in blood platelets has been observed,[55] as well as atrophy of the endocrine glands[56] and intestinal mucosa (Cramer).

In its solubilities vitamin A resembles the lipoids, but it is not identical with any of the known lipoids as isolated from tissue. It is found closely associated with the yellow pigmentary substances of fats, although not identical with the fat pigment, carotin.[57] Apparently it is not a fat or an ester because it resists saponification, and Takahashi believes it to be related to cholesterol,[58] a view that has been supported by recent experiments on the action of ultraviolet light on cholesterol (Hess, Steenbock). It resists such heating as occurs in ordinary cooking of foods. Since A is found in butter fat free from recognizable amounts of nitrogen and phosphorus, it has been thought that it must be a substance lacking these elements, but possibly these results merely depend on the small amount of material responsible for the vitamin effect. At one time it was thought

[51] The term implies, as Funk believed, that the vital agents are amines, but as it now seems certain that, whatever they may be, they are not amines, it has been generally agreed to drop the final *e* which carries the implication of the amine character, and the term *vitamin* is now generally used.

[52] References will ordinarily be given only to articles that have appeared more recently than the reviews quoted above.

[53] The fat-soluble A in cod liver oil is believed to be derived from marine algæ which are the fundamental food supply of marine animals (Drummond and Zilva, Biochem. Jour., 1922 (16), 518). Irradiation of animals with ultraviolet light, as well as of food stuffs, is said to confer antirachitic potency. (See Steenbock and Black, Jour. Biol. Chem., 1924 (61), 405).

[54] See Cramer and Kingsbury, Brit. Jour. Exp. Path., 1924 (5), 300.

[55] Cramer *et al.*, Brit. Jour. Exp. Path., 1923 (4), 37.

[56] See McCarrison[81] also Hintzelmann, Arch. exp. Path. u. Pharm., 1923 (106), 353.

[57] See Palmer, "Carotinoid and Related Pigments," Chemical Catalog Co., 1922.

[58] See Kagawa, Progres Méd., 1922 (37), 674.

that deficiency in A was responsible for rickets, but it is now generally agreed that there is a specific fat-soluble antirachitic vitamin, distinct from the A vitamin, which latter is related specifically to growth and to keratomalacia.[59] This property is conveyed to foodstuffs, to living animals, and even to pure cholesterol by exposure to ultraviolet light. The reproductive vitamin E is also fat-soluble.

Vitamin B (Water-soluble B, Antineuritic Vitamin).—Possibly two distinct vitamins are included in this group, one of which is necessary for normal growth,[60] lack of the other leading to polyneuritis in fowls and beriberi in man.[61] Animals deprived of B not only fail to grow and develop neuritis, but they suffer marked alterations in the body tissues, especially the endocrine glands, and a decreased desire for or capacity to utilize foods.[62] The water content of the tissues may be increased even without frank edema[63] and the nucleic acid content of the tissues is decreased.[64] There may be observed a fall in body temperature and an increased susceptibility to infection.[65] In rats, Hofmeister[66] found hemorrhages in the central nervous system without neuritis.

There is much conflicting evidence as to whether vitamins are essential for bacterial growth,[67] or even for the growth of yeasts,[68] and also as to whether bacteria produce vitamins that can be utilized by animals.[69] However, it seems to be established that yeasts may synthesize B, although neither B nor fat-soluble A could be demonstrated in tubercle bacilli by Cunningham.[70] An agent stimulating growth of yeasts was found in filtrates from cultures of tubercle bacilli by Borrel *et al.*[71]

B is found in all sorts of vegetable foods, especially abundantly in yeast, and to a less extent in meats and milk. Apparently less of B is stored in animal tissues than of vitamin A.

Vitamin B is soluble not only in water, but also in 70 per cent. alcohol, in olive oil, and oleic acid. It is stable in weak acids but not in alkali, and it resists boiling in neutral or weakly acid solution for some time. It is adsorbed readily by colloidal iron hydrate, fuller's earth, etc., and behaves as if of basic nature. This is the vitamin studied by Funk, and thought by him to be a compound of nicotinic acid and some pyrimidine

[59] See Steenbock *et al.*, Jour. Biol. Chem., 1923 (58), 59.

[60] Funk and Dubin (Jour. Biol. Chem., 1921 (48), 437) report the separation of the antineuritic vitamin from the substance which activates yeast and bacterial growth, which they call vitamin D. (Not accepted by McDonald, Jour. Biol. Chem., 1923 (56), 489). This substance seems to be the same as the "bios" of Wildiers.

[61] See Levene and Muhlfeld, Jour. Biol. Chem., 1923 (57), 341.

[62] See Cowgill, Amer. Jour. Physiol., 1921 (57), 420.

[63] Krause, Amer. Jour. Physiol., 1922 (60), 234.

[64] Findlay, Jour. Path. and Bact., 1921 (24), 175.

[65] Werkman, Jour. Infect. Dis., 1923 (32), 253; Findlay, Jour. Path. and Bact., 1923 (26), 485.

[66] Bioch. Zeit., 1922 (128), 540.

[67] See McLeod and Wyon, Jour. Path. Bact., 1921 (24), 205; Ayers and Mudge, Jour. Bact., 1922 (7), 449; Freedman and Funk, Jour. Metabol. Res., 1922 (1), 457.

[68] See Emmett and Stockholm, Jour. Biol. Chem., 1920 (43), 287.

[69] See Damon, Jour. Biol. Chem., 1923 (56), 895.

[70] Amer. Rev. Tuberc., 1924 (9), 487.

[71] Compt. Rend. Soc. Biol., 1923 (89), 191.

base. Several later observers have found evidence indicating to them that B is either a pyrimidine or a purine derivative.[72] Seidell[73] has obtained a crystalline picrate having the antineuritic properties of vitamin B, which fact speaks against the view once held that vitamins are enzymes or related thereto.

Vitamin C (Antiscorbutic Vitamin).—Scurvy in man and experimental scurvy in guinea pigs and monkeys results from deficiency of this agent. Defective tooth development is also a prominent feature, at least in experimental animals.[74] Probably less well-defined disorders of growth and health also result when C is not supplied in sufficient amounts.

It is well known that the juices of citrus fruits are rich in vitamin C, as also are tomatoes and cabbage, but all fresh vegetable foods contain more or less, while seeds and grains are almost or quite devoid of C. Milk and animal tissues contain less than most fresh vegetable foods, muscle being especially deficient. During sprouting of seeds much vitamin C appears, even although the seeds themselves were valueless as antiscorbutics,[75] so apparently C is either synthesized or formed from an inactive precursor during germination.

This vitamin is characterized by its ready destruction by drying and cooking of foods, and as the human organism apparently needs an abundant supply, a deficiency in vitamin C often occurs under conditions existing in modern life. It is particularly quickly destroyed by heating if the solution is at all alkaline, and is best preserved during heating if in weakly acid solutions, such as tomato juice. An abundant supply of oxygen during heating also hastens destruction. Vitamin C is not adsorbed by precipitates and colloids, as is B, and passes through parchment membranes. However, it is said that B and C exhibit similar qualities in respect to diffusion through collodion membranes of varying permeability, resembling in diffusibility such semicolloids as methylene blue, neutral red, and saponin.[76]

Other Vitamins.—Undoubtedly there exist numerous other specific agents that may be properly considered in our present state of ignorance as vitamins, some of which have not yet been recognized and others which have been detected. Among the latter is the antirachitic vitamin, this being a fat-soluble agent associated with but distinct from fat-soluble A, and described more fully under Rickets, p. 320. There is also evidence that, included in so-called water-soluble B, are two distinct vitamins, one antineuritic and the other with growth-promoting properties, which by some has been called vitamin D.[77] Unfortunately the name "Vitamin D" has been suggested for several different agents. Some observers (Funk and Dubin[60]) have used this to indicate what they believe to be a specific vitamin essential for the growth of bacteria, closely

[72] See Williams, Jour. Ind. Eng. Chem., 1921 (13), 1107.
[73] U. S. Public Health Reports, 1924 (39), 294; science, 1924 (60), 439.
[74] See Toverud, Jour. Biol. Chem., 1923 (58), 583.
[75] See Honeywell and Steenbock, Amer. Jour. Physiol., 1924 (70), 322.
[76] Zilva and Miura, Biochem. Jour., 1921 (15), 422.
[77] Not accepted by Tönnis, Zeit. physiol. Chem., 1924 (136), 89.

related to but distinct from B, while others have so designated the anti-rachitic vitamin.

Evans and Bishop[78] have described a vitamin necessary for fertility in female animals. Rats maintained on a properly balanced diet containing enough of both A and B, remain sterile unless provided with some other agent, found especially in green leaves and butter fat, but not identical with vitamin C. This deficiency seems to affect placental behavior more than ovulation. The vitamin responsible has been referred to as X, but this same designation has also been used for the antirachitic agent, and Sure,[79] who corroborates Evans, has christened the fertility vitamin as E, and finds this activity in the ether extract from green leaves, butter fat and wheat germ.

As yet we do not know the chemical nature of any of the vitamins.[80] Why the vitamins are essential and how they act are equally unknown. It is suggestive that they are found especially in cells with an active metabolism, but whether as a result of this activity or because essential for cell growth is undetermined. Vedder has suggested that the anti-neuritic vitamin is essential for growth and repair of the nervous tissue, and in its absence normal wear cannot be made good.[81] MacDougal[82] and Glanzmann[83] suggest that vitamins function by increasing the hydration capacity of cell colloids, which is necessary for growth of the cells. There is evidence that substances rich in the anti-neuritic vitamin stimulate growth in infants.[84] Moore[85] suggests that deficiency diseases may be the result of lack of something needed to neutralize toxic substances produced in metabolism or derived from outside sources, just as in poisoning with tri-nitro toluene and similar compounds there is no intoxication so long as the body can furnish sufficient glucuronic acid to neutralize the poisons. Dutcher[86] found some relation between catalase and vitamin content in experimental polyneuritis, and suggested that the vitamins stimulate oxidative processes which remove toxic substances. McCarrison[81] especially has laid emphasis on the possibility that vitamin deficiency may have an important indirect influence through the changes that he has observed to take place in the endocrine glands. Cramer[87] holds that the key of the vitamin action lies in their effect on the digestive tract, lack of A leading to atrophy of the intestinal mucosa while lack of

[78] See Science, 1922 (56), 650; Jour. Metabol. Res., 1922 (1), 319; 1923 (3), 201, 233.

[79] Jour. Biol. Chem., 1924 (58), 681.

[80] Review by Seidell, Science, 1924 (60), 439.

[81] McCarrison, ("Studies in Deficiency Disease," Oxford Med. Publn. 1921), has observed hypertrophy of the adrenal in pigeons with experimental beriberi, although the other organs are atrophied. He considers a general nuclear starvation from lack of necessary nuclear nutritive materials to be the essential condition. He found the sex glands particularly atrophied, and Houlbert (Paris Médicale, 1919 (9), 473), has found water-soluble vitamins essential for growth of sex glands.

[82] Proc. Soc. Exp. Biol. Med., 1920 (18), 39.

[83] Monatschr. Kinderh., 1923 (25), 178.

[84] Daniels *et al.*, Amer. Jour. Dis. Chil., 1919 (18), 546.

[85] British Publ. on Munitions, No. 11.

[86] Jour. Biol. Chem., 1918 (36), 634.

[87] Lancet, May 26, 1923.

B causes atrophy of the lymphoid elements; all the effects of vitamin deficiency he believes can be explained on the basis of secondary deficiency in absorption or increased local vulnerability to bacterial infection.

It is evident that more than one vitamin is necessary for maintaining normal conditions,[88] and deficiency of one causes beriberi, of another scurvy, for some dietaries lead to one disease and some to the other. Quite possibly minor, or less well-defined impairment in health may often result from quantitative deficiency in vitamin supplies. Apparently the body can store up more or less of the necessary vitamins in time of plenty to tide over periods of deficiency, the liver especially having been found to contain stored vitamins A, B,[89] and C.[90] There is disagreement as to how much influence deprivation of vitamins has on resistance to infection[91] or the development of antibodies during immunization,[92] but there is little doubt that a condition so deleterious as vitamin deficiency reduces the capacity to combat infection. This may depend either on local conditions, as in keratomalacia, or on decreased systemic resistance (See Cramer and Kingsbury).[54] A decrease in the bactericidal power of the blood has been observed in animals on a rickets-producing diet, without corresponding decrease in complement titer,[93] and complement was found increased in guinea pigs with experimental scurvy by M. L. Koch and Smith.[94] Wedgewood[94a] believes that a deficiency in vitamin B results from chronic infection, and that such deficiency, whether produced by dietary defect or disease, predisposes to anaphylactic sensitization. He also found that orange juice feeding protects guinea pigs against anaphylactic shock.

The main points concerning the most studied deficiency diseases may be summarized as follows:

Beriberi[95] occurs in two forms—the dry polyneuritic type and the edematous or wet beriberi, and mixed forms. The dry type resembles the experimental polyneuritis of birds, mentioned previously, for in the birds edema does not accompany the polyneuritis that can be produced experimentally by feeding polished rice. There now seems to be no doubt that human beriberi is the result of the absence of certain essential elements in the diet, observed especially when the diet is polished rice, but possibly occurring with other deficient diets, for the necessary vitamin is of course present in many other foods than rice. Not only has a condition closely resembling human beriberi been produced in animals, but also true beriberi has been experimentally produced in man by feed-

[88] See Groebbels, Zeit. physiol. Chem., 1924 (137), 14.
[89] Osborne and Mendel, Jour. Biol. Chem., 1923 (58), 363.
[90] See Parsons and Reynolds, Jour. Biol. Chem., 1924 (59), 731.
[91] See Stolte, Deut. med. Woch., 1922 (48), 1036; Smith and Watson, Jour. Immunol., 1923 (8), 195.
[92] See Werkman, Jour Infect. Dis., 1923 (32), 247; 1924 (35), 549.
[93] Smith and Wason, Jour. Immunol., 1924 (8), 195.
[94] Proc. Soc. Exp. Biol. Med., 1924 (21), 366.
[94a] Med. Bull. Univ. Cincinnati, 1924 (3), 48.
[95] Full discussion and early bibliography given by Vedder in his book "Beriberi," New York, 1913. See also Jour. Amer. Med. Assoc., 1916 (67), 1494.

ing on polished rice, as well as the repeated demonstration of both prevention and cure of the human disease by proper feeding or by administration of rice polishings or extracts thereof. From rice polishings has been obtained an impure crystalline substance, of which a dose of 20 to 30 milligrams will cure a polyneuritic bird. As stated above, the pure active substance has not been isolated and its exact nature is undetermined, so that it is designated as either "antineuritic vitamin" or "vitamin B."

Vedder believes that it is something that is needed for the repair of nervous tissue, so that in its absence the nervous tissues degenerate. The paralysis, he believes, depends more on central than peripheral nerve changes, since the degeneration of the nerves precedes the paralysis and may persist long after the paralysis has disappeared. As rice polishings relieve the cardiac symptoms, which are important features of beriberi, it is to be assumed that the vitamin is essential for the heart metabolism. Furthermore, heart muscle contains vitamin which will protect from polyneuritis birds fed on polished rice. This does not seem to be identical with the vitamin studied by Funk, for while it relieves the cardiac symptoms and dispels the dropsy of wet beriberi, it does not cure the paralytic symptoms of dry beriberi, according to Vedder. This author "has a growing belief that dry and wet beriberi are separate and distinct diseases, which are, however, generally associated." Rice polishings, he says, clear up beriberi dropsy quickly, but do not affect the paralysis unless the polishings have been hydrolyzed.

There is some evidence that a high proportion of carbohydrate in the deficient diet increases the effect of the vitamin deficiency[96], which explains the common relation of beriberi to diets composed chiefly of polished rice or white flour. Especially is there reason to believe that the wet form of beriberi depends on a relative excess of carbohydrates, as seen in famine edema or war dropsy. Other things being equal, an excessive demand for continued muscular activity in a person deficient in vitamin will favor appearance of the manifestation of the deficiency, and hence beriberi may appear in soldiers and sailors accomplishing heavy tasks when it does not appear in the general population on the same diet. Likewise, pregnancy and lactation often precipitate beriberi in mothers, whose children may also exhibit the same disease.

Certain it is that beriberi is a deficiency disease seldom seen except in those whose chief article of diet is rice with its high carbohydrate content. In this respect it may be compared with pellagra which seems also to be a deficiency disease, but which occurs chiefly among people whose staple food is maize. We do not commonly see beriberi among the pellagrous peasants of Roumania and Italy, nor do we often see pellagra among the people of Java and the Philippines where beriberi is common. Isolated cases of either disease may appear anywhere that suitable arrangement or utilization of foodstuffs produces the proper dietary deficiency, and presumably this may occur sometimes in unexpected places and under cir-

[96] See Walshe, Quart. Jour. Med., 1918 (11), 320.

cumstances difficult of explanation. The fishermen of Labrador and Newfoundland, who live chiefly on white flour during part of the year, have furnished numerous cases of beriberi. But, taken by and large, beriberi is a disease of rice eaters, and pellagra attacks the eaters of maize.

The disease itself offers no chemical changes of interest. Fleming[97] found normal basal metabolism, respiratory quotient and blood chemistry. In animals with experimental polyneuritis, the amount of cholesterol has been found increased except in the adrenals,[98] without changes in basal metabolism.[99]

Keratomalacia or Xerophthalmia,[1] a condition of opacity of the cornea, followed by ulceration and blindness, seems to be specifically due to lack of the fat-soluble vitamin A which is present in egg yolk, butter fats, green leaves, etc., but not in lard or in many vegetable oils. This disease can be produced readily in experimental animals by feeding diets free from proper fats, and is relieved by administration of small quantities of these fats. I have had the opportunity to observe numerous instances of xerophthalmia among the famine sufferers in Roumania, and to observe its prompt relief under cod liver oil feeding. It should not be confused with simple eye infections which are likely to occur in poorly nourished laboratory animals.[2] It is possible that the corneal changes are secondary to alterations in the glands lubricating the conjunctiva.[3] Other factors than lack of A may also be of importance in producing keratomalacia.[4] Nyctalopia (inability to see in reduced light) may also be dependent, at least in some cases, on vitamin deficiency.

Nutritional Dropsy. ("War Dropsy" or Famine Edema).[5] This condition, which was observed extensively during the war, especially among Russian prisoners in Germany, has been seen wherever famine occurs, and is undoubtedly caused by dietary deficiency. Apparently it is independent of scurvy. As it is often associated with xerophthalmia it has been thought to depend on absence of fat-soluble vitamins. It seems more probable, however, that it results from low caloric supply, although protein deficiency combined with excessive fluid and salt intake in the effort to maintain life with weak soups, are probably important factors.[6] Most of the adult subjects have been receiving only 800 to 1,200

[97] Philippine Jour. Sci., 1923 (23), 407.

[98] Hotta, Zeit. physiol. Chem., 1924 (136), 1; Verzár *et al.*, Klin. Woch., 1924 (3), 1723.

[99] Denel and Weiss, Proc. Soc. Exp. Biol. Med., 1924 (21), 456.

[1] Bloch, Ugeskr. f. Laeger., 1918 (80), 815.

[2] See Osborne and Mendel, Jour. Amer. Med. Assoc., 1921 (76), 905; and Amer. Jour. Physiol., 1924 (69), 543; Wagner, Arch. exp. Path. u. Pharm., 1923 (97), 441; Mori, Amer. Jour. Hyg., 1923 (3), 99.

[3] Lambert and Yudkin, Jour. Exp. Med., 1923 (38), 20.

[4] See Walker, Jour. Amer. Med. Assoc., 1922 (78), 273; McCollum *et al.*, Jour. Biol. Chem., 1922 (53), 313.

[5] Full Review by Schittenhelm and Schlecht, Zeit. exp. Med., 1919 (9), 1; Maver, Jour. Amer. Med. Assoc., 1920 (74), 934.

[6] See Guillermine and Guyot, Rev. Méd., Suisse Rom., 1919 (39), 115; Falta, Wien. klin. Woch., 1917 (30), 1637; Schittenhelm and Schlecht, Zeit. exp. Med., 1919 (9), 82.

calories per day, containing but 30 to 50 gms. of protein. It is much more likely to appear in undernourished persons who are compelled to work than in equally starved persons at rest since work, and also cold, increase the caloric deficiency.

Numerous studies of metabolism and blood chemistry in persons exhibiting war dropsy have given concordant results, which show the extreme depletion of the body in all nutritive reserves.[7] The blood shows hypoglucemia, decrease in potassium, fatty acids, phospholipins and residual nitrogen, with an increase in NaCl, and of acetone bodies and ammonia from starvation acidosis. There is also a decrease in the amount of protein in the blood even to one-half the normal amount, with hydremia and a less marked decrease in both red and white cells. The lack of reserve nitrogenous material, both in blood and tissues, is shown by the fact that when patients with war edema are fasted a few days the N excretion may fall to 2 to 3 gms. per day, while in absolute starvation of previously normal persons the N output usually is 10–12 gms. (Falta). Schittenhelm and Schlecht[7] suggest that the edema is merely the result of injury to the capillary endothelium, in common with all the other tissues, whereby their permeability becomes increased.

Famine edema seems to be closely related to the edema often observed in infants kept on a preponderatingly starchy diet, such as barley water, for long periods. Here most striking degrees of dropsy are observed, which seem in all respects similar to famine dropsy. There is probably also a close relation to the edema of pernicious anemia and cachexia. The relation of this form of edema to the edema of wet beriberi has not been determined, but it is highly probable that their origin has something in common. Both are dropsies due to diet deficiency, and it may well be that the deficiency is the same in each case. In both these conditions, as well as in the "Mehlnährschaden" of starch-fed babies, there is the common element of relatively excessive carbohydrate supply, which may have something to do with the dropsy. The clinical evidence is against the view that nutritional edema depends on a lack of specific vitamins.[8]

Experimental work supports the clinical evidence as to the etiology of nutritional edema. Miss Kohman[8a] has found that rats fed diets composed chiefly of carrots often develop a severe edema, which is prevented by supplying protein, but not by butter fat or starch. Evidently neither fat-soluble nor water-soluble vitamins are responsible. It was found that on a dry diet of equal caloric and protein deficiency the rats are not so likely to develop edema. Experiments done in my laboratory by M. B. Maver[5] agree fully with those of Miss Kohman. Apparently low protein and high fluid intake are the most essential factors, although relatively high carbohydrate must also be considered.

[7] See Schittenhelm and Schlecht, *loc. cit.;* Feigl, Biochem. Zeit., 1918 (85), 365; Jansen, Münch. Med. Woch., 1918 (65), 925; Bürger, Zeit. exp. Med., 1919 (8), 309.

[8] See Bürger, Zeit. exp. Med., 1919 (8), 309.

[8a] Denton and Kohman, Jour. Biol. Chem., 1918 (36); 240· Kohman, Amer. Jour. Physiol., 1920 (51), 378.

Scurvy[9] is now fully established as a deficiency disease, although there was until recently disagreement as to this, especially among those who have studied experimental scurvy in animals. There is reason to believe that more than one condition has been described as scurvy in experimental animals. Apparently guinea pigs, however, develop readily a disease which resembles scurvy very closely both anatomically and in its relation to dietary conditions.

On the other hand, rats and some other animals are immune to scurvy and may even synthesize the necessary protective substance.

Hess, who has studied especially infantile scurvy, found that orange juice given intravenously will relieve scurvy, and thus apparently disposes of all theories of gastrointestinal disorders as the responsible factor. Artificial "orange juice," (containing sugar, citric acid and inorganic salts in the proportions found in natural orange juice) is ineffective, so that apparently scurvy is the result of lack of some undetermined substance present in orange juice as well as in other fresh vegetable foods. As yet we have no evidence as to the character of this "vitamin," which has been designated as "water-soluble C," but it is probably quite distinct from either water-soluble B or fat-soluble A,[10] and Hess believes that our ordinary dietary probably does not contain any great excess of the antiscorbutic element, since scurvy so readily appears when the necessary vegetable foods are reduced in amount. It is chiefly the result of modern methods of nutrition with foods which have been deprived of this labile vitamin through the methods used in preservation, or of unavailability of fresh foods to sailors, explorers or prisoners.

According to Chick and Hume vitamin C is present in living vegetable and animal tissues, the largest amounts in fresh fruits and green vegetables, to a less extent in root vegetables and tubers, but peas and beans devoid of antiscorbutic potency develop it abundantly on germination. It is present in small amount in fresh meat and milk, and has not been detected in yeast, fats, cereals, pulses. The explorer, Stefansson,[11] has reported observations indicating the presence of antiscorbutic substances in raw meat, and their absence or deficiency in well-cooked meat and tinned foods. Evidently this antiscorbutic element is very unstable, since even drying vegetables at moderate temperatures,65–70°, and cooking or salting meats, destroys or greatly reduces their antiscorbutic value.[12] Mere aging of active fruit juices may destroy their potency, but tomato juice seems to retain its activity even when preserved as canned tomatoes, as do some other food stuffs.[13] Pasteurization of milk also reduces the preventive value of this food[14] which, in its raw state, contains in abundance all necessary factors for nutrition, but apparently little more of the antiscorbutic substance than is barely sufficient to maintain health.

[9] A full review is given by Hess, "Scurvy, Past and Present," Philadelphia, 1920. Metabolism in scurvy, see Hess, Endocrinology and Metabolism, 1924 (4), 883.

[10] Cohen and Mendel, Jour. Biol. Chem., 1918 (35), 425.

[11] Jour. Amer. Med. Assoc., 1918 (71), 1715.

[12] Givens and Cohen, Jour. Biol. Chem., 1918 (36), 127; Amer. Jour. Dis. Chil., 1919 (18), 30.

[13] See Kohmann, Jour. Amer. Med. Assoc., Jan. 17, 1925.

[14] See Hess, Amer. Jour. Dis. Chil., 1919 (17), 221.

Pellagra[15] probably belongs among the deficiency diseases, despite numerous attempts to account for it as an infectious disease. The work of Goldberger[16] is especially valuable in affirmative evidence of the relation of dietary deficiency to pellagra. Goldberger[17] once stated his conclusions as follows: "The pellagra-producing dietary fault is the result of some one, or, more probably, of a combination of two or more of the following factors; (1) a physiologically defective protein supply; (2) a low or inadequate supply of fat-soluble vitamin; (3) a low or inadequate supply of water-soluble vitamin, and (4) a defective mineral supply. The somewhat lower plane of supply, both of energy and of protein, of the pellagrous households, though apparently not an essential factor, may, nevertheless, be contributory by favoring the occurrence of a deficiency in intake of some one or more of the essential dietary factors, particularly with diets having only a narrow margin of safety. The pellagra-producing dietary fault may be corrected and the disease prevented by including in the diet an adequate supply of the animal protein foods, particularly milk, including butter and lean meat." More recently, Goldberger[18] has found evidence that neither minerals nor fat-soluble vitamins will prevent pellagra, and therefore believes that defective amino acid make up of the protein supply is chiefly responsible.

Whatever the deficiency in diet may be, pellagra seems to develop most often in persons whose diet is preponderatingly maize seed products. Only in countries where maize is the chief dietary staple does pellagra occur with any great frequency, and in those countries where part of the population lives chiefly on maize, and other groups live on other foods, pellagra occurs chiefly or only in the former group. I have had the opportunity to observe much pellagra in Roumania during a period of protracted and serious food shortage, and this relation to maize was most striking and convincing. The peasants of this country have for their chief food a thick mush of boiled, coarsely ground corn meal, called *mamaliga*, supplemented by such other foods as they can secure. Dwellers in the towns rely on bread from wheat flour as their chief carbohydrate supply, and have a much more abundant and varied list of accessory foods. Pellagra is prevalent in Roumania, but restricted to the maize-eating peasants, and in very definite relation to their inability to secure accessory food stuffs. While Roumanian physicians seemed generally inclined to accept the theory that spoiled maize is responsible for pellagra, my own observations would indicate that the chief difficulty is lack of accessory foods. The relation of maize to pellagra becomes particularly striking if we compare Roumania with a country where maize is not a staple food, such as Korea. Here for centuries a large part of the population has existed on

[15] Concerning metabolism in pellagra see Myers and Fine, Amer. Jour. Med. Sci., 1913 (145) 705. Chemical changes in the central nervous system described by Koch and Voegtlin, Hygienic Lab. Bull. 103, 1916. Reviews on etiology in "Endocrinology and Metabolism," 1924 (4), by MacNeal, p. 899 and McCollum, p. 911.

[16] Public Health Rep., 1914 (29), 1683; 1915 (30), 3117, 3336; 1920 (35), 648; 1922 (37), 462.

[17] Jour. Amer. Med. Assoc., 1918 (71), 944; Arch. Int. Med.. 1920 (25), 451.

[18] Jour. Amer. Med. Assoc., 1922 (78), 1676.

the verge of starvation, the chief food being rice. Although here beriberi is common enough, especially among those who can afford the luxury of polished rice, pellagra is not observed, despite a much greater deficiency in total food supply, both as regards calories and accessories, than prevails in Roumania.

It is possible that the deficiency of tryptophan in maize proteins may be a factor,[19] and it has been suggested that in pellagra there is adrenal insufficiency because of a lack of this aromatic compound from which epinephrin may be manufactured. Because of this low tryptophan content, it requires 105 grams of maize protein to replace 30 grams of meat protein in the diet (Wilson).[20]

Despite the abundant evidence of the relation of dietary deficiency there are those who interpret existing evidence as establishing or making probable that pellagra is nevertheless essentially an infectious disease.[21] The compromise view that pellagra is an infectious disease which can only manifest itself among those suffering from dietary deficiency has also been supported, especially by McCollum.[22] Jobling and Arnold[23] have suggested that the characteristic skin lesions, and perhaps other manifestations, may depend on the absorption of photodynamic substances, behaving like hematoporphyrin, produced in the intestines by bacteria or fungi whose growth is favored by an excessive carbohydrate diet, but this hypothesis fails to explain why pellagra does not develop more often in rice-eating countries.

The studies on pellagra among Turkish war prisoners added much evidence in support of the dietary basis and against an infectious etiology, but much importance was attributed to intestinal infection as a contributing cause through prevention of adequate assimilation of necessary food elements.[24] The blood of pellagrins shows a low non-protein N figure[25] and indolethylamine has been found in the urine,[26] while the sulfocyanate content of the urine and saliva is increased.[27] Intestinal putrefaction with increase of skatol and indol, and indicanuria, often marked, are frequently present, and may account for defective tryptophan absorption even when it is abundantly supplied in the diet.

Rickets.[28]—Extensive investigations carried on in the past few years have thrown an entirely new light upon the etiology of this disease, which is now firmly established as a deficiency disease. As is usually the

[19] See Chick and Hume, Biochem. Jour., 1920 (14), 135; Goldberger and Tauner, U. S. Publ. Health Reports, 1921 (37), 462.

[20] Jour. Hyg., 1921 (20), 1.

[21] See Jobling and Peterson, Jour. Infect. Dis., 1916 (18), 501.

[22] Proc. Amer. Philos, Soc., 1919 (58), 41; see also MacNeal, Amer. Jour. Med. Sci., 1921 (161), 469.

[23] Jour. Amer. Med. Assoc., 1923 (80), 365.

[24] Jour. Royal Army Med. Corps, 1919 (33); 1920 (34). See also Murlin, Hygienic Lab. Bull., 1920 (116), 45.

[25] Lewis, Hygienic Lab. Bull., 1920 (116), 37.

[26] Sullivan, Jour. Biol. Chem., 1922 (50), xxxix.

[27] Jour. Biol. Chem., 1921 (45), 473.

[28] Full review and bibliography by Park, Physiol. Reviews, 1923 (3), 106; Howland, Harvey Lectures, 1922–3 (18), 189.

story, rapid progress came when it was found possible to produce the disease experimentally at will in a laboratory animal, in this case the rat, which is so resistant to experimental scurvy. These experiments, together with exhaustive studies on human material, have shown, to quote from Park,

"that two factors exist, the one in radiant energy, the other in an unknown form in certain foods, either of which is capable of preventing rickets from developing or from continuing, if already established. It has become equally clear, also, that only when the organism is deprived of the influence of radiant energy and of *X*, can rickets develop. Recent advances in knowledge have failed entirely to disclose the nature of vitamins, or their mode of operation in the organism or the mode of operation of the ultra-violet radiations of the environment. They have revealed, however, certain of the effects upon the organism of radiant energy and of *X*; both exercise a stimulatory or a regulatory influence on the metabolism of the body, in particular of the calcium and phosphorus. One at least of their functions appears to be the protection of the organism from the dangers attendant on the absorption and entrance into the blood of substances which might disturb its salt balance. Moreover, increased knowledge has indicated that the role played by radiant energy and *X* in the maintenance of the normal salt metabolism is of the utmost importance, and that the organism is dependent on the energy of the sun's rays or of their equivalent in the food to an extent little appreciated. The facts enumerated indicate beyond a doubt that rickets is a deficiency disease."

The unknown, antirachitic agent is especially abundant in cod liver oil, and is generally found associated with the fat-soluble vitamin A, from which it is undoubtedly distinct. Of particular significance is the fact that exposure of food stuffs and even of pure cholesterol[29] to ultra violet light imparts to them the antirachitic function, even when they did not possess it before, just as exposure of the rachitic child or animal to the same actinic influence cures or prevents rickets. Also, that substances exhibiting the antirachitic effect emit ultraviolet rays.[30] Evidently the antirachitic element has the same effect as ultraviolet light in respect to maintaining a normal calcium and phosphate metabolism.[31] It has been shown that radiant energy or cod liver oil alike serve in some mysterious way to maintain the proper balance of calcium and phosphorus in the blood and tissues. In animals in which the calcium of the serum is low, cod liver oil causes the calcium to rise to the normal level, restoring a normal equilibrium. In animals in which the inorganic phosphorus in the serum is low, cod liver oil causes the inorganic phosphorus to rise to a normal level, establishing a normal equilibrium. Obviously cod liver oil acts as a regulator of the mineral metabolism, at least in so far as the calcium and inorganic phosphorus are concerned, and one may infer that a function of cod liver oil and also of radiant energy is to maintain the normal salt equilibrium of the body in the presence of salt combinations of different complexities which are continually being absorbed from the food. Neither cod liver oil nor radiant energy supplies the required calcium or the phosphorus. It is an extraordinary fact, however, that either one can

[29] Hess *et al.*, Jour. Biol. Chem., 1924 (63), 305; Jour. Amer. Med. Assoc., 1925 (84), 1033; Steenbock and Daniels, Jour. Amer. Med., Assoc., 1925 (84), 1093.

[30] Kugelmass and McQuarrie, Science, 1924 (60), 272.

[31] In congenital *fragilitas ossium* ultraviolet light may have the same effect (Ferri, Pediatria, 1925 (30), 20).

enable the rat, kept in a state of calcium or of phosphorus starvation to obtain so good a utilization of the minimal quantities of calcium or of phosphorus supplied in the food as to maintain the level of the calcium or of the phosphorus in the body fluids almost at the normal. Without supplying either phosphorus or calcium, radiant energy or the antirachitic vitamin cause the organism to operate as if a requisite or almost requisite quantity of phosphorus or calcium was supplied. Neither the histological studies of the bones nor the chemical examinations of the blood indicate that either cod liver oil or light bring new processes into operation, but rather permit the organism to have full use of processes which were natural to it all the time, but were not effective.

When there is an inadequate supply of the necessary vitamin or of radiant energy, rickets may be produced experimentally, and presumably also in human subjects, by inadequate or improperly balanced inorganic salt supply. According to Park, rickets has been produced:

1. By diminishing the phosphorus content of the diet and administering calcium in the form of the carbonate or lactate or chloride in disproportionately large quantities. The rickets produced in this way is characterized by a low blood phosphorus and a histological condition corresponding to that ordinarily found in the rachitic human being.
2. By diminishing the calcium and maintaining the phosphorus content of the diet at a higher level than that of calcium. The rickets resulting from diets of this kind has certain distinctive features.
3. By adding magnesium or strontium carbonate to the diet.

Experience has shown that not all diets deficient in antirachitic vitamin, and having unusual calcium phosphorus balances, regularly produce typical rickets. Undoubtedly there are many factors in the diet about which nothing is known. On the other hand, even in the absence of the antirachitic vitamin, rickets can be prevented by use of a diet which is adequate in respect to the proportion of inorganic salts and of necessary organic components.

(The chemical features of rickets are discussed in Chapter XIX.)

CHAPTER XV

DISTURBANCES OF CIRCULATION AND DISEASES OF THE BLOOD

THE COMPOSITION AND PHYSICAL PROPERTIES OF THE BLOOD

THE function of the blood being to maintain an equilibrium in the temperature, chemical composition, and osmotic pressure between all parts of the body, it follows that it is never of exactly the same composition in any two places or at any two times. To the extent that every tissue is continually giving off something to the blood, we may consider that every organ is a factor in its formation, and as a result of this multiplex origin of the blood, the substances it may contain are beyond enumeration. There are probably but few chemical substances occurring in the tissue-cells that do not also occur in greater or less amount in the blood. In addition to these there are also the substances characteristic of the blood itself, besides a host of substances of unknown nature, apparently manufactured in response to the stimulation of substances entering the body from outside; for we find that the blood of every adult individual contains substances that make him immune to a multitude of diseases that he has had in childhood, as well as substances that in later life protect him to a greater or less degree from infection by such organisms as the colon bacilli of his intestine, the pneumococci and streptococci in his throat, etc. What other substances of importance we may yet find in the blood is an open question. There are no apparent limits to the possibilities of the study of the blood, for it represents a little of every organ, and much that is characteristic of itself.

The composition of the blood in normal conditions is covered so fully in current texts on physiological chemistry that it will not be discussed here; beyond referring the reader also to the complete set of data compiled from the literature by Gram[1] on the physical properties and chemical composition of normal human blood. Because of their significance in pathological properties, however, we discuss briefly some of the physical qualities of blood,[2] namely, the reaction and the viscosity of the blood and the sedimentation rate of the red corpuscles.

Reaction.[3]—If we titrate the blood plasma with an acid, we liberate much of the alkali from the proteins, dissociate all the Na_2CO_3 present, as well as the $NaHCO_3$ and the sodium phosphate, and find in this way that the entire fresh blood contains *neutralizable alkali* corresponding to a solution of Na_2CO_3 of about 0.443 per cent. strength (Strauss). In other words the blood has a quantity of alkali in combination that can be drawn upon to neutralize acids to the extent indicated by the above

[1] H. C. Gram, Amer. Jour. Med. Sci., 1924 (168), 511.

[2] For bibliography see review on the physical chemistry of blood and lymph by Pincussohn, Handbuch d. Biochem., 1923 (4), 1.

[3] See review by Van Slyke, "Endocrinology and Metabolism," 1924 (4), 51.

figures. The *real alkalinity* of a fluid, however, is dependent upon the number of free OH ions in the solution; and Höber determined by physico-chemical methods that the concentration of OH ions in blood is but little greater than in distilled water. Michaelis[4] has found the H-ion concentration of the blood to be 0.45×10^{-7}, as contrasted with neutrality at 38° which is $H^+ = 1.5 \times 10^{-7}$. For convenience, the hydrogen-ion concentration (*c*H) of the blood is commonly expressed as its negative logarithm, using the term *p*H introduced by Sörensen. The *p*H of water is 7, (which means that the hydrogen ion concentration of water is 0.000,000,1 normal); solutions more acid than water have a *p*H less than 7, more alkaline solutions have a *p*H higher than 7, and each change of 1 in the *p*H corresponds to a tenfold change in the H-ion concentration. The *p*H of blood is close to 7.4, within a normal range of 7.3 to 7.5, the extreme range compatible with life being approximately 6.9 to 7.8. The difference between arterial and venous blood is only about 0.02.

The interchange between CO_2, phosphates, and carbonates in the blood is such that it is impossible for any considerable quantities of free H or OH ions to exist, and the protoplasm is thus protected from an excess of either; furthermore, the tissues themselves are well provided with the same protective mechanisms.[5] The capacity of the blood to neutralize acids and alkalies is referred to as its "buffer value." According to Henderson[6] not more than five parts of excess free H or OH ions can be present in ten billion parts of protoplasm. An alkalinity is impossible because this would cause an increased osmotic pressure which the kidneys would regulate; acidity is impossible because death would result from the inability of the blood to carry CO_2. The lungs remove excessive CO_2, the kidneys excrete either acids or alkalies in excess according to the state of the blood, and also form ammonia to neutralize acids, the blood and tissue proteins also can bind much of either H or OH ions, so that the preservation of neutrality is elaborately guarded. Presumably one important purpose of the exact regulation of reaction is to provide proper conditions for enzyme action.

In the tissues, because of the production of acids during metabolism, the H-ion concentration is slightly higher than that of the blood, being estimated by Michaelis at exact neutrality, 1.5×10^{-7}. Schade found the perimuscular fluid in exercise as low as *p*H 6.6, while Rous[7] found that, according to indicators, the inactive tissues such as fascia and tendons have a *p*H of 7.2 or slightly less.

The alkali of the blood exists in part as alkaline salts, carbonate and phosphate (the *diffusible alkali*), and partly combined with protein (*non-diffusible alkali*). As the corpuscles are richer in diffusible alkali than the plasma or serum, the number of corpuscles modifies the alkalinity of the blood decidedly. Much importance is attached to the question of the alkalinity of the blood for two reasons; first, in certain conditions of disease the blood contains so much of organic acids that the alkali is partly saturated and the power of the blood to carry CO_2 is lessened, with serious results (see "Acid Intoxication," Chap. XXII); and, second, the bactericidal power of the blood is found to vary according to its alkalinity. In fact, metabolic activity seems generally to be favored by certain degrees of alkalinity; for example, J. Loeb found that sea-urchin eggs develop with much greater rapidity if a small amount of OH ions is free in the sea-water. In cancer the titrable alkalinity is distinctly increased, and Moore and Walker[8] hold that this is due to an increased alkalinity of the proteins of the blood. (See also "Passive Congestion" for further discussion concerning the relation of alkalinity to bactericidal power.)

Viscosity of the Blood.[9]—Normal blood is about five times (4.5 times, Austrian) more viscous than water, chiefly because of the corpuscles and the dissolved proteins. This viscosity does not vary directly with the specific gravity or the hemoglobin, but is closely related to the number of red corpuscles (Burton-Opitz); laking the corpuscles increases the viscosity considerably. Most salts increase the viscosity, but some, especially iodides, are said to reduce it. Carbon dioxide increases viscosity greatly, even when in amounts possible in the circulating blood. Anemia decreases the viscosity, approximately in proportion to the number of corpuscles; polycythemia

[4] Deut. med. Woch., 1914 (40), 1170.

[5] See review on the neutrality regulations in the body by Wilson, Physiol. Reviews, 1923 (3), 295.

[6] Amer. Jour. Physiol., 1907 (18), 250; 1908 (21), 427.

[7] Jour. Exp. Med., 1925 (41), 451.

[8] Biochem. Jour., 1906 (1), 297.

[9] Review of literature by Bircher, Jour. Lab. Clin. Med., 1921 (7), 134; also Austrian, Johns Hopkins Hosp. Bull., 1911 (22), 9. See also Traube, Internat. Zeit. physik-chem. Biol., 1914 (1), 389.

is accompanied by a corresponding increase; leukemia, because of anemia, shows a decrease; in nephritis there may either be an increase or a decrease in the viscosity, not corresponding in any way to the blood pressure. Cardiac disease with edema shows low viscosity because of the anemia and hydremia, but if there is polycythemia and no edema the viscosity may be high. Jaundice causes an increase, diabetes gives variable results. Typhoid causes no characteristic change beyond that resulting from anemia, and in pneumonia the cyanosis and salt retention usually cause an increase (Austrian). Gullbring[10] found the viscosity to vary directly with the per cent. of neutrophiles. As blood viscosity depends largely upon the corpuscles, it increases with reduction in the size of the lumen of the tube through which it passes, unlike a true solution; hence with narrow capillaries the viscosity is abnormally high until we reach the point where the corpuscles plug the capillary.

Viscosity may be a factor in maintaining blood pressure[11] but probably it is not related to hypertension.

Spiro[12] defines as "specific viscosity" the viscosity of serum of known protein content as compared to normal serum of the same protein content, an abnormally high specific viscosity being found in general infections, cardiac and renal insufficiencies; normal viscosity was found with local inflammatory processes not accompanied by extensive tissue destruction; a lowered viscosity was not found in any condition, indicating that the plasma viscosity is normally kept at the minimum in order that the resistance to circulation may be as low as possible. The viscosity of the serum is from 1.7 to 2.0 times that of distilled water, that of plasma being 0.2 to 0.3 higher. Petschacher[13] found an increase in specific viscosity in dyspnea, and as the protein concentration was unchanged he concludes that alterations in the osmotic pressure and ionization of the protein must be responsible.

Sedimentation of Corpuscles.—Even in the days of Hewson (1771) and Hunter (1794) the increased rate of settling of red corpuscles, with formation of the *crusta phlogistica*[14] was recognized as a sign of inflammation. Much more recently it has been learned that if coagulation is prevented by oxalates, citrates, or hirudin, the red cells tend to agglutinate and fall out of suspension, the rate of sedimentation varying in different conditions but generally being in direct proportion to the speed of agglutination, which in turn depends on the proportion of globulin, and especially of fibrinogen,[15] in the blood. The red corpuscles being negatively charged, their mutual repulsion will be decreased by positively charged proteins of the serum, of which fibrinogen is the most important.[16] Albumin has less neutralizing effect on the negative charge of the corpuscles than globulin, which in turn has less than fibrinogen, and hence when the proportion of albumin is high and of globulin or fibrinogen low, sedimentation is delayed. An increased rate may also result from an increase of electropositive protein in the cells.[17] The rate of sedimentation is ordinarily increased with infectious diseases in which the globulin and fibrinogen content of the plasma is high,[18] *e. g.*, active tuberculosis,[19] syphilis, or surgical inflammations.[20] Cholesterol is said to hasten and lecithin to retard sedimentation,[21] but in practice their influence seems to be subordinate to that of the plasma proteins, except perhaps in pregnancy, when the cholesterolemia increases the sedimentation rate.[22] Polypeptids may also at times increase sedimentation.[23]

Rouleaux Formation.—The physical chemical mechanism of the formation of rouleaux by the red corpuscles is unknown.[24] Sellards found that heating serum at

[10] Beitr. klin. Tuberk., 1914 (30), 1.
[11] Lyon, Quart. Jour. Med., 1921 (14), 398.
[12] Kolloid Zeit., 1922 (31), 345; Klin. Woch., 1923 (2), 1744.
[13] Wien. Arch. inn. Med., 1924 (8), 369.
[14] See Gram, Arch. Int. Med., 1921 (28), 312.
[15] Seki, Biochem. Zeit., 1923 (143), 365; Schindera, Deut. Arch. klin. Med., 1924 (144), 113.
[16] See Höber and Mond, Klin. Woch., 1922 (1), 2412.
[17] Vorschütz, Zeit. klin. Med., 1923 (97), 39.
[18] Schürer and Eimer, Berl. klin. Woch., 1921 (58), 1251.
[19] Westergren, Beitr. klin. Tuberk., 1921 (46), 285; Morriss, Amer. Rev. Tuberc., 1924 (10), 431.
[20] Haselhorst, Deut. med. Woch., 1922 (48), 1100.
[21] Kürten, Arch. ges. Physiol., 1920 (185), 248.
[22] Kürten, Klin. Woch., 1924 (3), 1216.
[23] Abderhalden, Fermentforsch., 1920 (4), 230.
[24] See Swift, Jour. Lab. and Clin. Med., 1922 (7), 614.

60° C. greatly increases its power of producing rouleaux, which is lost on slight dilution. Dilution of the cells below 5 per cent. suspension also interferes. Defective rouleaux formation was observed by Swift with red cells from leukemia and Hodgkins disease, but not from pernicious anemia, nephritis, cancer, or infectious diseases.

HEMORRHAGE

Hemorrhages result from an altered condition in the vessel-walls, which may be due either to trauma or to chemical injuries. Of the chemical agencies causing hemorrhages, bacterial products are the most important practically, but many poisons, such as phosphorus, formaldehyde, *phytotoxins* (ricin, abrin, and crotin), and *zoötoxins* (snake venoms) cause numerous and abundant hemorrhages. Formerly, the tendency was to ascribe hemorrhages from the above causes to mechanical injury of the vessels by thrombi, or by emboli of agglutinated corpuscles, but the work of Flexner[25] has shown that venoms cause hemorrhages by injuring the capillary walls, so that actual rents are produced by the intravascular pressure, and it seems highly probable that hemorrhages are produced by other chemical substances in a similar way. We may, therefore, refer such hemorrhages to an *endotheliotoxic* action of the poison, or to a solvent effect upon the intercellular cement substance. In the case of ordinary chemical poisons the endotheliotoxic action is not specific, but with some of the toxins it seems to be quite so; for example, rattlesnake venom contains an endotheliotoxic substance (*hemorrhagin*), which seems to be a specific poison for endothelium, and which is the most dangerous constituent of the venom.

It is quite probable that the bacterial poisons that cause marked hemorrhagic manifestations likewise contain endotheliotoxins, although this matter does not seem to have been investigated.

Even *hemorrhage by diapedesis* seems to be due to, or at least associated with, chemical changes in the capillary walls, for Arnold[26] found that when capillaries from which diapedesis had occurred were stained by silver nitrate, dark areas were found between the endothelial cells. As silver nitrate is a stain for chlorides, and darkens intercellular substance because it is rich in sodium chloride (Macallum), it is probable that there is an increase in the amount or a difference in the method of combination of the chlorides of the cement substance between the endothelial cells at the places where red corpuscles escape. M. H. Fischer[27] suggests that diapedesis results from a change in the endothelial cells, which, under the influence of acids or other agents of metabolic origin, become excessively hydrophilic, swell, and become so softened that corpuscles may pass directly through the cell, just as a drop of mercury can pass through a sufficiently soft jelly without leaving a hole in the jelly.

Hemorrhage in cachetic conditions is often ascribed to changes in the vessel-walls due to malnutrition, but it is difficult to imagine capillary walls suffering from lack of nourishment, even with the poorest of blood, and it seems more probable that the hemorrhages are due, even in cache-

[25] Univ. of Penn. Med. Bull., 1902 (15), 355.
[26] Virchow's Arch., 1875 (62), 157.
[27] "Nephritis," New York, 1912, p. 78.

xia, to chemical constituents of the blood that injure the endothelium. Hemorrhages that follow re-establishment of the circulation after complete occlusion, however, may be the result of asphyxial changes in the capillary walls, presumably colloidal swelling of the cells.

If loss of blood by hemorrhage is rapid, the effects are naturally much more serious than when the loss is slow. The total quantity of blood in the average adult is estimated at about $\frac{1}{13}$ to $\frac{1}{15}$ the total body weight (therefore about 10 to 12 pounds), although this proportion does not hold for extremely obese or extremely thin individuals; in infants the proportion is lower—about $\frac{1}{20}$. When one-third of the total amount of blood is lost rapidly, a marked fall of blood pressure occurs; loss of one-half of the total amount may be fatal, and loss of more than that at one time usually is fatal. The chief cause of death following large hemorrhages is the low blood pressure rather than the loss of any of the constituents of the blood, hence the successful results of the use of physiological salt solution after severe hemorrhage. The number of corpuscles may be greatly reduced after several small hemorrhages, even to as low as 11 per cent. of the normal number (Hayem), without fatal results, because in the intervals between the hemorrhages enough fluid has been taken up by the blood to maintain the blood pressure within safe limits.

The changes which take place in the circulating blood after hemorrhage are considered under Secondary Anemia, (p. 332).

Changes in the Extravasated Blood.—These begin soon after its escape. In most situations sufficient fibrin ferment is formed to cause prompt clotting, but in the pleura and other serous cavities the blood may remain fluid for some time, possibly because of lack of cellular injury that might cause liberation of fibrin ferment.[28] If the blood does not become infected, the rapidity of subsequent changes depends chiefly upon the location and amount of blood. Blood confined in intact vessels between ligatures is hemolyzed much more slowly than extravasated blood. Small extravasations of blood into the tissues are subjected to the action of the tissue cells and of leucocytes emigrating freely from the capillaries; large masses of blood are but little affected by these agencies, the leucocytes within the mass soon die, and secondary changes go on very slowly. In small subcutaneous hemorrhages (*e. g.*, a bruise), enzymes from the invading leucocytes and tissue-cells soon dissolve the small quantities of fibrin present; even earlier the stroma of the red corpuscles is so altered that hemolysis occurs and the hemoglobin escapes and diffuses into the tissues.[29] This hemolysis may be brought about by the

[28] Denny and Minot (Amer. Jour. Physiol., 1916 (39), 455) believe that the blood really does clot, and that it remains fluid when withdrawn because the fibrinogen has been removed by clotting. Zahn and Walker (Biochem. Zeit., 1913 (58), 130), however, consider that the fibrinogen is altered by the pleural endothelium, so that it cannot clot, and they are supported by Van Herwerden (Nederl. Tijd. v. Geneesk., 1922 (66), pt. 1, 847) who says that even brief contact of fresh blood with pleural surfaces renders it incoagulable. Delrez (Arch. internat. physiol., 1921 (18), 147) does not confirm these views.

[29] Sometimes red corpuscles are found intact in bodies a surprisingly long time after death, seven months in a case observed by Strassmann (Berl. klin. Woch., 1919 (56), 994).

action of proteolytic enzymes on the corpuscles, or by the hemolytic action of the products of protein splitting. Soon the hemoglobin disintegrates, forming the masses of pigment so characteristic of old hemorrhagic areas, and also giving rise to the discoloration observed beneath the skin in the later stages of resorption of hemorrhagic extravasations.

It is generally said that the first products of the splitting of hemoglobin are: (1) The protein, *globin*, which constitutes 94 per cent. of the hemoglobin; and (2) the iron-containing coloring-matter, *hematin* (in the absence of oxygen the pigment is *reduced hematin* or *hemochromogen*). As hematin may be experimentally obtained by the action of proteases upon hemoglobin, the decomposition of the hemoglobin in the tissues is probably accomplished in a similar way by the proteases of the leucocytes, tissue-cells, and blood plasma; the globin is thus digested away and the soluble products carried off, while the insoluble hematin remains.[30] The hematin gradually undergoes further changes, forming an iron-free pigment (*hematoidin*) and an iron-containing pigment (*hemosiderin*). However, the persistency with which hematin may remain in the blood after its injection[31] has aroused doubt as to its being a normal intermediary in hemoglobin disintegration.

Hematoidin is nearly or quite identical with the bile-pigment, *bilirubin*, and is absorbed from hemorrhagic extravasations and eliminated as bilirubin in the bile and as *urobilin* in the urine. (See Chap. XX.) *Hemosiderin* seems to be relatively insoluble and, therefore, is more slowly removed, so that it may be found at the site of a hemorrhage after the other evidences of blood extravasation have disappeared. It may be easily demonstrated by staining with potassium ferrocyanide, the Prussian blue that is formed being readily distinguished. Unstained hemosiderin generally appears in the form of brown or yellowish-brown granules, never as crystals. After a time the hemosiderin is taken away, and deposited in the liver and spleen, either as hemosiderin or as some other iron compound. Eventually it is probably utilized to make new hemoglobin; at any rate, the iron liberated by the breaking up of hematin within the body does not appear to be eliminated.[32]

The changes in the red corpuscles described above are not at all peculiar to extravasated blood, but are quite the same as the changes that are going on continuously and normally in the blood.[33] Red corpuscles are short-lived,[34] being but non-nucleated fragments of cells, and they are continually disintegrating with the production of iron-free pigments that are excreted as the coloring-matters of the bile and urine, while the iron is worked over again into new hemoglobin after a varying period of storage in the tissues, particularly in the spleen

[30] More fully discussed in the consideration of "Pigmentation," Chap. XX.
[31] See Brown, Jour. Exp. Med., 1913 (18), 96.
[32] See Morishima, Arch. f. exp. Path., 1898 (41), 291.
[33] Full review on blood destruction by Rous, Physiol. Reviews, 1923 (3), 75.
[34] According to Ashby (Jour. Exp. Med., 1919 (29), 267) transfused red cells may persist thirty days or more.

and liver. The destruction of red corpuscles under normal conditions seems to take place chiefly in the spleen, bone-marrow, and hemolymph glands, where injured or decrepit corpuscles are taken out of the blood by the phagocytic endothelial cells, and decomposed by intracellular enzymes. In hemorrhagic extravasations the changes are essentially the same; some corpuscles are destroyed by phagocytes, but more by extracellular enzymes. The products of decomposition also seem to be no different from those formed during normal katabolism of hemoglobin, and they meet the same fate in the end.

If the hemorrhage into the tissue or closed cavities is very abundant, some hemoglobin may be absorbed as such and appear in the urine, but this probably seldom happens unless red corpuscles are also being destroyed in the circulating blood. An increased amount of iron accumulates in the liver, but if much blood has been lost by hemorrhage on free surfaces, the iron content of the liver is decreased, as it is taken away to form new hemoglobin (Quincke).[35] Excretion of bile-pigments is increased by destruction of blood (Stadelmann), but not greatly in the case of internal hemorrhages, for the blood is decomposed and absorbed too slowly. Schurig[36] found that hemoglobin injected into the tissues is partly decomposed *in situ* with formation of iron compounds, but the greater part enters the circulation as hemoglobin, and is partly converted into bile-pigment by the liver-cells, the rest being converted into simpler iron compounds by the spleen, bone-marrow, and renal cortex.

If the hemorrhagic extravasation forms a hematoma, the central portions of the mass are not soon, if ever, invaded by leucocytes or tissue-cells. Consequently the encapsulated blood is acted upon very slowly by the enzymes liberated by the leucocytes it contains itself, and by the small amounts of proteases in the serum. Furthermore, the products of decomposition are not soon absorbed, but accumulate in considerable amounts, so that we often find crystalline deposits of hematoidin, sometimes even of hematin, hemoglobin, or *parahemoglobin* (Nencki)[37] or *methemoglobin*. The least soluble constituent of the red corpuscles stroma, *cholesterol*, also accumulates in such extravasations as large, thin plates; after most of the other products of disintegration have been absorbed from such accumulations of blood, the most conspicuous part of the residue may be a mass of cholesterol crystals imbedded in proliferating connective tissue or giant cells.

HEMOPHILIA[38]

There are several pathological conditions associated with increased tendency to bleeding, notably scurvy and the various forms of pur-

[35] Deut. Arch. klin. Med., 1880 (25), 567; 1880 (27), 193.
[36] Arch. exp. Path. u. Pharm., 1898 (41), 29.
[37] Arch. exp. Path. u. Pharm., 1886 (20), 332.
[38] Literature and résumé given by Stempel, Cent. f. Grenzgeb. Med. u. Chir., 1900 (3), 753; Sahli, Zeit. f. klin. Med., 1905 (56), 294; Marchand, in Krehl and Marchand's Handb. allg. Pathol., 1912, II (1), 307. Also later references in this text.

puras, but especially the remarkable hereditary condition, hemophilia.[39] In the purpuric diseases various of the factors concerned in coagulation of the blood have been found altered,[40] notably the blood platelets,[41] but Howell found no change in either prothrombin or antithrombin in *purpura hemorrhagica* and other related conditions. Similar negative results were obtained in scurvy by Hess.[42] *Melena neonatorum* exhibits decreased prothrombin in the blood, while in leukemias and anemias there may be an excess of antithrombin,[43] leading to severe hemorrhage (see also Thrombosis).

Since hemophilia seems, superficially at least, to depend upon some alteration in a chemical property of the blood, namely, coagulability, it is frequently regarded as an example of hereditary transmission of a chemical abnormality. Various observers have described abnormal thinness, or increased cellularity, or fatty degeneration of the vessel-walls, but the findings have been far too inconstant to afford a satisfactory anatomical explanation of all the features of hemophilia. Likewise increased blood pressure can be ruled out, for although the left heart is frequently enlarged, there is usually no increased blood pressure demonstrable; furthermore, conditions of high blood pressure, such as nephritis, do not cause hemophilia. The theory of "hydremic plethora" is also without good foundation.

The most natural place to look for the fundamental fault is in the blood, but speaking strongly against this is the occasional occurrence of "local" hemophilia; *e. g.*, in this type of hemophilia wounds of the skin may behave as in normal individuals, whereas any injury of the mucous surfaces is followed by pronounced hemophilic bleeding;[44] in other cases the hemophilic bleeding is limited to regions above the shoulders; in still another class the bleeding is always from one organ, *e. g.*, the kidneys. Nevertheless, a great deal of investigation of the blood has been done, at first chiefly with negative results. There are no characteristic changes in the cellular elements of the blood, beyond the changes common to all secondary anemias, excepting possibly a decrease in the number of white corpuscles with a relative increase in the number of lymphocytes as observed by Sahli; the platelet count is normal. No changes of significance have been found in the erythrocytes,[45] and no constant alterations in the salts of the blood, calcium usually being normal,[46] and the proportion of water, fibrinogen and the several other proteins, the alkalinity, and the osmotic pressure of the

[39] Full discussion of heredity of hemophilia given by Bauer, Deut. Zeit. Chir., 1922 (176), 109.

[40] See Hurwitz and Lucas, Arch. Int. Med., 1916 (17), 543; Minot *et al.*, *ibid.*, 1916 (17), 101.

[41] See Lee and Robertson, Jour. Med. Res., 1916 (33), 323; Hess, Proc. Soc. Exp. Biol. Med., 1917 (14), 96.

[42] Amer. Jour. Dis. Children, 1914 (8), 386.

[43] Whipple, Arch. Int. Med., 1913 (12), 637.

[44] Abderhalden, Ziegler's Beitr., 1904 (35), 213.

[45] Bürger (Zeit. ges. exp. Med., 1921 (12), 161) found a high sugar content in the red cells.

[46] Klinger and Berg, Zeit. klin. Med., 1918 (85), 335, 406.

serum all seem to be normal. Metabolism is unchanged, except possibly for calcium loss in some cases.[47]

Since bleeding is normally stopped principally by coagulation, a deficiency in fibrin or its antecedents might be expected, but most studies on this point have shown a normal amount of fibrinogen in the blood of hemophilics,[48] the frequent formation of large tumors of clotted blood at the bleeding points supporting the experimental evidence that the blood contains an abundance of fibrinogen. The "bleeding time" following punctures in the skin is not excessive. As to the rate of clotting, Sahli,[38] who avoided a number of errors made in earlier investigations, found that in the intervals between the attacks of hemorrhage the rate of the coagulation of the blood is constantly much slower than normal. During an attack of bleeding the coagulation time approaches the normal; indeed, it may be faster than normal; apparently this is due to a reaction on the part of the organism to the loss of blood. If blood is collected directly from the site of bleeding the coagulation time is very rapid, because of the accumulation of fibrin ferment from the clot over which the escaping blood flows. Yet in spite of the normal coagulability of the blood and the rapid clotting after the blood escapes from the vessel, bleeding continues for long periods before it can be stopped. As he found no general change in the properties of the blood to account for the bleeding, and as local influences seem to be important in hemophilia, Sahli advanced the plausible hypothesis that the cause of hemophilia lies in hereditary deficiency of the fibrin-forming substances, thrombokinase or zymoplastic substance (see "Thrombosis"), in the vessel-walls, so that when the vessels are injured there is no local production of fibrin such as occurs normally. Local hemophilia may be explained readily as a local deficiency in fibrinoplastic material. In general hemophilia even the leucocytes may exhibit the same defect, in which case clotting of the blood is diminished even outside the tissues. This hypothesis seems to be in excellent agreement with many of the facts now known, but there yet remains to be demonstrated such a lack of fibrin-forming elements in the vessel-walls and other tissues of a hemophilic subject, and a single autopsy of a hemophilic subject gave, on the contrary, a very active thromboplastic extract from the vessels (Gressot).[49] The tissues of one case studied by Lowenburg and Rubenstone,[50] however, showed in the liver and thyroid a decreased capacity to accelerate coagulation.

With the improved methods of study of the factors in coagulation of blood introduced by Howell, it has been found by him and corrobo-

[47] Kahn, Amer. Jour. Dis. Children, 1916 (11), 103; Laws and Cowie, *ibid.*, 1917 (13), 236; Hess, Bull. Johns Hopkins Hosp., 1916 (26), 372. An elaborate study of urinary excretion in hemophilia by Berg (Zeit. klin. Med., 1921 (92), 331) showed that during the attacks there is an increased excretion of residual nitrogen and inorganic phosphates, while there tends to be a decreased excretion of urea, uric acid, P_2O_5, neutral and ester sulfur, chlorine and all inorganic bases except iron.

[48] See Wöhlisch (Münch. med. Woch., 1921 (68), 1382) who found fibrinogen content, thrombokinase, and osmotic resistance of the red cells normal.

[49] Zeit. klin. Med., 1912 (76), 194. Since corroborated by Minot and Lee.[51]

[50] Jour. Amer. Med. Assoc., 1918 (71), 1196.

rated by others[51] that *in hemophilia there is constantly a deficiency in prothrombin*, the other factors being practically normal in amount, and as in other hemorrhagic conditions there is no equal alteration in the prothrombin, they look upon this change as an essential characteristic of hemophilia. Fonio, and Minot and Lee,[51] however, find that the blood platelets of hemophilics are remarkably ineffective in causing coagulation of either normal or hemophilic plasma, although normal platelets cause normal coagulation of hemophilic plasma, and therefore conclude that there is some deficient activity on the part of the platelets in spite of their occurrence in normal numbers in hemophilia. According to Feissly[52] the platelets have a lessened capacity to agglutinate. Since retractility of blood clots seems to depend on the platelets, absence of this retraction leaves the clots soft and ineffective[53] and this may play a part in hemophilia.

The significance of platelets in stopping bleeding is shown especially clearly by the observation of Ledingham and Bedson[54] that antiplatelet serum will produce a purpuric condition when injected into animals of the species furnishing the platelets, although no similar effect is produced by antileucocyte or antierythrocyte serum. Hess[55] states that there may be an hereditary purpura, sometimes occurring in the females of hemophilic families, differing from hemophilia in a deficiency in the number of platelets, hemorrhages following local congestions or puncture wounds and exhibiting an increase in the "bleeding time."

ANEMIA AND THE SPECIFIC ANEMIAS[56]

The customary, although unsatisfactory and unscientific division of the anemias, is into—(*a*) *primary*, *i. e.*, those in which the anemia seems to depend upon some abnormality in the blood-forming organs or in the blood itself; and (*b*) *secondary*, embracing anemias the result of some obvious cause, such as hemorrhage, poisoning with blood-destroying poisons, cachexia, etc. In these various forms of anemia certain chemical differences prevail, but they are by no means so striking as are the histological differences in the formed elements of the blood.[57]

Secondary Anemias

As the simplest variety, anemia following a single large hemorrhage may be considered first. After severe hemorrhages the blood shows a

[51] Howell, Arch. Int. Med., 1914 (13), 76; Hurwitz and Lucas, *ibid.*, 1916 (17), 543; Minot and Lee, *ibid.*, 1916 (18), 474; Klinger, Zeit. klin. Med., 1918 (85) 335; Pettibone, Jour. Lab. klin. Med., 1918 (3), 275; these papers review recent work on hemophilia.

[52] Bull. Soc. Med. Hôp., 1923 (47), 1778.

[53] See Opitz and Schober, Jahrb. Kinderheilk., 1923 (53), 189.

[54] Lancet, Feb. 13, 1915; Jour. Path. and Bact., 1922 (25), 94; see also Watabiki, Kitasato's Arch. Exp. Med., 1917 (1), 195· Dyke, Lancet, 1924 (2), 714; Menne, Jour. Infect. Dis., 1922 (31), 455.

[55] Arch. Int. Med., 1916 (17), 203.

[56] Metabolism in anemia reviewed by Mohr, Handbuch d. Biochem., 1910 (IV (2)), 372.

[57] Concerning local anemia, see "Infarcts."

decrease in specific gravity and viscosity, an increase in surface tension and electrical resistance, and either increase or decrease of the freezing-point depression, all these changes being transient if the individual is otherwise normal. There is a rapid absorption of fluid from the tissues and tissue spaces, resulting in a dilution of protein and formed elements, but not of salts. For the same reason the density of the blood decreases in direct relation to the proportion of the total blood that has been lost.[58] The alkali reserve of the blood is somewhat lowered by severe hemorrhage,[59] but there is not a marked acidosis, although a measurable increase in H-ion concentration may occur.[60] This is soon compensated or over-compensated by increased acid excretion in the urine,[61] so that the blood comes to have a decreased *c*H and an increased alkali reserve.[61a] If the hemorrhage is sufficiently severe and rapid there occurs asphyxia with over-ventilation and decrease of CO_2 in the blood.[62] As the red cells are regenerated less rapidly than the plasma, there is a temporary increase in plasma volume.[63] Decreased oxygen consumption is not observed until 15 to 25 per cent. of the blood has been lost.[64]

The total nitrogen of the blood falls, of course, but there is a tendency for sugar, urea, amino-[65] and non-protein N to increase, and there is increased elimination of creatine in the urine, presumably from destruction of muscle tissue to replace the lost blood proteins. The bilirubin content of the blood is decreased.[66] There is said to be a decreased permeability of vessels, resulting in reduction of exudative processes.[67] The proportion of the several blood proteins is variably altered after repeated hemorrhages. There may occur a marked rise in the content of immune bodies, especially specific agglutinins,[68] but not of complement.[69] Rapid hemorrhages cause a decrease in the coagulation time[70] because of a decrease in antithrombin and a slight increase in prothrombin, in spite of a decrease in fibrinogen.[71] If the blood is withdrawn repeatedly in large amounts, centrifuged, and the washed corpuscles reinjected suspended in isotonic salt solution (*plasmapheresis*), life can be maintained even after 4 or 5 times the total volume of blood has been removed and washed.[72] This is possible because of rapid reformation of the plasma, and the blood shows the changes characteristic of secondary anemias. However, if the proportion of total serum proteins is carried

[58] Richet *et al.*, Compt. Rend. Acad. Sci., 1918 (166), 587.
[59] Buell, Jour. Biol. Chem., 1919 (40), 29; Tatum, *ibid.*, 1920 (41), 59.
[60] Crile, Arch. Surgery, 1923 (7), 154.
[61] Evans, Brit. Jour. Exp. Path., 1921 (2), 105.
[61a] Wilson, Physiol. Reviews, 1923 (3), 327.
[62] Henderson *et al.*, Jour. Amer. Med. Assoc., 1922 (78), 697.
[63] Carrier, Lee and Whipple, Amer. Jour. Physiol., 1922 (61), 138.
[64] Schlomovitz, Amer. Jour. Physiol., 1924 (69), 465.
[65] See Marino, Arch. farm. sper., 1923 (36), 88.
[66] Bernheim, Jour. Amer. Med. Assoc., 1924 (82), 291.
[67] Luithlen, Med. Klin., 1913 (9), 1713.
[68] See Hahn and Langer, Zeit. Immunität., 1917 (26), 199.
[69] Ecker and Rees, Jour. Infect. Dis., 1922 (31), 361.
[70] See Hanzlik, Jour. Lab. Clin. Med., 1920 (6), 59.
[71] Drinker, Amer. Jour. Physiol., 1915 (36), 305.
[72] Abel *et al.*, Jour. Pharmacol., 1914 (5), 625; 1915 (7), 129.

below 1 per cent., death may result.[73] Lipemia is often produced by severe or repeated hemorrhages, with an increase in the phospholipins of the plasma;[74] there is also an increased percentage and degree of unsaturation of the fatty acids,[75] but the cholesterol content is decreased if the hemorrhage is severe.[76]

The proportion of water increases at first more rapidly than the proportion of salts, and as a consequence the size of the red corpuscles is increased because of imbibition of water; indeed, it is possible that this may even be sufficient to cause hemolysis, which will happen if the isotonic strength of the blood becomes less than that of a 0.46 per cent. NaCl solution (Limbeck), while swelling may occur whenever the strength is below 0.8 per cent. The specific gravity of the erythrocytes is decreased;[77] the depression of the freezing point increases,[78] while the viscosity falls. The number of platelets is high.

Regeneration of the blood begins very soon, and for some time the number of corpuscles exceeds the proportion of hemoglobin. During this time the amount of iron in the liver and spleen is decreased, it being taken up to be used in the formation of new hemoglobin. The rate of regeneration[79] is much increased by a meat diet, by hemoglobin, by administration of red marrow and spleen,[80] but not by inorganic iron administration,[81] and abundance of vitamins is favorable.[82] The value of green plants in regeneration of blood may depend also in part on the chlorophyll furnishing pyrrol radicals for the regeneration of the hemoglobin,[83] and the same is perhaps true of tryptophan.[84] Rous[33] says that the production of stroma exceeds that of hemoglobin during recovery from hemorrhage. Red corpuscles do not regenerate in animals kept on an incomplete protein diet (gliadin).

If the hemorrhages are numerous and the condition of anemia prolonged, secondary changes in the viscera may occur, fatty metamorphosis being most marked. Indeed, many observers state that repeated bleedings greatly increase body weight by causing increased fat deposition, supposedly because of decreased oxidation, which, however, cannot be demonstrated by gas metabolism studies.

Metabolic Changes.—Gies[85] studied the *metabolism* of dogs after withdrawing a total amount of blood equal to 11.5 per cent. of the body weight during four bleed-

[73] Smith, Belt and Whipple, Amer. Jour. Physiol., 1920 (52), 54.
[74] Bloor, Jour. Biol. Chem., 1920 (45), 171; 1921 (49), 201. See also Horiuchi, Jour. Biol. Chem., 1920 (44), 363; Feigl, Biochem. Zeit., 1921 (116), 63.
[75] Bloor, Jour. Biol. Chem., 1925 (63), 1.
[76] MacAdam and Shiskin, Quart. Jour. Med., 1923 (16), 193.
[77] Bönninger, Zeit. exp. Path., 1912 (11), 1.
[78] Hoesslin, Hofmeister's Beitr., 1906 (8), 431.
[79] See review by Whipple, Arch. Int. Med., 1922 (29), 711.
[80] See Leake, Jour. Pharm., 1923 (22), 401; 1924 (23), 353.
[81] Hooper and Whipple, Amer. Jour. Physiol., 1918 (45), 573; 1920 (53), 151; Musser, Arch. Int. Med., 1921 (28), 638; Williamson, Trans. Assoc. Amer. Phys., 1924 (39), 285.
[82] Jencks, Amer. Jour. Physiol., 1922 (59), 240.
[83] See Scott, Biochem. Jour., 1923 (17), 157.
[84] Hirasawa, Jour. Osaka Med. Soc., 1922 (21), No. 9; from Jap, Med. World, 1923 (3), 36.
[85] American Med., 1904 (8), 155 (résumé of literature).

ings, and found that a slight and temporary increase in nitrogenous elimination followed the bleedings, owing to an increased protein katabolism. Basal metabolism may also be increased in anemia.[86] Sugar increases in the blood, while albumin and lactic acid appear in the urine. After each successive hemorrhage the proportion of fibrin and the coagulability of the blood increase, while the proportion of the ash obtained from both blood and serum remains practically unchanged (Meyer and Gies). Baumann[87] states that in regeneration after hemorrhage the serum albumins increase more rapidly than the globulins, while others have observed the opposite relation. The urine in secondary anemia shows the effects of increased protein katabolism, its specific gravity, total solids, and total nitrogen being raised; the total amount of urine is at first diminished because of lowered blood pressure, but it soon rises above normal and later falls back to normal. The view formerly held that oxidation is decreased in anemia has been considerably modified by more recent investigations;[88] in fact, respiration studies indicate heightened gas exchange in secondary anemia.[89]

Secondary anemia due to cachexia, or to malnutrition, is accompanied by a general decrease in all the elements of the blood, both cellular and chemical. The proteins of the plasma, particularly, show a decrease in starvation, being drawn on by the cells for food, and the total quantity of blood as well as of each of its constituents is decreased (Panum),[90] but the proportion of blood to body weight remains about normal. With protracted starvation there is only a slight loss of hemoglobin and an increased coagulability, but practically no other changes.[91] In aplastic anemias the prothrombin and platelet content are likely to be low, with normal fibrinogen.[92]

Anemia due to hemolytic agencies presents quite different features, in that red corpuscles are almost solely attacked and the products of their disintegration are present in the plasma. As a result, the plasma or serum may contain free hemoglobin, and if the hemoglobin is in large amounts, it may escape into the urine. Thus *paroxysmal hemoglobinuria* is probably due to the presence in the blood of hemolytic substances, which can be demonstrated in the blood of the patients during the attack. (See Chapter XI.) The products of the decomposition of the hemoglobin set free by hemolysis are present not only in the blood, but also in the organs, particularly the liver and spleen, which become rich in iron. In acute anemia produced by hemolytic sera, with destruction of more than half the blood in three days, nearly all the iron from the destroyed hemoglobin can be found in the liver, spleen, and kidneys, there being but little lost through the urine even in so severe an anemia as this (Muir and Dunn).[93] Excretion of bile-pigments increases, and hemolytic jaundice may result, the bile-pigments that are present in the blood being derived from the hematoidin of the hemoglobin molecule. Changes in metabolism occur which are quite similar to those

[86] Review by Tompkins *et al.*, Arch. Int. Med.. 1919 (23), 441.
[87] Jour. Physiol., 1903 (29), 18.
[88] See Mohr, Zeit. exp. Path., 1906 (2), 435.
[89] Grafe, Deut. Arch. klin Med., 1915 (118). 148.
[90] Virchow's Arch., 1864 (29), 241.
[91] Ash, Arch. Int. Med., 1914 (14), 8.
[92] Drinker and Hurwitz, Arch. Int. Med., 1915 (15), 733; Jour. Exp. Med., 1915 (21), 401.
[93] Jour. Path. and Bact., 1915 (19), 417. See also Dubin and Pearce, Jour. Exp. Med., 1918 (27), 479.

observed in other forms of anemia, with fatty changes in all the parenchymatous organs, increased protein katabolism, and an excessive quantity of pigmentary substances, particularly urobilin, in the urine. The plasma chlorides are increased,[94] and the protective power of the serum against hemolysis by saponin or oleates is reduced.[95]

Chlorosis

The characteristic feature of the blood in chlorosis is the relatively small amount of hemoglobin in proportion to the number of corpuscles. Apparently, therefore, the fault lies rather in the manufacture of hemoglobin than in either a destruction or a deficient formation of red corpuscles. Erben's[96] analyses of chlorotic blood showed that the total amount of protein is decreased, chiefly because of the deficiency of hemoglobin; the relation of serum globulins and serum albumin is unchanged, while the proportion of fibrinogen is increased. There is much more fatty substance than normal in both the serum and the erythrocytes, but the lecithin is decreased both in the serum and in the total blood, although somewhat increased in the red cells. Cholesterol is decreased in both serum and corpuscles. In the ash, phosphoric acid, potassium, and iron are decreased, while calcium and magnesium are both increased. An apparent increase in sodium chloride exists, but it is only apparent, being the result of the increase in the proportion of plasma in the blood. The total amount of plasma is greatly increased (polyplasmia). The decrease in hemoglobin is demonstrable chemically as well as microscopically, Becquerel and Rodier[97] having found the amount of iron in the total blood decreased in direct proportion to the apparent decrease in hemoglobin, which frequently falls to 30–40 per cent., and may drop to 20 per cent., or possibly less. The corpuscles are said to contain a larger proportion of water than normal, independent of the proportion of water present in the serum. Limbeck found their *isotonicity* (*i. e.*, the strength of NaCl necessary to prevent hemolysis) very low—about 0.38–0.4 per cent. NaCl.

Very few changes seem to occur in the organs of the body; the usual tendency to lay on fat, and the occurrence of fatty degeneration observed commonly in anemias, may be exhibited, and are correlated with Erben's observation of an increased fat content in the blood; but these changes are often absent. The hypoplasia of the aorta, upon which Virchow laid so much stress, is now considered to be of little or no significance. Thrombosis is a not infrequent complication of chlorosis,[98] and is probably favored by the increased platelet and fibrinogen-content of the blood and

[94] Steinfield, Arch. Int. Med., 1919 (23), 511.

[95] Clark and Evans, Bull. Johns Hopkins Hosp., 1921 (32), 113.

[96] Zeit. klin. Med., 1902 (47), 302. See also Frohmaier, Folia Hematol., 1915 (20), 115; Beumer and Bürger, Zeit. exp. Path., 1913 (13), 351.

[97] For literature see Krehl, "Basis of Symptoms," 1916, p. 106; Ewing, "Clinical Pathology of the Blood," 1901, p. 167; Kossler, Cent. f. inn. Med., 1897 (18), 657.

[98] See Schweitzer, Virchow's Arch., 1898 (152), 337, and Leichtenstern, Münch. med. Woch., 1899 (46), 1603.

the tendency to fatty changes in the vessel-walls. Studies of *nitrogenous metabolism* by Vannini[99] showed practically no alterations except a slight retention of nitrogen. Basal metabolism was found to be slightly lowered by Thiele and Nehring.[1]

Etiology.—As to the etiology of chlorosis, chemical findings indicate some possibilities and negative others, but decide nothing. That chlorosis does not depend upon a hemolytic poison is well established by the following facts: There is no free hemoglobin in the blood plasma, and even less iron in the serum ash than normal; lecithin and cholestesterol, important products of disintegration of erythrocytes, are both decreased in the serum; hematogenous icterus does not occur, and the amount of pigments in the urine and feces is decreased.

Apparently, therefore, *hematogenesis* is at fault, particularly the formation of hemoglobin, since this is more deficient than is the total number of red corpuscles. The rapid improvement in the condition that follows the administration of iron would seem to indicate that a deficient supply of iron is the cause of chlorosis, but there exist numerous objections to this hypothesis. Bunge advanced the idea that in chlorosis the iron taken with the ordinary food is precipitated in the intestines by sulfides or other products of intestinal putrefaction, and hence there results a deficiency in the amount of iron absorbed and available for the manufacture of hemoglobin. Many objections have been raised to this hypothesis, however, for competent observers have failed to find any abnormal putrefaction in chlorosis, and others have found that sulfide of iron itself gives good results in the treatment of chlorosis, while bismuth and other sulfur-binding substances are without effect. Furthermore, Bunge's contention that iron administered in medicinal form is not absorbed seems to have been completely disproved by several experiments.[2]

As a consequence of all these conflicting data we are at present completely in the dark as to the reason for that failure properly to manufacture hemoglobin which seems to be at the bottom of chlorosis. The hypothesis that iron and arsenic favor recovery by stimulating the hemogenetic tissues, which is urged by v. Noorden and others, is unsatisfactory in the extreme, and explains nothing. There is absolutely no question that administration of iron restores the composition of the blood to normal, usually quite rapidly, and this seems to leave as most probable the explanation that in some way an *iron starvation* is the fundamental cause of chlorosis. However, as Ewing says, any theory must be inadequate that fails to take into account the age of puberty, the female sex, and the function of menstruation.[3] A striking feature of the disease is the remarkable decline in its frequency since about the year 1900, few cases of typical chlorosis now being seen.[4]

Pernicious Anemia

In contrast to chlorosis many evidences of hematolysis may be found in pernicious anemia, particularly the increased amounts of iron in the liver, spleen, and kidneys; hemoglobinemia and hemoglobinuria, increase in urobilin, and not infrequently icterus.

Chemical Changes.[5]—Erben's[6] analysis of the blood in pernicious anemia gave the following results: The proteins are decreased, both in the serum[7] and in the blood as a whole; particularly in the latter, because of the great decrease in the number of

[99] Virchow's Arch., 1904 (176), 375.

[1] Zeit. klin. Med., 1896 (30), 41.

[2] Full reviews with bibliography by E. Meyer, Ergebnisse Physiol., 1905 (5), 698; Meinertz, Cent. Physiol. u. Path. Stoffwech., 1907 (2), 652; Grumme, Therap. Monatsh., 1919 (33), 421.

[3] von Jagic (Med. Klin., 1915 (11), 69) states that in chlorosis the Abderhalden test is positive with uterine and ovarian tissue.

[4] See Denecke, Deut. med. Woch., 1924 (50), 902.

[5] Review and bibliography by Squier, Jour. Lab. Clin. Med., 1917 (2), 552.

[6] Zeit. klin. Med 1900 (40), 266. Beumer and Bürger, Zeit. exp. Path., 1913 (13), 343.

[7] See also Heudorfer, Zeit. klin. Med., 1913 (79), 103.

corpuscles. The quantity of proteins in the individual corpuscles is increased, corresponding to their increased size. Fibrinogen is decreased in total amount, but is relatively normal as compared with the total proteins; albumin is normal; serum globulin much decreased. The proportion of water is much increased, both in the serum and in the corpuscles. Fat is present in normal amounts; cholesterol is decreased, although in relatively normal quantities in the corpuscles. Lecithin is decreased in the total blood, but increased proportionately in the corpuscles. The total ash is increased, owing chiefly to an excessively large proportion of NaCl and a slight increase in calcium and magnesium; potassium and phosphoric acid are decreased because of the small number of corpuscles; but the serum itself contains more P_2O_5 and potassium than normal. Although the total iron is, of course, much decreased, there is iron in the serum (indicating hemolysis) and the proportion of iron in the corpuscles is increased; but as Erben found the amount of iron in the corpuscles even greater than corresponds to the hemoglobin increase, he believed that either the hemoglobin in pernicious anemia is very rich in iron, or that the corpuscles contain iron bound in some form other than hemoglobin. The more recent studies of Leschke and Neufeld,[8] however, have indicated a normal proportion of iron in the hemoglobin.

The analyses of Rumpf[9] agree closely with those of Erben, and, taken jointly with other analyses in the literature, show the large proportion of water in the blood, the small amount of solids, the large amount of NaCl, and the decrease in potassium and iron. Rumpf also examined the brain, liver, heart, and spleen in one case. Water was found increased in the heart, decreased in the other organs, the solids not being decreased in any of the organs. There was little fat in any of the organs or in the blood, but NaCl was generally increased. The liver contained four or five times as much iron as normal; the spleen three or four times. Rumpf is inclined to lay stress on the general poverty of the body in potassium, and suggests its therapeutic application.

A study by Kahn and Barsky[10] of the functional capacity of various organs in three cases revealed the following conditions: Stomach: Gastric stasis seemed to be present; free acid and pepsin absent. The mucosa appeared to be nonfunctioning, the result of atrophy. Intestines: Digestion and absorption, especially of the proteins, were disturbed and below normal. Intestinal putrefaction was increased. Pancreas: The pancreatic enzymes were present in normal amounts and tests showed that the pancreas was functioning normally. Liver: The function of detoxication was deficient. Other functions, such as the glycogenic, ureogenic, biligenic, were normal. Pigments: Both pleochromia and urobilinocholia existed, showing that excessive hemolysis was going on. Kidneys: The excretory function of the kidneys was normal. Urine: Quantitative partition nitrogen determinations proved the normal output of the excretory nitrogenous substances, with the exception of a greatly increased oxyproteic acid.

Gettler and Lindeman[11] studied the blood chemistry in 32 cases and found that the nonprotein nitrogen, urea, and creatinine values are somewhat higher than normal. The uric acid is much above normal. The amino-acid content is greatly increased, supposedly because of excessive destruction of serum protein. The blood sugar is abnormally high. The alkaline reserve is subnormal. The refraction and specific gravity are both astonishingly low, indicating deficiency in serum albumin, serum globulin, and fibrinogen. In most instances the freezing point is very near to normal, but it is slightly raised in a small percentage of cases. This is due to the small increase of nonprotein nitrogenous substances and to the presence of normal or slightly increased amounts of inorganic salts. Peters and Rubnitz[12] found that although the total N is low because of loss of corpuscles, the plasma shows either normal or high total nitrogen.[13]

Denis[14] and Bloor[15] found the blood lipoids about normal unless the red cor-

[8] Klin. Woch., 1922 (1), 849.
[9] Berl. klin. Woch., 1901 (38), 477.
[10] Arch. Int. Med., 1919 (23), 334.
[11] Arch. Int. Med., 1920 (26), 453.
[12] Arch. Int. Med., 1920 (26), 561.
[13] Stephan (Zeit. exp. Med., 1921 (24), 407) found the tryptic enzyme of the blood increased.
[14] Jour. Biol. Chem., 1917 (29), 93.
[15] Jour. Biol. Chem., 1917 (31), 79; See also Gibson and Howard, Arch. Int. Med., 1923 (32), 1.

puscles were below 50 per cent.,[16] when there appear high fat and low lecithin and cholesterol in the plasma, but usually with normal corpuscular lipoid content.[17] The proportion of cholesterol free and as ester is normal. Bile pigment is frequently found in the blood, but so bound that it does not escape into the urine and it does not always cause evident jaundice. Bile salts may be found in the blood, either with or without pigment (Blankenhorn).[18] Fowell[19] found a considerable excess of iron in the blood over the amount combined with hemoglobin, but the urinary excretion of iron is not correspondingly increased (Klisch).[20] Schumm[21] could find no proteoses or other evidences of protein decomposition in the blood in a case of pernicious anemia, but he did find free hematin.[22] The tendency to hemorrhage observed in this disease may depend on a slight decrease in the prothrombin and a reduction in the number of platelets.[23] A high catalase content was found in the blood by Korallus,[24] but Krumbhaar[25] says that the catalase content of the blood varies according to the hemoglobin content. Bönninger found that the corpuscles themselves have practically normal specific gravity, while the decrease is chiefly in the serum.[26]

The plasma volume remains normal, but the loss of cells reduces the blood volume;[27] the specific electrical conductivity of the plasma remains normal.[28] The erythrocytes show an increased resistance to hemolysis by hypotonic salt solution, but this is said not to be true of the reticulated corpuscles, which, however, seem to undergo autolysis more than normal corpuscles or reticulated corpuscles from secondary anemia.[29] The crystal character of the hemoglobin is not altered in pernicious anemia,[30] and it contains the normal proportion of iron and has a normal combining power for oxygen.[31]

There is a marked increase in the urobilin output, corresponding in degree to the amount of hemolysis,[32] and the relative urobilin value of the duodenal contents is high.[33] Broun *et al.*,[34] found high values for plasma bilirubin in active periods of the disease, the reaction being of the delayed type as contrasted with the prompt reaction in hepatic jaundice. Hemoglobin and hematin are also present in the plasma during exacerbation of the disease, and an ether-soluble pigment of unknown character is found in late stages and after death. Iron may also appear in the urine in increased quantities,[35] and there may be enough iron deposited in the renal epithelium to interfere with function.[36] Reinwein and Thielmann[37] report finding in the urine histidine, methylguanidine, dimethylxanthine, γ-butyrobetaine and lactic acid. There is an increased elimination of oxyproteic acid nitrogen, other urinary nitrogen constituents being normal (Kahn and Barsky).[38] No constant metabolic changes follow splenectomy in

[16] Corroborated by Pacinni, Amer. Med., 1918 (13), 92; McAdam and Shickin Quart. Jour. Med., 1923 (16), 193.

[17] However, Koopman (Zeit. klin. Med., 1924 (100), 517) attributes the polychromophilia of pernicious anemia erythrocytes to a deficiency in lipoids.

[18] Arch. Int. Med., 1917 (19), 344.

[19] Quart. Jour. Med., 1913 (6), 179.

[20] Wien. Arch. inn. Med., 1922 (3), 283.

[21] Hofmeister's Beitr., 1903 (4), 453.

[22] Zeit. physiol. Chem., 1916 (97), 32; also by Van den Bergh, Berl. klin. Woch., 1915 (42), 1081.

[23] Drinker and Hurwitz, Arch. Int. Med., 1915 (15), 733.

[24] Deut. Arch. klin. Med., 1922 (139), 252.

[25] Jour. Amer. Med. Assoc., 1920 (75), 104; see also Tögel and Ceranke, Wien. Arch. inn. Med., 1924 (9), 300.

[26] Zeit. exp. Path., 1912 (11), 1.

[27] Denny, Arch. Int. Med., 1921 (27), 38.

[28] Buckman *et al.*, Jour. Med. Res., 1923 (44), 51.

[29] Buckman and Macnaugher, Jour. Med. Res., 1923 (44), 61.

[30] Amantea *et al.*, Arch. fisiol., 1923 (21), 411.

[31] Leschke and Neufeld, Klin. Woch., 1922 (1), 849.

[32] See Hausmann and Howard, Jour. Amer. Med. Assoc., 1919 (73), 1262; Sonnenfeld, Zeit. klin. Med., 1924 (100) 508,

[33] C. M. Jones, Arch. Int. Med., 1922 (29), 643.

[34] Jour. Clin. Invest., 1925 (1), 295.

[35] Kennerknecht, Virchow's Arch., 1911 (205), 89. Not confirmed by Queckenstedt, Zeit. klin. Med., 1913 (79), 49; bibliography.

[36] Stieglitz, Arch. Int. Med., 1924 (33), 58.

[37] Arch. exp. Path. u. Pharm., 1924 (103), 115.

[38] Denis, Arch. Int. Med., 1917 (20), 79.

pernicious anemia, but there may be an increased excretion of nitrogen and uric acid after blood transfusion.[39]

Extensive studies on the *protein metabolism* by Rosenquist[40] showed that there is a considerable destruction of tissue proteins, as indicated by nitrogen loss, but that at times nitrogen may be stored up for brief periods. At times there may also be an excessive elimination of purine nitrogen, indicating destruction of nuclear elements. Calorimetric studies show the metabolism to be slightly above normal.[41] In anemia due to *Bothriocephalus* quite similar changes have been observed.

In six cases of pernicious anemia, Stühlen[42] found abundant iron in the liver and spleen microscopically, and less constantly in the kidneys and bone-marrow. Iron is also found in the hemolymph nodes, sometimes more abundantly than in the spleen (Warthin).[43] Hunter[44] gives the following results of analysis of the liver, kidney and spleen for iron:

	Liver and Kidneys	Spleen
Pernicious anemia, seven cases, average	0.360 per cent.	0.125 per cent.
Other conditions (with anemia), average	0.079 per cent.	0.362 per cent.
Healthy organs	0.084 per cent.	0.090 per cent.

Etiology.[45]—Putting together the above findings, we see that in pernicious anemia we have every evidence that excessive hemolysis is taking place, and the fact that continued poisoning by toluylenediamine, phenylhydrazine (van den Bergh), and other hemolytic poisons, such as that of *Bothriocephalus*, may give rise to a condition resembling pernicious anemia very closely, indicates strongly that hemolytic poisons are the cause of pernicious anemia.[46] Histological studies show the same thing, and, as Warthin says:[47] "The hemolysis of pernicious anemia does not differ in kind from that occurring normally or in certain diseased conditions; the difference is one of degree only." The hemolysis seems to go on chiefly inside of phagocytic cells instead of in the blood, probably because the phagocytes pick up the corpuscles as soon as they have been injured by the hemolytic poisons. In some instances cholesterol administration improves the anemia, which suggests that the poison attacks the lipoids of the corpuscles, as so many hemolytic agents do,[48] but the cholesterol increase which follows transfusion is only temporary.[49]

Since the work of Faust and Talquist[50] it has been assumed that the anemia produced in infections with *Diphyllobothrium latum*, "*Bothriocephalus anemia*," is produced by hemolytic lipoids, presumably a cholesterol ester of oleic acid, which is present in large amounts in the bodies of these parasites. But Beumer[51] found that the cholesterol oleic

[39] Weicksee, Deut. med. Woch., 1924 (50), 1543.

[40] Zeit. klin. Med., 1903 (49), 193 (literature). See also Minot, Bull. Johns Hopkins Hosp., 1914 (25), 338.

[41] See review by Hurwitz, "Endocrinology and Metabolism," 1924 (4), 553.

[42] Deut. Arch. klin. Med., 1895 (54), 248 (literature).

[43] Amer. Jour. Med. Sci., 1902 (124), 674.

[44] Lancet, 1903 (1), 283; similar results obtained by Ryffel, Jour. Path. and Bact., 1910 (14), 411.

[45] Review on etiology of pernicious anemia given by Vogel, Jour. Amer. Med. Assoc., 1916 (66), 1012.

[46] See review on hemolysis in pernicious anemia by Panton *et al.*, Lancet, Mar. 15, 1924, p. 529.

[47] Amer. Jour. Med. Sci., 1902 (124), 674.

[48] See Reicher, Berl. klin. Woch., 1908 (45), 1838.

[49] Kipp, Jour. Biol. Chem., 1920 (32), 413.

[50] Zeit. klin. Med., 1907 (61), 427; Arch. exp. Path. u. Pharm., 1907 (57), 367.

[51] Biochem. Zeit., 1919 (95), 239.

acid ester is not hemolytic, and believes it improbable that a normal constituent of the body, such as oleic acid, can be responsible for the anemia. Many of the poisons which produce in animals a severe anemia, more or less resembling pernicious anemia, have little direct hemolytic effect *in vitro*, (*e. g.*, toluendiamine, pyrodin) and this is true for the poison obtained from the *Gastrophilus* larvæ (*Oestrus equi*) which seems to be responsible for a severe form of anemia in horses (Syderhelm).[52] The lipoids of this parasite dissolve red cells *in vitro* but have but slight effect when injected into animals. Syderhelm finds the same thing to be true of the poisons of *Bothriocephalus*, the lipoids being strongly hemolytic *in vitro*, but not *in vivo*, while a water-soluble product, which does not dissolve red corpuscles *in vitro*, produces a profound anemia in animals. He believes that similar, non-hemolytic poisons may be formed by intestinal bacteria which produce pernicious anemia, for he has found such poisons in human feces, equally whether the persons have pernicious anemia or not. This last observation he correlates with the known fact that only a very small proportion of persons infected with *Bothriocephalus* exhibit severe anemia, so that presumably, the poison is active under exceptional conditions when it is absorbed unchanged. Although in hemolytic anemias some have found an increased amount of unsaturated lipins in the blood,[53] Medak[54] did not find the isolated lipoids to be particularly hemolytic,[55] but the power of the serum to protect corpuscles from hemolysis by oleates and by saponin is decreased.[56] The origin and the nature of the specific hypothetical poisons have been sought in vain.[57] Some authors have referred them to infections of unknown nature, occurring perhaps in the mouth and gastro-intestinal tract (Hunter) or to hemolytic products of intestinal putrefaction,[58] or to faulty metabolism.[59] Others, with perhaps the best of

[52] Arch. Exp. Path. u. Pharm., 1914 (76), 149; Deut. Arch. klin. Med., 1918 (126), 95.

[53] See King, Arch. Int. Med., 1914 (14), 145; Csonka, Jour. Biol. Chem., 1918 (33), 401. Not corroborated by Rosenthal and Holzer, Biochem. Zeit., 1920 (108), 220. Gibson and Howard[15] found but little increase in unsaturated fatty acid groups. Bloor (Jour. Biol. Chem., 1925 (63), 1) found a marked increase in unsaturated fatty acids in secondary anemia following bleeding, and hence doubts their significance as an etiological factor in anemia.

[54] Biochem. Zeit., 1914 (59), 419.

[55] See McPhedran, Jour. Exp. Med., 1914 (18), 527.

[56] Clark *et al.*, Bull. Johns Hopkins Hosp., 1920 (31), 364; 1922 (33), 16.

[57] Ashby's (Jour. Exp. Med., 1921 (34), 147) observations on the behavior of transfused blood in pernicious anemia patients gave no evidence for the existence of a hemolytic poison nor of an intrinsic weakness of the pernicious anemia corpuscles, but these views are not accepted by Paton, Wearn, Rous and others.

[58] See Külbs (Arch. exp. Path. u. Pharm., 1906 (55), 73), who found the intestinal contents of patients with chronic intestinal disorders to contain hemolytic substances of undetermined character. Hemolytic lipoids in the intestinal contents have been described by Berger and Tsuchiga (Deut. Arch. klin. Med., 1909 (96), 252) and Lüdke and Fejes, *loc cit.*, but this observation failed of confirmation by Ewald (Deut. med. Woch., 1913 (39), 1293). As stated above, Syderhelm found in intestinal contents a lipoid hemolytic *in vitro* but not *in vivo*, and a poison causing anemia *in vivo* although not hemolytic *in vitro*. Herter (Jour. Biol. Chem., 1906 (2), 1) suggested a relation between pernicious anemia and intestinal infection with *B. aerogenes capsulatus*, which produces hemolytic substances, and Cornell (Canad. Med. Assoc. Jour., 1925

grounds would ascribe pernicious anemia to a multiplicity of causes, which produce a protracted slight hemolysis that continues until the hematogenetic organs give out, their exhaustion being perhaps hastened by the influence of the toxic substances in the blood, hematogenesis then becomes insufficient to replace the lost corpuscles, and the picture of pernicious anemia is established.[60] Familial occurrence[61] is sufficiently common to suggest that there must be some inherited predisposition to permit the exciting cause to be effective, or that the familial disease may be different from the usual type.[62] The relatively great proportion of the iron that is stored in the liver supports the view that the hemolysis takes place in portal territory, and many other facts point to the same conclusion, but it is not generally accepted that the spleen plays an essential role in causing pernicious anemia through excessive phagocytosis or production of hemolytic poisons.[63]

Whipple[64] takes a different view from most investigators, and doubts the hemolytic nature of pernicious anemia, holding that the amount of stercobilin production is too great to be explained in this way. He suggests that there is rather an excessive stimulus to pigment production with a deficiency in stroma formation, so that such red corpuscles as are formed are oversaturated with pigment, the excess of which is excreted.

Leukemia

In leukemia the chemical changes in the red corpuscles take a less prominent position, resembling either those of a secondary anemia or chlorosis, while the enormous number of leucocytes is the prominent feature and causes marked alterations in the composition of the blood. Large quantities of nucleoproteins and also of the intracellular enzymes

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(15), 26) has produced experimental and clinical evidence in support of this possibility. Syderhelm believes that invasion of the small intestine by the bacteria of the colon is of importance (Klin. Woch., 1924 (3), 1430). The occasional occurrence of pernicious anemia in cases of benign intestinal obstruction supports the intestinal origin (Meulengracht, Arch. Verd.-Kr., 1921 (28), 216). *Balantidium Coli* infection is also accompanied by a severe anemia (See Giffin and Bowler, Minn. Med., 1923 (6), 13). Wood has called attention to the resemblances between pernicious anemia and sprue (Amer. Jour. Med. Sci., 1925 (169), 28. Also Elders, Lancet, 1925 (1), 75).

[59] Iwao (Biochem. Zeit., 1914 (59), 435) reported that tyramine (*p*-oxyphenylethylamine) produces in guinea pigs a blood picture resembling pernicious anemia, but Koessler and Harris have been unable to confirm this (Unpublished experiments from this laboratory).

[60] See also Bunting, Johns Hopkins Hosp. Bull., 1905 (16), 222; Pappenheim, Folia Serologica, 1910 (10), 217.

[61] See review by Meulengracht, Folia Hematol., 1923 (28), 217; Amer. Jour. Med. Sci., 1925 (169), 177.

[62] No particular chemical changes are associated with the *sickle cell anemia* (review by Graham, Arch. Int. Med., 1924 (34), 778) first described by J. B. Herrick, a disease limited, as far as known, to negroes, and of a definitely hereditary character. There is bilirubinemia and urobilinuria, the erythrocytes show normal or slightly increased resistance to hemolysis by hypotonic salt solutions, and there is hypochlorhydria. In many respects this disease resembles hereditary hemolytic icterus (*q.v.*).

[63] See Hirschfield, Zeit. klin. Med., 1919 (87), 165.

[64] Arch. Int. Med., 1922 (29), 711.

are introduced into the blood by the excessive leucocytes. As the leucocytes are constantly breaking down, more or less of the products of their decomposition are present in the blood and appear in the urine. Because of the relatively slight metabolic activity of the lymphocytes the various chemical alterations are all less marked in lymphatic than in myelogenous leukemia. There is a notable reduction in antibody production in leukemia,[65] presumably because of the changes in the bone marrow; it is said that typhoid infection in leukemics may fail to result in agglutinin formation.[66]

Chemistry of the Blood.—Considering the quantitative alterations in the constituents of the blood, we find the specific gravity lowered, but not so much as it would be in a simple anemia with equally low hemoglobin, for the loss of hemoglobin is partly compensated by the increase in leucocytes and their products. Fibrinogen is usually increased in myelogenous leukemia.[67] The serum shows but slight change in specific gravity, a slight decrease in proteins[68] being compensated by an increase in the NaCl. The freezing-point of the blood is lowered (Cohn[69]), which is probably due to the increase in crystalloidal products of cellular decomposition. Erben[70] found that in lymphatic leukemia the serum contains less cholesterol than normal, although the fat content may be rather high. Calcium is frequently found increased, probably because of destruction of the bone tissue. In the red corpuscles the proportion of iron, protein, and potassium is decreased as is also that of the cholesterol, that of the lecithin and water being somewhat increased. The total amount of potassium and iron in the blood is decreased, but the P_2O_5 in the ash is increased because of the large amount of nucleoprotein in the blood. There is an increased excretion of iron in the urine and feces.[71]

The poor coagulation of leukemic blood has long been known, but the reason for it has not yet been ascertained. Some investigators have reported a deficiency in fibrinogen while others have found it increased. More recent reports, however, indicate that there is no marked change in either the amount of fibrinogen or of the fibrin-ferments. Erben[67] found a normal amount of fibrinogen in the blood in lymphatic leukemia; and in three cases of myelogenous and one of lymphatic leukemia, Pfeiffer[72] found the amount of fibrinogen nearly normal. This is quite remarkable in view of the fact that in ordinary forms of leucocytosis both the amount of fibrinogen and the rapidity of clotting are increased. It is, therefore, extremely difficult to understand the poor coagulability of leukemic blood. but study of the factors of coagulation by modern methods may clear this up, for in one case so studied Whipple[73] found an increase in antithrombin. The blood volume and plasma volume are increased, especially in chronic myelogenous leukemia.[74]

Decomposition Products.—Of particular interest is the finding in the blood of decomposition products of the leucocytes, which are probably produced by autolysis of the leucocytes. (See Leucocytic Enzymes, Chapter iii.) Normal leucocytes are rich in autolytic enzymes, which under ordinary circumstances seem to be held in check by the antienzymes of the blood. In leukemia this antienzyme action seems to be insufficient to prevent leucocytic autolysis, for even in freshly drawn blood, proteoses (or at least non-coagulable proteins) may be present.[75] According to Erben, this is true only of myelogenous leukemia, the fresh blood in lymphatic leukemia not only being free from non-coagulable protein, but furthermore this product

[65] Rotky, Zent. inn. Med., 1914 (35), 953.
[66] See K. Howell, Arch. Int. Med., 1920 (26), 706.
[67] Erben, Zeit. klin. Med., 1908 (66), 278; full details on composition of the blood in leukemia.
[68] Little change was found in the protein content of the serum by Heudorfer, Zeit. klin. Med., 1913 (79), 103.
[69] Mitteil. aus dem Grenzgeb. Med. u. Chir., 1906 (15), H. 1
[70] Zeit. klin. Med., 1900 (40), 282.
[71] Kennerknecht, Virchow's Arch., 1911 (205), 89.
[72] Cent. f. inn. Med., 1904 (25), 809.
[73] Arch. Int. Med., 1913 (12), 637.
[74] Keith, Amer. Jour. Med. Sci., 1923 (165), 174.
[75] For literature see Erben, Zeit. f. Heilk. (Int. Med. Abt.), 1903 (24), 70.

of proteolysis does not soon develop when the blood is kept aseptically at incubator temperature. This is, of course, what one would expect in view of the well-known enzyme-richness of the polymorphonuclear leucocytes and the scarcity of proteolytic enzymes in lymphocytes. Erben states that the neutrophiles seem to be the chief source of the proteoses, since their granules soon disappear in blood that is undergoing autolysis, whereas the eosinophiles preserve their granules well, and true proteoses are not present in blood rich in mast cells (*i. e.*, myeloma). The marrow, spleen and lymph nodes are found strongly proteolytic (according to the plate method), in myelogenous leukemia, but in lymphatic leukemia and pseudoleukemia, only the marrow shows a slight activity.[76] Schumm[77] found in the blood in a case of myelogenous leukemia several varieties of proteoses, most abundant being the so-called deutero-albumose; in another he also found peptone, leucine, and tyrosine. In addition he demonstrated the autolytic nature of the changes that occur in leukemic blood after death (see also "Autolysis in Leukemia," Chap. iii). Leukemic leucocytes show a reduced resistance to autolysis, but no autolysin for leucocytes is demonstrable in leukemic serum.[78] Most observers have failed to find *albumose* in the urine in leukemia but there is an increased amount of amino-nitrogen in the blood.[79] Because of the involvement of the bone marrow, small amounts of Bence-Jones protein, as well as Mörner's body, may be found in the urine.[80] Kolisch and Burian[81] not only found nucleoprotein constantly, and albumose frequently, but in one case of lymphatic leukemia they found histone in the urine, which undoubtedly came from nucleoprotein decomposition.

Metabolism.[82]—Stejskal and Erben[83] studied the metabolism of a case of myelogenous and of a case of lymphatic leukemia, and found the nitrogen loss much greater in the myelogenous form, although food-absorption was better than in the lymphatic; they consider that protein-destroying forces are at work in myelogenous leukemia, similar to those of cancer cachexia, so that nitrogeneous equilibrium cannot be attained.

The oxidase reaction is conspicuous in certain of the cells of myeloid leukemia, especially the large, non-granular cells of acute leukemia,[84] but it is not known that these oxidases influence the chemistry of the disease. In spite of the richness of leucocytes in lipases the serum shows no increased lipolytic activity.[85]

As the most characteristic products of decomposition of nucleoproteins are the purine bases, one would also expect to find them increased in leukemia, and early writers mention the finding of purine bases and uric acid in the blood and spleen. The urinary findings in this respect have been very variable. Ebstein[86] observed the complication of leukemia with gout which he considered a coincidence, and also noted uric-acid concretions in the urinary passages in four cases. Numerous other authors have described increased uric-acid elimination, while some have observed increase in the purine bases, either with or without uric-acid increase. Magnus-Levy[87] observed a particularly large uric-acid output in acute leukemias, but also found that the relation between the number of leucocytes and the uric acid is extremely variable. Sometimes the nitrogen loss is very great—even as much as 20 gm. per day—and, corresponding with the destruction of nucleoproteins and the resulting uric-acid formation, phosphoric-acid excretion is often greatly increased—even up to 15 gm. per day. On the other hand, the results obtained by many other writers have been in every respect extremely variable; some have found no increase in uric acid, some even report a decrease; likewise the P_2O_5 has been found even less than normal. For example, in a carefully studied case of lymphatic leukemia, Henderson and Edwards[88] found during six months no excessive excretion of uric acid or phosphoric acid. Zalesky and Erben found likewise no considerable increase in the uric acid

[76] Jochmann and Ziegler, Münch. med., Woch., 1906 (53), 2093.
[77] Hofmeister's Beitr., 1903 (4), 442; Deut. med. Woch., 1905 (31), 183.
[78] Szilard, Wien. Arch. inn. Med., 1924 (8), 33.
[79] Sandiford and Ross, Jour. Biol. Chem., 1924 (58), 845.
[80] Boggs and Guthrie, Bull. Johns Hopkins Hosp., 1913 (24), 368.
[81] Zeit. klin. Med., 1896 (29), 374 (literature on albuminuria in leukemia).
[82] See review by Hurwitz, "Endocrinology and Metabolism," 1924 (4), 533.
[83] Zeit. f. klin. Med., 1900 (39), 151.
[84] Dunn, Quart. Jour. Med., 1913 (6), 293.
[85] Caro, Zeit. klin. Med., 1913 (78), 286.
[86] For literature see résumé by Walz in Cent. f. Pathol., 1901 (12), 985.
[87] Virchow's Arch., 1898 (152), 107.
[88] Amer. Jour. of Physiol., 1903 (9), 417.

in lymphatic leukemia, but in myelogenous leukemia the uric acid was much increased; on the other hand, the amount of elimination of purine bases was reversed in the two forms, and creatine was decreased in both. Lipstein[89] found no excessive elimination of amino-acids even in myelogenous leukemia. An increase in calcium is quite constantly observed, and attributed to the bone destruction[85] occurring in this disease.

Undoubtedly these variations in results depend upon the known fluctuations in the course of the pathological processes of leukemia; the number of leucocytes, the size of the lymphatic organs, and the general condition of the patient all vary greatly from time to time, often with remarkable rapidity, and the excretion of products of metabolic activity must vary likewise. It can hardly be questioned that the enormous increase in the amount of lymphoid tissue in the body and blood must give rise to a greatly increased nuclein catabolism, with consequent appearance of its products (uric acid, purine bases, and phosphoric acid) in the urine. Furthermore, the presence of urate concretions in the urinary tract is a common occurrence in leukemia. In the blood there is a marked increase in uric acid and of nonprotein nitrogen, and the proportion of urea is relatively low, suggesting that there is present some unrecognized nitrogenous substance.[90]

Increased elimination of uric acid and purine bases, together with a general increase in the nitrogen output commonly follow the therapeutic use of *x*-rays in leukemia, which is attributed to the increased autolysis that *x*-rays are known to produce. Radium has a similar effect, increasing enormously the urinary total nitrogen, urea, ammonia, less markedly the uric acid, but especially the phosphates.[91] According to Rosenstern[92] the *x*-rays affect chiefly the leucogenic tissues rather than the adult leucocytes. Lipstein also found an excessive elimination of amino-acids in the urine of leukemic patients treated by *x*-rays. According to Curschmann and Gaupp,[93] the blood of leukemic patients who have been exposed to *x*-rays contains a specific *leucocytotoxin*, which may be produced by a process of autoimmunization against the leucocytic substance set free by the disintegrated leucocytes. Capps and Smith[94] have obtained similar results. *X*-rays seem not to alter the total metabolism appreciably.[95] (See also *x*-rays and radium.) Basal metabolism is increased in proportion to the number of immature white cells rather than according to the total leucocytes,[96] and it may be high in acute leukemia with relatively low leucocyte counts.[97]

Charcot's crystals (also called Charcot-Leyden and Charcot-Neumann crystals) represent a peculiar and striking product of nuclear destruction that has frequently been found associated with leukemia. These crystals were first observed by Robin[98] (1853) in leukemic tissues, but have been named after Charcot, who, with Robin, described their properties. They were described by Charcot as colorless, refractile, elongated octahedra; insoluble in alcohol, ether, and glycerol; soluble in hot water, acids, and alkalies; size variable, from 0.016 by 0.005 mm. up. Such crystals have been found not only in the tissues and blood of cadavers, but also occasionally in the freshly drawn blood of leukemics. They are strikingly resistant to putrefaction and autolysis. Poehl[99] believes them to be the same as Böttcher's *spermine crystals*, and derived from decomposed nucleins. Rosenheim[1] has found spermine present in many different organs and in yeast, and believes it to be a relatively simple aliphatic base with the nitrogen present in the amino form, the elementary composition probably being $C_{10}H_{26}N_4$.[2]

Crystals that appear similar are also found in asthmatic sputum, empyema, and ascites fluid, bone-marrow, and tumors, and in the stools in amebic dysentery

[89] Hofmeister's Beitr., 1905 (7), 527.
[90] Martin and Denis, Amer. Jour. Med. Sci., 1920 (160), 223.
[91] Knudson and Erdos, Boston Med. Surg. Jour., 1917 (176), 503.
[92] Münch. med. Woch., 1906 (53), 1063.
[93] Münch. med. Woch., 1905 (52), 2409.
[94] Jour. Exp. Med., 1907 (9), 51; see also Klieneberger u. Zoeppritz, Münch. med. Woch., 1906 (53), No. 18; Milchner u. Wolff, Berl. klin. Woch., 1906 (43), No. 23.
[95] Arch. Int. Med., 1917 (19), 890.
[96] Gunderson, Boston Med. Surg. Jour., 1921 (185), 785.
[97] Lennox and Means, Arch. Int. Med., 1923 (32), 705; 1924 (33), 576.
[98] Literature given by v. Leyden, Festschrift für Salkowski, Berlin, 1904, p. 1, and by v. Hoesslin, "Das Sputum," Berlin, 1921.
[99] Deut. med. Woch., 1895 (21), 475.
[1] Mary C. Rosenheim, Jour. Physiol., 1917 (51), Proc. VI.
[2] See Biochem. Jour., 1924 (18), 1253 and 1263; also Wrede, Zeit. physiol. Chem., 1924 (138), 119.

(Acton),[3] and it has been suggested that they are derived from or related to the oxyphile granules of the eosinophiles.[4] This view implies an agreement with Gumprecht's opinion that the crystals seen in bone-marrow, asthmatic sputum, etc., are not spermine, but of protein nature. As can be seen, the nature and significance of Charcot's crystals are, at the present time, quite undetermined, but their relation to the eosinophile granules receives growing support.

Summary.—The chemical changes observed in leukemia depend upon the excessive quantity of leucocytes and lymphoid tissue, which undergo processes of disintegration at irregular intervals with the result that the products of nucleoprotein destruction (uric acid, purine bases, and phosphoric acid) appear in the urine in increased quantities. As the large neutrophiles contain abundant autolytic enzymes, the products of cell autolysis (proteoses, amino-acids, and products of nucleoprotein destruction) may appear at times in the urine and in the blood; because of the small amount of such enzymes in the lymphocytes, these changes are all much less marked in lymphatic leukemia. Charcot's crystals, which are perhaps derived from leucocytic nucleoproteins, may be found in the blood and tissues. The changes in the red cells are chiefly those of a secondary anemia, with occasionally some chlorotic features. The chemical findings of leukemia throw no light whatever upon the cause of the disease.

Pseudoleukemia, and Hodgkin's disease (lymphogranulomatosis), show only the evidences of a secondary anemia, without the chemical changes of either leukemia or pernicious anemia. There seems to have been little study of the chemical processes of these diseases. Moraczewski[5] reports a study of metabolism in one case, designated by him as pseudoleukemia and so quoted in subsequent literature, although the only leucocyte count mentioned in the original article was 171,000. This case showed some retention of nitrogen and calcium, with little change in the phosphorus and purine bases in the urine.

HYPEREMIA

Active Hyperemia[6]

This condition is associated with but few chemical changes. Certain chemicals may cause active hyperemia; some locally, as in the case of irritants, such as alcohol, ether, ammonia, mustard, etc., which act either by producing a local vasodilator stimulus or by paralyzing the vasoconstrictors. Amino acids generally have a vasodilator effect.[7] Other substances may produce active hyperemia in special vascular areas, *e. g.*, cantharides causes active hyperemia in the kidneys, probably because of its elimination through these organs; pilocarpin causes active hyperemia in the salivary glands and skin, which is associated with increased function. In general, functional activity is associated with active hyperemia and Gaskell[8] has suggested that this is due to atonicity of the vascular

[3] Indian Jour. Med. Res., 1918 (6), 157.

[4] See Liebreich, Schweiz. med. Woch., 1921 (51), 275; Jacobsthal, Klin. Woch., 1923 (2), 194; Virchow's Arch., 1921 (234), 12.

[5] Virchow's Arch., 1898 (151), 22.

[6] Review by D. T. Harris, Proc. Roy. Soc. London, 1922 (93B), 384.

[7] Brouha and Fredericq, Compt. Rend. Soc. Biol., 1924 (91), 1169.

[8] Quoted by Lazarus-Barlow, "Manual of General Pathology," 1904, p. 126; corroborated by Atzler and Lehmann, Arch. ges. Physiol., 1922 (197), 221.

muscle, the result of decreased alkalinity of the lymph flowing away from the active organ along the vessel-walls, it having been found that alkalies cause a tonic contraction and acids an atonic dilation of arterial muscle.[9] According to Atzler[10] the maximum dilatation occurs, in experiments with frogs, at *p*H 5.65 to 6.6 which he says is the isoelectric range of the tissue proteins.

The study of capillary volume has indicated that capillaries play a more active part in determinining the amount of blood supply in the tissues than was formerly appreciated,[11] and hence it is significant that an increased H-ion concentration also leads to capillary dilatation (Schade)[12] while weak alkalies cause constriction (Stricker). Also that histamine, which may be formed where tissues disintegrate, is particularly active in causing capillary dilatation, the fatal shock produced by histamine being attributed to fall of blood pressure through capillary distension. Apparently heat, cold, and mechanical stimulation may cause capillary dilatation independent of any action on the muscular system of the arteries and veins; indeed, there often occurs a dilatation of the capillaries with contraction of arterioles, leading to capillary stasis as when the skin is "blue with cold."[13]

Pathological active hyperemia is seldom of long enough duration to lead to any alterations in the tissues in which it occurs. The blood itself remains unchanged, except that the venous blood going from the part contains much less CO_2 and more oxygen than usual, because more oxygen is brought to the tissues than can be used.[14]

Plethora.—Experimental plethora produced in animals by injecting blood from other animals, produces after a time a distinct anemia, because of an increased hematolysis, associated with deposition of large quantities of hemosiderin in the tissues.[15] Despite intravenous introduction of large quantities of N in the form of whole blood, the total N, urea, and NH_3 in the urine and feces are not raised for some time after the onset of plethora. The normal organism is apparently able to store large quantities of blood or its decomposition products. Upon the onset of "plethoric anemia," there is an increase in urinary total N and urea excretion, which is lowered during the course of the anemia.

Passive Hyperemia

Passive hyperemia is almost equally unassociated with chemical changes, especially in its etiology, as it depends solely upon mechanical factors. Some chemical alterations result, however, from the changes in the stagnating blood, which may, if the obstruction to outflow is severe, become of venous character in the capillaries of the con-

[9] See discussion by Woolley, Jour. Amer. Med. Assoc., 1914 (63), 2279; and by Adler, Jour. Pharm., 1916 (8), 297.

[10] Deut. med. Woch., 1923 (49), 1011.

[11] Full review by Hooker, Physiol. Reviews, 1921 (1), 112.

[12] Münch. med. Woch., 1924 (71), 1.

[13] See Bayliss, Science Progress, 1919 (14), 272.

[14] Polycythemia (Vaquez-Osler disease) is accompanied by an increase in the total nitrogen of the blood, in proportion to the number of erythrocytes; but the nitrogen content of the individual erythrocyte is decreased. (v. Jaksch. Zent. inn. Med., 1912 (33), 397.)

[15] See Krumbhaar and Chanutin, Jour. Exp. Med., 1922 (35), 847.

gested area. Oxidation in the tissues is, therefore, impaired, and some fatty changes may result, *e. g.*, in the center of congested liver lobules. Waste products accumulate, and possibly noxious products of metabolism are formed under lack of oxidation; either from these causes or solely from pressure and lack of nutrition there is a tendency to atrophy of the more specialized parenchymatous cells, and a proliferation of connective tissues. The atrophy of parenchyma is seen particularly in the liver, the increase of connective tissue in the spleen.[16] In the kidney neither atrophy nor stroma proliferations are pronounced, but the renal function is impaired, since it depends upon the amount and quality of the blood brought to the kidney.[17] Whether connective-tissue proliferation in hyperemia depends upon overnutrition or upon irritation by waste-products, or is compensatory to parenchymatous atrophy, may be looked upon as still an open question. Probably only the first two factors apply to the connective-tissue growth observed in the congested spleen, the clubbing of the fingers in congenital heart disease, or the thickening of the subcutaneous tissues in passive congestion of the lower extremities.

Changes in the Blood.—Venous blood differs from arterial, not only in its increased load of CO_2 and other waste products, but also in other ways. Venous blood generally clots less readily than arterial blood.[18] It contains more diffusible alkali because the CO_2 combines with and tears away part of the bases that are held by the proteins, especially in the corpuscles, and so alkaline carbonates are formed and enter the plasma. Blood from the jugular vein on this account contains 20–25 per cent. more diffusible alkali than carotid blood (Hamburger).[19] Since the bactericidal power of the blood has been shown to increase directly with the alkalinity, this property may be of importance in pathology. For example, the relative infrequency of infections in the right side of the heart may not depend solely upon lessened liability to endocardial damage, as generally considered, but is possibly due in part to the greater bactericidal power of venous blood. The same property probably explains the favorable results obtained in the treatment of local infections by artificially produced passive congestion.[20] Too severe a stasis, with resultant edema, probably favors local infection.[21] Hamburger has shown that the bactericidal power of the blood may be increased *in vitro* by shaking it with CO_2, as a result of the increased alkalinity, aided, perhaps, by some slight bactericidal power of the CO_2 itself; he also found the blood more strongly bactericidal in venous congestion

[16] See Christian, Jour. Amer. Med. Assoc., 1905 (45), 1615.

[17] See Rowntree and Geraghty, Arch. Int. Med., 1913 (11), 121; Nonnenbruch, Deut. Arch. klin. Med., 1913 (110), 162.

[18] Vierordt (Arch. f. Heilk., 1878 (19), 193) found coagulation faster in the blood in passive congestion than in normal venous blood; but Hasebrock (Zeit. f. Biol., 1882 (18), 41) found that if the stasis is protracted, the coagulation becomes delayed because of the excess of CO_2.

[19] Virchow's Arch., 1899 (156), 329; also, "Osmotischer Druck und Ionenlehre."

[20] See Bier, "Hyperæmie als Heilmittel," Leipsic, 1903.

[21] Glasewald, Cent. Grenz. Med. Chir., 1915 (18), 507.

than normally,[22] and the lymph from a congested part was also found more strongly bactericidal than normal lymph.[23] However, chemotaxis is, if anything, slightly decreased under the influence of CO_2, as also is phagocytosis;[24] large amounts of CO_2 may reduce the phagocytic power for coal particles by 25–50 per cent.

The blood in the veins and capillaries in passive congestion is generally richer in corpuscles than normal, perhaps because of some loss of water,[25] although this is not constant, applying particularly to more recent or more local processes; in long-continued stasis, as in congenital heart disease, the blood may be diluted.[26] In the concentrated blood of passive congestion the corpuscles may number six to eight millions per cubic millimeter, while the concentration of the solids of the serum may be at the same time reduced (Krehl). The viscosity of such blood is higher than that of normal blood.[27] In acute stasis the proportion of serum proteins, especially the albumin, increases with the duration of the stasis; no changes occur in the non-protein constituents of the blood (Rowe).[28]

THROMBOSIS

The chemistry of thrombosis in most respects resolves itself into the chemistry of fibrin formation, a subject which is so extensively considered in most treatises on physiological chemistry and physiology that it does not seem desirable to give here anything more than the essential principles involved in the clotting of the blood, as now understood, as an introduction to the consideration of the same process as it occurs under pathological conditions. In spite of innumerable investigations, our knowledge of the actual participants and processes involved in the formation of fibrin is in a very unsatisfactory and fragmentary state. Some facts seem well established, however, and we have a general idea of the subject that may be applied with advantage to the consideration of thrombosis.

Fibrin Formation[29]

Several different substances seem to be concerned in the formation of fibrin, of which the first of importance is its antecedent, *fibrinogen*. Fibrinogen is a simple protein, related to the globulins, and differing chiefly in its ready coagulability, not only by fibrin ferment, but also by heat, salts, and other coagulating agencies. By itself, however, it shows no tendency to coagulate spontaneously. According to Goodpasture,[30] fibrinogen is formed through the combined activity of the liver and

[22] Confirmed by Laqueur, Zeit. exp. Path. u. Ther., 1905 (1), 670.

[23] Schiller ascribes this not to increased alkalinity, but to disintegration of leucocytes with liberation of bactericidal substances (Beitr. klin. Chir., 1913 (84), 151).

[24] Hamburger Virchow's Arch., 1899 (156), 329.

[25] Grawitz, Deut. Arch. f. klin. Med., 1895 (54), 588.

[26] See Krehl, "Pathologische Physiologie," 1904, p. 201.

[27] Determann, Zeit. klin. Med., 1906 (59), H. 2–4.

[28] Jour. Lab. Clin. Med., 1916 (1), 485.

[29] For literature and full discussion see Hammarsten's or Mathew's Physiological Chemistry; Morawitz, the Handbuch d. Biochem., 1923 (4), 44; Howell, Harvey Lectures, 1917.

[30] Amer. Jour. Physiol., 1914 (33), 70.

intestines, although earlier writers have variously described its formation in the bone marrow, leucocytes, liver, or intestines. Hertzfeld and Klinger[31] have advanced the theory that the blood proteins constitute merely a continuous series of disintegration products of tissue proteins, of which the first step is the least dispersed of the blood proteins, fibrinogen. This theory accounts well for the increase in fibrinogen content with tissue injury, and the spontaneous coagulation of injured tissues.

The amount of fibrinogen present in the blood is actually quite small, the fibrin formed in normal clotting being but 0.1 to 0.4 per cent. of the weight of the blood. Acted upon by the fibrin-ferment, it yields the characteristic insoluble protein fibrin, in crystalline form under favorable conditions,[32] but we do not know definitely what changes the fibrinogen undergoes in this process. Fibrin resembles in its insolubility the proteins coagulated by heat, alcohol, etc., but when kept aseptically for some time, it becomes again dissolved; this process of *fibrinolysis* probably depends upon proteolytic enzymes, which fibrin, in common with other substances of similar physical nature, has the property of dragging out of solution and holding firmly. Undoubtedly entangled leucocytes are also an important factor in the fibrinolysis,[33] which is greatly increased in phosphorus poisoning and when the liver is excluded from the circulation, a fact suggesting that the liver may form inhibiting substances.

Theories of Fibrin Formation.—The great problem is the nature and the place and manner of origin of the fibrin-forming enzyme, generally called *fibrin-ferment* (also *plasmase*, *thrombin* and *coagulin*). The most fundamental theory of the origin and nature of fibrin-ferment is that of Alexander Schmidt, which may be briefly described as follows: The ferment, Schmidt believed, exists in the plasma in an inactive (*prozyme* or *zymogen*) form, which he called *prothrombin*. Upon disintegration of the leucocytes there is set free a substance, which, acting upon the prothrombin, converts it into the active thrombin; this activating agent Schmidt designated as the *zymoplastic* substance. With various modifications this stands to the present day as a basic theory.

It having been shown that calcium facilitates the formation of fibrin, Pekelharing advanced the idea that the prothrombin does not exist in the plasma, but is liberated from the leucocytes, and, combining with the calcium of the plasma, forms the thrombin. Morawitz considers three substances necessary for the formation of thrombin: (1) the *prothrombin* or *thrombogen*, which he believes originates in the blood-plates; (2) the *zymoplastic substance* or *thrombokinase*, which is liberated from the leucocytes into the plasma; (3) calcium salts. Howell,[34] however, explains coagulation as follows: Circulating blood normally contains all the necessary factors for fibrin formation, *i. e.*, fibrinogen, prothrombin and calcium. But there is also present an inhibiting substance, *antithrombin*,[35] which prevents the calcium from activating the prothrombin into thrombin. In shed blood there appears a *thromboplastin*, derived from the platelets or the tissues, which neutralizes the antithrombin and thus permits thrombin to form. Rettger[36] holds that the coagulation of the blood is not a true enzyme action at all, while Bordet and Delange[37] consider that thrombin is formed by the interaction of *cytozyme* from the platelets or tissue cells, and *serozyme* of the plasma. The cytozyme is believed to be lipoidal, perhaps lecithin;[38] the serozyme is of protein character.

Mathews follows Woolridge and considers the clotting of the blood as essentially the crystallization of a phospholipin-protein compound, *i. e.*, blood plasma, the stability of which compound is easily upset in many ways. The fibrin threads are essen-

[31] Biochem. Zeit., 1917 (83), 42; See Frisch and Starlinger, Zeit. ges. exp. Med., 1921 (24), 142.

[32] See Howell, Amer. Jour. Physiol., 1914 (35), 143; Hekma, Internat. Zeit. physik. chem. Biol., 1915 (2), 279.

[33] See Morawitz, *loc. cit.;* also Rulot, Arch. internat. d. Physiol., 1904 (1), 152; Rosenmann, Biochem. Zeit., 1922 (129), 101.

[34] Amer. Jour. Physiol., 1911 (29), 187.

[35] The antithrombin is formed by the action of a phosphatid from the liver (*heparin*) upon a pro-antithrombin (Howell and Holt, Amer. Jour. Physiol., 1918 (47), 328). Others consider antithrombins to be products of proteolysis (Pickering and Hewitt, Biochem. Jour., 1922 (16), 587).

[36] Amer. Jour. Physiol., 1909 (24), 406.

[37] Ann. Inst. Pasteur., 1912 (26), 657. See also Lee and Vincent, Arch. Int. Med., 1914 (13), 398.

[38] Full review of Bordet's theories given by him in the Bull. Johns Hopkins Hosp., 1921 (32), 213. Nolf's theory is given in Arch. néerland. physiol., 1922 (7), 348.

tially liquid crystals coming out of a saturated solution, the blood plasma, which is practically a dilute protoplasm.[39] It will not serve our purpose, however, to go further into the hypotheses[40] and disputes over these questions, which are detailed more fully in the literature previously cited, but it may be stated that numerous American observers have found Howell's theory to fit well with both experimental and clinical observations on the variations in the coagulability of the blood.

The question has been raised as to whether the leucocytes or platelets secrete their fibrin-forming constituent (be it thrombokinase or prothrombin is a matter of minor importance to the pathologist) or liberate it only after their disintegration. As far as pathological processes go, the latter seems to be the case, the disintegration apparently occurring whenever the leucocytes come in contact with a foreign body or with dead and injured tissues. The stroma of red corpuscles also contains thrombokinase.[41] Of the substances that may be isolated from tissues, *cephalin* is found especially active in producing thrombosis, and may be related to or identical with the thromboplastin.[42]

Tissue Coagulins.—Among the other points that are of importance in pathological conditions is the fact that not only the leucocytes, but also tissue-cells, can liberate fibrin-forming substances (*coagulins* is the non-committal term applied by Leo Loeb). Howell considers that the effect of the tissue "coagulins" is merely to neutralize the antithrombin of the blood, if such coagulins actually exist; possibly there is thromboplastin in the tissues. These coagulating agents are present in tissue extracts and are liberated whenever the tissues are injured; muscle is rich in coagulin, as are also the liver and kidney, and, which is particularly important, the blood-vessel wall (L. Loeb). Pieces of these tissues placed in contact with fibrinogen solution will bring about prompt clotting. Another important fact is that the coagulins, whether derived from leucocytes or from the tissues, have a certain degree of specificity—that is, they act solely or most rapidly with fibrinogen of blood of the species from which they are obtained.[43] In some instances this specificity is absolute, but more generally (particularly in the mammalia) it is only relative. Loeb also found that the amount of tissue coagulin was not decreased in organs altered by phosphorus poisoning, although during experimental autolysis the coagulins disappear. When tissue coagulins and blood coagulins act together, the effect is greater than the sum of their independent actions, indicating the probability that they combine in some way to produce a particularly active coagulin. The blood coagulins are quite different from the tissue coagulins in many important respects, and the coagulins cannot be looked upon as a single substance of different origins.

Mills[44] found that the phospholipins of tissues do not account for all the coagulin activity, combination with a protein element being essential. The purified coagulin obtained by him consisted of 41.6 per cent. of phospholipin and 58.4 per cent. of phosphoprotein. He believes that it consists of about thirteen phospholipin molecules united to one protein molecule, and that it unites with plasma fibrinogen to form fibrin, perhaps with calcium as the connecting link.[45] This tissue coagulin (or thromboplastin, thrombokinase or tissue fibrinogen), he believes, acts directly as above, rather than by removing an antithrombin according to Howell's theory, but if antithrombin is present the coagulin will neutralize it. Tissues deprived of their lipins were found to yield an anticoagulant globulin, which becomes a coagulant when united to cephalin.[46] The lung tissues seem to be especially rich in tissue coagulins which may be an important protective factor in an organ so vascular but of such a delicate structure.

Blood-platelets.—It is still undetermined just what part the platelets play in coagulation. The well-known observation that in thrombosis the fibrin is often

[39] There are several different types of coagulation structure which are different in different species, according to Kitamura, Arch. ges. Physiol., 1924 (203), 651.

[40] Physicochemical theories of fibrin formation have been advanced, among others, by Barratt (Biochem. Jour., 1920 (14), 189) and Amar (Compt. Rend. Acad. Sci., 1924 (178), 1628).

[41] Barratt, Jour. Path. and Bact., 1913 (17), 303.

[42] Howell, Amer. Jour. Physiol., 1912 (31), 1; MacLean, *ibid.*, 1916 (41), 250; 1917 (43), 586.

[43] Leo Loeb, Univ. of Penn. Med. Bull., 1904 (16), 382; Muraschew, Deut. Arch. klin. Med., 1904 (80), 187.

[44] Jour. Biol. Chem., 1921 (46), 135.

[45] Amer. Jour. Physiol., 1921 (57), 395.

[46] Amer. Jour. Physiol., 1922 (61), 42.

first formed about masses of platelets clinging to the wall of the vessel indicates that they participate in the process, and Bizzozero and others have maintained that the platelets and not the leucocytes are the source of the prothrombin. Numerous studies on the relation of the platelets to disease conditions, have indicated a certain parallelism between their number and the tendency to coagulation observed in the various diseases. Howell believes the platelets to be the chief source of thromboplastin, which neutralizes the antithrombin of the blood and thus causes clotting. Wright and Minot[47] find that a viscous metamorphosis of the platelets is intimately associated with the early stages of coagulation. Bordet and Delange[37] consider the platelets of more importance than the leucocytes in producing participants of the coagulating mechanism. The histological evidence of the importance of the platelets in thrombus formation is conclusive and Cramer and Pringle[48] state that coagulation cannot occur without platelets. However, the blood of fishes, birds, and reptiles clots, although no platelets are found in these animals. Human thoracic lymph also is devoid of platelets yet it clots; but it may contain products of platelet disintegration to explain this clotting (Jordan).[49] Kemp[50] concludes, from a thorough review of the subject, that the blood-platelets are usually normal or subnormal in number during acute infectious diseases, but increase rapidly if the disease terminates by crisis; in pernicious anemia the number is always greatly diminished, although in secondary anemias they may sometimes be increased; in *purpura hæmorhagica* the number of plates is enormously diminished, which is perhaps related to the slowness of the clotting of the blood in this condition. Gram[51] states that platelet counts below 100,000 generally accompany a tendency to bleed. He gives the normal figure as 200,000 to 500,000, but usually over 300,000.

If the platelet count is reduced artificially (by benzene, diphtheria toxin) a similar purpuric tendency is observed. Poisons that in large doses reduce the platelet count, will increase it if in small doses. Antisera prepared by immunizing animals against platelets, produce a purpuric condition when injected into normal animals (Bedson).[52]

Calcium Salts.—The exact significance of calcium in fibrin formation is still unsettled. Blood from which the calcium has been precipitated will not coagulate, and the addition of calcium salts will promptly cause it to do so. The various hypotheses advanced to explain the way in which calcium influences the clotting process are not in agreement. One hypothesis is that the calcium ions are necessary for the transformation of prothrombin into thrombin (Pekelharing, Hammarsten, Morawitz), the thrombin consisting of a compound of prothrombin, calcium salts, and thrombokinase. Howell considers that no kinase is necessary, the calcium activating the prothrombin whenever it is not inhibited by antithrombin. Vines[53] believes that during coagulation calcium is transformed from the combined to the ionized state, and that thrombokinase is an organic calcium compound. But Kugelmass[54] found the concentration of Ca ions to decrease during coagulation.

Modification of Coagulability.—If blood is drawn into a glass vessel well coated with oil or vaseline, through a cannula similarly protected, no coagulation will take place; but if any unoiled foreign substance enters, even particles of dust, coagulation begins at once. The explanation usually given is that the leucocytes do not liberate their coagulating substances until they have been injured by contact with some foreign body, but as plasma free from cells will also clot when in contact with unoiled glass, the process cannot depend solely on leucocytic injury, but seems to be more of a physico-chemical phenomenon (Bordet). How-

[47] Jour. Exp. Med., 1917 (26), 395.
[48] Quart. Jour. Exper. Physiol., 1913 (6), 1.
[49] Anat. Rec., 1918 (15), 37.
[50] Jour. Amer. Med. Assoc., 1906 (46), 1022.
[51] Arch. Int. Med., 1920 (25), 325.
[52] Jour. Path. and Bact., 1922 (25), 94.
[53] Jour. Physiol., 1921 (55), 86.
[54] Arch. internat. physiol., 1923 (21), 139. Full discussion of the physicochemistry of coagulation.

ever, the products of disintegration of leucocytes or any other cells greatly accelerate fibrin formation. Apparently contact with foreign bodies causes plasma to form thrombin and causes cells to liberate their coagulating agents.

The classical experiment of the ligation of a vein without injury to the endothelium, which permits the blood to remain stagnant for a long period without clotting, depends upon the same fact, namely, that normal endothelium neither liberates coagulin itself nor injures the leucocytes so that they disintegrate. Leo Loeb recalls the observation of Overton that lipoids are important constituents of the cell membranes, and suggests a similarity between the vessel lining and the oiled cannula, but analyses of aortic endothelium have shown a rather low lipin content (8.41–9.25 per cent.), although peritoneal endothelium has much more (13 to 15 per cent.).[55] The suggestion that the vessel walls contain an *anti-coagulin* could not be confirmed by Loeb. Since leucocytes are constantly undergoing disintegration in the blood and tissues under normal conditions, it might be asked why they do not cause clotting then and there. In explanation, Loeb advances his observation that the coagulins are destroyed during cell autolysis, and suggests that when leucocytes normally disintegrate, the coagulins are first destroyed by autolysis. It has also been shown that the cells and serum contain substances which inhibit or prevent coagulation, and it is possible that these play an important part under normal conditions in preventing coagulation by products of cell disintegration, much as other antienzymes are supposed to act in preventing autodigestion of living cells. Thus, the blood expectorated in phthisical hemoptysis is more or less incoagulable, and Magnus-Alsleben[56] attributes this to the presence of the products of autolysis in the tuberculous lung.

Coagulation of drawn blood may be *retarded* experimentally by removal of the calcium by precipitation as oxalate, fluoride, etc.; also by diminishing the oxygen and increasing the CO_2, by addition of solutions of neutral salts in large amounts, by diluting greatly with water, or by keeping the blood cold. Bile salts retard coagulation markedly, by interfering with the conversion of fibrinogen into fibrin.[57] Coagulation may be hastened by moderate heat, by whipping, exposure to air, by contact with much foreign matter, and by the addition of watery extracts from many different tissues and organs. Poisons that destroy the platelets reduce the coagulation (Duke).[58] Of particular interest pathologically is the retardation of coagulation that follows injections of proteoses (the so-called "peptone" solutions) and also various other protein-containing solutions, such as organ extracts, bacterial toxins, snake venoms, eel serum, extract of leeches or of *Uncinaria*, impure nucleo-protein solutions, or solutions of various colloids. Most of these substances *e. g.*,

[55] Tait, Quart, Jour. Exp. Physiol., 1915 (8), 391.
[56] Zeit. klin. Med., 1915 (81), 9.
[57] Haessler and Stebbins, Jour. Exp. Med., 1919 (29), 445.
[58] Jour. Exp. Med., 1911 (14), 265; Arch. Int. Med., 1912 (10), 445; Jour. Amer. Med. Assoc., 1915 (65), 1600.

peptone, eel serum, cause reduction of coagulability when injected into animals, and are without effect on blood removed from the body. A few, however, prevent coagulation of drawn blood (snake venom, extract of leeches). When substances of the first class are injected in sufficient quantities, there occurs first a period of accelerated coagulation which may, particularly in the case of organ extracts, cause prompt death from intravascular clotting; if the animal survives, there follows a period of decrease or total inhibition of coagulability of the blood, both within the vessels and after removal from the body.[59] The first period of increased coagulability undoubtedly depends upon the formation of a large amount of fibrin-ferment, but it has not yet been satisfactorily explained how the inhibition of coagulation is produced. Apparently the fibrin-ferment formed at first is rapidly destroyed, but it is thought by some that it is converted into a substance that neutralizes the fibrin-ferment that may be formed later, or that a true anticoagulin is formed. It is also among the possibilities that all the available prothrombin or thrombokinase is used up during the first stage of acceleration. As before mentioned, the blood and tissues contain substances that inhibit coagulation, and it may be that these are secreted in excessive amounts, a view which is receiving much support from recent observations. According to Davis,[60] injection of thrombin is followed quickly by an increase in the amount of antithrombin in the blood. It has been found that in animals deprived of the liver no coagulation-inhibiting substances are formed in the blood after injection of proteoses, hence Delezenne believes that the substances of this class act by causing a destruction of leucocytes, thus liberating a substance which increases coagulation and also another substance retarding coagulation; the first of these is destroyed by the liver, leaving the retarding substance to act unopposed.[61] Leech extract (*hirudin*) prevents clotting by means of an antiferment action, combining with the thrombin.[62] Snake venom, however, acts upon the thrombokinase (Morawitz).

Coagulability of the Blood in Disease.—In disease, the alterations in the coagulability of the blood depend upon much the same factors. In all conditions associated with suppuration and leucocytosis the amount of fibrinogen is increased. This is especially true of pneumonia.[63] The fluidity of the blood in septicemia is probably dependent upon the appearance of the coagulation-inhibiting phase that follows the action of the products of cell destruction, including among them proteoses. In this connection should be mentioned the observation of Conradi.[64]

[59] Menten (Jour. Biol. Chem., 1920 (43), 383) finds that the anticoagulating effects of peptone must be different from those of hirudin or cobra venom, since of these peptone alone causes a marked fall in the *p*H of the blood.

[60] Amer. Jour. Physiol., 1911 (29), 160.

[61] The manner in which gelatin injections affect the blood coagulability is not yet understood (see Boggs, Deut. Arch. klin. Med., 1904 (79), 539); Moll (Wien. klin. Woch., 1903 (16), 1215) found an increase in fibrinogen.

[62] Hirudin may contain antikinase (Mellanby, Jour. of Physiol., 1909 (38), 441).

[63] Dochez, Jour. Exp. Med., 1912 (16), 693.

[64] Hofmeister's Beitr., 1901 (1), 137.

who found that among the products of autolysis is a coagulation-inhibiting substance which is not destroyed by heat, diffuses readily, and in general behaves unlike the proteins. This or similar substances may well play a part in affecting coagulation in infectious diseases, and Whipple[65] has found a decreased coagulability in septicemia because of the presence of an excess of antithrombin. It may also be mentioned that animals soon acquire an immunity against proteoses, so that their inhibiting influence is no longer shown. This corresponds to the observation of Kanthack[66] that immune serum against venom neutralizes very effectively the anticoagulating principle of venom; an amount of antiserum altogether insufficient to neutralize the toxic properties of venom will neutralize its property of preventing clotting. The bacterial products may also modify coagulation, and L. Loeb[67] has found that different organisms are unequally effective in this respect, *Staphylococcus aureus* being much more powerful in causing coagulation than any others tested;[68] typhoid, diphtheria, tubercle, and xerosis bacilli and streptococci being without any apparent effect, while pyocyaneus, prodigiosus, and colon bacilli occupy an intermediate position. Furthermore, after the organisms are killed by boiling, this effect is greatly reduced, showing that it does not depend merely upon the mechanical action of the bacteria, but probably upon bacterial products contained in the culture-media.

After phosphorus poisoning the blood may become non-coagulable, which Jacoby[69] ascribed to an absence of fibrinogen in the blood, because of a fibrinogen-destroying ferment in the liver. Doyon[70] has made a similar finding in chloroform necrosis of the liver, but he attributes especial importance to an excess of antithrombin liberated from the liver in these conditions. Whipple has also found a decrease in fibrinogen with chloroform necrosis and cirrhosis of the liver.[71] In other instances of decreased coagulability the fibrinogen is present, generally in normal amounts. After death the blood becomes incoagulable because the fibrinogen is destroyed through a process similar to that of fibrinolysis;[72] this fibrinolysis may be complete as early as ten hours after death. The other proteins of the blood do not seem to be correspondingly attacked. Thrombokinase is also scanty in cadaver blood, but there seem to be no coagulation-inhibiting substances present. In anaphylactic shock the coagulability is reduced or abolished, associated with which is a leucopenia.[73]

[65] Arch. Int. Med., 1912 (9), 365.
[66] Cited by Lazarus-Barlow, p. 141.
[67] Jour. Med. Research, 1903 (10), 407.
[68] Much (Biochem. Zeit., 1908 (14), 143) states that staphylococcus contains thrombokinase.
[69] Zeit. physiol. Chem., 1900 (30), 175; also Doyon *et al.*, Compt. Rend. Soc. Biol., 1905 (58), 493.
[70] Compt. Rend. Soc. Biol., 1905 (58), 704; Jour. phys. et path., 1912 (14), 229.
[71] Bull. Johns Hopkins Hosp., 1913 (24), 207.
[72] Morawitz, Hofmeister's Beitr., 1906 (8), 1.
[73] The incoagulability of menstrual blood is ascribed to a lack of fibrin ferment by Bell (Jour. Path. and Bact., 1914 (18), 462) and to an excess of antithrombin by Dienst (Münch. med. Woch., 1912 (51), 2799).

Whipple[74] states that the antithrombin-prothrombin balance in the blood is in delicate equilibrium, but preserved by strong factors of safety. The prothrombin factor is rarely involved, most notably in melena neonatorum and aplastic anemia, and such conditions may be relieved by injecting normal blood, through the added prothrombin. The antithrombin factor is often excessive in hemorrhagic conditions, especially with hepatic injury or it may be lowered and lead to thrombosis from relatively slight injuries. Obviously the injection of normal blood will harm rather than help patients with hemorrhage due to excessive antithrombin. Antithrombin is often found increased in diseases of the blood-forming organs, *e. g.*, leukemia, possibly as a reaction to the products of disintegration of corpuscles; and hence hemorrhagic tendencies are noted in these diseases. In icterus the notable tendency to hemorrhage seems to depend upon the binding of the calcium of the blood by the bile pigments,[75] and administration of calcium may bring the coagulation time back to normal with a corresponding decrease in the hemorrhagic tendency.

There are numerous older analyses of the plasma for fibrinogen, which in general indicate an increase in acute infections with leucocytosis, especially in croupous pneumonia, which undoubtedly bears an important relation both to the characteristic fibrinous nature of the alveolar exudate in pneumonia and the striking amount of fibrin found in pneumococcus pleuritis, peritonitis, etc. More accurate methods have since been devised by Whipple and his colleagues, and the results are summarized by Foster[76] who found that normally women have a slightly higher fibrin content in the plasma than men, (average respectively 344 and 332 mg. per 100 cc.), increasing much in late pregnancy (average 415 mg.) and especially in pregnancy toxemias, falling in acute yellow atrophy of the liver even to undeterminable quantities. He also found high figures in pneumonia. In general, injury to the liver of severe degree decreases the fibrinogen content,[77] slight injury to the liver or injury to any other tissue usually increases the fibrinogen. The high figures for pneumonia (average about 1000 mg.,) drop rapidly to normal with an uncomplicated crisis, rising again if empyema or other infections exist. Severe hemorrhages cause a temporary fall in fibrinogen. Typhoid is one of the few infections with low fibrinogen.

The **coagulation time** determined by different methods, in which different conditions are presented, varies from 2 to 30 minutes; with most methods it is 5 to 8 minutes.[78] In general, coagulability is not constantly if at all altered by fever, cancer, diabetes, slight secondary anemias, or many other diseases, and in normal conditions it remains fairly constant. In infants the coagulation time is slightly shorter than in adults. The coagulation is hastened after considerable hemorrhages, in endocarditis, and perhaps in aneurism and thrombosis; and is commonly delayed in the acute exanthemata, in hemophilia, in *purpura neonatorum*, and occasionally in

[74] Arch. Int. Med., 1913 (12), 637.

[75] Lee and Vincent, Arch. Int. Med., 1915 (16), 59.

[76] Arch. Int. Med., 1924 (34), 201. See also Schultz, Nicholes and Schaefer, Amer. Jour. Path , 1925 (1), 101; McLester *et al.*, Arch. Int. Med., 1925 (35), 177.

[77] In dogs severe liver injury with $CHCl_3$ or CCl_4 may cause almost complete disappearance of fibrinogen.

[78] Full review and bibliography by Cohen, Arch. Int. Med., 1911 (8), 684 and 820.

some other diseases.[79] There is entire lack of agreement concerning the reputed acceleration of coagulation by oral administration of calcium salts, and retardation by citrates; and the supposed thromboplastic influence of gelatin cannot be shown consistently by direct observations. In jaundice, calcium salts probably have an effect, since here the cause of the deficient coagulation seems to be the fixation or precipitation of the blood calcium by the bile pigments. The bile salts also prevent the conversion of fibrinogen into fibrin.[57] It seems probable that the measurement of the time required for coagulation to take place *in vitro* does not exactly represent the tendency of the same blood to coagulate in the body of the person from whom it is obtained; for example, the injection of foreign serum has a notable effect in stopping hemorrhages, but the coagulation time of the recipient's blood is not correspondingly altered. Whipple's observation that with a low fibrinogen content the blood may coagulate in normal time, and yet the clots be too delicate to stop hemorrhage, explains at least part of the discrepancy; and of similar significance is the fact that with a very low platelet count the blood may coagulate as rapidly as normal, but the clots do not shrink and become firm (Duke). Hence even with a severe *purpura hemorrhagica* we may have a normal clotting time. In other conditions with normal coagulability, hemorrhages may result from excessive fibrinolysis which causes solution of the clot, especially in hepatic diseases.[80]

The Formation of Thrombi

If we apply the facts brought out in the preceding discussion relative to the factors in the coagulation of blood, to the manner and conditions under which thrombi are formed in the circulating blood, we find explanations for many of the features of thrombosis. Welch[81] describes the steps in the formation of a thrombus after injury to the vessel-wall, as follows: First, there is an accumulation of blood-platelets adhering to the wall at the point of injury. Leucocytes, which may be present in small numbers at the beginning, rapidly increase in number, collecting at the margins of the platelet masses and between them. Not until the leucocytes have accumulated does the fibrin appear. As Welch remarks, these findings afford no conclusive evidence as to whether fibrin-ferment is formed from the leucocytes or from the platelets, but since the fibrin does not appear until after the leucocytes have accumulated, and also since small thrombi may consist solely of platelets without fibrin, it seems probable that the leucocytes must be looked upon as the chief source of the ferment. If the blood is made incoagulable by injection of hirudin, injury to the vessel-walls causes the formation of thrombi composed entirely of platelets (Schwalbe). Sometimes small clots may form without the apparent participation of either platelets or leucocytes. These purely fibrinous thrombi seem to start from injured endothelial cells, particularly in inflammatory conditions, such as pneumonic lungs, and give the impression that the coagulin is derived from the endothelial cells. Zurhelle attributes by far the most important part to the platelets, an opinion shared by many, including Derewenko,[82] who holds that the coagulation of blood

[79] See Dochez (Jour. Exp. Med., 1912 (16), 693), who found some delay in coagulation in pneumonia. Corroborated by Minot and Lee, Jour. Amer. Med. Assoc., 1917 (68), 545.

[80] See Goodpasture, Bull. Johns Hopkins Hosp., 1914 (25), 330.

[81] Albutt's System, vol. 6, complete discussion of the general features of thrombosis; also see Küster, Ergeb. inn. Med., 1913 (12), 667; Hanser, Ergebnisse allg. Path., 1921 ($XIX_{(2)}$), 147. See Aschoff, Ziegler's Beit., 1912 (52), 205, and Arch. Int. Med. 1913 (12), 503, concerning the mechanics of thrombus formation.

[82] Ziegler's Beitr., 1910 (48), 123.

with entirely occluded vessels is quite distinct from true thrombosis because of the lack of platelets in stagnant blood. Clots formed in the absence of platelets do not shrink like proper thrombi (Duke).

The process of clotting in the stoppage of hemorrhage offers some differences from intravascular clotting, in that the coagulins of the tissue-cells also come into play. It is rather difficult to determine how much of the coagulation depends on these, and how much on the coagulins of the leucocytes, for the same conditions that favor liberation of tissue coagulins, *i. e.*, much laceration and destruction of the tissue, also favor the disintegration of leucocytes by offering large areas of surface for contact. Loeb is of the opinion, however, that of the two, the latter factor is the more important. It may be recalled that the joint action of tissue and blood coagulins is greater than the sum of their individual actions, which also must be an important factor in causing clotting in bleeding wounds.

As to the relative importance of stagnation and vessel injury in producing thrombosis, we know that total stasis in an uninjured vessel may not result in thrombosis, and, on the other hand, extensive injury or large calcified plaques in the intima of the aorta may also cause no thrombosis because of the rapidity of the blood flow; and, furthermore, clotting may occur even in intact vessels under the influence of substances liberating fibrin-ferment in the blood; *e. g.*, snake venoms, nucleoprotein injections, and possibly in disease. As the red corpuscles contain thromboplastic substances we may have thrombi formed when hemolytic agents are present in relatively stagnant blood, even without injury to the vessel-walls.[83] Presumably the clotting does not occur when the stream is rapid, because any fibrin-ferment that may be liberated by injured leucocytes or endothelium is swept away before fibrin can become attached to the vessel-wall; or, according to Howell's hypothesis, because the current brings an excess of antithrombin to the point where the thromboplastin is being formed. Naturally, the combination of an injured vessel-wall, a slow current, and a high coagulability offer the most favorable conditions, and we owe to Welch the appreciation of the fact that in a large proportion of all thrombi, even those caused by apparently purely mechanical agencies (*e. g.*, cardiac incompetence), bacteria are present and probably determine the injury to the vessel-walls and the liberation of fibrin-ferment.[84] We have previously referred to L. Loeb's observations on the effect of bacteria in causing coagulation of the blood.

Hyalin thrombi are frequently the cause of extensive degenerative lesions in the viscera, and although commonly formed of red corpuscles, they do not stain at all like normal corpuscles, presumably because a certain proportion of the hemoglobin has been altered or lost through hemolysis. Of particular interest is their reaction to Weigert's fibrin stain, by which they often, but not always, stain intensely, especially when hardened in Zenker's solution; a fact that has been the cause of much confusion in earlier studies. Flexner[85] first demonstrated the nature of these thrombi

[83] Deitrich, Cent. f. Path. (Verhandl.), 1912 (23), 372.
[84] Welch, Venous Thrombosis in Cardiac Disease, Trans. Assoc. Amer. Phys., 1900, vol. 15.
[85] Jour. Med. Research, 1902 (8), 316.

as originating from agglutinated red corpuscles, although Klebs, Ziegler, and others had earlier suggested that hyalin thrombi were formed from red corpuscles. Boxmeyer[86] independently arrived at the same conclusion as Flexner, in studying hyalin thrombi as the cause of necrosis in the liver of animals infected with the hog-cholera bacillus. Flexner produced hyalin thrombi by injecting corpuscles agglutinated by ricin, or by injecting ricin itself, or hemolytic substances such as ether or foreign serum. As the thrombi become old, the corpuscles lose their form and color and produce the typical hyalin appearance. Pearce[87] proved conclusively the dependence of the thrombus formation upon agglutination, for he secured the same results, including the liver necrosis, by injecting specific agglutinating serums. He states that fibrin threads may occasionally be found at the periphery of the larger thrombi, but never in the smaller ones. It is extremely probable, from Flexner's observations, that in the thrombosis produced by injecting various toxic substances into the blood, the so-called "*fibrin ferment thrombosis*," the thrombi are merely agglutinative thrombi, devoid of fibrin; this is undoubtedly true for many of the thrombi observed after poisoning with the powerfully agglutinative snake venoms (see Chap. vi). Bacterial hemagglutinins may also cause the formation of hyalin thrombi.[88] On the other hand, some, at least, of the hyalin capillary thrombi are undoubtedly composed of soft masses of fibrin which have not become fibrillar, although the successful staining by fibrin stain is not final proof of the fibrinous nature of a thrombus. The liver necrosis which follows ether injections in animals is caused by fibrinous thrombi which result from liberation of coagulins by the injured cells (L. Loeb).

Secondary Changes in Thrombi.—The changes that occur in thrombi after they have existed for some time are largely due either to ingrowth of new tissue or to calcification, the latter of which will be considered in a separate chapter. The only other change of interest from the chemical standpoint is the central softening which may occur in any large thrombus, but is particularly often observed in the large globular thrombi found in the heart. The center of the thrombus may be so completely softened that it resembles a sac of pus, the contents, according to Welch, consisting of necrotic fatty leucocytes, albuminous and fatty granules, blood-pigment and altered red corpuscles, and occasionally acicular crystals of fatty acids. Undoubtedly this softening is related to the process of fibrinolysis previously described, and depends upon digestion of the fibrin by leucocytic enzymes,[89] but the fact that the central portion alone undergoes softening is of interest, suggesting that the antibodies for leucocytic proteases, which Opie[90] found present in normal serum, prevent digestion at the surface of the clot. The same fact indicates that the tissue fibrinolysins[91] do not play an active part in softening clots.

Embolism

Emboli offer little of chemical interest, because of the purely mechanical nature of their origin and of the effects they produce.[92] An exception exists in the case of *fat embolism*, for the manner in which the fat is removed from the blood has invited considerable speculation.[93] Part of the fat is eliminated in the urine,[94] but the problem of how it escapes from the glomerular capillaries is not satisfactorily explained; large emboli undoubtedly lead to rupture of the capillary walls, and probably some fat also escapes through stomata or similar intercellular openings. Fat

[86] Jour. Med. Research, 1903 (9), 146.
[87] Jour. Med. Research, 1904 (12), 329; *ibid.*, 1906 (14), 541.
[88] Pearce and Winne, Amer. Jour. Med. Sci., 1904 (128), 669.
[89] Barker, Jour. Exp. Med., 1908 (19), 343.
[90] Jour. Exper, Med., 1905 (7), 316.
[91] See Fleisher and Loeb, Jour. Biol. Chem., 1915 (21), 477.
[92] Fat embolism may follow poisoning with potassium chlorate (Winogradow, Virchow's Arch., 1907 (190), 92).
[93] Full discussion by Beneke, Ziegler's Beitr., 1897 (22), 343.
[94] Discussed by Sakaguchi, (Biochem. Zeit., 1913 (48), 1) who finds a little fat in the normal urine. It may also be found in pyonephrosis (Rosenbloom, Jour. Lab. Clin. Med., 1923 (8), 621).

may also escape in the bile, and some is probably taken up by the tissue and endothelial cells by phagocytosis. Beneke found that the fat becomes partly emulsified by the mechanical action of the blood current, aided to a slight extent by saponification. The larger droplets after lodging in the capillaries are surrounded by leucocytes, to which Beneke ascribes an active part in the removal of the fat as fine droplets by phagocytic action. We may well believe, however, that the lipase of the plasma is an important agent in disintegrating the emboli, although its action is limited because of the relatively small surface which the large drops offer for attack. After fat droplets have been taken into the cells, they presumably are utilized in metabolism like normally acquired fat, as described previously. It may be of significance that lung tissue, where fat emboli first lodge, is particularly active in lipolysis of injected fats.[95]

The amount of fat free in the blood in fat embolism may be surprisingly large. Bissell[96] found from 2 to 6.5 per cent. in the venous blood of several typical cases, although sometimes figures within normal limits (0.2 to 0.6 per cent.) were found. The higher quantities represent such a great amount of free fat in the blood, even without considering the quantity held in the capillaries, that it is scarcely possible for it all to have come from the fractured bones. Fat normally present in the blood may form microscopically visible droplets, which may be mistaken for fat emboli.[97]

Air embolism presents some features of interest from the chemical standpoint, especially in those cases following sudden decrease in atmospheric pressure in persons who have been exposed for some time to pressures considerably higher than that of the atmosphere (diver's palsy, caisson disease, etc.). This form of air embolism is due to the fact that fluids can dissolve much more gas at high pressures than at low pressures; consequently when the abnormally great pressure to which divers, caisson workers, etc., are subjected is too suddenly reduced to that of the atmosphere, the excessive gas that was absorbed during the period of high pressure by the blood and tissue fluids is released, and forms bubbles in the tissues and blood. The bubbles in the nervous tissues may cause paralyses of various sorts, or death; those in the blood may, if in sufficient amount, cause serious or fatal capillary obstruction. The bubbles consist chiefly of nitrogen, because the power of the blood to hold oxygen in combination is so great that not much of this gas becomes freed.[98] The body fluids of normal persons contain about 675 c.c. of nitrogen, all told, but at 22 pounds pressure this is increased to 1,350 c.c., while but about 50 c.c. of free oxygen would be present (Langlois). Carbon dioxide is so readily combined in the blood that none is free even at high pressure, although McWhorter[99] reports that the gas collected from the right side of the heart in a fatal case contained 20 per cent. CO_2 and 80 per cent. nitrogen. Possibly some oxygen may also be released from solution during decompression.[1] At body temperature fats can dissolve five times as much nitrogen as serum or plasma,[2] which probably accounts for the severity of the changes in the nervous system with its rich lipoid content and delicate structure. Air embolism following obstetrical operations or surgical operations about the neck and chest presents chiefly mechanical

[95] Sicard *et al.*, Compt. Rend. Soc. Biol., 1923 (88), 564; Roger and Binet, *ibid.*, p. 1079.

[96] Jour. Amer. Med. Assoc., 1916 (67), 1926.

[97] See McKibben, Amer. Jour. Physiol., 1919 (48), 331.

[98] This subject is fully discussed by Leonard Hill in "Recent Advances in Physiology and Biochemistry," London, 1906.

[99] Amer. Jour. Med. Sci., 1910 (139), 373; Erdman, *ibid.*, 1913 (145), 520.

[1] Hill and Greenwood, Proc. Royal Soc. (B), 1907 (79), 284.

[2] Vernon, *ibid.*, p. 366; Quincke, Arch. exp. Path. u. Pharm., 1910 (62), 464.

features,[3] and large quantities of air must be present to cause dangerous obstruction to circulation.[4] Gas-bubbles may be produced in the blood soon after death by *B. aërogenes capsulatus*, but it is not probable that they are formed before death and cause air embolism.

INFARCTION

The changes that occur in infarcted areas are of much interest in connection with the study of autolysis, for the absorption of the necrotic tissue of aseptic infarcts is purely a matter of autolysis. Jacoby[5] found by ligating off a portion of a dog's liver, and keeping the dog alive for some time afterward, that in the infarcted tissues so produced, leucine and tyrosine could be detected, just as they are found in liver tissue undergoing autolysis outside the body. So, too, proteoses may appear in the urine when any considerable amount of tissue is cut off from its blood-supply. The processes of autolysis which occur in ordinary sterile infarcts are, however, extremely slow, and it is doubtful if enough of the products are ever in the blood or urine at any one time to be detected or to cause noticeable effects. For example, in an infarct of the kidney which was known to be almost exactly fourteen weeks old, there still remained a layer of necrotic cortex one millimeter thick, quite unabsorbed during this time. If we examine such aseptic infarcts in various stages, we get the impression that the digestion is accomplished by leucocytes acting on the periphery of the infarct, and not entering the dead area deeply, presumably because of a lack of chemotactic substances in the dead cells. On the other hand, it seems probable that the tissue enzymes themselves play an important part in the autolysis, for if we imp ant into animals pieces of tissue in which the enzymes have been destroyed by heating to boiling, it will be found that the cells and their nuclei remain unaffected for many weeks; whereas if sterile unheated pieces of tissue in which the enzymes are still active are implanted, they lose their nuclear stain and begin to disintegrate relatively soon, without apparent participation by the leucocytes.[6] Ribbert[7] found that in experimentally produced anemic infarcts in the kidneys of rabbits the nuclei retain their staining property well for nearly twenty-four hours, becoming then small and deeply stained, undergoing karyorrhexis, and in large part disappearing from the convoluted tubules inside of forty-eight hours, although some nuclei may persist in the glomerules for three or more days. In human infarcts, Ribbert believes, the process goes on faster, for he has observed here a loss of nuclei within twenty-four hours. These nuclear changes undoubtedly depend upon autolysis, but it is probable that the enzymes concerned reside in the cytoplasm rather than in the nucleus for I have observed that cells of lymphoid type, with practically no cytoplasm, generally retain their nuclear stain much longer than cells with more cytoplasm;

[3] Review of literature by Wolff, Virchow's Archiv., 1903 (174), 454.
[4] See Hare, Amer, Jour. Med. Sciences, 1902 (124), 843.
[5] Zeit. physiol. Chem., 1900 (30), 149.
[6] Wells, Jour. Med., Research, 1906 (15), 149.
[7] Virchow's Arch., 1899 (155), 201.

this is particularly noticeable in splenic infarcts, where the Malpighian corpuscles retain their staining affinities much longer than the pulp elements. Whether the destruction of the nuclei is accomplished by the ordinary intracellular proteases, or by special nucleoprotein-splitting enzymes (nuclease, etc.), remains to be determined. It is quite possible, however, that the first changes consist of a splitting of the nucleoproteins of the nucleus by the autolytic enzymes, liberating the nucleic acid, which gives the nuclei the characteristic intense staining with basic dyes (*pycnosis*) observed in areas of early anemic necrosis. The nucleic acid may then be further decomposed by the nuclease or similar enzymes. Taken all together, then, it would seem that the nuclear and cellular alterations that make up the characteristic picture of anemic necrosis are brought about by the intracellular enzymes—an autolytic process. The removal of the dead substance, however, seems to be accomplished rather by the invading leucocytes, through heterolysis. The relatively small part taken by the intracellular enzymes may possibly be due to the seeping through them of alkaline blood-plasma, for autolytic enzymes are not active in an alkaline medium; the leucocytic enzymes, however, act best in an alkaline medium.[8]

About the periphery of infarcts is usually observed more or less fat deposition (Fischler),[9] particularly in the endothelial cells (Ribbert). This is not peculiar to infarcts, however, for Sata[10] found a similar peripheral fatty metamorphosis common to all necrotic areas. The basis of this is possibly the persistence of the cell lipase, which synthesizes fatty acid and glycerol diffusing into the necrotic area with the plasma, unchecked by normal oxidative destruction of these substances. (See "Fatty Degeneration," Chap. XVIII.)

Hemorrhagic infarcts offer in addition to the changes common to anemic infarcts, the alterations occurring in the blood-corpuscles. Panski[11] found that after ligation of the splenic vein of dogs the red corpuscles begin to give up their hemoglobin in about three hours. After twelve hours fibrin formation begins in the tissues, the corpuscles continue to give up hemoglobin and become cloudy in appearance. Later, iron-containing pigment is formed in the cells beneath the capsule, but in the deeper tissue even the iron normally present in the spleen tissue seems to disappear;[12] this possibly depends upon the fact that pigment reacting for iron, hemosiderin, is formed only in living cells under the influence of oxygen, or it may be that acids formed during autolysis dissolve it. During autolysis *in vitro*, however, Corper[13] found no evidence of removal of iron from insoluble or coagulable compounds. The hemolysis is probably produced either by the action of autolytic products, which are notoriously hemolytic, or perhaps also by direct attack of tissue and blood proteases upon the corpuscles.

Other retrogressive changes that may occur in infarcts, such as septic softening and calcification, are not greatly different from the same processes occurring in other conditions, and will be considered with the discussion of these processes.

[8] More fully discussed by Wells,[6] *loc. cit.*, and under necrosis, Chap. XVII.
[9] Cent. f. Path., 1902 (13), 417.
[10] Ziegler's Beitr., 1900 (28), 461.
[11] "Untersuchungen über den Pigmentgehalt der Stauungsmilz," Dorpat, 1890.
[12] See also M. B. Schmidt. Cent. f. Path., 1908 (19), 416.
[13] Jour. Exper. Med., 1912 (15), 429.

CHAPTER XVI

EDEMA[1]

It was formerly considered that the term edema indicates the excessive accumulation of lymph (which may be either normal or modified in composition) in the cells, intercellular spaces, or serous cavities of the body, and on this basis the problems of edema are closely connected with the consideration of the processes of physiological formation and removal of lymph. If by "lymph" is meant only the fluid found free in the lymph vessels and cavities of the body, then lymph formation constitutes but a subsidiary feature of edema, for the intracellular accumulation of fluids is in many forms of edema the most important stage of the process, and, except perhaps in inflammatory edema, the free edema fluid found in the spaces of the body may represent merely fluid that has been extruded from edematous cells. The principle that the primary site of edema lies in the tissues rather than in the vessels or the fluids of the body, as emphasized particularly by Martin Fischer,[2] has received striking support from the observations of McClure and Aldrich[3] who have found that intradermally injected salt solution disappears much more rapidly in patients with general edema, or in local areas of edema from various causes, than it does in normal conditions. This fact, which has been repeatedly verified, is in harmony with the view that edema occurs because the tissues have a heightened affinity for water. Hence in edema the fluid may accumulate in the cells, with or without the presence of an excess of free "lymph," and lymph formation is not an essential feature of edema. Nevertheless, because of its historical relation to the study of edema, and because there usually is an increased amount of lymph present in edematous tissues, constituting commonly the most obvious, although probably not the most important aspect of edema, and also because of the relation of lymph formation to some aspects of edema, we may properly begin this chapter with a discussion of

THE FORMATION AND REMOVAL OF LYMPH[4]

For many years the study of these processes has been a favorite field of investigation by physiologists, and the great battle-place of the "vitalistic" and "mechanistic" schools; and to this day the forces that deter-

[1] Early bibliographies are given by Meltzer, American Medicine, 1904 (8), 19 *et seq.;* also by Klemensiewicz, in Krehl and Marchand's Handbuch d. allg. Path., 1912, II (1), 341. More recent reviews are given by Leo Loeb, "Edema," Medicine Monographs, III, 1923; McLean, "Endocrinology and Metabolism," 1924 (4), 153.

[2] See "Oedema and Nephritis," M. H. Fischer, first Ed., 1910; 3d Ed. 1921.

[3] Jour. Amer. Med. Assoc., 1923 (81), 293; 1924 (82), 1425; see also Baker, *ibid.*, 1924 (83), 1566.

[4] Review by Schulz, Handb. d. Biochem., 1923 (4), 143.

mine the formation of lymph and its subsequent absorption have not been completely understood. By the application of the principles of physical chemistry to the problem, however, great advances have been made, which seem to render our understanding of both lymph-formation and its pathological accumulation in the tissues much clearer and more nearly accurate than they were before. Before discussing the physiological formation of lymph, however, we first must consider:

The Composition of Lymph.—Lymph consists of material derived from two chief sources. The greater part consists of fluid passing out of the capillaries into the tissue spaces; here it is modified by the addition of products of metabolism derived from the tissue-cells, and by the subtraction of materials that the cells utilize in their metabolism. It is, therefore, essentially a modified blood plasma, and the modifications the plasma undergoes are so slight, that, under ordinary conditions, lymph shows on analysis no considerable differences from blood plasma, except a relative poverty in proteins, due chiefly to the relative impermeability of the capillary walls for colloids. Its quantitative composition varies greatly, depending upon the conditions under which it is collected, whether during activity or rest, etc. The following tables of analyses have been collected by Hammarsten:

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0.4	2.2
Protein	42.7	42.8	34.7	
Fat, Cholesterol, Lecithin	3.8	9.2	.....	35.0
Extractive bodies	5.7	4.4	.....	
Salts	7.3	8.2	7.2	7.5

1 and 2 are analyses of lymph from the thigh of a woman, 3 is from the contents of sac-like dilated vessels of the spermatic cord, 4 is lymph from the neck of a colt.

Chyle differs from lymph chiefly in the presence of large quantities of fat; during starvation the lymph and the chyle are of practically the same composition.

Normal lymph contains much less fibrinogen than does the blood plasma, and hence coagulates slowly. Lipase and other enzymes have been found in the lymph, as in the plasma. Under pathological conditions the lymph may be greatly altered, becoming poorer in solids under some conditions of edema, and becoming rich in proteins and blood-corpuscles under inflammatory conditions, until it partakes of the characteristics of an inflammatory exudate (see analyses of transudates and exudates).

An important fact to consider is, that *of the entire water of the body but about one-tenth is in the blood.* About two-thirds of the entire weight of the body is water, which is mostly in the cells and tissues, firmly bound by the colloids, only an unknown but smaller portion being free movable fluid, and even here always associated with more or less colloid. A body weighing 60 kilos will, therefore, have 40 kilos of water, of which but about 4 kilos is in the blood, and most of this is probably not free, but firmly united to the colloids of the blood.

Different investigators have recognized the existence of various possible factors or mechanisms in lymph formation, and have interpreted or valued them differently, so that various theories have been developed, which are discussed in the following paragraphs:

Filtration Theory.—The simplest possible conception of lymph formation is that it is merely the result of filtration of the liquid constituents of the blood through the capillary walls under the influence of the blood pressure. This "filtration theory" was supported origi-

nally by Ludwig, and it was a prominent factor in the early applications of mechanical principles to biological processes. In support of this theory were advanced the results of numerous experiments in which it was shown that increasing the blood pressure by means of ligating the veins, or by causing arterial dilatation, resulted in an increase of the lymph flowing out of the lymph-vessels of the part. Also, when the blood pressure is raised by epinephrine or by other means, a large proportion of the fluid leaves the blood vessels; conversely, when the blood pressure is suddenly lowered by hemorrhage there is a rapid passage of fluid from the tissues into the blood. The experimental results were not always favorable to the theory, however, particularly in the experiments in which blood pressure was raised by arterial dilatation; often the flow of lymph was little increased, even when the arterial flow and pressure were greatly increased. Furthermore, *some of the most extreme edemas are seen in tissues in which the arteries have been occluded, leading to extremely low blood pressure in the area in which the edema develops.*

Nevertheless, the filtration theory held for many years, not only as an explanation of lymph formation, but also as an explanation of urinary secretion and of the secretion by other organs. However, after a time it became clear that filtration alone could not account for all the phenomena of secretion. For example, in many lower forms with undeveloped circulatory systems, and almost no blood pressure, secretion goes on vigorously; the pressure of glandular secretions may be much higher than the blood pressure within the capillaries; the activity of secretion is by no means in proportion to blood pressure, etc. If in glandular secretion, therefore, fluids are removed from the blood and transferred into an excretory duct through the action of some force other than that of the blood pressure, it is probable that lymph formation is equally independent of blood pressure. On this basis Heidenhain advanced his—

Secretory theory of lymph formation, in which he suggested that lymph is the product of an active secretion by the endothelial cells of the capillaries, just as saliva is the product of the activity of the glandular cells. He showed that certain chemical substances may stimulate lymph flow, independent of blood pressure, just as pilocarpine and other drugs may stimulate the secretion of saliva. These lymph-stimulating substances, which he named *lymphagogues*, fall into two distinct classes. One, which includes such substances as peptone, leech extract, strawberry juice, extracts of crayfish, mussel or oysters, and numerous other tissue extracts, are characterized by causing the secretion of a lymph which is rich in proteins, even richer in proteins than the blood plasma; and, furthermore, there is no simultaneous increase in urinary secretion. Heidenhain considered that these substances caused lymph secretion by stimulating the capillary endothelium in a specific manner; as they caused no appreciable rise in blood pressure the increased lymph secretion certainly could not be attributed to filtration. That lymph flow does not depend on blood pressure is most conclusively shown by *postmortem lymph secretion;* for example, Mendel and

Hooker[5] observed lymph flow for four hours after death, in a dog that had received an injection of peptone eight minutes before being killed.[6]

The *second class* of lymphagogues includes crystalloidal substances, such as sugar, urea, and salts.[7] Lymph secreted under the influence of these substances is poorer in protein than ordinary lymph, and at the same time an increased urinary secretion is produced. With these crystalloidal lymphagogues the amount of effect is in inverse proportion to their molecular weight, which means that their effects depend upon the number of molecules in solution rather than upon their nature; in other words, the stimulation of lymph by crystalloids is dependent upon the osmotic pressure of the crystalloids, and they must be injected in hypertonic solution to produce their effects.[8] Heidenhain explained their action as follows: The crystalloids are secreted into the lymph-spaces by the action of the capillary endothelium, and there, owing to their raising osmotic pressure, cause a flowing of water out of the vessels. The difficulty here is to explain why the crystalloids while still in the vessels do not attract the fluids from the lymph-spaces into the blood, and so cause rather *lessened* lymph secretion.

While admitting that in pathological conditions (*e. g.*, passive congestion) pressure and filtration *may* play an important part, Heidenhain considered that an active secretion by the endothelial cells is the chief factor in the normal formation of lymph. The means by which the cells perform this function was unknown; it was considered an example of "vital activity," Heidenhain meaning by this term such chemical and physical forces of living cells as are unknown or not understood at the present time, rather than any metaphysical conception of living matter, such as many vitalists assume.

Other observers, corroborating Heidenhain's results for the most part, have modified, or amplified his theory. Asher and his collaborators, for example, ascribe the work done in causing lymph formation to the cells of the various tissues and organs, rather than to those of the capillary wall. The increased flow of lymph from the salivary gland that occurs during its activity they consider due to the work of the gland cells, and its function the removal of products of metabolism. The action of such a lymphagogue as peptone they ascribe to its stimulation of cellular activity, particularly in the liver, where it causes an increased formation of bile. Gies[9] and Asher also observed that after an injection of crystalloidal lymphagogues, such as sugar, a prolonged flow of lymph occurred after the death of the animal, proving completely that such lymphagogic action is independent of blood pressure. From what we now know concerning

[5] Amer. Jour. of Physiol., 1902 (7), 380.

[6] A fact not sufficiently taken into account is that blisters filled with serum, *i. e.*, an inflammatory edema, may be produced in dead bodies by burns or scalds. (See Leers and Raysky, Virchow's Arch., 1909 (197), 324).

[7] The action of many other substances has been investigated by Yanagawa, Jour. Pharmacol., 1916 (9), 75.

[8] See Jappelli, Arch. sci. biol., 1920 (1), 193; Beck, Johns Hopkins Hosp. Bull., 1924 (35), 206, (also discusses the forces responsible for lymph flow).

[9] Amer. Jour. Physiol., 1900 (3), p. xix; Zeit. f. Biol., 1900 (40), 207.

the effect of crystalloids on colloidal solutions, it seems probable that lymphagogues of the second class act by altering the colloidal state of the plasma and tissues, so that water is set free.[10]

Potocytosis.—In explanation of the process by which the cells, whether endothelial or tissue-cells, pass fluids through themselves from one place to another, Meltzer[1] has made an interesting suggestion, as follows: Considering the property of endothelial cells to act as phagocytes, MacCallum[11] has shown that solid granules (*e. g.*, coal pigment, carmin) are taken through the walls of the lymphatics by the phagocytic activity of their endothelial cells.[12] Meltzer suggested that in a similar way the endothelial cells may transport through the vessel-walls not only solid particles, but also, by the same mechanism, substances in solution; and for this hypothetical process he suggested the name "*potocytosis.*" There can be little question that cells do take up substances in solution, and sometimes this is done in an apparently selective manner; *e. g.*, the taking up of bacterial toxins and vegetable poisons in the peritoneal cavity by the leucocytes. Presumably the mechanism of "potocytosis" is not different from that of phagocytosis, chemotactic forces determining the occurrence of the process. No experimental evidence has been advanced as yet for this very plausible hypothesis.

Permeability of Capillaries.—In explanation of the variability in the amount and composition of the lymph Starling[13] introduced the factor of altered permeability of the capillary walls, which presumably depends upon the number and size of the pores. He found that normally the lymph coming from the lower extremities contains only 2 per cent. to 3 per cent. of proteins, while lymph from the intestines contains 4 per cent. to 6 per cent., and lymph from the liver contains 6 per cent. to 8 per cent. of proteins; hence he considers that the liver capillaries are the most permeable, *i. e.*, have the largest pores, so that more of the large colloid molecules can escape from them. The effect of lymphagogues of the first class (peptones, etc.) he attributes to their poisonous properties, and the consequent injury to, and alterations in, the capillary wall. The crystalloidal lymphagogues, he believes, act by first attracting fluids from the tissues into the blood with a resulting "hydremic plethora," which in turn leads to increased blood pressure and consequent filtration of a watery fluid out of the vessels. He considers, therefore, that the amount and quality of the lymph produced in any part are determined solely by two factors, the intracapillary blood pressure and the permeability of the capillary walls.

In connection with this question of the permeability of the capillary walls, Meltzer suggested that the contractility and irritability of the endothelium may be a potent factor in determining the size of the pores in the capillary walls. When in a tonic condition, the endothelium is firmly contracted about the pores, keeping their size small; when the endothelial cells become relaxed by any cause, such as poisons, high blood pressure, poor nourishment, etc., the pores are enlarged, and increased escape of fluid results.[14] It must be borne in mind, however, that most histolo-

[10] See Fischer, loc. cit.[2]; also Meyer, Klin. Woch., 1924 (3), 1352.
[11] Johns Hopkins Hosp. Bull., 1903 (14), 1.
[12] See also Cunningham, Amer. J. Physiol., 1922 (62), 248.
[13] Lancet, 1896 (i), May 9, *et seq.*
[14] Evidence of the contractility of capillary walls is discussed by Krogh, Jour. Physiol., 1919 (52), 457; also "The Anatomy and Physiology of Capillaries," August Krogh, Yale Univ. Press, 1922.

gists do not now admit that capillary walls contain pores. Krogh has shown that when the capillaries are widely distended their permeability is much increased, not only for substances in solution but also for particles as large as soluble starch particles ($5\mu\mu$). Cunningham[15] holds that despite these observations the permeability of the endothelium is entirely a factor of the cytoplasm. However, it does not seem to have been proved that there are no open spaces or pores between the endothelial cells in capillaries distended by pressure.

M. H. Fischer holds that the endothelial cells undergo changes in consistency through changes in the amount of water held by the cell colloids; especially under the influence of acids the endothelium may become much more fluid and of greater permeability. Adolf Oswald[16] says that the normal capillary wall is somewhat permeable for the less viscous blood proteins (albumin and pseudoglobulin), and in inflammation this permeability becomes increased so that the more viscous euglobulin and fibrinogen can pass through.

Osmotic Pressure.—Still another possible factor in causing fluid to leave the vessels is osmotic pressure. Heidenhain refers to this cause the transudation produced by crystalloid lymphagogues, although in a rather unsatisfactory manner. As a result of the more recent studies of physical chemistry, and its application to biological processes, we have learned to appreciate the possible importance of osmotic pressure in cell activities, and in the question of lymph formation it occupies a particularly important place. We may consider it as follows: In the blood we have certain proportions of readily diffusible crystalloids and of non-diffusible colloids. If no metabolic processes were going on in the tissues, the diffusible substances would pass through the vessel-walls until an osmotic equilibrium is established in the tissues and in the blood. As a matter of fact, however, the blood proteins are not absolutely non-diffusible, but small quantities do pass through the capillary walls, and so lymph under such a hypothetical condition would consist of a mixture of the same osmotic concentration as the blood plasma, with about the same proportion of crystalloids, but a smaller proportion of proteins; this, it will be noticed, is just the composition of normal lymph. During life, however, the cells of the tissues are causing metabolic changes in the extra-vascular constituents, and these changes consist chiefly in breaking down large molecules of proteins, carbohydrates, and fats into much smaller molecules. Now the osmotic pressure of a solution is dependent upon the *number* of molecules and ions it contains, hence by breaking down these few large molecules with very little osmotic pressure into many small molecules, the osmotic pressure in these cells and tissues becomes raised above that of the blood-vessels, and consequently water flows out of the vessels because of the increased pressure. We see here the probable explanation of the stimulating influence of metabolic products upon the formation of lymph, noted by Hamburger, Heidenhain, and others. For suggesting

[15] Amer. Rev. Tuberc., 1924 (9), 491. Review on endothelial permeability.
[16] Zeit. f. exp. Path., 1910 (8), 226.

and urging the importance of osmotic pressure in the formation of lymph we are indebted particularly to Heidenhain, v. Korányi,[17] J. Loeb,[18] and Roth.[19] Loeb shows very clearly the relative greatness of the water-driving force of osmotic pressure as compared to that of blood-pressure, by his statement that the osmotic pressure of a physiological salt solution is about 4.9 atmospheres, which is *twenty times as great as the blood pressure* with which we have to do in ordinary physiological experiments. In varying osmotic conditions we may readily see an explanation for the increased lymph flow that occurs during tissue activity; namely, it may be due to the increased formation of metabolic products. Many of the lymphagogues may act similarly by stimulating metabolic activity, with resulting increase in the formation of osmotic pressure-raising products of metabolism in the organs; *e. g.*, the increased lymph flow from the thoracic duct that follows stimulation of hepatic activity by injection of peptone (Heidenhain). As we shall see later in considering edema, osmotic pressure may play an important part in the pathological formation of lymph. It must be admitted, however, that there are many difficulties in the way of accepting unqualifiedly the original views as to the importance of osmotic pressure in lymph formation.[20] For example, the lymph contains more chlorides and may have a much higher osmotic pressure than the serum of the same animal (Hamburger, Carlson, *et al.*).[21]

Such conditions, however, may be explained by the Donnan equilibrium, which has been presented in Chapter I. By his studies, which indicate that proteins combine with acids and alkalies to form true ionizable salts, J. Loeb has not only been able to furnish support for Procter's theory of swelling as based on the Donnan equilibrium, but has also been able to show that the potential differences across a membrane separating a solution of a protein salt from pure water, fully support Donnan's theory. He says that the reason that osmotic pressure, viscosity of protein solutions, and the swelling of protein gels are all influenced in a similar way by electrolytes, is that all three properties are in the last analysis functions of one and the same property, namely, osmotic pressure.[22] In the body the capillary wall, being impermeable to protein, gives rise to a Donnan equilibrium and in support of this is the fact that the tissue fluids contain more chloride than the blood.[23] Especially significant is the observation that when ascites fluid and the serum of the patient are

[17] Zeit. f. klin. Med., 1897 (33), 1; 1898 (34), 1.

[18] Pflüger's Arch., 1898 (71), 457.

[19] Englemann's Arch., 1899, p. 416.

[20] Gunzberg (Arch. néerland physiol, 1918 (2), 364) has advanced the theory that the passage of water into the intercellular spaces is due to the electrical properties of the membrane separating the circulating fluid from the tissues. The element potassium and the ions H and OH play an important part in this electrical osmosis which is able to drive the fluid in the opposite direction to osmotic pressure. Thus, a dialyzing sack containing Ringer solution minus K immersed in Ringer solution loses weight. Perfusion of frogs with Ringer solution minus K produces marked edema.

[21] Amer. Jour. Physiol., 1907 (19), 360; 1908 (22), 91.

[22] An explanation of the Donnan equilibrium, which does not depend on osmosis, has been advanced by Fodor and G. H. Fischer, Kolloidch. Beihefte, 1923 (18), 129.

[23] See R. Loeb, Atchley and Palmer, Jour. Gen. Physiol., 1922 (4), 591.

separated by a membrane there is no alteration in the original composition, showing that in the living body the capillary walls have accomplished the same equilibrium as the collodion membranes produce *in vitro*. According to Lehmann[24] the composition of the aqueous humor, when compared with that of the blood, is just what would be expected on the basis of Donnan's equilibrium, an increase of protein in the fluid being accompanied by a decrease in the chlorine ion and an increase in the H-ion concentration.

Variable Capacity of Colloids for Water.—Colloids of the type of the tissue proteins, *i. e.*, hydrophil colloids, imbibe water with great avidity, until a certain proportion of water is present, the proportion varying under different conditions. The importance of this force in the production of edema and related processes was first pointed out by Martin H. Fischer, and has been developed extensively by him.[2] The amount of water which a given hydrophil colloid, such, for example, as gelatin, or fibrin, will take up, is greatly modified by the reaction of the solution and by its content of electrolytes. Very small concentrations of acids or alkalies will greatly increase the amount of water absorbed, while salts reduce it, and the different acids and salts vary in their effects; thus, hydrochloric acid causes a greater swelling of colloids than a corresponding strength of sulfuric acid, and calcium chloride depresses the swelling more than potassium chloride.[25] The effect of the salts is made up of their constituent ions. Non-electrolytes have relatively little effect. The forces developed by this affinity of colloids for water are enormous; thus, to prevent the taking up of water by starch requires a pressure of over 2500 atmospheres, dried gelatin will take up 25 times its weight of water, and fibrin as much as forty times. Different colloids differ greatly in their affinity for water and in the way in which this affinity is modified by electrolytes, and change in a colloid may greatly alter its capacity for swelling; thus, β-gelatin, which can be formed from ordinary gelatin by the action of proteolytic enzymes, has greater capacity for swelling than the original gelatin. Gies especially lays stress on this factor, that is, the alterations of the hydrophilic tendencies of the tissue colloids by enzymes.[25] In the plant world we find striking examples of this character; thus, the succulence of some plants results from the conversion of poly-saccharides with little hydration capacity into hydrophilic pentosans and mucilages.[26]

On the basis of the facts briefly summarized above, the proportion of water present in any cell or in any fluid of the body which contains colloids is assumed by Fischer to be determined by certain factors, namely (1) the character of the colloids themselves; (2) the proportion and nature of acids or alkalies present in the fluids in and about the colloids; (3) the proportion and nature of the salts. All these factors are changeable, and therefore the amount of water present in the cell or fluid varies

[24] Klin. Woch., 1924 (3), 1028; Arch. ges. Physiol., 1924 (205), 210.

[25] A definite and clear-cut example of the swelling of a tissue under the influence of acid of metabolic origin is shown in the muscle cell in Zenker's waxy degeneration (Wells, Jour. Exper. Med., 1919 (11), 1).

[26] MacDougal and Spoehr, Plant World, 1918 (21), 245.

accordingly. Thus, if a cell through its metabolism develops from such a non-electrolyte as sugar (which has no considerable effect on the water content of the protoplasm), an organic acid, such as lactic acid, which has a large effect in increasing the affinity of the colloids for water, the cell will, presumably, take on more water, perhaps to a degree to cause intracellular edema. The acids diffusing from the cell into the intercellular spaces or into the lymph will cause equally well an increased affinity for water in the colloids here present, leading to intercellular edema. Conversely, neutralization of acids present in a colloidal solution, by alkaline salts brought by the blood, will decrease the affinity of the colloids for water which will escape from the colloids as they shrink.

This theory, which introduces a formerly unappreciated factor into the considerations of lymph formation and edema, is of the utmost importance. It practically eliminates osmotic pressure, (unless colloidal imbibition be a manifestation of osmosis), also the cell membranes so essential for the efficiency of this force, and in view of the difficulties that have arisen in trying to fit the cell membrane hypothesis and osmotic pressure to many facts of normal and pathological biology, an alternative hypothesis is welcome. As pointed out above, the forces involved in the swelling of colloids are so large as to be of great significance, and the amounts of electrolytes necessary to cause considerable variations in colloidal swelling are not more than can be present under normal and pathological conditions; consequently the possible influence of colloidal swelling must be taken into account in all consideration of pathological processes. Whether or not it is capable of as universal application as Fischer maintains, remains to be demonstrated. Certainly we cannot entirely exclude the influence of osmotic pressure as now understood, since according to the work of Procter and J. Loeb this is the basis of the swelling pressure of colloids.

The hydrophilic force of proteins is, according to J. Loeb, chiefly a result of the unequal distribution of the ions in the Donnan equilibrium. The observed osmotic or swelling pressure results from the difference in the osmotic pressure in the inner and outer solutions. The greater the quantity of ions bound to proteins, the greater will be this pressure and, as shown by Loeb, this is a function of the H-ion concentration. Being practically zero at the isoelectric point, at which there are no ions bound to the protein, the more the H-ion concentration of a protein solution deviates from the isoelectric point the more binding of ions and the greater the osmotic or swelling pressure of a protein solution. Because the *p*H of blood plasma is on the alkaline side of the isoelectric point of the proteins, cations and not anions are united to the proteins. At the isoelectric point no ions are bound by proteins and hence they exert scarcely any noticeable swelling pressure. As serum albumin has a lower isoelectric point than the globulin or fibrinogen, at the *p*H of plasma the albumin will exert more pressure than the other proteins, and so the higher the fibrinogen and globulin content, the less the osmotic- or swelling-pressure of the plasma.

One feature of colloidal swelling that is entirely out of harmony with the idea that acids are ordinarily responsible for tissue swelling, is the fact that the closer to the isoelectric point a colloid is the less water it will take up. Now, as pointed out by Loeb, since the fluids in the body are always more alkaline than the isoelectric points of the chief proteins of the cells, the blood, and the lymph, any addition of acid should *reduce* the amount of cations bound by the proteins and hence reduce their swelling capacity. This, of course, is not in harmony with the Fischer theory, but it must also be admitted that it is not in harmony with the numerous observations that when tissues are made more acid they *do* swell. For example, the red corpuscles are measurably increased in diameter in acidosis, and even the change in *p*H between arterial and venous blood is sufficient to produce a distinct increase in their size.[26a]

At this writing a satisfactory solution has not yet been agreed upon for these various discrepancies, but in any event it may be said that variations in the hydrophilic tendency of colloids, however brought about, is a fundamentally important conception in considering the phenomena of edema.

Summary.—We see from the above discussion that numerous theories have been advanced to explain the normal formation of lymph, and as their basis exist several different possible factors. Filtration, active secretion by the capillary endothelium, attraction by the tissue cells, osmosis in response to formation of crystalloids outside the vessels, and changes in the affinity of colloids for water; all have been shown to be possible causes of lymph formation. It is highly probable that in a certain way all are involved, particularly if we accept the view of the physical school that "secretion" and "attraction" by the cells are merely the outcome of physical forces; the causes of lymph formation then reduce themselves to absorption, filtration and diffusion. It is generally accepted that at least some of the lymph does escape from the vessels through simple filtration, for the pressure inside the capillaries is presumably greater than outside, the capillary walls are not water-tight and they are not impermeable to the substances dissolved in the plasma. Likewise, osmotic exchanges surely go on between the vessels and the tissue-cells, and the conditions which determine the water content of our colloid solutions constantly vary. The question that remains is, do these few factors account for all of the lymph formation, and are they sufficient by themselves to explain the physiological regulation and the pathological variations in the lymph flow? They are purely physical or mechanical causes, and the "vitalist" school will claim that they are inadequate and that "vital activities" of the cells play the deciding rôle. But at present the evidence that is being accumulated seems to point more and more strongly to the conclusion that these "vital activities" are but the result of simple well-known physical forces acting under very complex conditions—complex because of the large number of different chemical compounds occurring together, and the varying influence of circulation, food supplies, cell structure, etc.

[26a] Weichmann and Schürmeyer, Deut. Arch. klin. Med., 1925 (146), 362.

ABSORPTION OF LYMPH

By no means all the fluid that escapes from the vessels, nor all the products of cell metabolism, are carried away in the lymph—a considerable and perhaps the greater part of them is absorbed back into the capillaries directly. A classical proof of this is the experiment of Magendie, who observed that if poisons were injected into the leg of an animal, which had been separated from the body entirely except for the blood-vessels, that poisoning developed in the usual manner. In such experiments the lymph-vessels are severed and probably largely occluded; hence it does not solve the question as to whether substances are absorbed by the blood-vessels under normal conditions. Orlow found, however, that during absorption of fluid from the peritoneal cavity there is no perceptible increase in the lymph flow from the thoracic duct. Addition of sodium fluoride, a protoplasmic poison, was found to interfere with this absorption, for which and other reasons Heidenhain and Orlow considered that the absorption depended upon the "vital activity" of the cells.[27] More nearly reproducing normal conditions were the experiments of Starling and Tubby, who found that methylene-blue or indigo-carmine injected into the pleura or peritoneum appeared in the urine long before it colored the lymph in the thoracic duct.[28] Adler and Meltzer found evidence, however, that not all the absorption is accomplished by the blood-vessels, for obstruction of the thoracic duct retards absorption. That the absorption is not dependent solely upon the circulation and blood pressure is shown by the fact that absorption from the peritoneal cavity occurs in dead bodies (Hamburger, Adler and Meltzer).

The nature of the mechanism by which fluids are taken into the blood-vessels is still unknown. We can easily understand the entrance of injected poisons and coloring-matters from the tissues into the blood, because they are more concentrated at the point of injection than in the blood, hence they may diffuse directly through the capillary wall. Likewise we can understand the diffusion of water from a hypotonic solution into the blood, but how a solution of the same concentration as that of the blood can enter the blood is difficult to explain. Cohnstein and also Starling attribute this absorption to the proteins of the blood, in the following manner: After a fluid is injected into the tissues or serous cavities there occurs a diffusion exchange between this fluid and the blood, until the concentration of the crystalloids in each is equal; but the proteins of the blood cannot diffuse, and as they exert a positive although very slight osmotic pressure, this difference in osmotic pressure in favor of the blood causes diffusion of the extravascular fluid into the blood. Fischer would ascribe the passage of fluid to the relative affinity of the colloids of the blood and of the tissues for the fluid, and this would be towards the blood whenever the blood colloids had, from whatever possible cause, a greater affinity for the fluid than the tissue colloids. The application of the Donnan equilibrium, however may clear up this problem.

[27] See also Hara, Biochem. Zeit., 1921 (126), 281.
[28] See Mendel, Amer. Jour. Physiol., 1899 (2), 342.

Passage of the fluid from the tissues into the lymph stream was very easy to understand in the light of the older conception of the lymphatic circulation, namely, that the lymph-vessels were merely continuations of the interstitial spaces; we could then assume that as soon as the fluid left the blood-vessels it was practically within the lymphatic system, and was crowded along the lymphatic channels by the *vis a tergo*, aided by the valves of the lymph-vessels and the intrathoracic vacuum. But it now seems, particularly through the studies of MacCallum,[29] that the lymphatic vessels form a closed system, not in communication with the interstitial spaces. This being the case, we have to explain the passage of the lymph through the walls of the lymphatic vessels, and this is a problem which is not by any means a simple one, and which has yet to be investigated. It is significant that the thoracic lymph has a higher osmotic pressure than the blood of the same animal (Luckhardt),[30] so that the lymph which enters the duct must do so against the osmotic pressure. There is evidence that in the serous cavities, at least. absorption does take place through pores.[31]

THE PATHOGENESIS OF EDEMA

With the facts and hypotheses mentioned in the preceding paragraphs in mind, we may consider their bearing on the production of abnormally large accumulations of fluid in the tissues, that is, edema. We can imagine any one of the following factors as causing or helping to cause such a pathological accumulation:

1. Obstruction to outflow through the lymph-vessels.
2. Increased blood pressure.
3. Decreased extravascular pressure.
4. Increased permeability of the capillary walls.
5. Increased filterability of the blood plasma.
6. Osmotic pressure changes—either an extravascular increase or an intravascular decrease.
7. Changes in the affinity of the colloids for water.

These may be taken up one by one, and considered in relation to their bearing upon the general problem of edema.

1. Obstruction to Outflow through the Lymph-vessels.—Because of the very abundant anastomosis of the lymphatic vessels it is extremely difficult or impossible to cause any appreciable obstruction to the lymphatic circulation by occlusion of lymphatic trunks in the limbs or organs of the body, and in pathological conditions this possible cause of edema is seldom actually observed. The chief instance of edema from lymphatic obstruction is observed after occlusion of the thoracic duct by tumors, tuberculous processes, animal parasites, or thrombosis; such occlusion is usually followed by rupture of the duct or its tributaries with the production of *chylous ascites* or *chylothorax*, and *chyluria*. Filaria or their

[29] Johns Hopkins Hosp. Bull., 1903 (14), 105.
[30] Amer. Jour. Physiol., 1910 (25), 345.
[31] Magnus, Deut. Zeit. Chir., 1923 (182), 325.

ova may occupy so many of the lymphatic channels of an extremity (leg) or part (scrotum) that the anastomotic channels are thoroughly blocked, with a resulting local edema that in course of time is followed by the production of inflammatory connective tissue and elephantiasis.[32] Chronic lymphangitis or plugging of the lymph vessels by cancer cells may also result in lymphatic obstruction to such an extent that chronic edema results. It would seem, from Opie's experiments,[33] that the acute edemas may at times depend upon lymphatic obstruction, for he found that experimental edema of the liver, produced by cantharidin, seems to be determined by inflammatory processes which occlude the sinuses of the lymph glands through which the hepatic lymph passes.

Another way in which edema may be caused or influenced by lymphatic obstruction is generally overlooked, but it is possibly of great importance; namely, from *pressure upon the lymph channels* by dilated vessels in hyperemia, or by cellular exudates and swollen tissues in inflammation. We see evidence of this in the rapid absorption of exudates that frequently follows the removal of but a part of the fluid in a chest cavity; apparently the decrease in pressure frees the paths of absorption and permits them to take up the remaining fluid. In inflammatory edema the lymphatic obstruction is probably not great, for Lassar found that the amount of lymph escaping from an edematous extremity is much greater than from a normal one; but in the case of strangulated hernias or other conditions in which edema results from circular constriction, obstruction of the lymphatic vessels may be a factor of no mean importance. In general stasis the increased pressure in the veins of the neck may interfere with the passage of the fluid out of the thoracic duct into the blood.

There is no difficulty in understanding edema from the above causes—it is simply a passive congestion of the lymphatic circulation, and no chemical factors are involved. The nature of the fluid found in such forms of edema will be discussed later.

2. Increased Blood Pressure.—This takes us back to the filtration theory of lymph formation, and as it is generally conceded that more or less fluid escapes from the vessels by this mechanical process,[34] the questions to be decided are: Can and does increased blood pressure, alone and without other aiding factors, cause edema? If not, does it play an auxiliary part in producing edema, and how important a part may this be? Many experiments have been performed with the object of answering these questions, with more or less conflicting results. Cohnheim demonstrated that vasodilatation (active hyperemia) alone will never bring on an edema; and many observers state that ligation of the femoral or other large veins will not cause edema in animals. However, when the vein is occluded, and the arteries are dilated by cutting their vasoconstrictor nerves, then edema may result (Ranvier, Cohnheim);

[32] Manson, Allbutt's System, 1897 (ii), 1082.

[33] Jour. Exper. Med., 1912 (16), 831.

[34] A rise of blood pressure leads to an increase in proportion of the hemoglobin of the blood, presumably because the fluid is forced out into the tissue spaces (Scott). Amer. Jour. Physiol., 1917 (44), 298.

but whenever venous outflow is impeded, we have other factors than simply increased pressure to consider, for the nourishment of the parts is decidedly impaired, and, as we shall see later, this may be of much greater importance than is the associated rise in blood pressure. To produce edema in the lungs by mechanical forces it is necessary to ligate the aorta and its branches, or the pulmonary veins (Welch). As such high pressures do not occur in any pathological conditions, it is safe to assume that increased pressure alone is not capable of causing by itself the pulmonary edema so frequently observed clinically. On the other hand, occlusion of the bronchial arteries which supply the oxygen to the lung tissues, leads to edema despite the low blood pressure (Fischer).

In the edema of passive congestion, increased blood pressure would seem to be a factor, and there is no doubt that with an increased pressure of the degree observed in such conditions some increase in the lymph flow would result; but from the evidence at hand it is improbable that the amount of lymph so secreted would ever be more than the lymph-vessels could carry away. Even the added obstruction to lymphatic flow due to pressure upon the lymph capillaries by congested blood-vessels, and the resistance to the lymph escaping from the thoracic duct offered by the increased pressure in the subclavian vein, would not satisfactorily account for the edema of cardiac incompetence. Not to go into details here, it may be stated that the impression prevails that uncomplicated rise in blood pressure is not sufficient by itself to produce edema. Some of the reasons for belittling this factor will be brought out in the subsequent discussion.

3. Decreased Extravascular Pressure.—This factor is particularly prominent in the so-called "*edema ex vacuo*," which occurs after the absorption of an area of tissue so located that the surrounding tissues cannot contract or fall in to fill the gap, *e. g.*, brain softening, serous atrophy of fat. A still better example, however, is the edema that follows local decrease in atmospheric pressure in "cupping." In these instances the edema depends partly upon increased transudation, and partly on the retention of the fluid in the tissues, because it cannot well leave them against the atmospheric pressure. The idea advanced by Landerer that decreased elasticity of the tissues was a possible cause of edema has been attacked by Böninger.[35] During the early stages of edema the elasticity of the skin may be measurably decreased,[36] even when no edema is demonstrable by palpation, but this is not evidence that any loss of elasticity occupies a causative relation to the edema. The tissues can take up water until as much as six kilos has been added to the weight of the entire body before any edema can be detected by palpation (Widal). Edema *ex vacuo* is again an illustration of edema due to purely mechanical causes, but it is of little practical importance.

4. Increased Permeability of the Capillary Walls.—The importance of this factor in the production of edema was first demonstrated by

[35] Zeit. exp. Path. u. Ther., 1905 (1), 163.
[36] Schade, Zeit. exp. Path., 1912 (11), 369.

Cohnheim and Lichtheim, who found that the production of an enormous increase in the amount of fluid in the blood (hydremic plethora) by injecting large quantities of salt solution, caused an edema of the viscera and serous cavities, but not any subcutaneous edema until the skin had been irritated by some means, such as hot water, iodine, etc. By this irritation the capillary walls are injured, and an excessive escape of the blood fluids follows. Magnus also showed that poisoning with arsenic, which injures the vessels, favors the experimental production of edema by transfusion; but we must recognize that in such experiments not only the vascular tissues are injured, but also the extravascular tissues, and hence the observed edema may not depend so much on the vascular injury as Magnus assumed (Aldrich).

Starling, as noted before, observed that the permeability of the capillaries varies normally in different organs and tissues, which determines quantitative and qualitative differences in the lymph normally flowing from various vascular areas. Heidenhain's "lymphagogues of the first class," which are all poisonous substances, probably act by increasing the permeability of the capillaries, and in this way they produce *local urticaria*, which is often observed as a result of poisoning by these same lymphagogues, *e. g.*, shellfish and strawberry poisoning. Just what changes are produced in the capillary walls that render them more permeable we do not know. Possibly in some instances it is a partial solution of the intercellular cement substances, possibly an enlargement of the stomata through loss of tonicity of the endothelium (Meltzer), sometimes it may be actual death of the endothelial cells, or, as Heidenhain and Cohnheim thought, there may be a stimulation of the endothelial cells to increased secretory activity. Fischer believes that a change in the hydrophilic tendency of the colloids, induced especially by acids formed in asphyxiated conditions of the cells, alter their structure and with that their permeability. Lipoid-soluble poisons seem especially effective in increasing the permeability of cells for fluids, leading both to passage of fluid from the vessels and imbibition of water by the cells;[37] the poison of the gas gangrene bacilli which produces the characteristic edema, owes its properties to its affinity for lecithin (Wuth).[38]

A mere quantitative increase in capillary permeability could of itself scarcely cause edema, for the passage would be equally augmented both into and out of the vessels. Under pathological conditions a qualitatively increased permeability of the capillary walls is, however, probably one of the chief factors in the production of certain forms of edema. We see evidence of it particularly in inflammatory edema, with its protein-rich exudate. It cannot be doubted that in such conditions actual physical alterations take place in the capillaries, when we see that the slightly diffusible proteins escape from the vessels in the same proportions as they exist in the plasma; especially when we note the indistinguishable transition of such an inflammatory exudate into one containing leucocytes and

[37] B. Fischer, Frankf. Zeit. Path., 1922 (28), 201.
[38] Bioch em. Zeit., 1919 (93), 289.

red corpuscles, which must pass through openings of some kind in the vessels. Edema due to inflammation and poisoning certainly depends to a large degree upon alterations in the vessel-walls. The question remaining is, do edemas that are not associated with distinct inflammatory or toxic influences depend also upon the vascular permeability?—does increased permeability ever lead to the formation of protein-poor transudates? Cohnheim was inclined to attribute nearly all edema to this cause, for in passive congestion, or nephritis, or any of the common causes of edema, it is easy to find reason for the belief that poisons may be present in the blood; and as there was good evidence that the blood pressure alone could not account for the edema, it was natural to ascribe all these forms of edema to the action of toxic substances upon the capillary walls, leading to increased permeability; or, what might amount to the same thing, increased secretory activity of the endothelium, as understood by Heidenhain. It is impossible at this time to eliminate as nonexistent this secretory-activity doctrine, but, as we hope to show later, there exist other factors in all these non-inflammatory edemas that are sufficient to account for the edema without our having recourse to this hypothesis. For the present, therefore, we may consider altered capillary permeability as an essential factor in edemas characterized by protein-rich fluids (exudates), and state that the influence of altered permeability in the production of protein-poor fluids (transudates) is not proved, and is perhaps not of importance, although the evidence of recent studies on experimental nephritis seems to point more and more to the importance of vascular changes in the edema of acute nephritis, at least.[39]

5. Increased Filterability of the Blood Plasma.—This takes us back to Richard Bright's conception of renal dropsy. He imagined that through the great loss of protein in the urine, the blood became so thinned and watery that it could filter through the vessel walls, while normal plasma, he thought, was too thick and viscid to do so. The same idea was applied to the edemas of cachexia in cancer, etc., and all forms of edema associated with a decrease in the corpuscular or protein elements of the blood. Undoubtedly changes in the amount of colloids in the blood greatly modify the amount of fluid held in the blood; *e. g.*, gelatin and gum acacia have been used in intravenous injections because they hold in the blood vessels a large amount of fluid by virtue of their hydrophilic character.

Stewart and Bartels considered that in renal dropsy the increased filterability of the plasma was not due so much to the loss of protein as to retention of water, which caused an hydremic plethora. But this factor was soon eliminated, for it was found that complete anuria, produced by ligating both ureters, does not ordinarily cause edema; and also that to produce an edema by increasing the water of the blood it was necessary to increase it many times as much as it can ever be increased by disease. Simply increasing the proportion of water by removing part of the blood and injecting a corresponding amount of salt solution does

[39] See Schmid and Schlayer, Deut. Arch. klin. Med., 1911 (104), 44.

not cause edema (Cohnheim and Lichtheim). In the presence of other factors for the production of edema, however, the amount of fluid in the vessels is important; thus Pearce[40] found that in experimental uranium nephritis, hydremia exerted a marked influence on the production of edema.

Although all cases of nephritis with edema snow a fall in the concentration of protein in the plasma, the plasma protein may be below normal in cases without edema,[41] and the greatest decrease in plasma proteins does not correspond to the greatest edema. It is characteristic of the tubular or nephrotic type of nephritis to exhibit marked decrease in the blood protein concentration, but this decrease is present whether there is edema or not; neither is there a constant increase in the plasma protein concentration with disappearance of edema.[42] The amount of protein lost in the urine does not account fully for the decrease in the protein content of the blood in nephritis. Certainly the results of blood analyses do not support the hypothesis that low plasma protein is responsible for edema, nor do they support the old ideas of an hydremic plethora or an increase of plasma volume in the edema of nephritis.[43] According to Kempmann[44] during nephritic edema with excretion of acid urine, the blood shows hydremia, whereas under alkali therapy as the edema undergoes reduction the protein concentration of the blood increases, indicating that the kidneys are removing water set free from the colloids by the alkali. There is reason to believe that the greater part of the water in the blood and tissues is bound by the colloids, and possibly by the crystalloids also to some extent, and that blood and protoplasm may be looked upon as hydrates. It is only the free water which can be readily excreted as urine and sweat, and which migrates readily from vessels to tissues and back as the need arises. Such an hypothesis serves to explain fever as a lack of free water, so that the usual temperature regulation capacity through evaporation is impaired (Balcar, Sansum and Woodyatt).[45] It also accounts for diuresis as the result of an increase in the amount of free water in the blood. On this basis, in edema the tissues bind more water than normally, and in some cases possibly the blood does the same. Under such conditions the kidneys lack free water to excrete, and oliguria results. When the tissue and blood colloids begin to lose their abnormal affinity for water the water set free is available for excretion, with resulting polyuria. The actual proof that there is such a variable "free water" has not yet been furnished, but the hypothesis has many items of indirect evidence in its favor. Beckmann[46] has made a few observations on the filterability of the blood under pressure through collodion filters (ultrafiltration) and although he found some relationship of ultrafiltrability

[40] Arch. Int. Med., 1908 (3), 422.
[41] See Wordley, Quart. Jour. Med., 1920 (14), 88.
[42] See Linder, Lungsgaard and Van Slyke, Jour. Exp. Med., 1924 (39), 887, 921.
[43] See also Brown and Rowntree, Arch. Int. Med., 1925 (35), 129.
[44] Klin. Woch., 1924 (3), 182.
[45] Arch. Int. Med., 1919 (24), 116.
[46] Deut. Arch. klin. Med., 1924 (145), 22.

and diuresis, his material was not satisfactory for the purpose of establishing the relation of filtrability and edema.

Schade and Claussen[47] have devised a method for measuring the "swelling pressure" or "osmotic pressure" of the blood colloids, which they find to be normally about 2.5 cm. of mercury. In nephritis with edema this pressure was found to be lowered regularly, ranging from 1.98 to 1.10 cm., whereas in nephritis without edema this reduction in swelling pressure was not demonstrable, and normal pressures were also obtained in cardiac edema without nephritis. Therefore, they believe that nephritic edema does not depend on an increased affinity of the tissue colloids for water, but, on the contrary, to a reduced affinity of the blood colloids for water. On this basis the nephritic kidney is one that has lost in part its ability to force water out of the blood colloids, wherefore the retained water causes a true hydremia, and nephritic edema is to be looked upon as an hydremic edema dependent upon an increase in the "free water" of the blood. This conception is, of course, a reversion to the theory of edema as the result of an increased filterability of the blood, and in consequence has opposed to it all the evidence that has been arrayed against that theory. Nevertheless, Schade and Claussen having introduced a new method for the study of the water affinity of the blood colloids, we can expect many new developments that may serve to settle the relation of swelling force to edema formation.

In nephritic edema there is a marked tendency for the plasma fibrinogen and globulin fractions to increase at the expense of the albumin. Rusznyák[48] gives the following figures for the partition of the blood proteins in blood from normal and edematous blood:

	Normal		Various edemas	
	Relative, per cent.	Absolute	Relative, per cent.	Absolute
Albumin	60.2–69.2	3.25–4.39	26.5–47.5	1.11–2.75
Globulin	25.9–36.9	1.25–2.07	15.6–55.3	0.90–4.53
Pseudoglobulin	25.1–35.3	1.16–2.01		
Euglobulin	1.0– 1.8	0.06–0.09		
Fibrinogen	2.7– 4.9	0.12–0.24	11.5–37.1	0.79–2.15

He does not accept the view of Kollert that this increase in fibrinogen is responsible for edema, since fibrinogen has a lower affinity for water than the other plasma proteins, but believes that because of the high isoelectric point of fibrinogen it alters the Donnan equilibrium effect in such a way as to reduce resorption of fluid into the blood.

6. Disparity of Osmotic Pressure in Favor of the Tissues and Lymph over the Blood.—On preceding pages we have already con-

[47] Zeit. klin. Med., 1924 (100), 363.
[48] Zeit. ges. exp. Med., 1924 (41), 532.

sidered the means by which changes in osmotic pressure in the tissues are brought about, and how they may lead to an accumulation of fluid. The importance of osmotic pressure in causing pathological edema was suggested by J. Loeb[49] in his studies on the physiological action of ions. He stated that edema occurred when the osmotic pressure was higher in the tissues than it was in the blood and lymph, and the cause was to be sought in conditions that lowered the osmotic pressure of the blood and lymph or raised that of the tissues. This condition he found in the accumulation of metabolic products:—In the case of muscle, tetanization of a frog's muscle for ten minutes raised the osmotic pressure over one atmosphere; separating a muscle from its blood-supply led to such an increase in osmotic pressure that it took up water from a 4.9 per cent. NaCl solution, which has a pressure of *over thirty atmospheres.* When we consider that in his studies on lung edema Welch was able by ligation of the aorta to raise the pulmonary blood pressure less than $\frac{1}{10}$ atmosphere, we begin to appreciate how much more powerful are the physico-chemical forces that are at work in the body than is the blood pressure, even of the aorta itself.

Loeb found that whenever oxidation is impaired in a tissue its osmotic pressure rises, which he ascribed to the accumulation of incompletely oxidized metabolic products, particularly acids, and as a result of the increased osmotic pressure the tissue takes up water and becomes edematous. On this basis we might explain the edema of venous stagnation as due to accumulation of products of metabolism, partly because of impaired oxidation, partly, perhaps, because of their slow removal by the blood on account of the stasis. The so-called "neurotic" edemas may possibly be explained by local increase in metabolic activity brought about by nervous stimuli, which causes increased formation of substances raising osmotic pressure in the stimulated tissues. It has also been thought that in renal edema the retention of water may depend rather on osmotic pressure than on circulatory disturbances or alterations in the vessel-walls, for retention of chlorides is associated with the dropsy in some cases. The chlorides accumulating in the tissues might lead to an increased osmotic pressure and cause the abstraction of water from the blood and its retention in the tissues. (The details of this subject will be considered later.) Conversely, Meltzer and Salant found that salt solution is absorbed from the peritoneal cavity more rapidly in nephrectomized rabbits than in normal rabbits, perhaps because metabolic products accumulate in the blood and raise its osmotic pressure above normal; and it was observed by Fleisher and L. Loeb,[50] that the rate of absorption of fluid from the peritoneal cavity is increased when the osmotic pressure of the blood is raised. Injection of strongly hypertonic salt and sugar solutions have been used to reduce edemas (*e. g.*, glaucoma,[51] cerebral

[49] Pflüger's Arch., 1898 (71), 457.
[50] Jour. Exper. Med., 1910 (12), 510.
[51] Sansum, Jour. Amer. Med. Assoc., 1917 (68), 1885.
[52] Weed and McKibben, Amer. Jour. Physiol., 1919 (48), 531.

edema),[52] which effect may depend on osmotic diffusion of fluid from the edematous tissue into the blood, or upon the dehydrating effect of these crystalloids on the tissue cells.

There are some difficulties, however, in applying the influence of osmotic pressure as an explanation of all edemas. For example, in edema of the lungs, what is the force that drives the fluid into the empty air-cells? Equally difficult to explain as the result of osmotic disturbance is the distribution of fluid that is seen in cardiac dropsy. The fluid does not accumulate in the tissues where metabolism is greatest, or where the most oxygen is used; but rather in the inactive subcutaneous tissues and in the serous cavities. Possibly the original transudation does occur in the muscles and solid viscera, and the fluid is then mechanically forced out of them into the surrounding tissue-spaces, later settling according to the laws of gravity or according to the distensibility of the tissues. It is important to take into consideration the fact that palpable edema does not manifest itself until a very large quantity of fluid has been retained by the body—as much as six kilos, according to Widal. Probably palpable edema merely means the presence of fluid in the interstices between the tissue elements which can be crowded out by pressure, for as long as the fluid is intracellular it will not be readily displaced by pressure, and as long as it is bound to colloids it cannot be displaced by any ordinary pressure.

J. Loeb directed attention to the biological importance of the "Donnan equilibrium" (*q. v.*) and Schade, Rusznyák and others have attempted to apply it to the explanation of edema, as mentioned on previous pages. Possibly this hitherto unconsidered phenomenon may be found to explain some of the difficulties now existing in our understanding of edema.

7. Increased Hydration Capacity of the Tissue Colloids.—According to Fischer's theory this factor is of greater importance than any of the preceding, and of chief importance in increasing the amount of water present in the tissues are organic acids formed during metabolism. For example, the great power of asphyxiated muscle to take up water from a strong salt solution, which J. Loeb ascribed to the osmotic pressure of the acids formed in asphyxia, is attributed by Fischer to the influence of these acids upon the capacity of the colloids for water, and this explanation seems to be in better agreement with the facts, especially since Overton has shown that even if all the proteins, carbohydrates and fats in a muscle were split into the greatest possible number of simple molecules and ions, the resulting osmotic pressure would not be sufficient to account for the amount of water taken up. Furthermore, when cells with demonstrable semi-permeability die, they at once lose their semi-permeability, and in consequence their osmotic pressure falls—but dead cell and tissues often exhibit great power of taking up water and becoming edematous.[53]

[53] The secreted fluid of postmortem thoracic lymph flow differs from normal thoracic lymph in being more cloudy, often bloody, contains more solids, has a higher molecular concentration with decreased electrical conductivity (Jappelli and d'Errico, Zeit. f. Biol., 1907 (50) 1), all of which findings are in agreement with the hypothesis that post mortem lymph flow depends upon asphyxial changes in the cells.

It is an indisputable fact that edema is especially associated with conditions of asphyxiation, and the attempt to explain this by the increased osmotic pressure of the products of incomplete oxidation seems to harmonize with the facts far less successfully than the application of the principle of colloidal swelling.[54] A common error of the critics of this theory is that of assuming that *free* acid must be present to cause swelling. This is not at all true. An amount of acid far less than enough to saturate the acid-binding property of a protein or to be detected by indicators will greatly increase the amount of water which this protein will combine. Neither does Fischer maintain that increased acidity is the only possible factor in changing the hydration capacity of tissue colloids, as many writers seem to assume.

Fischer's theory of edema, in his own words, is this: "A state of edema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water is increased above that which we are pleased to call normal. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids, or through the conversion of colloids which have but little affinity for water into such as have a greater affinity, must also be borne in mind." In support of this theory he advances evidence which he interprets as indicating that: (1) "An abnormal production or accumulation of acids, or conditions predisposing thereto, exist in all states in which we encounter the development of an edema. (2) The development of an edema in tissues is antagonized by the same substances which decrease the affinity of the (hydrophilic) emulsion colloids for water (salts) and is unaffected by the presence of substances which do not do this (nonelectrolytes). (3) Any chemical means by which we render possible the abnormal production or accumulation of acids in the tissues is accompanied by an edema."

There are many features of lymph formation and edema with which this theory seems to harmonize well, and others with which it does not seem to agree so well, if at all, so that at this time it is a fair statement that the theory is under consideration, but the limitations of its applicability have not yet been agreed upon. It has met with much adverse criticism, some of which was poorly founded, but the fact cannot be disputed that the amount of water that colloids will hold varies greatly with changes in the colloids. We may not know absolutely, at present, whether the changes that take place in the colloids during life are great enough to alter their water content appreciably, but it is highly probable that they are. In many instances the principles of colloidal hydration

[54] Experiments on absorption of fluid by tissues *in vitro* (*e. g.* Schade and Menschel, Zeit. klin. Med., 1923 (96), 279) give experiments of doubtful value since they occur under conditions of profound asphyxia.

offer the best explanation of observed conditions, and their application often elucidates matters more satisfactorily than any other working hypothesis. Certainly they cannot be disregarded in considering the factors that may come into play in producing edema.

A common source of misinterpretation of Fischer's theory lies in the failure to understand the difference in the significance of the water which lies in the cavities and tissue spaces and the water which is bound in the tissue elements. The fluid in the tissue spaces is commonly looked upon as constituting the edema, when actually it may merely represent a late phase of the edematous process, except in inflammatory edema. In other forms of edema the essential feature is the taking up of water by the tissue elements, and this water is for a certain period of time not free, as shown by the fact that puncture or incision of such edematous tissues is not followed by escape of fluid. After a time there is an escape of fluid from these highly hydrated colloids, after the order of the "syneresis water" formation so familiar as exhibited by agar slants in test tubes. This setting free of water from the hydrated colloids may come as a result of any change which reduces the hydration capacity of the colloids from the maximum point that they have reached, and this is probably sometimes the neutralization of the organic acids formed in metabolism, which were responsible for the original swelling of the tissues, or the dehydrating effect of sodium chloride or other salts. In this way may be explained the development of puncturable edemas following administration of alkalies to diabetics, the so-called bicarbonate edema.[55] Water escaping from hydrated tissues in this way accumulates in the nearest tissue spaces, constituting the phase of edema which is most readily recognized, although by no means the only or even the most important phase of the process. The abstraction and fixation of the water takes place in the tissues where there is active metabolism; its free accumulation occurs in the tissue spaces after it leaves these tissues, and the site of puncturable edema is not necessarily or even ordinarily the place in which the active process has taken place.[56]

Strongly supporting this conception of edema is the fact, frequently observed in applying the McClure-Aldrich[3] method of studying the affinity of the tissues for water, that when edema is developing, and even before it is demonstrable by palpation, the tissues may show a greatly increased avidity for water, whereas when edema is beginning to subside the tissue affinity for water decreases, despite the fact that at this stage there may be a large amount of fluid in the tissue spaces and a profound palpable edema. These observations are in harmony with the statement

[55] Haldane (Lancet, 1924 (1), 536) says that acidosis, by bringing the colloids nearer the isoelectric point, causes them to give up water and diuresis results. This may be seen in the edema following excessive administration of $NaHCO_3$, when $CaCl_2$ or NH_4Cl are given to reduce the excess alkalinity.

[56] An excellent investigation of the applicability of M. H. Fischer's theories to the actual conditions of clinical edema is reported by A. Fodor and G. H. Fischer, (Zeit. ges. exptl. Med., 1922 (29), 465,509) who find the theory well in harmony with the facts. These authors also point out the misinterpretations on which many of the criticisms of Fischer's work are based.

of Hülse[57] that during the development of edema the large lymph channels are empty, and they do not become distended until the oversaturated tissues begin to give up their "syneresis water" and puncturable edema is present. He says that, when examined microscopically, tissues show no spaces filled with fluid while the edema is developing. Although numerous writers speak as if the fluid in subcutaneous edema were largely in the collagen fibers, the histological appearances indicate that the fluid is chiefly between the fibers, which are widely separated but not noticeably swollen. Another common error in critique is to fail to distinguish between edemas dependent on increased affinity of the tissues for water and inflammatory edemas dependent in large measure upon injury to the vessel walls of such a degree that the plasma leaks out. Such an inflammatory edema is undoubtedly a fundamentally different thing from the edemas with transudation or cell swelling, and the behavior of an excised piece of tissue that has been treated with a violent capillary poison[58] can not throw light on the problems of other types of edema.

Summary.—We find that a number of factors may be considered as responsible for edema, some of them being prominent in one instance, some in another, but *in few cases can we consider one factor alone as the sole cause.* In most of the forms of edema, such as those due to renal disease and cardiac disease, it now seems probable that either osmotic pressure changes or changes in the affinity of the tissue colloids for water, play the most important part; whereas in inflammatory edema it would seem that alteration in the capillary walls is a more important factor. But the mechanical factor of blood pressure cannot be disregarded, although by itself seldom sufficient to cause edema; associated with other factors it is undoubtedly an important agency in some cases. Hydremia and hydremic plethora may almost be disregarded, except in so far as they may cause altered metabolism in the tissues, injury to vessel-walls, over-saturation of the blood colloids, and decreased osmotic pressure within the vessels. Lymphatic obstruction is possibly a factor of some secondary importance, especially if we consider that distended vessels and tense tissues may occlude the lymph capillaries.

Special Causes of Edema

We may now consider which of the above factors are at work in bringing about edema under the conditions in which it is usually observed clinically. Before taking up the detailed consideration of edematous conditions, however, it may be well to call attention to the fact that our knowledge of edema, and especially its clinical recognition and study, has been handicapped by the lack of a suitable objective method of detecting and measuring edema. We are in the same position in respect to edema that we were to blood pressure when the only measure was the clinician's forefinger. An attempt to remedy this defect has been made by Schade,[36] whose "elastometer" reveals and measures degrees of edema not discerni-

[57] Klin. Woch., 1923 (2), 63.
[58] Such experiments as those of Hirschfelder, Amer. Jour. Physiol., 1924 (70), 507.

ble by the palpating finger. A study of edema with this instrument in the hands of Schwartz[59] has revealed many interesting facts, but as yet the apparatus is too complicated for general clinical use. The absorption time of intracutaneous salt solution injections, as applied by McClure and Aldrich,[3] is a distinct advance as giving an objective measure of the affinity of the tissues for water.

"Cardiac" Edema.—Passive congestion introduces nearly all these aforementioned factors, for in addition to the increased blood pressure there is also an opportunity for changes in the capillary wall, either from stretching and thinning of the cells and cement substances, or from "loss of tone" in the endothelium surrounding the stomata (Meltzer), or from toxic injury by accumulated products of tissue metabolism. When the stasis is nearly complete, or if it is complete for a time and then relieved, the endothelium may be injured through lack of nourishment or oxygen. As the edematous fluid in chronic passive congestion is usually of a watery type, poor in proteins, the edema is probably less dependent upon capillary permeability than upon other factors, except in the case of acute stasis, when the fluid partakes of the character of the exudates. Also, there is probably more or less obstruction to lymphatic outflow because of the increased pressure on the lymphatic channels, and perhaps, also, in the case of cardiac incompetence, obstruction to the discharge of lymph from the thoracic duct into the subclavian vein against the high intravenous pressure.

Presumably the accumulation of crystalloids within the tissues also plays a part in this form of edema, as the osmotic pressure is raised in tissues having deficient oxygen supply. But Fischer holds that the reduction in oxidation acts chiefly by increased production of acids, which greatly increase the affinity of the tissue colloids for water and at the same time alter the colloidal state of the capillary endothelium so that the capillaries become more permeable. That there is a distinct and rapid increase in the formation of lactic acid in asphyxiated tissues is shown by the observation that when blood is drawn from a vein engorged by applying an elastic constrictor to the arm, there is a demonstrable increase in the lactic acid.[60]

McClure and Aldrich[3] have found that in cardiac decompensation the affinity of the skin for water is always greater in the dependent parts of the body than in the skin above the heart level, even when there is no palpable edema. Also, when the edema is clearing up, the water affinity of the skin may decrease while there is still a marked palpable edema. These and other observations indicate that the variations in the hydration capacity of the tissue colloids play a major role in the development and behavior of cardiac edema.

Renal Edema.[61]—We must recognize under this heading two different types of edema. In acute nephritis (*e. g.*, in scarlatina) toxic

[59] Arch. Int. Med., 1916 (17), 396 and 459.
[60] Mendel, Engel and Goldscheider, Klin. Woch., 1925 (4), 306.
[61] Review of literature by Veil, Ergeb. inn. Méd. u. Kinderheilk., 1923 (23), 648.

materials appear to be the chief cause, and, as Senator contends, injure alike the capillaries of the renal glomerules and of the subcutaneous tissues; in each case there results an increased permeability which is manifested by albuminuria as a result of the injury to the renal capillaries, and by edema as a result of the injury to the tissue capillaries. This sort of edema is allied to that produced by peptone and similar lymphagogues, and we might well imagine that the mechanism consisted merely in an injury to the capillaries through which excessive fluid is driven by the blood pressure, were it not for such observations as those of Mendel and Hooker,[62] who found that postmortem flow is increased by these lymphagogues also. We can hardly account for the force exhibited in postmortem lymph flow on any other ground than that it is furnished by osmotic pressure or colloidal absorption unless we wish to fall back upon "vital activity" of the surviving cells.

We shall undoubtedly be more nearly correct if we modify Senator's statement to include not alone or even primarily the capillaries of the kidneys and tissues, but all the cells, since the seat of edema is primarily in the cells and not merely in the capillaries. Thus, Baker[3] has observed that in scarlatina the affinity of the skin for water parallels the evidence of toxic injury to the kidneys, supporting the view that *acute toxic nephritis is usually a general tissue injury* which is called nephritis merely because changes in the urine give evidence of injury to the renal cells, while the injury to other cells has not been so easily recognized before the intradermal salt test made possible a recognition of conditions in at least the surface tissue elements. Stieglitz[63] has found that normally the renal epithelium is alkaline when secreting an acid urine and acid when secreting alkaline urine, but in acute experimental nephritis the renal epithelium is acid irrespective of the reaction of the urine, indicating the importance of local acidosis for the renal injury, and presumably equally well for the other tissues injured in acute toxic nephritis. Rous[63a] has shown that the intracellular reaction during life is independent of the reaction of the fluids about the cells; *e. g.*, the cells in a peritoneal exudate may be full of granules with a *p*H at about 3, although the fluid is nearly neutral.

Marriott and Clausen[64] summarize the evidence that the edema of so-called acute nephritis does not depend upon the kidney, as follows: (1) Complete nephrectomy does not lead to the edema. (2) When lesions morphologically identical with those of acute Bright's disease are produced by certain toxic agents, edema does not occur. (3) Edema may occur before there is any evidence whatsoever of renal involvement. (4) The edema is not due to retention of salt by the kidneys. On the other hand, there is definite salt retention by the tissues. (5) That there is a change in the permeability of body cells is shown by the fact that when a tourniquet is placed on the arm of a patient with Bright's disease fluid passes

[62] Amer. Jour. of Physiol., 1902 (7), 380.
[63] Arch. Int. Med., 1924 (33), 483.
[63a] Jour. Exp. Med., 1925 (41), 379, 399.
[64] Trans. Amer. Ped. Soc., 1923 (35), 205.

from the blood into the tissues.[65] (But this latter observation, I believe, is better interpreted as indicating an increased affinity of the asphyxiated tissues for water.)

These authors found that the colloid osmotic pressure of blood was low in patients with acute Bright's disease, paralleling the fall in protein content of the serum, but not all patients with decreased blood colloid and low osmotic pressure have edema. They found that the surface tension of the blood serum was invariably low in the case of patients with nephrosis suffering from persistent edema. With disappearance of the edema the surface tension was observed to return to normal. In the urine from such patients was found an unknown substance, apparently colloidal, which lowers surface tension, and as it was not present in patients with cardiac edema or other forms of nephritis it may be that this effect on surface tension may have an influence in the production of the edema of acute Bright's disease.

In the more common edema of chronic tubular nephritis or "nephrosis" we have to consider, among other factors, the blood pressure. That this is not an essential or even important cause, however, is shown by the fact that edema is usually much less marked in interstitial nephritis with high blood pressure than it is in parenchymatous nephritis with a much lower pressure. It is recognized that edema of chronic glomerular or interstitial nephritis is usually the result of circulatory failure, and hence a cardiac rather than a renal edema. That renal edema is not the result of any primary failure of the kidney to excrete water is proved by the absence of edema when there is total anuria from bilateral nephrectomy or ureteral occlusion. No constant changes in the physico-chemical and noncolloidal chemical constituents of the blood are found to accompany either the appearance or disappearance of edema,[66] which negative finding also supports the conception that edema does not depend on changes in the blood but on changes in the tissues. That *in nephritic edema the tissues have an increased avidity for water* is shown by the fact that if the vessels of the arm are completely occluded for ten minutes in a person with edema from nephrosis, the blood in the occluded veins becomes somewhat concentrated, whereas in normal persons the blood under the same conditions shows a dilution of 4 to 6 per cent. (Molnár).[67]

Toxic substances are, of course, also present in the blood even in chronic nephrosis and may alter capillary permeability; these toxic substances may account for the localized edemas and erythemas sometimes observed in nephritis. But probably most important is the action of the crystalloids which the kidney does not excrete, and which seem to be stored up in the tissues, where they cause transudation of water under the influence of their osmotic pressure or their effect on the hydration capacity of the colloids. For example, Rzentkowski[68] found that the

[65] Denecke, Deut. Arch. klin. Med., 1922 (140), 179.
[66] Atchley *et al.*, Arch. Int. Med., 1923 (31), 606, 611, 616.
[67] Wien. Arch. inn. Med., 1924 (8), 587.
[68] Berl. klin. Woch., 1904 (41), 227.

average lowering of the freezing-point by the edematous fluid in nephritis was 0.583°, in cardiac dropsy it was 0.548°, and in tuberculous pleuritis 0.526°. This indicates that the osmotic concentration of the fluid is highest in renal dropsy, and supports the belief that here and in cardiac dropsy osmotic pressure plays a more important part than it does in inflammatory exudation.[69] Of the crystalloids that cause accumulation of fluid in the tissues, sodium chloride seems to be the most important, but it must also be considered that urea especially increases the hydration capacity of colloids. Perhaps this may explain why Morawitz and Denecke[70] found that agar jelly saturated with Ringer's solution loses weight after 24 hours implantation in the subcutaneous tissues of normal animals, but gains weight in animals with ligated ureters or with uranium nephritis.

Retention of Chlorides in Edema.[71]—From the investigations made by numerous clinicians, especially the French, it appears that—(1) in nephritis with edema a retention of sodium chloride frequently occurs; (2) that elimination of the chlorides is often increased during periods of improvement of the edema; (3) that a reduction of the amount of chlorides in the diet sometimes causes a great improvement in the edema, while administration of chlorides may make the edema much worse. There are, however, observations that also indicate that chloride retention does not account for many cases of renal dropsy, for commonly the above-mentioned conditions are not fulfilled.[72] Nevertheless, it cannot be denied that chloride retention is sometimes an important causative factor in the edema of nephrosis or parenchymatous nephritis.[72a] If the retained chlorides obeyed the ordinary laws of diffusion, we should expect them to become distributed alike in the blood and tissues, so that they would merely cause an equal increase in the fluids of the blood and of the tissues; that is to say, there would be an hydremic plethora due to retention of water in the body by the accumulating chlorides. But, according to a number of observers, there is a specific retention in the tissues, which Strauss calls "*historetention*," and which explains the local edema. The way in which the historetention is produced is, however, not clearly understood, and not all observers accept this hypothesis. If chlorides do bear a causative relation to edema, the predilection of the subcutaneous tissues for edematous accumulations may be explained by the observation that when salt is given to an animal an undue proportion (28–77 per cent.) accumulates in the skin.[73] In many conditions other than nephritis, there is also a chloride retention (*e. g.*, pneumonia, cardiac incompetence, sepsis, typhoid), and the edemas observed in these diseases may possibly depend upon chloride retention, as many French authors suggest. Rumpf,[71] indeed, often found more chlorides in edematous fluids of non-nephritic origin than in nephritic edema. Apparently it is the sodium rather than the chlorine ion that is responsible for the NaCl effect; other cations do not have the same effect as sodium. Fischer[72] holds that the retention of chlorides in edema is secondary and not primary, for he

[69] Epstein (Amer. Jour. Med. Sci., 1917 (154), 638) calls attention to the decrease of serum proteins (sometimes 60 to 70 per cent.) and ascribes the edema to lowered osmotic pressure of the blood from loss of colloids. Low protein content of the blood might more probably favor edema by reducing the amount of fluid which the blood can hold as a hydrophile colloid.

[70] Biochem. Zeit., 1922 (127), 47.

[71] Literature, résumé by Widal and Javal, Jour. Physiol. et Pathol., 1903 (5), 1107 and 1123; Rumpf. Münch. med. Woch., 1905 (52), 393. Review in Albu and Neuberg's "Mineralstoffwechsel," Berlin, 1906, pp. 171–178; Georgopulus Zeit. klin. Med., 1906 (60), 411; Christian, Boston Med. and Surg. Jour., 1908 (158), 416; Palmer, Arch. Int. Med., 1915 (15), 329.

[72] See Blooker, Deut. Arch. klin. Med., 1909 (96), 80: Fischer, "Œdema and Nephritis."

[72a] See Borchardt, Deut. med. Woch., 1912 (38), 1723.

[73] Schade, Zeit. exp. Path. u. Ther., 1913 (14), 1. Also gives an interesting discussion of the relation of the skin to edema.

found that tissues made to take up more water through acidification, also take up an increased amount of chlorides.

Diabetic Edema and "Soda Dropsy."—In severe cases of diabetes edema often occurs without evidence of renal injury to account for it, and it is seen especially after alkali therapy for diabetic acidosis. During the development of such a "bicarbonate edema" or "soda dropsy"[47] there is observable a retention of sodium chloride, and Widal and others have believed this salt to be responsible.[75] According to Fischer's theory the soda edema seen in diabetic acidosis is merely the expression of the setting free into the tissue spaces of water which had been accumulated in the tissue colloids under the influence of the acidosis. Its appearance after administration of alkali is readily explained by the reduction of the hydration capacity of the tissue colloids through the neutralization of the organic acids of the tissues and also the dehydrating effect of the salts on the hydrated colloids. According to Falta[76] the visible edema may be made to disappear by substituting potassium for sodium salts, presumably because potassium proteins hold more water than sodium proteins.

The fact that the weight of patients increases with the administration of the alkali does not fit with the hypothesis that the fluid is merely released tissue fluid. That the edema is dependent on the acidosis is shown by the observations of Földes,[77] who believes it to be the result of a combination of acidosis, sudden reduction in the diuresis because of reduction in sugar excretion, and overloading of the body with sodium salts with their tendency to cause water retention. There is always a marked hydremia in diabetic edema, with resulting low protein figures. The red corpuscles increase in volume with the acidosis, being always swollen in diabetic edema, returning to normal when the urine is made alkaline by alkali therapy (Földes), even when this leads to edema. This is good evidence that there is a marked swelling of tissue colloids in diabetic acidosis. Földes says that small doses of alkali, not sufficient to make the urine neutral, lead to marked edema in the acidotic diabetic, but if enough alkali is given to make the urine alkaline promptly no edema results. The production of a soda edema in persons not the subject of acidosis, and the influence in edema of NaCl as well as of $NaHCO_3$, is explained by Fischer as due to the replacement of Ca and Mg in the tissues by Na, for Na proteins have a much greater water affinity than Ca and Mg proteins.

Inflammatory Edema.—Although here the alterations in the capillary walls play an essential rôle, as shown by the protein-rich nature of the exudates, yet most of the other factors are added. Increased blood pressure is prominent; lymph outflow is impeded by plugging of the lymphatic channels by clots and leucocytes, and by pressure on the outside; there is, undoubtedly, an excessive formation of metabolic products

[74] See Breitmann, Zent. inn. Med., 1913 (34), 633.
[75] See Magnus-Levy, Zeit. klin. Med., 1921 (90), 287.
[76] Wien. Arch. inn. Med., 1923 (5), 581.
[77] Wien. Arch. inn. Med., 1922 (3), 469.

in the tissues, to cause exosmosis, and the asphyxial conditions in inflamed tissues favor acid formation which may cause in the colloids an increased affinity for water.[78] Schade[79] has found that in inflamed areas, especially those with suppuration, the accumulation of products of tissue disintegration may become so great that the osmotic pressure is raised to as high as eleven atmospheres, and at the same time the H-ion concentration is greatly increased, as much as fifty times the normal.

According to Oswald,[80] the permeability of the vessels for proteins becomes specifically altered in inflammation, so that not only the less viscous albumin and pseudoglobulin pass through their walls, but also the more viscous euglobulin and fibrinogen. To this class of edemas belong also the urticarias which follow the ingestion of various toxic substances, many of which can be shown experimentally to be lymphagogues. A good example is the urticaria which often follows the injection of antitoxic or other foreign serums, particularly their repeated injection; in experimental animals such a serum may cause death very quickly by acute pulmonary edema. All these poisons probably produce urticarial edema by injury to the capillary walls in the subcutaneous tissues, and possibly changes in the hydrophilic properties of the tissue colloids are also produced by the poisons, which are assumed to be present but the nature of which is unknown. If the anaphylactic poisons are amines, as has often been suggested, then the marked effect of amines in increasing the hydration of colloids may be of significance. In the action of vesicants especially, it may well be questioned if changes in the capillary walls and active hyperemia are not supplemented by local metabolic alterations. The edema which follows the sting of insects, which are known to secrete into the wound such acids as formic, seems to be a particularly good illustration of the production of edema by the influence of acids on the tissues (Fischer).

Schade[81] points out that in suppurating areas the osmotic pressure may reach high figures ($\Delta = 0.7 - 1.4$). Here the osmotic pressure undoubtedly greatly outweighs the mechanical forces. "Against osmotic pressure differences of even one tenth degree lowering of freezing point, which equals 91 cm. Hg in mechanical equivalents, the swelling pressure differences and mechanical differences in blood pressure cannot compete, except perhaps with marked alterations in the tissue colloids through chemical disintegration." He believes that inflammatory edema has primarily an osmotic origin. However, not only is there a great increase in the osmotic pressure and H-ion concentration in the inflamed tissue, but also in inflammatory edemas blood pressure seems to play a distinct rôle, for in experimental edema produced with mustard oil,[82] or in the

[78] Inflamed tissues have less ability to take up fluid *in vitro* than normal tissues (Hauberrisser, Zeit. ges. exp. Med., 1922 (29), 200), but the explanation of this observation or its significance are not known.

[79] Münch. med. Woch., 1924 (71), 1.

[80] A. Oswald, Zeit. exp. Path., 1910 (8), 226.

[81] Zeit. klin. Med., 1923 (96), 324.

[82] Hirschfelder, Amer. Jour. Physiol., 1924 (70), 507.

remarkably localized edemas of the head of rabbits injected with paraphenylene diamine,[83] reduction of blood pressure prevents or inhibits the development of edema, presumably because in these edemas the process is mainly a leaking out through capillary walls injured by the poisons.

Neuropathic Edema.—Until we understand better than we now do the manner in which nervous impulses modify metabolism, it will be difficult to estimate properly the importance of nervous impulses in the production of edema. That nervous control is a possible factor is well shown by many experiments; for example, simple ligation of the femoral vein in animals does not cause edema, but if the sciatic nerve is cut the vasoconstrictors are paralyzed, and edema may follow (Ranvier).[84] In this case the nervous influence is only indirect through its vasomotor effects. Similarly, stimulation of vasodilator fibers may cause edema. It is furthermore possible that nervous stimulation may lead to excessive metabolic activity, with an accumulation of crystalloidal products and acids sufficient to cause edema when supplemented by active congestion and some resulting pressure upon the lymph-vessels. There are certainly many intances in which edema seems to depend upon nervous disturbance; for example, edema in the area of distribution of a neuralgic nerve, or sudden joint effusions in tabetic arthropathy. However, there is a strong probability that many of the cases formerly considered to be "angioneurotic" edema[86] are actually examples of allergic urticaria.[85] Even the urticarias of apparently mechanical origin (*urticaria factitia*), show evidence of a toxic action, in that there occurs a severe nuclear fragmentation (Gilchrist).[87]

Hereditary Edema.—In a number of families there has been observed a peculiar inherited tendency to the occurrence of acute attacks of local edema, which not infrequently have proved fatal when involving the glottis.[88] It has been generally assumed that these instances of hereditary edema depend upon a nervous affection of some kind, being practically an angioneurotic edema; but how the edema is produced, and what the nature of the nervous alteration may be, are as mysterious as are most other so-called "nervous inheritances." It is highly probable that many of these cases represent merely hereditary allergy, with urticaria.

There also are cases of *congenital edema*, which may occur repeatedly in the fetuses of the same mother and cause habitual miscarriage;[89] and still another class of cases in which the children are born apparently

[83] Tainter and Hanzlik, Jour. Pharmacol., 1924 (24), 179.

[84] Similarly, pulmonary edema follows experimental hydremia only when the vagi are cut (F. Kraus, Zeit. exp. Path., 1913 (14), 402).

[85] See Schulmann, Bull. Soc. Med. Hôp., 1920 (44), 939; Phillips, Jour. Amer. Med. Assoc., 1922 (78), 497.

[86] Metabolism in angioneurotic edema is discussed by Miller and Pepper, Arch. Int. Med., 1916 (18), 551.

[87] Johns Hopkins Hosp. Bull., 1908 (19), 49.

[88] Literature, see Fairbanks, Amer. Jour. Med. Sci., 1904 (127), 877; Hope and French, Quart. Jour. Med., 1908 (1), 312; Crowder, Arch. Int. Med., 1917 (20), 840.

[89] W. Fischer, Berl. klin. Woch., 1912 (49), 2403; Capon, Jour. Obst. Gyn. Brit. Emp., 1922 (29), 239.

healthy, but develop fatal dropsy when a few weeks old.[90] Nothing is known as to the cause of this condition. It has been suggested that blockage of the main lymphatic channels may be responsible.[91] Patein[92] has analyzed the fluid in a case of congenital ascites and found it somewhat more like an exudate than a transudate.

Nutritional Edema ("War Dropsy" or Famine Edema).—(Discussed under Deficiency Diseases, Chapter XIV.)

Anhydremia.[93]—This condition, the opposite of edema, is accompanied always by a loss of water from the tissues throughout the body as well as from the blood. Of this the greater part is lost by the muscles and skin, whereas the more important organs lose less. The concentration of the blood is readily observed because of the high corpuscle count, but the increase in serum protein is relatively even greater, not infrequently increasing over 50 per cent. All the changes in the blood are most marked when the anhydremia develops rapidly. The inorganic salts of the blood increase relatively little, phosphates being retained more than chlorides. There is a high non-protein nitrogen, partly through decreased renal function and partly from increased destruction of body protein, so that sometimes figures as high as those of uremia may be observed. Blood sugar is usually somewhat increased. There is a diminished alkali reserve and oftentimes lactic acid and ketone bodies in excess. Viscosity is increased because of the concentration. Excretion of urine is reduced, eventually to anuria, because of the lack of free water available for excretion. For the same reason fever is often observed (Balcar, Sansum and Woodyatt[94]). In traumatic shock and in poisoning with war gases, intestinal obstruction, and severe burns, anhydremia has been observed as well as in conditions where the cause of the water loss is more obvious, *e. g.*, Asiatic cholera, hyperemesis.[95]

COMPOSITION OF EDEMATOUS FLUIDS[96]

The composition of edematous fluids varies greatly according to the cause of the edema and the place where it occurs. In general, non-inflammatory edemas (transudates) contain much less protein than do the inflammatory exudates, as is shown by the following table of analyses by Halliburton[97] and by Bernheim's[98] determinations of proteins in ascitic fluids.

TABLE I

	Sp. gr.	Parts per 100 of fluid			
		Total protein	Fibrin	Serum-globulin	Serum-albumin
Acute pleurisy	1.023	5.123	0.016	3.002	2.114
Acute pleurisy	1.020	3.4371	0.0171	1.2406	1.1895
Acute pleurisy	1.020	5.2018	0.1088	1.76	3.330
Hydrothorax Aver. of 3 cases	1.014	1.7748	0.0086	0.6137	1.1557

[90] Edgeworth, Lancet, 1911 (181), 216.
[91] Clark, Anat. Record, 1921 (21), 127.
[92] Jour. Pharm. et Chim., 1910 (102), 209.
[93] Full review by Marriott, Physiol. Reviews, 1923 (3), 275.
[94] Arch. Int. Med., 1919 (24), 116.
[95] Concerning water metabolism see review by Rowntree, Physiol. Reviews, 1922 (2), 116; also Schade, Handbuch. d. Biochem., 1923 (8), 149.
[96] Many data are given by Gerhartz, Handbuch der Biochemie, 1908, II (2), 137.
[97] Adami, Allbutt's System, 1896 (1), 97.
[98] Quoted by Hammarsten, "Physiological Chemistry."

TABLE II

Ascitic fluid in	Parts of protein to 1000 c.c. fluid		
	Max.	Min.	Mean
Cirrhosis of the liver	34.5	5.6	9.69–21.06
Bright's disease	16.11	10.10	15.6–10.36
Tuberculous and idiopathic peritonitis	55.8	18.72	30.7–37.95
Carcinomatous peritonitis	54.20	27.00	35.1–58.96

The specific gravity varies nearly in direct proportion to the amount of proteins, that of transudates usually being below 1.015, and exudates above 1.018, although there are many exceptions. Indeed, it is often very difficult to decide whether a given fluid is an exudate or a transudate,[99] there being no definite line of demarcation either etiologically or chemically. The more acute and severe the inflammatory process the more closely the composition of the exudate approaches that of plasma, and with hemorrhagic exudates the composition may differ from that of whole blood only in the different proportions of red corpuscles and their disintegration products.

Transudates, even when produced by the same cause, vary in composition in different parts of the body, presumably because of variations in the permeability of the vessels in different vascular areas; just as pleural, pericardial, peritoneal, and meningeal fluids normally differ from one another. Thus, C. S. Schmidt[1] found the composition of the transudates in different parts of the body of a patient who died of nephritis to have the following composition:

TABLE III

	Pleural	Peritoneal	Subarachnoid	Subcutaneous
Water	963.95	978.91	983.54	988.70
Solids	36.05	21.09	16.46	11.30
Organic matter	28.50	11.32	7.98	3.60
Inorganic matter	7.55	9.77	8.48	7.70

[99] Rivalta (Rif. Med., 1903; Biochem. Centr., 1904 (2), 529) has suggested the following test to distinguish between exudates and transudates: Into a beaker containing 200 c.c. of water with 4 drops of glacial acetic acid, let fall a few drops of the fluid to be tested. If an exudate, a bluish-white line is left transiently behind the sinking drops, supposedly due to precipitation of the euglobulin and fibrinogen. It may depend on a substance similar to chondrotin sulfuric acid (Alzona, Policlinico, 1922 (29), 1353). This test, and also certain modifications (see Rivalta, Policlinico, 1910 (17), 676), seem to give quite reliable results. (See Ujihard, Berl. klin. Woch., 1914 (51), 1112.) Not confirmed by Daumas and Lautier, Compt. Rend. Soc. Biol., 1923 (88), 1147. With tuberculous effusions Rivalta's test is positive, but not Morelli's test, which consists in dropping the fluid into saturated $HgCl_2$ solution, a yellowish ring of albuminate forming with non-tuberculous exudates, and a granular precipitate with transudates. (See Zannini, Gaz. degli Osped., 1914 (4), 461.) Memmi (Clin. Med. Ital., 1905, No. 3) suggests the larger content of lipase as a means of distinction of exudates. Sugar is found more often in transudates (Sittig). Sochanski's test (decolorization of phenolphthalein), is said to give a reliable differentiation. (See Larsen and Secher, Hospitalst., 1920 (63), 289.)

[1] Hoppe-Seyler's Physiol. Chemie.

As in this case, the general rule is that while the proportion of salts remains nearly constant, the proportion of protein in edematous fluids in different localities varies in decreasing order as follows:[2] (1) pleura; (2) peritoneum;[3] (3) cerebrospinal; (4) subcutaneous. In the last-named location the specific gravity of edematous fluids may be as low as 1.005, and the proteins even less than 0.1 per cent. (Hoffmann[4]). An increase in solids occurs after the effusion has existed for some time, presumably because of absorption of water, leaving a slowly increasing proportion of proteins. Furthermore, the composition of the patient's blood has considerable influence on the composition of the effusion; this is particularly true in the case of ascites from portal obstruction, the contents of the blood coming from the intestine during digestion modifying the composition of the ascitic fluid.[5] Thus Müller,[6] in a case of portal vein thrombosis, found in the ascitic fluid of a patient on an ordinary mixed diet, 0.179 per cent. nitrogen; on a protein-rich diet, 0.2494 per cent. N; on a protein-poor diet, 0.1764 per cent. N. In cachectic conditions the proportion of proteins is less than in stronger individuals, and, as in the blood plasma, the albumin decreases more rapidly than the globulin as the cachexia advances (Umber).[7]

Physical Chemistry of Edema Fluids.—The differences between transudates and exudates depend almost solely on their protein contents, for the non-protein elements are almost identical with those of the lymph and blood-serum, which naturally must be so since any original or temporary deviation in osmotic pressure must be rapidly equalized by diffusion. Thus Bodon[8] found the concentration of the electrolytes nearly constant in spite of considerable differences in composition of various edema fluids, indicating that the serosa permits passage of inorganic salts always in the same concentration, while holding back the organic substances. Transudates contain an excess of NaCl over other electrolytes, while in exudates the proportion of electrolytes other than chlorides is increased over the findings in transudates.[9] When the equilibrium conditions between the blood and the serous effusions are examined in the same patient, it has been found by R. Loeb, Atchley and Palmer[10] that the edema fluid contains more Cl and less K than the blood serum; while the Na, HCO_3, Ca, urea, glucose and non-protein nitrogen exist in approximately the same concentrations in the serum and

[2] Corroborated by Epstein, Jour. Exp. Med., 1914 (20), 334. Javal (Jour. phys. et path., 1911 (13), 508) places the fluids in this order: serum, peritoneal, pleural, subcutaneous, cerebrospinal.

[3] Composition of normal peritoneal fluid is given by Vogt, (Arch. Gynäk., 1923 (120), 40; Med. Klin., 1923 (19), 943), as protein, usually 0.2 to 0.7 per cent., rarely up to 2 per cent; sugar, traces only; sp. gr., 1.018 to 1.025.

[4] Deut. Arch. klin. Med., 1889 (44), 313.

[5] See Denis and Minot, Arch. Int. Med., 1917 (20), 879.

[6] Deut. Arch. klin. Med., 1903 (76), 563.

[7] Zeit. klin. Med., 1903 (48), 364.

[8] Pflüger's Arch., 1904 (104), 519; also see Galeotti, Lo Sperimentale, 1901 (55), 425.

[9] Gruner, Biochem. Jour., 1907 (2), 383.

[10] Jour. Gen. Physiol., 1922 (4), 591.

in the edema fluid. The freezing point is also the same in both fluids, while the specific conductivity is constantly higher in the edema fluid. The above mentioned variations between the edema fluid and the serum, appear to be related to the difference in the concentration of protein in the two solutions, and to result from a simple membrane equilibrium, influenced in part by the proteins present. The surface tension of exudates is lower than that of transudates,[11] depending chiefly upon the globulin content.

The H-ion concentration is usually the same as, or slightly lower than that of the blood, being lowest in acute infectious effusions. In general, tissue fluids are intermediate in H-ion concentration between the cell juice and the blood plasma. Schade and his co-workers[12] determined the reactions of many exudates electrometrically; pus from acute infections varied in *p*H between 5.96 and 6.57; pus from chronic inflammations, such as tuberculosis, between 6.58 and 7.00; serous exudates, as in tuberculous pleurisy, between 7.00 and 7.09; and noninflammatory transudates between *p*H 7.17 and 7.24. In joint effusions the sterile exudates vary from *p*H 7.27 to 7.42, infected effusions being as low as 6.19 (Botts and Cullen).[13] However, the alkali reserve of edema fluids has been found equal to or slightly greater than that of the plasma.[14]

Rzentkowski[15] found some slight differences in molecular concentration as indicated by the freezing-point; in tuberculous pleurisy the average lowering was 0.523°, that of the serum being −0.56°; in cardiac dropsy the subcutaneous fluid gave −0.548°, and in renal dropsy −0.583°; tuberculous peritonitis, −0.523°; cirrhosis −0.536°; carcinomatous edema −0.547°. Of these figures, the most significant is the comparatively high molecular concentration of the fluid in nephritis, purulent exudates may show a high molecular concentration (−0.84° in one case), due to decomposition of the proteins into crystalloids. Tieken[16] has found the results in transudates, exudates, and other body fluids shown in Table IV.

The very high figures for effusions in nephritis and cardiac decompensation indicate the concentration of crystalloids in these fluids, and support the belief that in the formation of both, osmotic pressure is a factor.[17]

[11] Trevisan, Zeit. exp. Path., 1911 (10), 141.
[12] Schade, Neukirch and Halpert, Zeit. ges. exp. Med., 1921 (24), 11.
[13] Jour. Exp. Med., 1922 (36), 405.
[14] See Gollwitzer-Meier, Klin. Woch., 1923 (2), 1827; Carlier, Compt. Rend. Soc. Biol., 1923 (89), 1313.
[15] Virchow's Arch., 1905 (179), 405; and also Berl. klin. Woch., 1904 (41), 227.
[16] Amer. Medicine, 1905 (10), 822.
[17] Meyer and His (Deut. Arch. klin. Med., 1905 (85), 149) claim that the lowering of the freezing-point is less than that of the blood in exudates while forming, the same as the blood while stationary, and greater during absorption, which they consider ndicates a "vital process" on the part of the cells.

TABLE IV

Nature of fluid	Sp. gr.	Freezing-point of effusion, −° C.	Freezing-point of blood, −° C.	Disease
Pleuritic effusion	1,016	−0.55	−0.56	Pneumonia, lobar.
Pleuritic effusion	1,018	−0.55	−0.55	Pneumonia, lobar.
Pleuritic effusion	1,018	−0.54	−0.56	Tuberculosis.
Pleuritic effusion	1,020	−0.55	−0.56	Tuberculosis.
Pleuritic effusion	1,016	−0.55	−0.56	Tuberculosis.
Pleuritic effusion	1,018	−0.64	−0.56	Valvular heart disease.
Pleuritic effusion	1,030	−0.60	−0.58	Empyema; cyanosis.
Pericardial effusion	1,018	−0.55	−0.56	Pericarditis.
Pericardial effusion	1,016	−0.56	−0.56	Pericarditis.
Pericardial effusion	1,012	−0.56	−0.56	Hydropericardium.
Ascitic fluid	1,024	−0.60	−0.56	Cirrhosis of liver.
Ascitic fluid	1,020	−0.57	−0.56	Cirrhosis of liver.
Ascitic fluid	1,018	−0.58	−0.56	Tuberculous peritonitis.
Ascitic fluid	1,013	−0.62	−0.56	Organic heart disease.
Ascitic fluid	1,035	−0.65	−0.58	General peritonitis.
Hydrocele fluid	1,016	−0.56	−0.56	Tuberculosis.
Cerebrospinal fluid	1,018	−0.62	−0.58	Uremic coma.
Cerebrospinal fluid	1,016	−0.64	−0.68	Uremic coma.
Cerebrospinal fluid	1,020	−0.64	−0.64	Uremic coma.
Cerebrospinal fluid	1,014	−0.56	−0.56	Tuberculous meningitis.
Cerebrospinal fluid	1,017	−0.56	−0.56	Epidemic meningitis
Cerebrospinal fluid		−0.56	−0.56	Epidemic meningitis.

Protein Contents.—As indicated in the tables given previously, these vary greatly in quantity in various fluids;[18] the quantitative relations of the different varieties of proteins have been less studied. Serum-albumins and globulins constitute by far the largest part of the proteins, fibrinogen being scanty except in some inflammatory exudates, so that coagulation very seldom occurs spontaneously; pneumococcus exudates seem particularly rich in fibrinogen, which coagulates rapidly and firmly. The differences in the proportion of different serum proteins in transudates is attributed by A. Oswald[19] to the relative viscosity of these proteins, which determines their ability to pass through the capillary walls. The vicosity of serum proteins varies in the following increasing order: albumin, pseudo-globulin, euglobulin, and fibrinogen; hence in transudates we may find only the first two or perhaps only the albumin, while in exudates the two latter appear. Joachim[20] found in pleural transudates and exudates that the proportion of albumin, euglobulin, and pseudoglobulin is always lower in hydrothorax than in pleurisy. In the pulmonary edema produced by irritant gases the fluid may approximate plasma in composition.[21]

Of different forms of ascites, the largest proportion of globulin and the smallest of albumin occur in cirrhosis; while with carcinoma the pro-

[18] See also v. Jaksch, Zeit. klin. Med., 1893 (23), 225; Rzentkowski (*loc. cit.*).[15]
[19] Zeit. exp. Path., 1910 (8), 226.
[20] Pflüger's Arch., 1903 (93), 558.
[21] See Mayer and Morel, Bull. soc. chim. biol., 1921 (3), 520.

portions are reversed. In general the albumin is more abundant than the globulin,[22] but even so, the ratio of globulin to albumin is almost always higher than in the normal blood serum. Umber[7] has found that the proportion of albumin sinks more rapidly in cachexia than does the globulin, corresponding to the similar changes in the blood proteins. The amount of protein lost in exudates is strikingly shown by one of Umber's cases of cancerous ascites; during one year the fluid removed by paracentesis contained not less than three kilos of pure protein, the patient weighing but 55.5 kilos. With repeated aspiration and reaccumulation the fluid becomes progressively poorer in protein, unless there is acute inflammation superadded.[23]

Several authors have found, in inflammatory ascitic exudates, a protein having physical and chemical properties much resembling mucin; it has been especially studied by Umber[24] who finds it quite similar to the synovial mucin isolated in arthritis by Salkowski, and calls it serosamucin. Hiruma[25] reports that exudates contain a special protein, absent from transudates, which is precipitated by acetate buffer mixtures at the isoelectric point of 0.36×10^{-4}.

Non-protein Organic Contents.—Proteoses,[26] leucine, and tyrosine may be present in small quantities in exudates, being produced by autolysis[27] (Umber); and also mucoid substances (Hammarsten). Nucleoproteins may be present from leucocytic disintegration in exudates, as well as the products of their further splitting, such as purines and phosphates. Allantoin is said to have been found in exudates (Moscatelli),[28] but this is doubtful.

The salt content is discussed on p. 389. Magnus-Levy[29] found the calcium content somewhat higher in pleural exudates than in the blood serum or in other effusions, and effusions contain less magnesium than the serum. In the effusions of cardiac dropsy, however, the calcium content has been found to be about 55 to 70 per cent. that of plasma, corresponding to the fraction that is diffusible.[30] Sulfates are less abundant than in the blood.[31] Absorption of iodides injected into pleural effusions is about equally rapid with either exudates or transudates, except when interfered with in chronic pleuritis with dense exudates.[32]

All the other innumerable components of plasma may be found in edematous fluids. The amount of urea varies usually but not always as it does in the blood of the same individual,[33] and it seems probable that all the crystalloid substances present in the blood pass freely into and from inflammatory exudates, so that an equilibrium between blood and exudates is approximated.[34] Sugar is said sometimes to be greater in amount in transudates than in the blood, but in exudates it is usually, if not always,

[22] See Epstein, Jour. Exp. Med., 1914 (20), 334.

[23] See Natali, Riv. crit. clin. Med., 1921 (22), 349.

[24] Zeit. klin. Med., 1903 (48), 364; also Holst, Upsalalakar. Forhand., 1904, p. 304.

[25] Biochem. Zeit., 1923 (142), 506.

[26] Opie, Jour. Exp. Med., 1907 (9), 391.

[27] Histidine and arginine were found in a carcinomatous exudate by Wiener (Biochem. Zeit., 1912 (41), 149).

[28] Zeit. physiol. Chem., 1899 (13), 202.

[29] Zeit. klin. Med., 1919 (88), 1.

[30] Salvesen and Linder, Jour. Biol. Chem., 1923 (58), 617.

[31] Huebner and Meyer-Bisch, Biochem. Zeit., 1921 (122), 120.

[32] Cobet and Ganter, Deut. Arch. klin. Med., 1921 (135), 146.

[33] Rosenberg, Berl. klin. Woch., 1916 (53), 1314. Sherrill and Allen, Trans. Assoc. Amer. Phys., 1920 (35), 231.

[34] Wells and Hedenburg, Jour. Infect. Dis., 1912 (11), 349; Scheel, Nord. Med. Laeg., 1916 (77), 610.

lower than 0.1 per cent.[35] Hiruma[25] found the average sugar of exudates to be 0.0672 as against 0.1145 per cent. in transudates. By using more accurate methods than have been employed by most of the observers quoted above, Denis and Minot[36] found urea, uric acid, and creatinine to occur in exudates and transudates in the same concentrations as in the blood, but the sugar content of ascitic fluids is somewhat higher than that of the blood. Some authors, however, have found the edema fluid of nephritics to contain more non-protein N than the blood.[37] Landsberg[38] finds an increased ratio of free amino-acids to protein in exudates undergoing absorption, in direct proportion to the rate of absorption. Creatine, fats and cholesterol are much lower in transudates than in exudates in which they approach the concentration in the blood. In ascitic fluid the urea, uric acid, and cholesterol are influenced by the diet.

Lipins.—Lecithin is always present, partly bound to globulin and partly free (Christen).[39] Cholesterol is present particularly in fluids that have been standing for a long time in the body, appearing often as visible crystals shining in the fluid; it probably originates from degenerating cells. Ruppert has described a case of pleural effusion with 1.129 per cent. of cholesterol when tapped the first time, 0.22 per cent. the second and 0.05 per cent. the third. Hedstrom reported finding in an old pleural effusion, 4.5 per cent. of cholesterol; one year later there was but 0.09 per cent. Zunz[40] has described a carefully studied case in which 14 aspirations were made; the cholesterol content was about 3 per cent. at first, but fell suddenly to 0.48 per cent. and then remained between 0.5 per cent. and 0.28 per cent. Lecithin varied from 0.1 to 0.04 per cent. As there did not seem to be enough cells present in the fluid to yield the obtained cholesterol through their disintegration, Zunz suggests that it may have been secreted by the walls of the cavity. Weems[41] has described a similar case, with 1.39 per cent. in the first fluid drawn, but smaller amounts in fluids withdrawn later; this patient had a marked hypercholesterolemia. Arnell[42] found 0.41 per cent. of cholesterol in a tuberculous pleurisy. In most of these cases some fats have been present, Weems finding 0.33 per cent. and Ruppert 0.36 per cent.

Toxicity.—Contrary to earlier ideas, transudates are not demonstrably toxic, even in nephritis (Baylac,[43] Boy-Teissier,[44] Lafforcade[45]), and therefore the toxic manifestations frequently observed after reduction of edema in nephritis, and ascribed to absorption of poisons in the transudates, are probably due to some other cause. In inflammatory exudates, of course, the causative agents as well as the products of cell destruction render the fluids poisonous, and it is possible that edema fluids serve to dilute poisons in the tissues in uremia and the toxemias of pregnancy.[46]

Enzymes and Immune Bodies.—All the enzymes of the plasma may appear in edematous fluids, being in all cases probably more abundant in exudates than in transudates. According to Carrière,[47] oxidases are inconstant, even in exudates. Lipase is said to be much more abundant in exudates than in transudates.[48] (Concerning proteolytic enzymes see "Autolysis of Exudates," Chap. iii.) The various immune bodies seem to pass freely into both transudates and exudates, and their presence is not characteristic of either,[49] but as a rule the proportion is much higher in exudates.[50] Peptid-splitting enzymes are usually found in such fluids,[51] especially

[35] Hegler and Schumm, Med. Klinik, 1913 (9), 1810.
[36] Arch. Int. Med., 1917 (20), 879.
[37] Strisower, Wien. Arch. inn. Med., 1922 (4), 115.
[38] Wien. Arch. inn. Med., 1921 (2), 467.
[39] Cent. f. inn. Med., 1905 (26), 329.
[40] Travaux Ambulance de L'Ocean, La Panne, 1918, Tome II, Fasc, 1.
[41] Amer. Jour. Med. Sci., 1918 (156), 20.
[42] Hygiea, 1917 (79), 737.
[43] Compt. Rend. Soc. Biol., 1901 (53), 519.
[44] *Ibid.*, 1904 (56), 1119.
[45] Gaz. heb. Med. et Chir., Jan. 28, 1900.
[46] See Aldrich, Jour. Amer. Med. Assoc., 1925 (84), 481.
[47] Compt. Rend. Soc. Biol., 1899 (51), 561.
[48] Zeri, Il Policlinico, 1903 (10), No. 11; Memmi, Clin. med. Ital., 1905, No. 3; Galletta, Clin. med. Ital., 1911 (50), 143.
[49] Granström, Inaug. Dissert., St. Petersburg, 1905.
[50] Not corroborated by Lüdke, Cent. f. Bakt., 1907 (44), 268. See also Delrez, Bull. Acad. Roy. Med. Belg., 1919 (29), 733.
[51] Hall and Williamson, Jour. Path. and Bact., 1911 (15), 351.

tuberculous exudates,[52] and these enzymes seem to be different from both erepsin and trypsin. Probably this type of enzyme is more often present than trypsin. Antitryptic activity is usually high, unless exhausted by the presence of much trypsin from cell-rich exudates.[53] Purulent fluids are usually poor in opsonins,[54] in non-purulent fluids the opsonin content varies with the amount of proteins.[55] Turpentine exudates may sometimes be more strongly bactericidal than the serum of the same animal.[56] Exudates usually contain about as much complement as the serum, but in suppuration the complement disappears; transudates contain little of either complement or hemolysins.[57]

Precipitin Reactions, Etc.—Edematous fluids have often been used as a source of material in immunizing animals against human proteins. The precipitins thus formed are specific for human serum or for the proteins of the effusion, but can not be used to differentiate a transudate from an exudate, or a hydrothorax fluid from an ascites fluid (Quadrone).[58] Immune bodies, complement, agglutinins, and antitoxins are present in effusions; *e. g.*, the once common use of blister fluid for the Widal test. Furthermore, according to Hamburger,[59] edema fluid is distinctly more bactericidal than normal lymph.

Varieties of Edematous Fluids[60]

On the preceding pages have been mentioned the chief differences in the characters of the effusions in the usual sites,[61] with their variations in protein content, which variation agrees with Starling's statement that the permeability of the capillary wall for proteins differs normally in different localities. Some of the other effusion fluids not mentioned previously have particular properties of some interest.

Meningeal Effusions.[62]—Normal meningeal fluid differs from all other serous fluids in being clear and watery, in its low specific gravity (1.004 to 1.007), and in containing but a trace of protein which is chiefly globulin. There is normally in the adult from 60 to 150 cc., and Frazier estimates that from 360 to 720 cc. is secreted daily. Halliburton gives the following analyses of pathological accumulations of such fluids:

Table V (Spina bifida)

	Case 1	Case 2	Case 3
Water	989.75	989.877	991.658
Solids	10.25	10.123	8.342
Proteins	0.842	1.602	0.199
Salts	9.626	0.631	3.028
Extractives		7.890	5.115

The percentage of solids in spina bifida is thus a little higher than in normal meningeal fluids. In hydrocephalus the percentage of solids is rather greater, as seen in Table VI.

[52] See H. Koch, Zeit. Kinderheilk., 1914 (10), 1.
[53] Zunz, Ambulance de l'ocean, 1918 (2), 249.
[54] Opie, Jour. Exper. Med., 1907 (9), 515.
[55] Böhme, Deut. Arch. klin. Med., 1909 (96), 195.
[56] Rastaedt, Zeit. Immunität., 1912 (13), 421.
[57] Aronstamm, Cent. f. Bakt., 1914 (74), 326.
[58] Cent. f. Bakt. (Ref.), 1905 (36), 270.
[59] Virchow's Arch., 1899 (156), 329.
[60] Chemistry of Pus and Sputum are discussed under Inflammation, Chapter XIII.
[61] Literature and résumé on tuberculous exudates, see Wells, DeWitt and Long, 'Chemistry of Tuberculosis," Williams and Wilkins, 1923, Chap. IX.
[62] Full review by Levinson, "Cerebrospinal Fluid in Health and Disease," 2nd Ed., Mosby, St. Louis. Review on the formation and circulation of cerebrospinal fluid by Weed, Physiol. Reviews, 1922 (2),171.

TABLE VI (Hydrocephalus)

	CASE 1	CASE 2	CASE 3
Water	986.78	984.59	980.77
Solids	13.22	15.41	19.23
Proteins and extractives	3.74	6.49	11.35
Salts	9.48	8.92	7.88

(1) *Physical Chemistry.*—By gas chain measurements, Levinson[63] found the spinal fluid almost neutral (pH = 7.4 – 7.6); in epidemic meningitis it is 7.3 – 7.4, but lower figures (pH = 6.9) have been found by others[64] in meningitis. The alkali reserve is nearly constant in systemic diseases, except diabetes (McClendon),[65] and is practically the same as that of the blood. According to Fuchs and Rosenthal[66] the average freezing-point of the cerebrospinal fluid is lowered about the same in all diseases ($\Delta = -0.52°$ to $-0.54°$) except in tuberculous meningitis, where it is much less (average $-0.43°$). The amount of potassium is about the same as in the blood,[67] and not increased in degenerative diseases of the central nervous system;[68] after death the amount is much increased by post-mortem changes.

Pathological fluids show also specific alterations in their colloidal property of preventing precipitation of colloidal suspensions by electrolytes (the "Goldzahl" of Zsigmondy);[69] apparently the typical curve of syphilis depends on a balanced increase in both the euglobulin and the pseudoglobulin of the fluid.[70] The surface tension is higher than that of the serum and is not characteristically altered in disease.[71] The viscosity increases with the protein and cell content.[72] In epidemic meningitis there is more positively charged protein, while in tuberculous meningitis there is more negatively charged protein, which can be distinguished by suitable precipitants (Tashiro and Levinson).[73]

(2) *Inorganic Constituents.*—Critchley and O'Flynn[74] found the calcium practically constant at 6.2 mg. per 100 cc. and not showing any characteristic change in disease, except a marked fall in acute tetany and a rise with Froin's syndrome. Chlorides are slightly decreased from the normal (NaCl = 0.72 per cent.) in meningitis (Neuda).[75] Spinal fluid normally contains more sodium chloride than the blood or than other body fluids,[76] and also more total sulfates.[77] The inorganic phosphorus content is normally 1.25–2 mg. per 100 cc., being increased more in all forms of meningitis than in chronic nervous diseases (Cohen).[78] On the whole the changes in P_2O_5[79] content in disease are not characteristic.

(3) *Enzymes.*—In general the inflammatory fluids in the spinal canal resemble exudates elsewhere, but usually the concentration of the different components is relatively low, except the chlorides.[80] Normal cerebrospinal fluid contains no antiprotease (for leucoprotease), as does the fluid in many cases of chronic inflammations; in acute inflammation proteases may appear (Dochez).[81] Peptid-splitting enzymes

[63] Jour. Infect. Dis., 1917 (21), 556.
[64] Shearer and Parsons, Quart. Jour. Med., 1921 (14), 120.
[65] Jour. Amer. Med. Assoc., 1918 (70), 977.
[66] Wien. med. Presse, 1904 (45), 2081 and 2135.
[67] Myers, Jour. Biol. Chem., 1909 (6), 115, literature.
[68] Rosenbloom and Andrews, Arch. Int. Med., 1914 (14), 536.
[69] Lange, Zeit. Chemother., 1912 (1), 44; Spät, Zeit. Immunität., 1915 (23), 426; Vogel, Arch. Int. Med., 1918 (22), 496; Bloch and Biberfeld (Deut. med. Woch., 1924 (50), 461) discuss the principles of this reaction.
[70] See Mellanby and Anwyl-Davies, Brit. Jour. Exp. Path., 1923 (4), 132; Reznikoff, Jour. Lab. Clin. Med., 1922 (8), 92.
[71] Kisch and Remertz, Münch. med. Woch., 1914 (20), 1097.
[72] Soda, Jour. Nerv. Mental Dis., 1921 (54), 227.
[73] Jour. Infect. Dis., 1917 (21), 571.
[74] Brain, 1924 (67), 337.
[75] Wien. klin. Woch., 1923 (36), 447.
[76] See Sherrill and Allen, Trans. Assoc. Amer. Phys., 1920 (35), 231.
[77] Meyer-Bisch, Biochem. Zeit., 1924 (150), 23.
[78] Quart. Jour. Med., 1924 (17), 289.
[79] Apelt and Schumm, Arch. Psychiat. u. Nervenkr., 1908 (44), 845.
[80] Javal, Jour. phys. et path. gén., 1911 (15), 508.
[81] Jour. Exp. Med., 1909 (11), 718.

are relatively abundant in meningitis,[82] and amylase is increased especially in tuberculous meningitis,[83] not bearing any constant relation to the cell count.[84] Antibodies pass from the serum into the cerebrospinal fluid only in minimal amounts or not at all, except when inflammatory exudation occurs, and even then the antibody concentration is usually low.[85] The amount of catalase depends on the cell and protein content, normal fluid containing none,[86] the same being true for oxidase and peroxidase (Sabatini),[87] but there may be a marked increase in hemolysins.[88] The increased organic matter of pathological fluids raises the permanganate reduction index.[89]

(4) *Lipins.*—According to Levinson[90] normal fluid contains no demonstrable cholesterol, nor do most fluids giving positive Wassermann reactions, but traces are usually present in meningitis, more in cases of cerebral hemorrhage, occasionally 6 to 19 mg. per 100 cc. being found in such conditions. According to Weston[91] cholesterol can be found in all cases of mental disease, the amount not bearing any relation to the type of psychosis; ordinarily 0.2 to 0.7 mg. per 100 cc. is found.

(5) *Proteins.*—Under pathological conditions the amount of protein varies greatly and to some extent characteristically. Thus, in syphilis the euglobulin is so greatly increased that it is readily identified by various precipitation methods,[92] including specific antiglobulin precipitins,[93] while in more acute inflammations fibrinogen appears.[94] Denis and Ayer[95] found normal spinal fluids to contain 35–100 mg. total protein per 100 cc.; inactive syphilis, 50 to 125; tabes, 100–200; acute syphilis of nervous system, and paresis, 200 to 600; lethargic encephalitis, 100 to 200; tuberculous meningitis, 200 to 1,000; acute meningitis, 400 to 1,300; cerebral hemorrhage or thrombosis, 100 to 300. No other chemical changes are found characteristically in syphilis.[96] The globulin content is especially increased in tuberculous meningitis, but may also be high in uremic convulsions and spasmophilia in children (Baar).[97] The presence of fibrinogen means an inflammation, but the amount bears no constant relation to the number of cells or to the formation of a pellicle.[98] According to Mott[99] the fluid is especially rich in nuclein in progressive paralysis, and lipoids are increased in the fluid with degenerations of the central nervous system.

(6) *Other Constituents.*—Even simple chemicals enter the normal spinal fluid but very little,[1] except perhaps alcohol.[2] Normally the sugar content is 50 to 65 mg. per 100 cc.; it is not altered in paresis, but averages about 80 mg. in lethargic encephalitis[3] and dementia precox;[4] it is, of course, very high in diabetes, and may be raised in uremia and decreased in meningitis.[5] Goodwin and Shelley[6] say that the spinal fluid

[82] Major and Nobel, Arch. Int. Med., 1914 (14), 383.
[83] Saito, Japan Med. World, 1920 (10), No. 29.
[84] Leschke and Pincussohn, Deut. med. Woch., 1917 (43), 8; Katakura, Kyoto Jour. Med. Sci., 1916 (13), 1.
[85] Lemaire and Debré, Jour. physiol. et path. gén., 1911 (13), 233.
[86] Levinson and Becht, Jour. Amer. Med. Assoc., 1920 (74), 1310.
[87] Pediatria, 1922 (30), 863.
[88] Schleissner, Med. Klin., 1921 (17), 1206.
[89] See Hoffman and Schwartz, Arch. Int. Med., 1916 (17), 293.
[90] See Levinson, Landenberger and Howell, Amer. Jour. Med. Sci., 1921 (161), 561.
[91] Jour. Med. Res., 1915 (33), 119.
[92] See Noguchi, Jour. Exp. Med., 1909 (11), 604.
[93] Neymann and Hektoen, Jour. Nerv. and Mental Dis., 1922 (56), 16.
[94] See Mestrezat, Rev. d. Méd., 1910, p. 189; Kaffka, Deut. med. Woch., 1913 (39), 1874.
[95] Arch. Int. Med., 1920 (26), 436.
[96] Seham and Nixon, Arch. Int. Med., 1921 (28), 561.
[97] Wien. klin. Woch., 1921 (34), 614.
[98] Levinson and Kirschenbaum, Jour. Infect. Dis., 1923 (33), 193.
[99] Lancet, July 9, 1910.
[1] See Rotky, Zeit. klin. Med., 1912 (75), 494.
[2] Schottmüller and Schumm, Neurol. Zb., 1912 (31), 1020.
[3] See Thalheimer and Updegraff, Jour. Neurol. Psych., 1922 (8), 15.
[4] Weston, Jour. Med. Res., 1916 (35), 199; Kraus and Corneille, Jour. Lab. Clin. Med., 1916 (1), 685; Alpers *et al.*, Arch. Neurol. Psych., 1924 (11), 653.
[5] Leopold and Bernhard, Amer. Jour. Dis. Chil., 1917 (13), 34; discussion of chemistry of spinal fluid in children. See also Wilcox and Lyttle, Arch. Pediat., 1923 (40), 215; Steiner, Jahrb. Kinderheilk., 1923 (52), 173.
[6] Arch. Int. Med., 1925 (35), 242.

sugar is between 45 and 65 per cent. of that of the blood, except after an alimentary hyperglucemia when there is for a time an excessively high proportion of sugar in the spinal fluid. In early cerebrospinal syphilis and in meningitis, whether tuberculous or acute, the sugar content is low both actually and in proportion to the blood sugar.

Spinal fluid contains normally from 2 to 4 mg. of amino-N per 100 cc., or about half that in the blood, without definite changes in syphilis.[7] There is almost the same amount of urea as in the serum of the same person, *i. e.*, 20 to 42 mg. per 100 cc.[8]

In uremia the non-protein constituents of the spinal fluid increase with those of the blood, but to a less degree. Brun[9] found about half as much residual N in the spinal fluid as in the blood normally, but in uremia the proportion may become much higher, and he found as much as 353 mg. per 100 cc. of spinal fluid in a case of uremia with 376 mg. nonprotein-N in the blood; the proportion was generally higher in uremia without than with convulsions. According to Bernhard[10] there is about one-fifth as much uric acid in the spinal fluid as in the blood, there being more in children than in adults, and no particular change except in uremia. However, Brogsitter and Krauss[11] found normally one half as much uric acid, two-thirds as much creatinine, and somewhat more chloride in spinal fluid than in the blood; in uremia the amount of uric acid, creatinine, and total residual N, lags behind the rise in the blood. Substances giving the ninhydrin test appear in meningitis,[12] but Rosenberg states that even with the highest indicanemia[13] no indican is found in the spinal fluid. In tuberculous meningitis the fluid may give positive reactions for free tryptophan and tyrosine.[14] In diseases associated with destruction of brain tissue, such as general paralysis and epilepsy,[15] *choline* or some other base[16] may be found in the spinal fluid (See "Choline," Chap. xxiii).

(7) *Xanthochromia.*—In cases of retention of spinal fluid, usually in a lumbar cul-de-sac, it may assume a yellow color although free from blood pigment, containing much globulin and numerous mononuclears, and coagulating spontaneously (Froin's syndrome). In one case Denis and Ayer found 2 per cent. of protein in this condition. The color is apparently due to concentration of plasma held for some time in the spinal canal, and may be from bilirubin.[17] Most usually this condition accompanies tumor of the spinal cord,[18] but simple xanthochromia, without the other features of Froin's syndrome, often occurs in meningitis or other conditions with escape of red corpuscles, without obstruction to the spinal canal.[19]

Subcutaneous Effusions.[20]—When of non-inflammatory origin these are very watery, having ordinarily a protein content of from 0.1 to 0.2 gm. per 100 cc., there being more globulin in nephritic than in cardiac dropsy. The non-coagulable nitrogen and chloride content are not so high as in the blood of the same patients, but the ash is the same as that of the serum. The specific gravity may be as low as 1.005, but the solids increase with the duration of the edema.

Hydrocele and Spermatocele Fluids.—These have been studied particularly by Hammarsten, who found the average result of analyses of seventeen hydrocele fluids and four spermatocele fluids as follows:

[7] Ellis *et al.*, Jour. Amer. Med., Assoc., 1915 (64), 126.
[8] Ellis and Cullen, Jour. Biol. Chem., 1915 (20), 511.
[9] Acta Med. Scand., 1919 (52), 367; full discussion of spinal fluid in nephritis.
[10] Jour. Lab. Clin. Med., 1924 (9), 753.
[11] Deut. Arch. kl. Med., 1924 (143), 297.
[12] Nobel, Münch. med. Woch., 1915 (62), 1355, 1786.
[13] Berl. klin. Woch., 1916 (53), 1314.
[14] Aiello, Policlinico, 1922 (29), 537.
[15] Concerning spinal fluid in epilepsy see Larkin and Cornwall (Jour. Lab. Clin. Med., 1919 (4), 352).
[16] Kaufmann, Zeit. physiol. Chem., 1910 (66), 343; Laignel-Lavastine and Lasusse, Compt. Rend. Soc. Biol., 1910 (68), 803.
[17] Bauer and Spiegel, Deut. Arch. klin. Med., 1919 (129), 18.
[18] Review by Sprunt and Walker, Bull. Johns Hop. Hosp., 1917 (28), 80; Elsberg and Rochfort, Jour. Amer. Med. Assoc., 1917 (68), 1802.
[19] See Wallgren, Acta Med. Scand., 1920 (53), 303; Levinson, Arch. Int. Med., 1920 (26), 459; Leschke, Deut. Med. Woch., 1921 (47), 376.
[20] See Epstein, Jour. Exper. Med., 1914 (20), 334.

TABLE VII

	HYDROCELE	SPERMATOCELE
Water	938.85	986.83
Solids	61.15	13.17
Fibrin	0.59	
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether-extractive bodies	4.02	
Soluble salts	8.60	10.76
Insoluble salts	0.66	

Marchetti[21] found in ten specimens of hydrocele fluid rather higher results for the solids than did Hammarsten. He found 57.8 to 104.2 p. m. of solids, containing organic substances 48.8 to 95.02, and inorganic substances 8.10 to 9.56; proteins 33.5 to 90.19; ratio of globulin to albumin as 2.56 to 9.11. Among the proteins is found 1 to 4 p. m. that is not precipitated by heat. Corresponding with the analytic results, the specific gravity of hydrocele fluid is higher, 1.016 to 1.026 as against 1.006 to 1.010 for spermatocele fluid. Cholesterol is often abundant in hydrocele fluids, appearing to the naked eye as glistening scales. Patein[22] found sugar in most specimens of hydrocele. Apparently hydrocele fluid stands intermediate in properties between transudates and exudates.[23] Usually it contains but little of the immune bodies from the blood (Delrez).[50]

Wound secretions obtained from large aseptic wounds, mostly amputation stumps, have been studied by Lieblein.[24] The reaction is generally alkaline, globulin and albumin abundant, but fibrinogen scanty, total nitrogen being less than that of the blood and decreasing from day to day; the proportion of albumin increases and globulin decreases as healing progresses. Occasionally albumoses were found, but only on the first day in aseptic wounds; if found later, they generally were antecedent to suppuration (concerning suppuration see "Inflammation," Chap. XIII).

Blister fluid is generally rich in solids and proteins (40–65 p. m.). In a burn blister Mörner[25] found 50.31 p. m. proteins, among which were 11.59 p. m. globulin and but 0.11 p. m. fibrin; also a substance reducing copper oxide, but not pyrocatechin. By refractometric determinations the amount of protein in blister fluids is in direct proportion to the amount in the blood.[26] Residual-N is about the same as in the blood but sugar is higher (Gänsslen).[27] Antibodies of all sorts seem to pass readily into blister fluids[28] although the complement-fixation reaction is not so strong as with the blood.[29]

Hydrosalpinx, according to Jägerroos,[30] contains from mere traces of protein up to 6.75 per cent., the ratio of albumin to globulin varying, but generally being low; the specific gravity varies from 1.006 to 1.022 and the character is generally more like a transudate than an exudate.

Hydrops of Gall Bladder.—The watery fluid contains 99 per cent. water, a mucin-like substance, but no other proteins and no bile acids.[31]

Fetal Bronchiectasis.—The fluid resembles closely liquor amnii.[32]

Chylous Effusions.[33]—Fat may be present in effusions in sufficient quantity to cause a milky appearance, either from escape of chyle from a

[21] Lo Sperimentale, 1902 (56), 297.
[22] Jour. pharm. et chim., 1906 (23), 239; also Compt. Rend. Soc. Biol., 1906 (60), 303.
[23] Vecchi, Gaz. Med. Ital., 1912 (63), 211; Epstein, Jour. Exp. Med., 1914 (20), 344.
[24] Beit. klin. Chir., 1902 (35), 43.
[25] Hammarsten, "Physiological Chemistry."
[26] Engel and Orszag, Zeit. klin. Med., 1909 (67), 175.
[27] Münch. med. Woch., 1922 (69), 263.
[28] Eisenberg, Deut. med. Woch., 1909 (35), 613.
[29] Buschke and Zimmermann, Med. Klinik, 1913 (9), 1082.
[30] Arch. Gynäk., 1921 (114), 328.
[31] Sjöquist, Svenska Läk. Handl., 1916 (42), 1291.
[32] Koeckert, Amer. Jour. Dis. Chil., 1919 (17), 95.
[33] Literature by Gandin, Ergeb. inn. Med., 1913 (12), 218.

ruptured or obstructed thoracic duct, or through fatty degeneration of the cells in the effusion or the lining of the walls of the cavity. The former are designated as chylous, the others as chyliform or adipose fluids, but it is not always easy to distinguish between them, and, according to Blankenhorn it is doubtful if any such distinction should be made, since in all cases the turbidity is due to emulsified fat.[34] The composition of the fluids in chylous exudates will vary according to the food taken and the amount of fat the food contains, and will resemble the composition of chyle, except to the extent that it is modified by the effusion or absorption going on in the cavity. They are characterized by strong bactericidal powers, as evidenced by lack of putrefaction after long standing.

Analyses of human chyle are scanty. Panzer[35] found 90.29–94.53 per cent. water; 5.47–9.71 per cent. solids; 0.80–1.04 per cent. inorganic salts; 2.16 per cent. coagulable protein; 6.59 per cent. ether-soluble material; also diastatic enzymes, soaps, and occasionally traces of cholesterol, lecithin, and sugar. Carlier,[36] in a specimen from a child, obtained very similar results, except that the salts were much less abundant. The proteins and fats vary greatly with the diet; thus Sollmann[37] found variations in the proteins from 1.85 to 6.5 per cent.

Edwards[38] found that of 31 definitely established cases of chylous or chyliform ascites studied at autopsy, in 21 there was established the existence of a rupture in the thoracic duct or lacteals. Chylous ascites fluid often, but not always, contains sugar,[39] but it may disappear after having once been present; the amount of fat is small, usually about 1 per cent., and the fluid is rich in solids. If due to a ruptured thoracic duct, it may be possible to detect special fats taken in the food, *e. g.*, butter-fats (Straus).[40] The reaction is usually alkaline or neutral, and some specimens coagulate spontaneously. Specific gravity varies from 1.007 to 1.040, the average being about 1.017. Perhaps the most important characteristic is the variation produced by changes in diet.[41] Zdarek[42] found in a chyle-cyst 2.7 per cent. of fats, 7.2 per cent. of proteins, and 0.05 per cent. of sugar; feeding of fats increased their amount in the cyst and starvation decreased it. Schumm[43] found in the solids of such a cyst 35.76 per cent. of fat, some of which was in the form of calcium soap.

Chylothorax fluid is, of course, quite similar to that of chylous ascites. Thus, Buchtala[44] found 91.34 per cent. of water, 8.66 per cent. solid, 4.86 per cent. protein,

[34] Arch. Int. Med., 1923 (32), 129.
[35] Zeit. physiol. Chem., 1900 (30), 113.
[36] British Med. Jour., 1902 (ii), 175.
[37] Amer. Jour. Physiol., 1907 (17), 487; see also Hamill, Jour. Physiol., 1906 (35), 151.
[38] Medicine, 1895 (1), 257; also see "Chem. u. morph. Eigenschaften fetthaltige Exsudaten," St. Mutermilch, Warschau, 1903.
[39] For example, v. Tabora (Deut. med. Woch., 1904 (30), 1595) found as high as 0.864 per cent. of sugar in a typical case.
[40] Arch. Physiol. et Pathol., 1886 (Ser. 3, vol. 8), 367.
[41] A sample of the composition of 1 liter of chylous ascitic fluid is shown by the analysis in the case studied by Comey and McKibben (Boston Med. Surg. Jour., 1903 (148), 109). Specific gravity, 1.010; solids, 21 gm.; protein, 9.75 gm.; urea, 1.28 gm.; fat, 1.45 gm.; inorganic matter, 8 gm.; peptone (?) and sugar, present; fibrinogen, mucin, nucleo-albumin, and uric acid absent.
[42] Zeit. f. Heilk., 1906 (27), 1.
[43] Zeit. physiol. Chem., 1906 (49), 266.
[44] Zeit. physiol. Chem., 1910 (67), 42.

2.5 per cent. fat, 0.26 per cent. cholesterol, and 0.94 per cent. ash. Similar figures were obtained by Salkowski[45] and others.

Chyluria,[46] which seems to depend upon an abnormal communication between the lymphatics of the receptaculum chyli and the kidney,[47] shows no particular chemical features beyond those of an admixture of a considerable amount (100 to 1000 c.c. per day) of chyle with the urine. Carter[48] found the amount of fat in the urine to rise with increase of fat in the food. Pecker[49] observed a rise from a former average of 1.5 gm. fat per liter to 9.75 gm. after eating oils and milk. The lipins are mostly neutral fats, and there is commonly found a protein which is precipitated with acetic acid (Patein).[49] In some cases chyle escapes directly into the bladder or ureter from the lymphatics, in others the fat may be excreted directly from the blood, independent of lymphatic abnormality; in some cases the fluid entering the urine is true chyle and in others it is lymph.

"Ascites adiposus" is characterized by the absence of sugar and by a higher percentage of fat, the maximum observed being 6.4 per cent. It is ascribed to fatty metamorphosis of cells, particularly in carcinomatous and tuberculous exudates; Edwards was able to show experimentally that a transudate may change from serous to cellular, and later come to contain fat.

"Pseudochylous effusions" are also observed, not only in the abdominal and thoracic cavities, but even in the fluid of the edematous legs and scrotum; these resemble chylous fluids in being turbid or milky, but are said to contain little or no fat. The turbidity is ascribed chiefly to lecithin, which is largely combined with the pseudoglobulin of the fluid (Joachim),[50] but there may be some cholesterol.[51] Possibly in some cases the turbidity is partly or largely (Poljakoff)[52] due to poorly dissolved proteins. Strauss[53] has noted the occurrence of this form of ascites particularly in chronic parenchymatous nephritis, but believes the turbidity has a local origin. Hammarsten has observed turbidity due to mucoid substances, as also have Gouraud and Corset.[54] The pseudo-chylous effusions have a lower freezing point, a lower specific gravity, a lower fat and greater lecithin content than typical chylous ascites. Gandin,[33] however, questions the possibility of always differentiating the three types of turbid fluids as above indicated. Collecting all the recorded analyses in the literature he finds wide discrepancies, as indicated in the following table: (The maximum and minimum percentage figures are given for each component determined quantitatively, with the average in parentheses).

	Chylous	Adipose (Chyliform)	Pseudochylous
Ether extract	0.065 – 9.2 (1.65)	0.1 – 4.3 (1.15)	0.007 – 1.86 (0.25)
Cholesterol	+ in 7, – in 2	+ in 4	+ in 3, – in 2
Lecithin	+ in 4, – in 1	+ in 3	+ in 20, – in 2
Sugar	+ in 46, – in 28	+ in 1, – in 4	+ in 15, – in 14
Dry residue	3.1 – 10.6 (6.2)	1.6 – 11.7 (5.1)	1.2 – 7.6 (2.9)
Protein	0.9 – 7.7 (3.5)	0.6 – 6.8 (3.0)	0.1 – 4.2 (1.4)
"Pepton"	+ in 6, – in 4	+ in 1, – in 2	+ in 1, – in 5
Ash	0.1 – 1.0 (0.59)	0.45 – 1.03 (0.65)	0.49 – 0.90 (0.73)

It is quite evident that although the pseudochylous fluids usually contain little fat, they often contain more than the minimal content found in the other forms. Each type of fluid overlaps the others in one respect or another. Gandin states

[45] Virchow's Arch., 1909 (198), 189; also Tuley and Graves, Jour. Amer. Med. Assoc., 1916 (66), 1844; patein, Jour. pharm. chim., 1915 (11), 265; Welker and Williamson, Science, 1920 (51), 397.

[46] Review of literature by Sanes and Kahn, Arch. Int. Med., 1916 (17), 181.

[47] See Magnus-Levy, Zeit. klin. Med., 1908 (66), 482.

[48] Arch. Int. Med., 1916 (18), 541.

[49] Jour. pharm. chim., 1917 (16), 139. See also Patein, ibid., 1917 (16), 230.

[50] Münch. med. Woch., 1903 (50), 1915; also Christen, Cent. f. inn. Med., 1905 (26), 329; Wallis and Schölberg, Quart. Jour. Med., 1910 (3), 301; 1911 (4), 153.

[51] Gibson and Howard, Amer. Jour. Med. Sci., 1923 (166), 80.

[52] Fortschr. d. Med., 1903 (21), 1081; also Haushalter, Compt. Rend. Soc. Biol., 1910 (68), 550.

[53] Note to Poljakoff's article;[52] also Biochem. Centr., 1903 (1), 437.

[54] Compt. Rend. Soc. Biol., 1906 (60), 23.

that to produce a turbid fluid but 0.01–0.1 per cent. of finely emulsionized fat is necessary, and he believes that milky fluids always mean admixture of chyle, rejecting the terms pseudochylous and chyliform as unwarranted. He admits that fluids may contain droplets of fats not emulsionized, and hence not milky, which may be properly called adipose fluids. There are no characteristic chemical differences in the fats extracted from the different types of fluids. Blankenhorn[34] also holds that the chief difference of pseudochylous or chyliform from chylous effusions is that the latter have larger fat particles and are often more concentrated, and doubts the existence of turbidity due to anything else but fat

Chemistry of Pneumothorax

In connection with the subject of exudates the above topic may appropriately be considered. The composition of the gases found in the pleural cavity in pneumothorax will necessarily vary greatly according to the cause. If the pleural cavity is in free communication with the exterior, the gas will be simply slightly modified air; for example, Ewald[55] found the following proportions in the gases in such a pneumothorax: CO_2, 1.76 per cent.; O, 18.93 per cent.; and 79.31 per cent. N. Here the proportion of CO_2 is even a little less than in ordinary expired air, which contains 3.3–3.5 per cent. When air enters a closed pleural cavity and no effusion follows, it is slowly absorbed until a mixture of about 90 per cent. N, 4 per cent. O and 6 per cent. CO_2 results; but if there is a serous effusion the oxygen disappears nearly or quite completely (Tobiesen).[56] Grass and Meiners[57] give the average in closed dry pneumothorax as 3.67 per cent. O and 6.95 per cent. CO_2; with exudation the CO_2 may rise to 10 per cent. while the oxygen falls to 1 per cent. In general, in a closed pneumothorax the gases come to an equilibrium with the gases of the blood and tissues, except when there is inflammation which causes a rise in CO_2 and a fall in O.[58] In a seropneumothorax Ewald found 8.13 per cent. of CO_2, 1.26 per cent. of O, and 90.61 per cent. of N, which is quite similar to the proportions of the gases in dry pneumothorax. Purulent pneumothorax generally shows more CO_2 than the serous form, the average in the former being 15–20 per cent., in the latter 7.5–11.5 per cent. The average of the analyses in six cases of pyopneumothorax is given by Ewald as 18.13 per cent. CO_2, 2.6 per cent. O, and 79.81 per cent. N. In open pyopneumothorax the gas approaches more closely the composition of air, but usually shows a slight excess of CO_2; it is thus possible by a determination of the carbon dioxide to learn whether a given pneumothorax is in communication with the outside air. The transformation of a purulent into a putrid pneumothorax is accompanied by an increase of CO_2, even as high as 40 per cent. having been found. The products of decomposition by the putrefactive saprophytes also are present, one analysis having shown 4.3 per cent. of hydrogen, 6.25 per cent. of methane, and traces of hydrogen sulfide.

Infection of a pleural effusion by gas-producing organisms may also convert it into a pneumothorax, although this is not a common occurrence. The gases then present are the same as the organisms produce in similar culture-media, modified somewhat by absorption. The anaërobic gas-producing organisms have been found as the cause of such gaseous accumulations; it is questionable if the ordinary pathogenic organisms can cause a pneumothorax, since they are for the most part not capable of producing gas. Complete quantitative analyses of the gas in this form of pneumothorax seem not to have been made, but May found about 20 per cent. of CO_2. The combustibility of the gas has frequently been noted, and is probably due to hydrogen and methane.[59] The colon bacillus produces gas in sugar-containing media, but the amount of sugar in the pathological exudates is too small to yield any considerable amount of gas; an exception is the pleural effusion in diabetes, and pneumothorax from infection of the pleural effusion in a diabetic by *B. coli* has been reported.

[55] Early literature and résumé given by Clemens, in Ott's "Chem. Path der Tuberculose," Berlin, 1903, p. 406.

[56] Beitr. z. Klin. d. Tuberk., 1911 (19), 451; 1911 (21), 109; Deut. Arch. klin. Med., 1914 (115), 399.

[57] Beitr. klin. Tuberk., 1922 (51), 134.

[58] Hill and Campbell, Brit. Med. Jour., 1923 (1), 752.

[59] *Intestinal Cystic Pneumatosis.*—In this condition Pascual found CO_2, 0.6–5.6; O, 0.0–10.4; N, 78.9–89 per cent.; in one case 14.6 per cent. of H was present (Anales soc. españ. fís. quím., 1924 (22), 506).

CHAPTER XVII

RETROGRESSIVE CHANGES (NECROSIS, GANGRENE, RIGOR MORTIS, PARENCHYMATOUS DEGENERATION)

NECROSIS

We recognize that a cell is alive through its reproducing, functioning, and taking on and utilizing nutritive substances; yet at the same time we appreciate that a cell may do none of these things and still be alive. For example, a bacterial spore is quite inert physically, and exhibits no chemical activity, yet it is by no means dead, since it still possesses the latent power to assume again an active existence under suitable conditions. In pathological conditions we are accustomed to recognize the fact that a cell is dead by certain alterations in its structural appearance, particularly disintegrative changes in the nucleus; but this is exactly equivalent to recognizing that an animal is dead by the appearance of postmortem decomposition, for most of the characteristic histological changes of necrosis are merely postmortem changes in the cell. A cell may be dead and show absolutely none of these microscopic disintegrative changes, either because it has not been dead long enough for them to have taken place, or because the changes have been prevented by some means, just as we can prevent the appearance of postmortem decomposition by embalming. For example, if we examine microscopically the mucous membrane of the stomach of a person who has died immediately after taking a large quantity of carbolic acid, although to the naked eye this mucous membrane is hard, white, and definitely necrotic, yet we find the histological picture presented by the cells almost absolutely unchanged from the normal. The cells are dead, but they have been so "fixed" that postmortem changes could not affect their structure. All cells examined by ordinary histological methods are, of course, dead—killed by the fixing agents outside of the body, in the same way that the carbolic acid fixes them within the body. It is evident, therefore, that it may be very difficult to determine always whether a cell is dead or not. Part of the difficulty, perhaps, lies in our failure to appreciate that not all parts of a cell die at the same time; *i. e.*, the different chemical processes of the cell depend on its different intracellular enzymes, and these are not necessarily destroyed at the same rate by the same agents. Even considerable respiratory activity may be exhibited by cells that have been killed.[1]

We recognize that after an animal is dead as a whole the various cells of its body do not die for some time, as shown by the following examples:

[1] See Haas, Bot. Gazette, 1919 (67), 347.

(1) We can cause the heart to beat for a considerable period after its removal from the body; (2) if we perfuse a mixture of glycine and benzoic acid through the kidney of a recently killed animal, synthesis of these substances into hippuric acid will occur; and (3) the epithelium of the skin can be removed from the body of an animal long after death and transplanted successfully on another animal. So, too, in ordinary cell death (necrobiosis) not all the enzymes are destroyed together. When all are destroyed at once, as by strong chemicals or by heat, the customary disintegrative changes do not take place. If, however, not all the enzymes are thrown out of function, then the others may be able to act, producing the disintegrative changes by which histologists ordinarily recognize cell death. These disintegrative changes are, for the most part, apparently brought about by the intracellular proteases, that is, through autolysis. This may be shown as follows:[2] If we take two pieces of fresh normal tissues from an animal, and in one kill the enzymes by heating to 100° C., then implant both aseptically into the abdominal cavity of an animal of the same species, it will be found that the changes that follow in the two will be very unlike. In the unheated tissue nuclear changes soon occur, so that they lose their capacity for taking up basic stains, the cytoplasm becomes granular and fragmented, the tissue becomes friable so that it is difficult to secure good sections, and the changes are in general similar to those seen in areas of necrosis. The boiled tissue, on the other hand, retains its capacity for nuclear staining for months, except at the periphery, where it is slowly attacked by leucocytes and the enzymes of the blood plasma. Therefore it would seem that the characteristic changes of necrosis depend chiefly upon the intracellular enzymes, rather than upon the infiltrating plasma as Weigert[3] and other early writers imagined. In areas of anemic necrosis (see "Infarcts") we have another case, in which the oxidizing enzymes are thrown out of function through lack of oxygen, while the other enzymes are, presumably, at first unaffected. From studies of infarcts it would seem that the intracellular proteases bring about the subsequent nuclear and cytoplasmic alterations, but that the eventual digestion of the area is accomplished by the invading leucocytes working slowly inward from the periphery. Apparently when the supply of materials from outside ceases, and when the oxidation processes of the cells no longer accomplish necessary steps of synthetic reactions or destroy products of protein catabolism, the proteases continue to split proteins without the balancing by the above-mentioned factors, with a resulting disintegration of the cells.

Karyolysis and *karyorrhexis* are, then, the result of an autolytic process, which is perhaps due to intracellular proteases that act specifically on nucleoproteins, and which may be designated as *nucleases*.[4] Nuclear staining by the usual methods depends upon an affinity of the acid nucleoproteins (in which the nucleic acid is not completely saturated by pro-

[2] Wells, Jour. Med. Research, 1906 (15), 149.
[3] Cent. f. Path., 1891 (2), 785.
[4] See Purine Metabolism, Chap. XXV.

teins) for basic dyes. Presumably in karyolysis the first step consists in a splitting of the nucleoprotein of the chromatin into nucleic acid and protein; this can be accomplished, according to Sachs, by trypsin, and presumably, therefore, by the trypsin-like enzymes of the cell. Corresponding with this change we should expect the free nucleic acid to give an intense staining with basic stains, and this has frequently been described by those who have studied the cytological changes in anemic necrosis,[5] in the condition called *pycnosis*. As supporting this view still further may be quoted Arnheim's[6] observation that in alkaline solutions the nucleus soon stains diffusely and weakly, and not at all after twelve to eighteen hours; this is to be explained by the fact that nucleic acid is both dissolved and neutralized by alkaline solutions. Acids developed in injured cells may, by combining with the basic elements of the nucleoproteins, render them still more acid and highly basophilic; thus, in muscles showing waxy degeneration from accumulation of lactic acid the muscle nuclei will be found pycnotic (see waxy degeneration). After the nucleic acid has been freed from the protein by the autolytic enzymes, it is still further decomposed by the "nuclease" or similar intracellular enzymes that have the property of splitting nucleic acid into the purine bases that compose it[7]—corresponding with this change the hyperchromatic nucleus loses its affinity for stains, and *karyolysis* is complete. When extensive necrosis occurs there will result, therefore, an increased elimination of purines, as was found by Jackson and Pearce[8] in animals with severe hepatic necrosis from hemotoxic serum.

A careful analytical study of the changes taking place in the autolyzing spleen, for the purpose of correlating the chemical and microscopical changes, has been made by Corper,[9] which corroborates the interpretation of necrosis advanced above. He found that during the stage when pycnosis is the chief feature there is no appreciable change in the nucleus; that is, the nucleic acid has not been split into free purines and the rest of its components; at this stage but little change has occurred in the lecithin, and a very slight amount of proteolysis is demonstrable. During the stage of karyorrhexis and karyolysis the most active disintegration is taking place, about one-fourth of the nucleic acid becoming disintegrated by the time all nuclear structures have disappeared; in the same period nearly half the lecithin (phosphatids) is hydrolyzed, while about one-fourth the coagulable protein has been hydrolyzed into non-coagulable compounds. After this stage the changes are very slow. It is somewhat surprising to find that when no vestige of nuclear substance remains in stainable form, there still remains three-fourths of the nucleic acid in an intact condition. Corper publishes a series of plates, together with the chemical details, thus establishing a standard whereby the histological changes can be interpreted in terms of the chemical changes which cause them.

Autolysis of aseptically preserved tissues outside the body is much more rapid than is the autolysis of infarcts and similar aseptic necrotic areas within the body. This may be due to either or both of two factors:[10]

[5] Schmaus and Albrecht, Virchow's Arch., 1895 (138), supp., p. 1; Ergeb. allg. Pathol., 1896 (3), 486 (literature).

[6] Virchow's Arch., 1890 (120), 367.

[7] According to Bauer (Virchow's Arch., 1920 (228), 526) granules of free purines can be stained in necrotic cells.

[8] Jour. Exper. Med., 1907 (9), 569.

[9] Jour. Exper. Med., 1912 (15), 429.

[10] Literature and more complete discussion under "Autolysis."

First, autolysis is much slower in neutral than in acid media; outside the body autolyzing tissues develop an acid reaction which favors their autolysis; within the body this is checked by the plasma. Second, the plasma contains inhibiting substances, which also may interfere with self-digestion in the body. In corroboration of the above may be recalled the fact that large necrotic areas show autolysis first in the center, where the antagonistic body fluids presumably cause the least effect. It has been found by Wells[11] that the histological changes of autolysis proceed much faster in tissues placed in serum that has been heated to destroy the antibodies than in unheated serum. Leucocytes, as Opie has shown, contain autolytic enzymes acting best in an alkaline medium; hence they perform their digestive function readily at the periphery of necrotic areas, and coagulated tissue proteins, when acted upon by body fluids, produce chemotactic substances which attract leucocytes to dead areas.[12]

When a cell dies, certain *physical changes* occur that are probably of considerable importance. Bechhold says: "With the occurrence of death, protoplasm gelatinizes, Brownian movement of the smaller particles ceases, and the structure of the gel appears in the ultramicroscope as a conglomeration of many reflecting platelets. It makes a substantial difference whether the protoplasm dies slowly or is suddenly killed by a fixative (alcohol, formalin, etc.). In the first instance there is a precipitation (flocculation), whereas, in the latter there is a stiffening; this difference may be readily recognized under the ultramicroscope." The permeability of the cell wall is almost immediately increased, so that all diffusible substances readily pass through, *i. e.*, its semipermeable character is lost. This we see particularly in plant cells, which lose their turgor with their semipermeability, and therefore the plant wilts. The cell structure is also disintegrated, and as a result coördination of the cell chemistry is at once destroyed.[13] Intracellular enzymes escape into the blood from areas of local death of cells,[14] or as an agonal manifestation in general death.[15] Various dyes which cannot penetrate living cells may stain dead or dying cells.[16]

These changes depend on alterations in permeability, and as permeability determines electrical resistance, Osterhout has used the resistance of plant cells as an indicator of vitality.[17] He finds that normal cells have a rather constant resistance, which is reduced by anything that lowers the vitality of the cell, and in direct proportion to the degree of injury or loss of vitality. The temperature coefficient is also considerably lower in dead than in living tissue. When secondary disintegrative

[11] Jour. Med. Research, 1906 (15), 149.
[12] Bürger and Dold, Zeit. Immunität., 1914 (21), 378.
[13] See v. Prowazek, Biol. Centrbl., 1909 (29), 291. Pictet suggests that in dead proteins, aldehydes and amino radicals unite with one another to form cyclic compounds (Arch. sci. phys. nat., 1915 (40), 181).
[14] Mandelbaum, Münch. med. Woch., 1914 (61), 461.
[15] Schultz, Münch. med. Woch., 1913 (60), 2512.
[16] See Steckelmacher, Beitr. path. Anat., 1913 (57), 314.
[17] Full review in "Injury, Recovery and Death in Relation to Conductivity and Permeability," W. J. V. Osterhout, Lippincott, 1922. Phila.

changes occur in the protoplasm, with the formation of many small molecules from the large molecules of the cell, both osmotic pressure and electrical conductivity increase rapidly. Changes in the permeability of cell protoplasm, however, may be of considerable degree without necessarily indicating serious injury of the cells (Osterhout). Death is accompanied by changes of the character of a monomolecular reaction, which is continually going on even in a normal actively growing cell, and which is accelerated by the toxic agent. Up to a certain point the reaction seems to be reversible.

The loss of the oxidative function of the cell with cell death may be recognized by various colorimetric reactions, *e. g.*, the reduction of meta-dinitrobenzol to meta-nitrophenylhydroxylamine is stopped when death occurs,[18] but dead tissues continue to absorb oxygen.[19] Immediately after death the reaction becomes more acid, through formation of lactic acid because of defective oxidation, and there is a setting free of potassium and phosphates, presumably in a partial neutralization of this acid.[20]

Colloidal Changes and Senescence.—A principle of colloid chemistry, the alteration of colloids with time, has an interesting bearing on the question of aging and natural death of tissues.[21] It is characteristic of colloidal solutions (which, of course, is what cells are), that they continuously change in their properties, the change being generally in the direction of aggregation of the disperse colloidal particles, with a resulting tendency to precipitation or coagulation; the gels tend to decrease in elasticity and to become more turbid, associated with which are alterations in their permeability to crystalloids. A gelatin mass possesses its maximum elasticity three or four hours after it is first formed; and crystalloids penetrate fresh, quickly-formed gels at first more rapidly than later. As Bechhold says, we can imagine a relation of such facts to(1) the greater elasticity of young tissues; (2) to a presumably greater permeability for crystalloids and hence more rapid metabolism; (3) to the decreasing water of the tissue with age (94 per cent. of water in the fetus of three months, 69–66 per cent. at birth, and 58 per cent. in adults); (4) to the demonstrated greater permeability of young nerve tissues for vital stains, etc. "In general we can say that the tissue colloids decrease in their water affinity (*Quellbarkeit*) both in animal organisms, which become poorer in water with age, and in plants, as shown by the hardening of older plant tissues."

Dehydration of the colloids results in a decrease in their surfaces and with this come lowered energy and electrostatic capacity.[22] Ruzicka[23] has named this progressive increase in the condensation products with age, "protoplasmic hysteresis," and says that it can be measured by the changes in the *p*H and other physical values. Moulton,[24] however, finds that the changes of later life depend chiefly on a progressive increase in the fat content of the tissues, the proportion of water, protein, and salts remaining practically constant after reaching a certain point if calculated on a fat-free basis. The water content of mammals decreases very rapidly from conception to birth, when approximately 4.5 per cent. of the total life cycle has been completed, alike in all species. The bearing of these principles on the problem of senility and degeneration of elastic tissue, regeneration and many other subjects is obvious.

[18] Rostock, Arch. ges. Physiol., 1923 (199), 217.
[19] Bauer, Bull. Johns Hopkins Hosp., 1923 (34), 422.
[20] See Menten, Jour. Med. Res., 1922 (43), 323.
[21] See H. Bechhold, "Die Kolloide in Biologie und Medizin," Dresden, 1912, p. 65.
[22] See Rocasolano, Kolloid chem. Beihefte, 1924 (19), 441.
[23] See Deut. med. Woch., 1922 (48), 931; Arch. Mikr. Anat. Entwick, 1924 (101), 459.
[24] Jour. Biol. Chem., 1923 (57), 79.

Causes of Necrosis

Anemia.—After the cutting off of blood-supply, cells soon undergo morphological changes that we recognize as indicating their death, and after a time they also become incapable of returning to their normal condition when the blood-supply is re-established, probably because of these structural changes. In just what way lack of nourishment causes death has not been determined, but, as has been before suggested, it seems probable that it is because catabolic processes are no longer balanced by anabolic processes, and with these latter, oxidizing enzymes seem to be inseparably associated as far as our present knowledge shows us. That the loss of oxygen alone, with other materials presumably supplied to the cells in adequate amount, may cause necrosis, is shown by the presence of marked hepatic necrosis in animals kept a week in atmospheres extremely low in oxygen (5–9 per cent.).[25] Life may be maintained by merely supplying oxygen in solution in the blood after the hemoglobin has been saturated with carbon monoxide,[26] so that evidently it is the oxygen as such that the tissues utilize.

The most highly specialized cells usually die first when oxygen is removed, presumably because their more active metabolism sooner produces lethal alterations; *e. g.*, anemia of the kidney causes death of the cortical tubular epithelium when the other elements are intact.[27]

Studying small fragments of rat tissues kept in neutral red solutions, and using special criteria as to death of cells, Lewis and McCoy[28] found that nerve cells die most quickly (1 hour) whereas tissue macrophages survived 240 hours, actively functioning epithelial cells dying much more rapidly than protective epithelium, cartilage, endothelium or smooth muscle cells.

Acid formation is probably the chief factor in producing anemic necrosis; *e. g.*, Stieglitz[29] found that in nephritic kidneys the damaged cells always present an acid reaction irrespective of the reaction of the urine. It is known that after shutting off of oxygen, there is a rapid development of acidity in tissues, which is enough to account for lethal alterations in the protoplasm, and also to incite autolysis; *e. g.*, five minutes after excision of a piece of liver its reaction had changed to *p*H 4.6, and lactic acid sufficient for isolation had formed.[30] Meyerhoff found that, at least in muscle, lactic acid is formed whether oxygen is present or not, but in the presence of oxygen it is then burned or otherwise removed, hence the high acidity present in asphyxiated muscle. Were it not that the proteolytic enzymes continue in action after nutrition is shut off, the cells might remain in a condition capable of resuming function when nourishment is again supplied, which is decidedly contrary to the facts.

[25] Martin, Loevenhart and Bunting, Jour. Exp. Med., 1918 (27), 399.
[26] Haldane, "Oxidation and Environment," Yale Univ. Publ., 1917, p. 45.
[27] See Chandler, Proc. Soc. Exp. Biol. Med., 1920 (18), 23.
[28] Bull. Johns Hopkins Hosp., 1922 (33), 284.
[29] Arch. Int. Med., 1924 (33), 483.
[30] Morse and Goldberg, Jour. Biol. Chem. (Proc.), 1922 (50), xxxviii.

(The general features of anemic necrosis have been already discussed in the preceding paragraphs, and also under the subject of infarction.)

Thermic Alterations.—These have been studied particularly in connection with the cells of the lower organisms.[31] While some unicellular organisms can survive a temperature of 69°, most of them are killed at from 40°–45°. For the great majority of metazoa the maximum temperature lies below 45°, and in the case of marine species below 40°.[32] The heating is accompanied by the appearance of granules in the cytoplasm, which become larger until the condition of "heat rigor" sets in. Kühne, in 1864, showed that in muscle cells, at least, there is contained a protein which becomes turbid through partial coagulation at 40°, and Halliburton[33] has found that in nearly all tissues are globulins coagulating at from 45°–50°; it is probable, therefore, that the granules formed in heated cells are produced through coagulation of these proteins. It has been difficult, however, to reconcile the temperatures at which coagulation of proteins take place and the relatively low temperatures at which cell life ceases. Heilbrunn[34] has found that, at least in sea urchin eggs, coagulation of the protoplasm precedes death of the cell, this coagulation not being a protein coagulation as it is reversible and apparently associated with some change in the state of the fatty materials of the cells.

The importance of this coagulation in determining death is not yet fully established, but it would seem to be very great. Halliburton has observed that in both muscles and nerves to which heat is applied, contractions occur at various temperatures, corresponding exactly with the temperatures at which the several varieties of the proteins of the cells coagulate.[35] Furthermore, Mott[36] has found that the temperature that is immediately fatal to mammals (47°) is exactly the same as the coagulating temperature of the lowest coagulating protein of nerve-cells. This fact is undoubtedly of great practical importance in causing death from fever, for although 47° C. (117° F.) is probably never reached in man, yet application of much lower temperatures, even 42° (108° F.), for a few hours, will cause coagulation of these proteins (all proteins coagulate at less than their ordinary coagulation point if the heating is continued for a long time). It would seem from the above observation that heat may cause cell death through coagulation of the proteins. Whether the cell death is in any way dependent upon destruction of the enzymes by heat has not been ascertained; but as most enzymes are not destroyed much below 60°–70°, it seems improbable that they are greatly injured at the temperatures at which cells are killed. It is possible, however, that

[31] Literature, see Davenport, "Experimental Morphology," New York, 1897; Schmaus and Albrecht, Ergebnisse der Pathol., 1896 (3, Abt. 1), 470.

[32] The adaptation of animal cells to high temperatures is an interesting topic, especially in view of such results as those of Dallinger, who, by raising the temperature gradually during several years, caused flagellata, with a normal maximum of about 21°–23° to become capable of living at 70° (see Davenport).

[33] "Biochemistry of Muscle and Nerve," Phila., 1904.

[34] Amer. Jour. Physiol., 1924 (69), 190.

[35] Not confirmed by Meigs, Amer. Jour. Physiol., 1909 (24), 178.

[36] Quoted by Halliburton.[33]

under the conditions in which enzymes exist in the cell they may be more susceptible to heat than under other conditions. Presumably coagulation of cell protoplasm determines the death of a cell by altering the physical conditions of the cell. Ordinarily we have in the cell an equilibrium between colloids in solution and colloids in the solid or gel state; if the colloids are rendered insoluble by heat, or by any other cause, so that this equilibrium is destroyed, serious alterations in the mechanism of all metabolism must result. Other chemical reactions will also have their point of equilibrium altered by changes in temperature, and such alterations might well have disastrous results.

Different tissues show unequal susceptibilty to heat. Werhovsky[37] found the blood most affected by raising the temperature of living animals, next the liver, kidneys, and myocardium in order, the other tissues being little or not at all structurally injured. Animals exposed to heat show a fall in the leucocyte count, followed by a rise in lymphocytes which persists; there is an extensive degeneration of cells in the spleen and lymph glands, followed by marked mitotic proliferation in the germinal centers.[38]

Cold[39] is well withstood by unicellular forms, and relatively poorly by more complex organisms, particularly by those with a highly developed circulatory system; this is because individual cells are not greatly affected by freezing, whereas the circulatory channels are readily blocked by this cause. Bacterial cells are not killed by exposure for long periods to the temperature of liquid air[40] ($-190°$). Reduction of the temperature of plant cells to $-13°$ may result in a granular transformation of the cytoplasm, often with rather serious structural alterations. Cytoplasm seems to be more affected than the nucleus, for mitosis may occur slowly in plant cells at $-8°$, and Uschinsky[41] noted that in animal tissues the nuclei were less affected by cold than the cytoplasm. Blood seems little affected by freezing temperature, for du Cornu found that dog's blood kept on ice for five to ten days could be employed for transfusion without causing hemoglobinuria. Grawitz saw motion persist in human ciliated epithelium kept for seven to nine days on ice. Ciliated epithelium from the mouth of the frog may survive cooling to $-90°$ and frog eggs are not killed by $-60°$. In many cells, however, the physical changes produced by freezing, and also by the subsequent thawing, are sufficient to render them incapable of further existence.[42] Cells devoid of or poor in water cannot be killed by freezing, hence it is probable that the currents set up about the crystals of ice in thawing, the concentration of the cell constituents because of the freezing out of the water, as well as the rapid contraction and expansion under the influence of the cold and the ice formation,

[37] Ziegler's Beitr., 1895 (18), 72.

[38] Murphy and Sturm, Jour. Exp. Med., 1919 (29), 1.

[39] Systemic effect of cold reviewed by Foord, Jour. Infect. Dis., 1918 (23), 159; review on gangrene from cold by Nägelsbach, Deut. Zeit. Chir., 1920 (160), 205.

[40] MacFadyen, Lancet, 1900 (i), 849.

[41] Ziegler's Beitr., 1893 (12), 115.

[42] In plant cells it is the freezing and not the thawing that causes the harm (Maximow, Berichte Deut. Bot. Gesell., 1912 (30), 504).

are the causes of the effects of freezing, which, therefore, are not dependent upon chemical, but upon physical, alterations.

Exposure of the entire body of animals to cold brings about changes in the blood similar to those of foreign protein shock.[43] Cooling of the surface of the body of man causes ischæmia in the nasopharyngeal mucosa, which may be a factor in the production of colds.[44]

In the case of warm-blooded animals, the gangrene following freezing depends not so much upon the freezing of the cells themselves as upon the formation of hyalin thrombi in the injured vessels (v. Recklinghausen, Hodara).[45] Kriege[46] found that if the freezing is transitory, the thrombi may again disappear; if over two hours in duration, they are persistent. Rischpler,[47] however, considers that cell death is due primarily to the effect of the cold upon the cells, and Lake[48] found that with both isolated cells in culture and living tissues with intact blood supply, death occurred at −6° C., this being the temperature at which protoplasm freezes. On the other hand, Steckelmacher[49] found that freezing of liver tissue produced the same changes as ligation of the hepatic artery, *i. e.*, increased permeability of the cell wall followed by similar changes in the nucleus, suggesting that the changes produced by freezing depend on the vascular changes. Therefore gangrene probably is the result of thrombosis of vessels following degenerative changes in the frozen intima.

Light.[50]—Light may affect tissues seriously, apart from the effects of accompanying heat, although the experiments of Aron[51] indicate that *insolation* does not depend on the light rays, but solely on the heat. In the treatment of lupus by the Finsen method with concentrated light rays, the action is largely a stimulating one, but associated with, or subsequent to, a certain degree of cell injury. Ogneff[52] found that moderate action of electric light, rich in violet and ultraviolet rays, causes mitotic cell division; if the action is stronger, the cells undergo amitotic division and then become necrotic. Blue rays have but slight cytotoxic action, and rays further towards the red end of the spectrum are without demonstrable effect. Light baths are said by Oerum[53] to increase greatly the quantity of corpuscles and hemoglobin, while residence in the dark reduces these elements. Normal blood absorbs light rays in large amounts, as Finsen showed, and it is quite possible that changes in the chemistry of the blood result from the light rays. An increase in the

[43] Widal *et al.*, Compt. Rend. Acad. Sci., 1921 (173), 207.
[44] Mudd and Grant, Jour. Med. Res., 1919 (40), 53.
[45] Münch. med. Woch., 1896 (43), 341.
[46] Virchow's Arch., 1889 (116), 64.
[47] Ziegler's Beitr., 1900 (28), 541.
[48] Lancet, Oct. 13, 1917.
[49] Beitr. path. Anat., 1913 (57), 314.
[50] Review by Janet Clark, Physiol. Reviews, 1922 (2), 277. See discussion of the principles of the action of light on tissues by Bovie, Amer. Jour. Tropical Dis., 1915 (2), 506.
[51] Philippine Jour. Sci., B, 1911 (6), 101.
[52] Pflüger's Arch., 1896 (63), 209.
[53] Pflüger's Arch., 1906 (114), 1.

number of blood platelets may also be produced.[54] Exposure to the sun may cause a general leucocytosis with relative lymphocytosis.[55] In animals this lymphocytosis is due entirely to rays shorter than 330$\mu\mu$.

The destruction of bacteria by light is a well-known phenomenon,[56] but it has been suggested that their destruction depends rather upon the action of substances produced in the culture-medium under the influence of light than upon the effect of the light upon the bacterial cells themselves. In view of the fact that enzymes and antibodies in solution are quite readily weakened or destroyed by the action of light, it is possible that intracellular enzymes may be similarly destroyed by light, with resulting cell death.[57] However, in the case of bacteria, at least, the effects of light seem to depend upon oxidation processes, for in the absence of oxygen, bacteria are not seriously injured by light, and D'Arcy and Hardy[58] found that "active oxygen" is formed by the same portion of the spectrum that is most active in destroying bacteria.[59] Light may also alter the solubility of cell proteins, especially in the presence of various organic and inorganic substances that act as sensitizers, such as silicates, sugar, lactic acid or urea.[60] In this may lie the cause of cataract, especially diabetic cataract.

The bactericidal action of light is confined to the ultraviolet region of the spectrum, beginning at 350 and extending to 185.6$\mu\mu$, these limits coinciding with the absorption of ultraviolet light by bacteria;[61] but the shorter the rays the more active they are, different species of bacteria not differing in their susceptibility to different wave lengths. As the spectrum of sunlight reaches only to 290$\mu\mu$ in the ultraviolet, it is evident that sunlight has but little effect on bacteria as compared with lights giving more of the shorter rays. As Clark[50] says, there is in all the physiological actions of light, a very marked difference between the effect of light from 400 to 300$\mu\mu$ (near ultraviolet) and light from 300 to 100$\mu\mu$ (far ultraviolet). Light greater than 300$\mu\mu$, being our normal environment, it is obvious that any organism, ordinarily exposed to this light and easily injured by it, would have perished long since. Light less than 300$\mu\mu$ is an unnatural environment and produces in all living cells strong and often very harmful reactions. Since the effect of light is probably due to the photochemical reactions produced when light energy is absorbed, it is not surprising to find that the various constituents of protoplasm begin to absorb light strongly in the neighborhood of 300$\mu\mu$.

[54] Cramer and Drew, Brit. Jour. Path., 1923 (4), 271.

[55] Aschenheim, Zeit. Kinderheilk., 1913 (9), 87; Taylor, Jour. Exp. Med., 1919 (29), 41.

[56] Literature given by Wiesner, Arch. f. Hyg., 1907 (61), 1.

[57] Kreibich (Virchow's Arch., 1916 (222), 28) finds that ultraviolet light destroys intracellular oxidase and peroxidase and delays catalase action.

[58] Jour. of Physiol., 1895 (17), 390.

[59] See also Agulhon, who found that ultraviolet rays may attack enzymes to some extent in the absence of oxygen (Ann. Inst. Pasteur., 1912 (26), 38).

[60] Schanz, Biochem. Zeit., 1915 (71), 406; Arch. Ophthal., 1918 (96), 172; Burge, Amer. Jour. Physiol., 1916 (39), 335; Neuberg and Schwarz, Berl. klin. Woch., 1917 (54), 84.

[61] Bayne-Jones and van der Lingen, Johns Hopkins Hosp. Bull., 1923 (34), 11.

We do not know the exact nature of the photochemical reactions produced in protoplasm by ultraviolet light, although various clues have been suggested. Bovie finds that paramœcia exposed to a sublethal dose of ultraviolet light are so sensitized to heat that they cannot stand, even for sixty seconds, a temperature which is an optimum for the controls. He concludes that death from ultraviolet is due to heat coagulation following sensitization by radiation. Others have found that the effect of ultraviolet light on protein solutions is to make them less soluble, as indicated by their easier precipitation.

Light exerts some influence on body metabolism, as is shown by a number of results indicating a change in the amount of CO_2 expired, a change in rate and depth of respiration, and increased rate of growth in the light compared to the dark. Since ultraviolet light is a powerful oxidizing and reducing agent it seems strange that it does not produce more marked changes in metabolism than it does. The small penetration of the chemically active rays is, of course, our protection against them. As far as the change in CO_2 output is concerned, it is visible light and ultraviolet light greater than $330\mu\mu$ which is responsible for the effects reported so far, as the animals experimented on were always exposed to light under glass. Recent experimental work on rickets (*q. v.*) has given evidence that light is concerned in the phosphorus and calcium metabolism of the body.

According to Neuberg[62] the general effect of light acting on organic substances present in plant and animal cells, is to produce from carbonyl-containing materials, aldehyde or ketone compounds, whose reactivity and availability for important synthetic changes are conspicuous. Whether oxidative processes are the cause of death in animal cells following injury by light is not known, but we are familiar with many chemical reactions of various sorts that are initiated or checked by the action of light.[63] Thus, bilirubin is oxidized into biliverdin when acted upon by sunlight, even when not in contact with air; many vegetable oils are oxidized by sunlight, and it is probable that the oxidizing action of light upon organic compounds is of wide-spread occurrence. It is, therefore, quite possible that such oxidative changes may be the cause of necrosis produced by the action of light rays, especially as Bering[64] has found that chemically active light rays have a direct action on oxidizing enzymes.

According to Hertel[65] the ultraviolet rays cause oxygen to split off the easily oxidizable compounds of protoplasm, and Bovie[66] found that they coagulate proteins; they also have a destructive effect on enzymes[59] and serum complement,[67] but not on opsonins of rayed animals.[68] Even

[62] Biochem. Jour., 1908 (13), 305.
[63] See Davenport, "Experimental Morphology," 1897, p. 162.
[64] Münch. med. Woch., 1912 (59), 2795.
[65] Zeit. Augenheilk., 1911 (26), 393.
[66] Science, 1913 (37), 24; see also Young, Proc. Roy. Soc. London, 1922 (93B), 235; Mond, Arch. ges. Physiol., 1922 (196), 540.
[67] Brooks, Jour. Med. Res., 1918 (38), 345.
[68] Albela, Deut. med. Woch., 1922 (48), 1347.

the visible spectrum has some inhibiting effect on complement.[69] However, Burge,[70] found that exposure of living cells to ultraviolet radiation of sufficient intensity to kill the cells does not decrease to any appreciable extent the activity of the intracellular enzymes; the cell death he attributes to coagulation of protoplasm. Harris and Hoyt[71] advance evidence that the susceptibility of protoplasm to ultraviolet light is conditioned by selective absorption of the toxic rays by the aromatic amino-acids of the proteins.[72] Toxins are reduced in activity by ultraviolet rays.[73] As stated in discussing vitamins, these rays compensate for loss of certain of the fat-soluble vitamins,[74] and confer the qualities of these vitamins on inert foods and upon the tissues of the rayed animals. They also stimulate protein metabolism.[75]

Photodynamic Sensitization.—Although visible light is apparently without effect except in producing vision, heat, and possibly some change in metabolism, it is possible to sensitize living cells so that they become as sensitive to visible rays as normal cells are to ultraviolet rays. This is definitely true in the case of individuals or animals with certain pigments in their blood, notably hematoporphyrin (*q. v.*). In them, not only may skin eruptions result from relatively small exposure to light, but mice may be so sensitized that a few moments of exposure to light is fatal.[76] Artificial fluorescent substances, such as eosin, also sensitize tissues and proteins to light,[77] although the photodynamic action is not directly proportional to the degree of fluorescence. In higher animals no other sensitizer has been found to be so active as hematoporphyrin.

Clark says that light energy, absorbed by a body, may increase the molecular motion, thereby producing a rise in temperature. "If the incident light has small enough wave lengths to produce vibrations in the electrons, instead of in the molecules and atoms, the absorption of light may result in the escape of electrons from the atoms, with a consequent change in valency, resulting in chemical action. We may regard it as practically certain that the first stage in any photochemical reaction consists in the separation of valency electrons under the influence of light. In the true photo-electric effect the electrons are lost from the substance, in fluorescence they return to their original atom, in photochemical reactions they attach themselves to some other atom, or group of atoms. For light of wavelength less than 300$\mu\mu$ we get intense reactions, due primarily, we may suppose, to the ionization of photo-active elements in the skin and blood of higher animals, and in the protoplasm of lower animals. Subsequent chemical action on these ionized substances produces changes which, in certain cells, result in the formation of the pigment melanin. Ordinarily, light of longer wavelength is ineffective in producing physiological changes, but the living cell, like the photographic plate, can be sensitized. In the presence of certain substances, such as eosin and hematoporphyrin, the photo-electric threshold of the

[69] Ecker, Jour. Infect. Dis., 1922 (31), 356.
[70] Amer. Jour. Physiol., 1917 (43), 429.
[71] Science, 1917 (46), 318; Univ. Calif. Publ. (Pathol.), 1919 (2), 245.
[72] Tyrosine and phenylalanine protect complement against ultraviolet rays (Hill and Schmidt, Jour. Infect. Dis., 1919 (25), 335).
[73] Hartoch *et al.*, Zeit. Immunität., 1914 (21), 643.
[74] See also Cramer and Drew, Brit. Jour. Exp. Path., 1923 (4), 271.
[75] Koenigsfeld, Zeit. klin. Med., 1921 (91), 159.
[76] Hausmann, Biochem. Zeit., 1914 (67), 309. A similar light sensitivity has been observed in man, independent of hematoporphyria (Duke, Jour. Amer. Med. Assoc., 1923 (80), 1835).
[77] Full review on photodynamic action of light by Sellards, Jour. Med. Res., 1918 (38), 293; See also Iodlbauer and Haffner, Biochem. Zeit., 1921 (118), 150.

cell, or of some of its constituents, shifts towards longer wavelengths and we get the same chemical changes initiated by visible light that we usually get only with ultraviolet. *In vivo* it happens only rarely, hematoporphyrin alone being capable of creating any marked sensitivity to visible light. There is considerable evidence to show that diseased tissue is more susceptible to radiations than normal tissue. To push the photographic analogy further, we may assume that normal tissue acts like a slow plate, and diseased tissue like a fast one. Light shorter than 300$\mu\mu$ acts on the living cell by ionizing its photo-electric constituents, and thereby leading to photochemical action. Light longer than 300$\mu\mu$ acts in the same way in the presence of sensitizers, which so affect the surface conditions of these constituents that their photoelectric threshold is shifted into the visible, and they therefore become ionized, with resulting chemical action, when illuminated by visible or near ultraviolet light."

X-rays[78] stimulate cell growth when applied in small amounts,[79] but larger amounts produce necrosis, which is peculiar in that an interval of several days, or even weeks, may elapse after the exposure before the necrosis manifests itself. Ellis[80] considers that the amount of necrosis is out of proportion to the changes in the vessels, which some have believed to be the cause of *x*-ray gangrene, and therefore that the cells must be directly injured, a view supported by Casemir's[81] experiments with plant cells. The extensive studies of the Hertwigs and others show that the chromatin is chiefly affected, which presumably explains the fact that immature cells, and cells in active division, are more sensitive to *x*-rays than adult cells,[82] and that monstrosities develop from eggs exposed to radiant energy. Inheritable abnormalities have also been produced in mammals through raying of the ovaries of the mothers[83] and it is probable that human defects may be produced in the same way.[84]

Lieber[85] suggests that *x*-rays influence the Donnan equilibrium of the cell membranes, leading to changes in their osmotic behavior with resulting changes in the composition of the cell contents. The permeability of the cell wall is also apparently increased,[86] but Weber[87] found no effect on the viscosity of the cytoplasm of plant cells. After a preceding radiation, proteins are coagulated by lower degrees of heat than before, and cells are made more sensitive to heat by previous radiation. As far as histological studies show, hard rays produce less but quite the same changes as soft rays. Edema of the cells and decrease of mitochondria are observed in the rayed tumors,[88] and the connective tissue of inflamed areas shows marked nuclear abnormalities in the fibroblasts, since these are actively dividing (Maximow).[89]

[78] Full review by Colwell and Russ, "Radium, X-rays and the Living Cell," 2d Ed. London. Also see L. Loeb, Jour. Cancer Res., 1922 (7), 229.

[79] See Schwarz, Münch. med. Woch., 1913 (60), 2165. However, Holzknecht (Münch. med. Woch., 1923 (70), 761) says that roentgen rays never stimulate but are destructive in even the smallest doses.

[80] Amer. Jour. Med. Sci., 1903 (125), 85.

[81] Med.-Naturw. Arch., 1910 (2), 423; résumé on *x*-rays.

[82] See I. Levin, Amer. Jour. Radiol., 1920 (7), 552.

[83] Little and Bagg, Amer. Jour. Roentgenology, 1923 (10), 975; Amer. Jour. Physiol., 1924 (33), 119.

[84] See Naujoks, Monat. Geb. Gyn., 1924 (68), 40.

[85] Wien. klin. Woch., 1924 (37), 979.

[86] Packard, Proc. Soc. Exp. Biol. Med., 1923 (21), 226.

[87] Arch. ges. Physiol., 1923 (198), 644.

[88] Prigosen, Jour. Cancer Res., 1924 (8), 305.

[89] Jour Exp. Med., 1923 (37), 319.

That *x*-rays have a marked effect on metabolism has been abundantly established.[90] According to Musser and Edsall,[91] the effect of *x*-rays upon metabolism is unequalled by any other therapeutic agent, and is manifested by excessive elimination of the products of protein destruction, which arise particularly from the lymphatic structures.[92] These changes have been studied, therefore, particularly in connection with the treatment of leukemia (*q. v.*). In consequence of the injury to the blood-forming tissues, resistance to bacteria is decreased (Läwen).[93] The renal epithelium seems also to suffer injury in some cases,[94] but does not seem to be particularly sensitive (Whipple).[95]

Exposure of the entire body of animals, or large areas of hematopoietic tissue in man, leads to profound changes. Chief of these are destruction of lymphoid cells, pigmentation of the spleen, destruction of bone marrow cells, primary rise in polymorphonuclear cells followed by a fall to below normal, steady decline in lymphocyte count, and an increased resistance of the red cells to radiation.[96] Even slight exposure causes an initial fall in lymphocytes which is followed by a decided rise (Murphy). Fibrinogen content and coagulability of the blood are increased because of injury of the liver,[97] but there seems to be little effect on existing antibodies.[98] So marked may be the effect of *x*-rays on the marrow and spleen that antibody formation is greatly depressed (Hektoen).[99] After heavy doses of hard rays in dogs, marked metabolic changes occur which indicate a profound intoxication, there being vomiting and diarrhœa, high non-protein N in the blood and a great increase in the urinary N (Hall and Whipple).[95] These authors also observed necrosis in the intestinal epithelium which seems to be especially sensitive to the *x*-rays. Presumably these reactions are similar to those observed following superficial burns, and depend on disintegration of tissue proteins with production of toxic substances.

Schmitz[1] found generally 40 to 50 per cent. increase in the non-protein-N of the blood after radiation of tumors, which he attributes to

[90] See Harvey (Jour. Path. and Bact., 1908 (12), 548), concerning the effects of *x*-rays.

[91] Univ. Penn. Med. Bull., 1905 (18), 174; also Edsall and Pemberton, Amer. Jour. Med. Sci., 1907 (133), 426.

[92] A selective action for the generative cells is also shown by *x*-rays, which cause marked atrophy of the ovaries and testicles. In the latter it affects chiefly the germinative cells, sparing the cells of Leydig. (See Albers-Schönberg, Münch. med. Woch., 1903 (50), 1850; Frieben, *ibid.*, 1903 (50), 2295; Specht, Arch. f. Gyn., 1906 (78), 458; Thaler, Deut. Zeit. f. Chir., 1905 (79), 576; Reifferscheid, Zeit. f. Gyn., 1910 (34), 593.)

[93] Mitt. Grenz. Med. u. Chir., 1908 (19), 141.

[94] See Schulz and Hoffman, Deut. Zeit. f. Chir., 1905 (79), 350; Warthin, Amer. Jour. Med. Sci. 1907 (133), 736.

[95] Amer. Jour. Med. Sci., 1919 (157), 453; Jour. Exp. Med., 1922 (35), 187. 225.

[96] Résumé by Gudzent, Strahlentherapie, 1913 (2), 467. See also Taylor *et al*, Jour. Exp. Med., 1919 (29), 53.

[97] See Frisch and Starlinger, Zeit. ges. exp. Med., 1921 (24), 142; Kaznelson and Lorant, Münch. med. Woch., 1921 (68), 132.

[98] Hartley, Brit. Jour. Exp. Path., 1924 (5), 306.

[99] Jour. Infect. Dis., 1918 (22), 28.

[1] Amer. Jour. Obst. Gyn., 1924 (7), 449.

products of autolysis of the tumor tissue. However, Dodds and Webster,[2] who have made a careful study of metabolism in patients given *x*-ray and radium treatments, found no changes following exposure of any part of the body except the abdomen, with doses used in therapeutics, and no retention of non-protein nitrogen but merely some signs of acidosis and evidence of a temporary inhibition of function of the abominal viscera. Even in cases of *x*-ray sickness no metabolic changes were found.[3] Other observers have found a marked chloride retention in such cases.[4] Exposure of blood to either radium or roentgen rays increases its toxicity.[5]

The long-continued action of *x*-rays upon the skin has, in many cases, led to the formation of cancer, apparently because the proliferation stimulated by the rays progresses until it exceeds normal bounds.[6] Likewise leukemia and aplastic anemia have been observed several times in roentgenologists, presumably produced in the same way.[7] Tumor cells, and apparently to some extent normal tissue cells, acquire after repeated exposure a varying degree of immunity to *x*-rays, and may become entirely refractory. On the other hand some people develop a hypersensitivity to the rays.[8]

As the metabolic changes produced by *x*-rays indicate an extremely high rate of autolysis, one may ascribe the effects either to a stimulating effect of *x*-rays upon autolytic enzymes,[9] or as Neuberg[10] does, to an inhibitive action of *x*-rays and radium rays upon the other intracellular enzymes without a corresponding deleterious effect upon the autolytic enzymes.[11] This hypothesis agrees with the facts at hand, but more details concerning the effects of these rays upon various enzymes are needed; for example, Wels[12] found that the growth-inhibiting effect was not associated with the processes of oxidation. The long latent period before the appearance of necrosis after exposure to *x*-rays is difficult to explain and agrees rather with the hypothesis of slow proliferative and obstructive changes in the blood-vessels.

Radium, which shares with *x*-rays the power of causing tissue necrosis, does not have so marked an effect upon the blood,[13] nor do the ultra-violet rays (Linser and Helber).[14] When red corpuscles are exposed

[2] Lancet, Mar. 15, 1924, p. 533. Review of literature.

[3] In rabbits Hussey (Jour. Gen. Physiol., 1922 (4), 511) found changes resembling those of a severe alkalosis after very heavy *x*-ray dosage.

[4] See Cameron and McMillan, Lancet, Aug. 23, 1924, p. 365.

[5] Macht and Hill, Jour. Gen. Physiol., 1924 (6), 671.

[6] See review by Wyss. Beitr. z. klin. Chir., 1906 (49), 185; Porter and Wolbach, Jour. Med. Res., 1909 (21), 357.

[7] See Jagic and Schwarz, Berl. klin. Woch., 1911 (48), 1220; Faber, Ugesk. f. Laeger, 1923 (85), 8.

[8] See de Courmelles, Gaz. des Hôp., Paris, 1922 (95), 954.

[9] See Hajos and Hofhauser, Biochem. Zeit., 1924 (146), 204.

[10] Zeit. f. Krebsforschung, 1904 (2), 171; also Meyer and Bering, Fortschr. Roentgenstrahlen, 1911 (17), 33; Richards, Amer. Jour. Physiol., 1914 (36), 400.

[11] Some authors have believed certain of the effects of *x*-rays to be produced by choline liberated through the decomposition of lecithin. (See Benjamin and Reuss, Münch. med. Woch., 1906 (53), 1860.)

[12] Arch. ges. Physiol., 1924 (203), 262.

[13] See Millet and Mueller, Jour. Cancer Res., 1918 (3), 127.

[14] Deut. Arch. klin. Med., 1905 (83), 479.

directly to heavy doses of radium emanation, however, hemolysis followed by stromatolysis is brought about, chiefly by the α-rays.[15] In general, radium has much the same effect on tissues as x-rays,[16] and the metabolic effects are quite the same with gamma rays as with x-rays (Dobbs and Webster).[2] Radium seems to stimulate the action of most enzymes,[17] autolysis, however, is not increased (Brown).[18] Radium partially destroys the growth-promoting "vitamins" of yeast, which may account for some of its effects on tumors (Sugiura and Benedict),[16] and the effects of exposure of animals to radium resemble those of avitaminosis.[19] It is said that the radioactivity exhibited by living tissues disappears with their death.[20] Radium also causes severe skin lesions and a general lymphocytosis in those exposed to it for long periods.[21] Active deposit of radium emanation injected intravenously into animals is highly toxic, even small doses causing fatty degeneration in the liver associated with giant cell formation and hyperchromatic nuclei; larger doses cause multiple hemorrhages and death with severe enteritis. Lesions also occur in the kidneys, lungs, spleen, and bone marrow,[22] and there is increased output of nitrogen, especially as ammonia.[23] Persons handling radium may show leucopenia,[24] but radium seems to have much less effect on the testicles than roentgen rays, although it does attack the ovaries.[25] In proper amounts radium stimulates plant metabolism (Gager). Packard[26] finds that the permeability of *Paramecium* is increased by brief exposure to radium (the slowest β rays being chiefly concerned), which leads to an acceleration of the division rate. Thorium-x attacks specifically the leucocytes,[27] so that by proper dosage an animal may be made practically leucocyte-free,[28] which result has been used for experimental studies on the functions of the leucocytes.

Electricity.—The effects of the electric current upon cells are described by Davenport as follows: A weak constant current causes a centripetal flowing of the protoplasm (in *Actinosphærium*); if the current is increased or long continued, the cytoplasm of the pseudopodia becomes varicose, and droplets are formed which soon burst, causing a collapse of

[15] Redfield and Bright, Amer. Jour. Physiol., 1923 (65), 312.

[16] Review by Richards, Science, 1915 (42), 287. Bibliography by Sugiura and Benedict, Jour. Biol. Chem., 1919 (39), 421.

[17] Loewenthal, Berl. klin. Woch., 1910 (47), 287; Kionka, Med. Klinik, 1911 (7), 685. Denied by Gudzent, Zeit. Strahlenther., 1914 (4), 666.

[18] T. R. Brown, Arch. Int. Med., 1912 (10), 405.

[19] Cramer *et al.*, Lancet, 1923 (1), 963.

[20] Nodon, Compt. Rend. Acad. Sci., 1924 (178), 486.

[21] See Ordway, Jour. Amer. Med. Assoc., 1916 (66), 1.

[22] Bagg, Jour. Cancer Res., 1920 (5), 1, and 301; Hektoen and Corper. Jour. Infect. Dis., 1922 (31), 305.

[23] Theis and Bagg, Jour. Biol. Chem., 1920 (41), 525.

[24] Mottram and Clark, Proc. Roy. Soc. Med. Electrother., 1920 (13), 25.

[25] Sugiura and Failla, Jour. Gen. Physiol., 1922 (4), 423.

[26] Biol. Bull., 1924 (46), 165.

[27] See Plesch *et al.*, Zeit. exp. Path., 1912 (12), No. 1; Schweizer, Münch. med. Woch., 1916 (63), 341.

[28] There is no increase in antitrypsin from this leucocyte destruction (Rosenow, Zeit. exp. Med., 1914 (3), 377).

the protoplasmic framework. Finally, the protoplasm on the anode side begins to disintegrate, and the loose particles move toward the positive electrode; eventually the cell structure may be entirely destroyed. A similar disintegration of the anode side of ameba has been observed by McClendon,[29] which he attributes to anions which cannot pass through the cell wall, and therefore accumulate on that side of the organism. If an alternating current is used, both anode and cathode sides of the cell are affected. In moving organisms, electric currents determine direction of motion, even certain vetebrates (tadpoles, fish) being made to orient themselves according to the current. The nucleus seems to be more susceptible to harm by electric currents than the cytoplasm (Pfeffer),[30] and there seems to be no oxidation-process involved in cell destruction by electricity (as is the case with light rays), for the effects are much the same in the absence of oxygen (Klemm). Schmaus and Albrecht state that the effect of electricity upon protoplasm depends upon a loosening of the cohesion and a solution of the constituents of the cell (vacuolization), which last is, perhaps, due to direct chemical alterations. It may be suggested that the electric current causes a migration of ions toward one or the other pole of the cell, in this way separating the movable inorganic ions of the ion-protein compounds of the cell from the immobile colloidal proteins, with consequent serious alterations in the chemistry of the cell. Zeit[31] found that continuous currents kill bacteria through the production of antiseptic substances in the culture-medium, but do not harm them directly.

Jellinek[32] has studied extensively the cause of death after severe electric shocks, and finds that there are produced intracerebral hemorrhages and degeneration of the nerve-cells, which are sufficient to explain the death of the individual without having recourse to the more indefinite idea of "shock." Cunningham[33] considers fibrillary contraction of the heart as the cause of death.[34] Spitzka and Radasch[35] find changes in the brains of electrocuted criminals, which indicate a sudden liberation of gas about the blood vessels along which the current passes. The amperage seems to be far more important in determining the effect of a current than the voltage or wattage.[36]

Chemicals cause cell death whenever they are of such a nature as either to coagulate the cell proteins or to destroy its enzymes. The action of such substances as sulfuric acid, strong caustics, etc., hardly calls for explanation. Phenol (carbolic acid) may cause necrosis and gangrene even when in very dilute solution; this appears to be due more to the production of hyaline thrombi of agglutinated red corpuscles in the capil-

[29] Pflüger's Arch., 1911 (140), 271.
[30] Literature given by Davenport, "Experimental Morphology."
[31] Jour. Amer. Med. Assoc., 1901 (37), 1432, literature.
[32] Virchow's Arch., 1902 (170), 56; Lancet, 1903 (i), 357.
[33] New York Med. Jour., 1899 (70), 581.
[34] Full discussion by Jelliffe in Peterson, Haines, and Webster, "Legal Medicine and Toxicology," 1923 (1), 261.
[35] Amer. Jour. Med. Sci., 1912 (144), 341.
[36] Jellinek, Wien. klin. Woch., 1913 (23), 1793.

laries than to direct action upon the cells. In some unpublished experiments on the subject of "carbolic acid gangrene," I found this action of phenol very striking when dilute solutions were placed on the web of a frog's foot, under the microscope; as soon as the solution penetrated to a capillary, stasis with fusion of the corpuscles occurred in a very few seconds. Similar results have been obtained by Rosenberger.[37] Probably many other poisons, *e. g.*, formaldehyde, cause necrosis through agglutination thrombi.[38]

Some poisons seem to cause necrosis without destroying the autolytic enzymes, in which case the cells are rapidly digested; at least, such an hypothesis seems to explain best the changes seen in the liver in chloroform poisoning, acute yellow atrophy, eclampsia, etc.[39] It is quite probable that many of the poisons act by interfering with the oxidative capacity of the cells; and even bacterial poisons (diphtheria and typhoid) were found by Pitini[40] to decrease the oxidizing power of the cells.

Not all poisons, by any means, cause cell death—tetanus toxin, morphine, and other alkaloids cause death of the individual as a whole, usually without coausing primary necrosis of any of the cells. Cell death does not necessarily depend upon destruction of *all* the cellular enzymes, as has been pointed out previously. Thus, bacteria may be killed by many chemicals which seem not to affect their autolytic enzymes seriously. Any considerable excess of either H or OH ions is incompatible with cell life, and it is possible that at times the production of acids within a cell may be sufficient to cause death[41] *e. g.*, in the kidney in acute nephritis (M. H. Fischer)[42] or in the muscle in waxy degeneration (Wells).[43] In tissue cultures, at least, fibroblasts are killed by a *p*H of 5.5, and at 4.6 there is visible coagulation of the nucleus, but this coagulated condition is reversible, at least for a short time; *p*H of less than 3.8 is fatal, and *p*H of 4.3 is required to coagulate the cytoplasm (Lewis).[44] At *p*H 6–9 the cytoplasm becomes fluid, at *p*H 10 the nucleus dissolves, and after solution of the nucleolus has occurred the process is no longer reversible. Lewis found that the acid formed in dead cells is even more effective in causing gelatinization of cells than most of the ordinary laboratory acids and it also has a high toxicity for cells. There is reason to believe that some chemicals produce necrosis through liberating acids in the tissues; for example, tartrates produce necrosis in the renal cells through which they are eliminated;[45] chloroform and such halogenized fat-soluble substances as

[37] Verh. Phys. Med. Gesellsch. z. Würzburg, 1900, vol. 34.
[38] See Jacobi, Arch. exp. Path. Pharm., 1924 (102), 93.
[39] Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.
[40] Biochem. Zeit., 1910 (25), 257.
[41] The partial protection afforded by a rich carbohydrate diet against the necrogenic action of chloroform, phosphorus, and renal poisons, as observed by Opie and Alford (Jour. Exp. Med., 1915 (21), 1), may depend on the antiketogenic effect of carbohydrates.
[42] See also Stieglitz, Arch. Int. Med., 1924 (33), 483.
[43] Jour. Exp. Med., 1909 (11), 1.
[44] M. R. Lewis, Bull. Johns Hopkins Hosp., 1923 (34), 373.
[45] Underhill, Wells and Goldschmidt, Jour. Exp. Med., 1913 (18), 322.

phosgene and mustard gas (dichlordiethylsulfide) seem to cause necrosis because they split off the free halogen acid within the cells.[46]

The term "protoplasmic poison," has been variously used and defined. Kunkel says that a protoplasmic poison "is a poison which, without producing directly evident alterations, harms or kills all living protoplasmic structure." $HgCl_2$ is such a poison, whereas H_2SO_4, bromine, and similar substances that destroy all life through their strong chemical action are not included in this category. The protoplasmic poisons presumably act by combining with one or more of the constituents of cell protoplasm; *e. g.*, $HgCl_2$ probably combines with the proteins, chloroform with the cell lipoids (physically?).

The rapidity of action of the heavy metals is remarkable, Menten[47] having found that minute doses cause visible cellular changes in the kidney within five minutes, accompanying which the potassium is set free in the protoplasm. By means of his special technic Barber[48] is able to introduce minute quantities of poisons into living cells and observe their effect on the cytoplasm; $HgCl_2$ is thus found to be most toxic, while As_2O_3 is relatively inert. Mathews[49] has shown that the toxicity of ions depends on the ease with which they part with their electrical charges, and the toxicity of a salt is a function of the sum of the toxicity of the ions; hence the toxicity of a salt is in inverse proportion to its decomposition tension. Kunkel suggests that oxalic acid and fluorides are poisons because they combine the cell calcium, and barium salts may be poisonous because they precipitate the SO_4 ions. We can readily imagine that the combining of any one of the essential constituents of the cell may so upset the normal chemical processes that the cell no longer takes up substances to repair its waste, and hence necrosis ensues. Presumably alteration in the colloidal state of the protoplasm is accomplished by many of the poisons, with disastrous results to the cell life.[50] It is also suggested that alterations in the radio-active elements in the cells may greatly modify their integrity and behavior.[51] The action of protoplasmic poisons is accompanied by marked increase in protein katabolism, as shown by the nitrogen elimination.[52]

Physical agents may cause necrosis, usually in ways too obvious to require explanation. With most cells, large portions of the cytoplasm can be destroyed without serious results, for so long as the nucleus is intact the cytoplasm can be reconstructed. The fact that necrosis frequently follows relatively slight injuries of the nucleus is perhaps best explained by considering that injury to the nuclear membrane modifies the permeability of the nucleus for substances in solution, which might readily affect its metabolic activities to a serious degree. It is possible,

[46] Graham, Jour. Exp. Med., 1915 (22), 48; Marshall *et al.*, Jour. Pharm. Exp. Ther., 1919 (12), 265.
[47] Jour. Med. Res., 1922 (43), 315.
[48] Jour. Infect. Dis., 1911 (9), 117.
[49] Amer. Jour. Physiol., 1904 (10), 290; Nicholl, Jour. Biol. Chem., 1909 (5), 453.
[50] See Shackell on phenol poisoning, Jour. Gen. Physiol., 1923 (5), 783.
[51] Zwaardemaker, Arch. neerl. physiol., 1920 (4), 177.
[52] Glaubitz, Zeit. ges. exp. Med., 1921 (25), 230.

also, that solvents of lipoids, such as chloroform, etc., produce much of their deleterious effects by modifying the permeability of the cell, for the semipermeability of cell membranes depends largely upon the lipoids they contain.[53]

Physical injury of even slight degree may bring on severe alterations in cells, and indeed may cause severe chemical alterations. We know that many chemical reactions can be brought about by slight mechanical disturbances, *e. g.*, the explosion of fulminate, nitrogen iodide, etc., and it is quite possible that mechanical disturbances can, likewise, cause chemical changes in the protoplasm. Mechanical injury of cells while under the microscope results in an apparent increase in the acid reaction of the part involved (Chambers)[54] and likewise traumatized nervous tissues develop an acid reaction (Moore).[55] Many lower animals, devoid of a nervous system, respond to mechanical stimuli by chemical activity; *e. g.*, the production of phosphorescence by marine organisms when agitated by an oar, etc. Possibly, the secretion of thrombokinase by the leucocytes, which occurs whenever they come in contact with a foreign body, is an example of a similar reaction to a mechanical stimulus. Even in *urticaria factitia* the simple mechanical irritation which suffices to produce the wheals is followed very quickly by extensive nuclear fragmentation,[56] but it may be that unknown poisons are present in the hypersensitive skin and cause the karyorrhexis, and not the trauma alone. We have no good evidence that mere contact with a chemically inert foreign body unaccompanied by cellular injury, can cause death of tissue-cells.[57] However, Chambers[58] states that simple trauma, even mere compression, of the eggs of *asteria*, may cause them to coagulate into a solid mass. Furthermore, what seem to be but slight changes in the colloidal state of protoplasm may have marked physiological effects, as shown by the extreme toxicity developed in blood plasma merely by adding distilled water to it (Novy and DeKruif).[59] Hence we may suspect that corresponding physicochemical changes in cells may produce toxic products which injure the cells themselves, or at least disturb their functions.

Extreme changes in osmotic pressure may lead to cell death, either by causing structural alteration in the cell (*e. g.*, the bursting of plant-cells in water), or concentration of the electrolytes may become so great that the colloids are thrown out of solution, as in the ordinary salting-out processes of the laboratory. It is doubtful, however, if osmotic changes *per se* ever become so abnormal within the animal body (except in experimental conditions) as of themselves to cause cell necrosis.

[53] See Pascucci, Hofmeister's Beiträge, 1905 (6), 552.
[54] Amer. Jour. Physiol., 1917 (43), 1.
[55] Proc. Soc. Exp. Biol. Med., 1917 (15), 18.
[56] Gilchrist, Bull. Johns Hopkins Hosp., 1908 (19), 49.
[57] Meltzer (Zeit. f. Biol., 1894 (30), 464) has shown that bacteria may be killed by violent agitation, which causes disintegration of the cells.
[58] Trans Roy. Soc. Canada, 1918, p. 41.
[59] Jour. Amer. Med. Assoc., 1917 (68), 1524.

Varieties of Necrosis

Coagulation Necrosis.[60]—This name is applied to necrotic areas that are firm, dry, usually pale yellowish in color, and observed principally in areas of total anemia or tuberculosis. The question has been long disputed as to whether a true coagulation occurs in such tissues or not. Necrosis produced by heat, carbolic acid, corrosive sublimate, etc., is naturally a coagulation necrosis, the cells of the affected area having undergone true coagulation; *i. e.*, the conversion of their soluble colloids (*sols*) into the insoluble "*pectous*" modification. Whether the same change occurs in areas of anemic necrosis is not so well established. If the part contains a fair amount of plasma, the liberation of the tissue coagulins from the dead cells will cause a conversion of the fibrinogen into fibrin—this can usually be demonstrated microscopically, but the presence of fibrin is not constant, and its quantity is usually insufficient to explain satisfactorily the condition of coagulation necrosis in infarcts, etc., as Weigert maintained.[61] Schmaus and Albrecht believe that a true coagulation of the cell proteins does occur in anemic infarcts, etc., for they found that the cells of kidneys with ligated vessels contain at first granules soluble in water and salt solution; after forty-eight hours the granules cannot be dissolved in these solvents or in weak acetic acid, but are soluble in 2 per cent. KOH; after five to six days the granules are insoluble even in KOH. Beyond these experiments, we seem to have no proof of the occurrence of intracellular coagulation within areas of coagulation necrosis due to anemia; exact chemical studies on this point are much needed. Since tissue-cells contain coagulins for fibrinogen, it is possible that they also contain coagulins for cell-proteins, but this remains to be established. Hertzfeld and Klinger[62] have advanced the theory that fibrinogen is always a product of tissue disintegration, which, if true, would account for coagulation of injured tissues. We do not know whether Chambers' observations on the spontaneous coagulation of traumatized *asteria* eggs[58] are applicable to other cells.

Bacteria produce substances coagulating milk and fibrinogen. Bergey[63] calls attention to the coagulation of serum by enzymes and acids produced by bacteria, and Ruppel[64] found that the tubercle baccillus produces substances precipitating proteins; hence coagulation necrosis in bacterial infections may be brought about in this way, and Schmoll[65]

[60] Literature by Jores, Ergebnisse der Pathol., 1898 (5), 16.

[61] Weigert believed that the dead area becomes permeated by plasma containing fibrinogen, which is coagulated in and between the cells. He put much weight on an increase in size of the necrotic area, which is by no means constant, as he intimated; necrotic areas are inelastic, and when death occurs they do not shrink with the fall of blood pressure as the surrounding tissues do, and hence they may appear to project from the surface of the dead organ when they did not do so during life. According to Moos (Virchow's Archiv., 1909 (195), 273) the plasma does not permeate infarcted areas to the extent that Weigert assumed.

[62] Biochem. Zeit., 1917 (83), 42.

[63] Jour. Amer. Med. Assoc., 1907 (49), 680.

[64] Zeit. physiol. Chem., 1898 (26), 218.

[65] Deut. Arch. klin. Med., 1904 (81), 163.

has shown that the necrosis occurring in tubercles is associated with an almost complete coagulation of the cell-proteins.

Necrosis associated with inflammatory exudation is, of course, accompanied by coagulation of the fibrinogen of the exudate (*e. g.*, diphtheria); this type of coagulation necrosis is chemically a simple fibrin-formation and readily understood. The peculiar hyaline degenerations of parenchymatous cells (*e. g.*, Zenker's degeneration of muscles) are often included under this class, but it would seem more probable that this process consists rather of the fusion of the structural elements of the cell into a homogeneous substance than a true coagulation. When necrosis is produced by chemical means, more or less coagulation of some of the soluble proteins probably takes place; even in plant cells this coagulation of dead protoplasm is described.[66]

Liquefaction necrosis occurs particularly in the central nervous system, where the cell substance seems not to undergo the coagulative changes described in the preceding paragraphs. Whether this is due to a lack of tissue-coagulins or to a difference in cell composition cannot be said, but the large proportion of lipoids in brain tissue is probably an important factor. Probably "edema *ex vacuo*" is responsible for much of the accumulation of fluid, due to the anatomical conditions that prevent a shrinking or collapse of the tissues to fill in the gap, and the lack of connective-tissue formation. Aseptic softening in general may be safely ascribed to digestion of proteins by cellular enzymes, either from the dead cells or from the leucocytes. Suppuration is merely a form of liquefactive necrosis, in which such digestion is particularly rapid because of the large number of leucocytes. Necrosis of the gastric mucosa or of the pancreas is also followed by rapid liquefaction, through the action of the digestive enzymes of these tissues. When necrosis is accompanied by edema (as in superficial burns), the fluid enters the cells in large amounts, and in this way another form of liquefaction necrosis may be produced. Bacterial enzymes may be a factor in producing liquefaction of dead tissues, but with most pathogenic forms there is little proteolytic activity.[67]

Caseation.[68]—This term is applied to a form of coagulation necrosis in which the dead tissue has an appearance quite similar to that of cheese. If we bear in mind the fact that cheese is a mixture of coagulated protein and finely divided fat, and that in caseation we have a coagulation of tissue proteins associated with the deposition of considerable quantities of fat, the reason for the gross resemblance of the product of this form of necrosis to cheese is apparent. Schmoll[69] has analyzed caseous material, and found it almost entirely free from soluble proteins or proteoses.[70] The protein material is almost solely coagulated protein, which in its

[66] Gaidukov, Zeit. chem. Kolloide, 1910 (6), 260; Lepeschkin, Ber. Deut. Bot. Gesell., 1912 (30), 528.

[67] See Bittrolff, Beitr. path. Anat., 1915 (60), 337.

[68] Discussed more fully in "Chemistry of Tuberculosis," Wells, DeWitt and Long, Baltimore, 1923, p. 123.

[69] Deut. Arch. klin. Med., 1904 (81), 163.

[70] Corroborated by Jobling and Petersen, Jour. Exp. Med., 1914 (19), 251.

elementary composition is related to the simple proteins or to fibrin, and not at all to the nucleoproteins. The extremely small amount of phosphorus present in the caseous material indicates that the products of disintegration of the cell nuclei must diffuse out early in the process. Caseation is, therefore, characterized by a coagulation of the proteins and a dissolving out of the nuclear components. Schmoll does not explain the cause of coagulation, however. It may be that it is the same as in the coagulation of anemic infarcts (since tuberculous areas are decidedly anemic), or possibly the tubercle bacillus produces substances coagulating proteins, as Ruppel states is the property of "tuberculosamin." Indeed, Auclair[71] claims that the fatty substance that can be extracted from tubercle bacilli by chloroform is the cause of the caseation. Ray and Shipman,[72] however, have failed to find evidence that any of the fatty materials that can be extracted from tubercle bacilli produces true caseation, although these lipins, as well as defatted tubercle bacilli and lipins from other bacteria, all are capable of producing typical tubercles without caseation.

Comparison of the chemical composition of bovine and human tuberculous lesions with the corresponding normal tissues has been made by Caldwell,[73] and gave the following results among others:

> Cholesterol forms about 6.5 per cent. of the lipins from normal bovine lymph glands or about 1.5 per cent. of the dry weight. The lipins from the walls of lymph node and liver tubercles contain, in every case, 2–3 times as much cholesterol as do the lipins from the normal tissues. The caseous material contains even a larger percentage of cholesterol than do the tubercle walls. Phospholipins constitute about 32 per cent. of the lipin fraction of normal bovine lymph nodes, or about 7.9 per cent. of the dry weight; the corresponding values for normal liver are 41.2 per cent. of the fats, or 14 per cent. of the dry weight. The phospholipin content of the fats from the tubercle walls is slightly less than that of the normal tissues, while there is a very marked reduction in the phospholipin content of the lipins from caseous material of bovine origin. In the specimen of caseous material from human lymph nodes, phospholipins formed 30.9 per cent. of the total lipins. The iodine numbers obtained from the fats of the tuberculous lymph nodes are higher than those from the normal tissues. In the liver there is no difference noted between the iodine numbers obtained for the lipins from normal and tuberculous specimens, although the values are practically the same as those from the fats from the lymph node tubercles. The amount of purine nitrogen in the walls of lymph node tubercles is only slightly more than half that of normal lymph tissue, and the amount is apparently much less in the caseous material. In the residues from the walls of liver tubercles, purine nitrogen is present in only slightly higher percentage than in the normal liver. The results here obtained would seem to indicate that the purines are even more abundant in the caseous residues of liver tubercles. The amount of material which enters the water solution during extraction is distinctly less from caseous material than from the residues of normal tissues.

The abundance of fat seen in caseous material on microscopic examination is very striking. In addition to the figures obtained by Caldwell, Bossart[74] found from 13.7 per cent. to 19.4 per cent. of the dry substance of caseous material soluble in alcohol and ether. In the scrapings from

[71] Arch. méd. exper., 1899, p. 363.

[72] Amer. Rev. Tuberc., 1923 (7), 88.

[73] Jour. Infect. Dis., 1919 (24), 81. Full review on composition of tuberculous tissues.

[74] Quoted by Schmoll, *loc. cit.*[69]

tuberculous bovine nodes I have found 22.7–23.9 per cent. of the organic material soluble in alcohol and ether.[75] Of this soluble material, Bossart found 25 to 33 per cent. of cholesterol, and Leber[69] found 38.31 per cent. of lecithin which is a much higher phospholipin proportion than Bossart detected. Caldwell found cholesterol higher and phospholipins lower in caseous than in normal tissues. The total amount of lipins, however, constituted a smaller percentage of the dry weight than in the normal tissues from which the caseous material originated. Presumably these fatty materials are derived chiefly from the disintegrated cells; this is probably true of the phospholipin and cholesterol, but the fact that in histological preparations most of the fat is found about the periphery of the caseous area,[76] supports the belief that it has wandered in from the outside.[77] A certain proportion of the fat is possibly derived from the bodies of the tubercle bacilli, which usually contain about 40 per cent. of fatty matter; but it has not been determined whether the fat from this origin forms an appreciable part of the fatty matter of caseous material.

Caseous areas persist for extremely long periods of time without undergoing absorption, which indicates that the autolytic enzymes are destroyed early in the process, presumably by the toxins of the tubercle bacillus; corresponding to this Schmoll found autolysis very slight indeed in caseous areas, and even when the caseous material breaks down to form a "cold abscess" the fluid differs from true pus in containing less free amino-acids; *e. g.*, tyrosine is missing.[78] Caldwell also obtained lower figures for extractives in caseous than in normal tissues. Because of a lack of chemotactic substances no leucocytes enter to remove the dead material, in consequence of which caseous material gives no evidence of containing proteases, according to the Müller-Jochmann plate method. That the failure of absorption is not due to a modification of the proteins into an indigestible form is shown by the rapid softening of caseous areas when, through mixed infection, chemotactic substances are once developed and leucocytes enter. Jobling and Petersen[79] suggest that in caseation the autolysis is inhibited by the soaps of fatty acids, which are abundant in caseous areas and have a marked antitryptic effect.

Fat Necrosis[80]

Through usage this term has come to indicate a specific form of necrosis of fat tissue, which is characterized by a focal, circumscribed arrangement, and by the splitting of the fat in the necrotic area into fatty acids and glycerol, the latter disappearing, the former combining with bases

[75] Wells, Jour. Med. Research, 1906 (14), 491.

[76] Sata, Ziegler's Beitr., 1900 (28), 461.

[77] Fischler and Gross (Ziegler's Beitr., 1905 (7th suppl.), 344) could find no fatty acids in caseous areas by histological methods.

[78] See Müller, Cent. inn. Med., 1907 (28), 297.

[79] Jour. Exp. Med., 1914 (19), 239; Zeit. Immunität., 1914 (23), 71.

[80] General literature will be found in the articles cited in the text; also in Opie's "Diseases of the Pancreas;" and in Truhart's "Pankreas-Pathologie," Wiesbaden, 1902; Gross and Guleke, "Die Erkrankungen des Pankreas," 1924.

to form soaps.[81] In practically all cases fat necrosis is produced by the action of pancreatic juice upon fat tissue, presumably through the action of the enzymes it contains, and the condition can be produced experimentally by any procedure that causes escape of the pancreatic juice from its natural channels. Similar-appearing forms of necrosis of fat have, however, been observed in the subcutaneous tissues,[82] independent of pancreatic lesions, sometimes traumatic and sometimes of unknown etiology. Such lesions are also sometimes observed in animals slaughtered for food, and attributed to trauma during shipment.

Langerhans[83] made the first studies of the nature of the changes in pancreatic fat necrosis and established the fact that the fat of the cells is split into its components, and that the fatty acids combine (at least in part) with calcium. Dettmer[84] found that, although fresh pancreatic juice caused fat necrosis, a commercial preparation of trypsin did not do so, and, therefore, he concluded that probably the lipase of the pancreatic juice was the active agent. Flexner[85] supported this contention by demonstrating the presence of a fat-splitting enzyme in foci of fat necrosis, which was corroborated by Opie.[86] The latter[87] was also able to demonstrate the presence of lipase in the urine of a patient with fat necrosis,[88] and the highest values for amylase in the blood and urine are found in pancreatitis (Stocks).[89]

In a study of the pathogenesis of fat necrosis, particularly with reference to the question whether the lipase or the trypsin of the pancreatic juice was responsible, Wells[90] found that typical fat necrosis could be produced by injecting extracts of fresh pancreas into animals, either of the same species as that from which the pancreas was obtained, or a foreign species. Commercial "pancreatins" were also quite effective, whether in weak acetic acid or weak alkaline solutions. The power of these materials to cause fat necrosis was reduced by heating to or above 60° for five minutes, and completely destroyed at 71°, indicating that the active agent is an enzyme. But, as in the same material trypsin was injured by temperatures above 60°, and destroyed at between 70° and 72°, and lipase was weakened above 50°, and destroyed above 70°, it was

[81] The fatty acids form masses of crystals in the fat-cells, and they can also be demonstrated microchemically by Benda's method (Virchow's Arch., 1900 (161), 194), which consists of staining with a copper acetate mixture, blue-green copper salts of the fatty acids being formed.

[82] See Fabyan, Johns Hopkins Hosp. Bull., 1907 (18), 349; Farr, Ann. of Surg., 1923 (77), 513; Lee and Adair, Ann. of Surg., 1924 (80), 670.

[83] Virchow's Arch., 1890 (122), 252.

[84] Dissertation, Göttingen, 1895.

[85] Jour. Exper. Med., 1897 (2), 413.

[86] Johns Hopkins Hosp. Rep., 1900 (9), 859.

[87] Opie, "Diseases of the Pancreas," Lippincott, 1903, p. 156; Johns Hopkins Hosp. Bull., 1902 (13), 117.

[88] It yet remains to be seen if this is a constant occurrence, and also if the lipase so excreted comes from the pancreas, for Zeri (Il Policlinico, 1905 (12), 733) has found lipase in the urine in hemorrhagic nephritis and inflammation of the urinary tract; also Pribram and Loewy, Zeit. physiol. Chem., 1912 (76), 489.

[89] Quart. Jour. Med., 1916 (9), 216.

[90] Jour. Med. Research, 1903 (9), 70.

impossible to determine, by heating pancreatic preparations, whether the lipase or the trypsin was the essential factor. By permitting pancreatic extracts to digest themselves it was found that the power to produce fat necrosis decreased, *pari passu*, with the decrease in lipolytic strength. Preparations strongly tryptic, but very weak in lipase, produced no fat necrosis, and, on the other hand, extracts of pig's liver or of cat's serum, both rich in lipase but devoid of trypsin, were equally ineffective. Furthermore, mixtures of liver or serum lipase and trypsin were incapable of causing fat necrosis. Fresh pancreatic extracts from fasting dogs, containing lipase but almost no trypsin (which in fresh extracts is still in the form of inactive trypsinogen), produced abundant fat necrosis, whereas after the trypsinogen in such extracts had been activated by enterokinase, no fat necrosis could be produced. It therefore seems certain that trypsin alone cannot produce fat necrosis, and that the decrease in strength of lipase in a pancreatic extract is associated with a corresponding decrease in power to produce fat necrosis. But, on the other hand, lipase of liver or blood-serum alone, or when mixed with trypsin, will not produce fat necrosis. The possibility remains that pancreatic lipase is different from liver or serum lipase, and can by itself cause fat necrosis; more probably, however, the production of fat necrosis depends upon a double action, trypsin causing the death of the cells, and lipase splitting the fats.[91] The fatty acids alone will not cause necrosis of fat-cells, and it was shown that the first steps in the process consist of a necrosis of the surface endothelium, extending thence into the connective and fat tissue; this may occur in a few minutes, while evidence of fat-splitting can be obtained only after about three hours, and the splitting occurs only in cells that have already become necrotic; hence the fat-splitting is not the cause of the necrosis, but occurs subsequent to the necrosis. After about four hours a substance which stains with hematoxylin, probably calcium, appears in the decomposed fat.

Fat necrosis may be produced by any means that will cause the escape of pancreatic juice from the natural channels within the gland.[92] In human pathology it has followed trauma and acute infection of the gland, and the blocking of the ampulla of Vater by gall-stones which permits the bile to back up into the pancreatic duct,[93] where it produces an acute inflammation of the pancreas (Opie).[94] Flexner[95] has shown that it is

[91] When fat tissue dies in the body from other causes, the lipase normally contained within the fat tissue does not cause the changes seen in fat necrosis. It is possible, therefore, that the combining of newly split fatty acids by the alkali of the pancreatic juice is responsible for the formation of the large amount of soaps found in fat necrosis. Otherwise we might expect the lipase to produce only an equilibrium, and that, in the case of fat, seems to exist when most of the substance is neutral fat. In support of this idea I found that strong alkalies injected into fat tissue sometimes caused changes very closely resembling areas of fat necrosis in the early stages.

[92] Perforation of the intestine may lead to abdominal fat necrosis (Wulff, Berl. klin. Woch., 1902 (39), 734; Richter, Northwestern Univ. Med. School Bull., Dec. 1910).

[93] It is anatomically possible for a calculus lodged in the ampulla to cause a reflux of bile up the pancreatic duct in 65 per cent. of persons (Cameron and Noble, Jour. Amer. Med. Assoc., 1924 (82), 1410).

[94] Bull. Johns Hopkins Hosp., 1901 (12), 182.

[95] Jour. Exp. Med., 1906 (8), 167.

the bile salts that cause the inflammation, and also that this effect is decreased or prevented by the presence of large amounts of colloids. Other authors attribute less importance to bile reflux in causing pancreatitis.[96]

Much emphasis is laid by some authors[97] upon the necessity of enterokinase passing up the ducts to activate the trypsinogen (an idea first advanced by Starling and Bayliss in 1902), but it should be remembered that there are kinases present in leucocytes, and that kinases develop in the pancreas itself during autolysis, which can activate the trypsinogen; hence the presence of *entero*-kinase is not essential for sufficient activation of trypsinogen to account for pancreatitis and fat necrosis. Lattes[98] believes that fresh pancreatic juice, which digests tissues very slowly, can produce typical fat necrosis but not the characteristic intoxication; this results from the action of juice which has been activated by enterokinase, or by products of pancreatic autolysis which have a similar effect. The kinases of leucocytes were found unable to activate pancreatic trypsinogen sufficiently to make it highly toxic. These observations indicate that in pancreatic necrosis it is the kinase liberated from the autolyzing necrotic tissue which is responsible for the activation and resulting toxic effects of the trypsinogen. As a result of injury by bile salts, or any other agent that produces cell death, the dead and injured cells are digested by the pancreatic juice, which is thus further activated and makes its escape into the surrounding fat tissue. Kestner[99] found that pure fistula pancreatic juice from dogs does not produce fat necrosis when injected intraperitoneally, but an intestinal juice containing a mixture of pancreatic juice, intestinal secretion, and bile produced typical effects. Intravascular injection of non-lethal doses of intestinal juice produced no fat necrosis.

My own observations[90] showed that the lesions of fat necrosis may be produced in three to five hours, large enough to be visible to the naked eye; their form and size depend solely upon the area of fat tissue exposed to the action of the pancreatic juice. The process progresses for only a few hours, the extension seeming to be limited by surrounding leucocytes. The lesions may appear at remote points in the thoracic and pericardial cavities or in the subcutaneous tissues, the causative agent probably being carried by the lymphatic vessels, possibly in the form of emboli of pancreas cells.[1] There may even be some splitting of the fats in the liver in these cases, with intrahepatic necrosis.[2] Fat necrosis itself is not dangerous to the affected organism, the associated pancreatitis[3] (and

[96] See Mann and Giordano, Arch. Surg., 1923 (6), 1.

[97] Pólya, Mitt. Grenz. Med. u. Chir., 1911 (24), 1; Rosenbach, Arch. klin. Chir., 1910 (93), 278.

[98] Virchow's Arch., 1913 (211), 1.

[99] Virchow's Arch., 1923 (246), 305.

[1] Payr and Martina, Deut. Zeit. Chir., 1906 (83), 189.

[2] See Berner, Virchow's Arch., 1907 (187), 360.

[3] Full review on pancreatitis in "Die Erkrankungen des Pancreas" O. Gross and N. Guleke, Springer, Berlin, 1924.

peritonitis) causing all the symptoms.[4] There is no evidence that sufficient quantities of soaps (which are toxic) are absorbed from the necrotic areas to cause appreciable intoxication. The soaps that are formed in the necrotic areas, indeed, are probably not much absorbed, but are precipitated as calcium soaps; in such areas at least as high as 85 per cent. of the soaps may be insoluble.[5] Healing follows rapidly in case of recovery; the foci may disappear as early as eleven days after their formation (in experimental animals).

In the urine of persons with pancreatitis is frequently found a substance forming an osazone, which has been the subject of much investigation because of its possible diagnostic value. Cammidge,[6] who first described a reaction, based on this observation, considers that the substance is a pentose,[7] derived from the nucleoproteins of the pancreas; it bears no relation to the fat necrosis, but is commonly found with fat necrosis because of the associated pancreatitis. Presumably cell necrosis elsewhere than in the pancreas may at times cause the same reaction to appear.[8] In pancreatitis with fat necrosis, or whenever there is any injury to the pancreas, there may be found an increase in the amount of diastase in the blood and urine, sufficient to be of diagnostic value according to Y. Noguchi.[9] The blood also commonly shows increase in urea and sugar.[10] The peritoneal exudate in acute pancreatitis is not toxic, contains no free trypsin, and is no more lipolytic than normal serum, presumably because of neutralization of the enzymes and poisons by the exuded plasma.[11]

Self-digestion of the pancreas occurs soon after death, and the pancreatic juice may in this way bring about a portmortem fat digestion that resembles somewhat the intravital fat necrosis in its gross appearances,[12] and Wells[90] found that the same

[4] Guleke (Arch. klin. Chir., 1908 (85), 615) considerst he intoxication of *acute pancreatitis* as an intoxication with trypsin, which can be checked by antitrypsin. Doberauer (Beitr. klin. Chir., 1906 (48), 456), Egdahl (Jour. Exp. Med., 1907 (9), 385), Petersen, Jobling, and Eggstein (*ibid.*, 1916 (23), 491), and Cooke and Whipple (*ibid.*, 1918 (28), 222), however, look upon the products of cellular disintegration as the source of the intoxication. v. Bergmann (Zeit. exp. Path. u. Ther., 1906 (3), 401), states that the toxicity is not due to either the enzymes or to albumoses; and that it is a true autointoxication which can be prevented by previous immunization with either pancreas extracts or commercial trypsin. (See also Fischler, Deut. Arch. klin. Med., 1911 (103), 156; and v. Bergmann and Guleke, Münch. med. Woch., 1910 (57), 1673.) The histones and protamines liberated from the digested tissue, and which are very toxic, have been suggested as a possible factor by Schittenhelm and Weichardt (Zeit. Immunität., 1913 (14), 609), while the beta-nucleo-proteins are included among the toxic elements by Goodpasture, Jour. Exp. Med., 1917 (25), 277.

[5] See Frugoni and Stradiotti, Berl. klin. Woch., 1910 (47), 386.

[6] Proc. Royal Soc. Med., 1910 (III, pt. 2), 163, bibliography; Lancet, 1914, Sept. 26.

[7] Weber believes that it is a hexose (Deut. med. Woch., 1912 (38), 166), and it may be urinary dextrin (Pekelharing and Van Hoogenhuyze, Zeit. physiol. Chem., 1914 (91), 151).

[8] See Whipple, *et al.*, Johns Hopkins Hosp. Bull., 1910 (21), 339; Karas, Zeit. klin. Med., 1913 (77), 125.

[9] Arch. klin. Chir., 1912 (98), 544. See also Permin, Revue d'chir., 1924 (62), 341.

[10] Rabinowitch, Canad. Med. Assoc., Jour., 1924 (14), 1057.

[11] Whipple and Goodpasture, Surg., Gyn. and Obst., 1913 (17), 541.

[12] Chiari, Zeit. f. Heilk., 1896 (17), 69; Pförringer, Virchow's Arch., 1899 (158), 126; Liepmann, *ibid.*, 1902 (169), 532; Wulff, Berl. klin. Woch., 1902 (39), 734.

changes might be produced by injecting pancreatin into the bodies of dead animals, or by keeping fat tissue in pancreatin solutions. Wulff found that fatty acids were demonstrable by Benda's method in the pancreas of nearly all cadavers. The process differs from the *intra vitam* form in being less sharply circumscribed, and microscopically by the absence of cellular and vascular reaction. That the essential changes of fat necrosis can be produced postmortem is final proof that they are due to enzymes, rather than to circulatory or cellular action.

GANGRENE

This term indicates merely that certain marked secondary changes, either putrefaction or desiccation, have occurred in necrotic areas of some size. Hence we have the chemical changes of putrefaction added to those of necrosis in the case of moist gangrene, whereas in dry gangrene nearly all the chemical changes are brought to a standstill through the desiccation. In the latter it is only at the line of demarcation, where some moisture remains, that chemical changes still go on; these consist chiefly of autolysis of the dead tissues, and also of their digestion by leucocytes, which results eventually in the separation of the dead tissue from the living; this is best seen after surface burns, carbolic-acid gangrene, etc.

Moist gangrene is accompanied by the dual action of the cellular enzymes and of the putrefactive organisms that are growing in the dead tissue, and as a result such tissue contains all the innumerable products of the decomposition of proteins and fats. Thus Ziegler mentions as morphological elements that may be present in gangrenous tissue: Fat needles, the so-called "margarin" crystals (a mixture of stearic and palmitic acids), fine acicular crystals of tyrosine, globules of leucine, rhombic plates of triple phosphate, black and brown masses of pigment, and crystals of hematoidin. In solution we also have, beyond a doubt, all the substances formed in the decomposition of proteins, from proteoses and peptones down through the different amino acids to such final products as ammonia and its salts, while CO_2 and H_2S are abundantly given off. In addition occur, undoubtedly, many of the ptomaines which are formed by the action of the bacteria upon the amino acids derived from the proteins.[13] In the sputum from pulmonary gangrene there is but little soluble protein, most of the nitrogen, of which there is much, is in the formed elements.[14] The fetid plugs which occur in the bronchioles in gangrene, the "Dittrich's plugs," were found by Traube to be composed chiefly of fatty acid crystals, and Schwartz and Kayser[15] ascribe their formation to the action of lipolytic staphylococci.

If the necrotic tissue is in contact with living tissue over a considerable area, enough of these products of autolysis and putrefaction may be absorbed to cause intoxication (*sapremia*). At the same time, the formation of such large quantities of crystalloids from the proteins of the dead tissue, leads to a diffusion of water into this area, with consequent swelling, and often a lifting up of the skin in the form of blisters.

[13] An interesting observation concerning gangrene of the lung has been made by Eijkman (Cent. f. Bakt., Abt. 1, 1903 (35), 1), who found in this condition bacteria that secrete an enzyme dissolving elastic tissue.

[14] Orszag, Zeit. klin. Med., 1909 (67), 204.

[15] Zeit. klin. Med., 1905 (56), 111.

Emphysematous gangrene,[16] usually produced by gas-forming anaërobic bacteria, including *B. aërogenes capsulatus*, may also possibly be produced by *B. coli communis* in diabetic patients in whose blood and tissues there may occur sufficient sugar to permit of gas-formation. Hitschmann and Lindenthal found that the gas produced in cultures by an anaërobic organism which they isolated from a case of emphysematous gangrene, consisted of 67.55 per cent. hydrogen, 30.62 per cent. carbon dioxide, and traces of ammonia and nitrogen; this corresponds to the statement of Welch and Nuttall that the gas in the tissues of infected animals is inflammable. Dunham[17] found that the gas produced by *B. aërogenes capsulatus* in cultures has the following composition: Hydrogen, 64.3 per cent.; carbon dioxide, 27.6 per cent.; other gases, probably chiefly nitrogen, 8.1 per cent. Grown in a medium of muscle and water, Wolf[18] found 70–75 per cent. of CO_2 produced by *B. sporogenes*, while *B. Welchii* produced 38 per cent. of CO_2, the rest being chiefly H. The former bacillus is very actively proteolytic, the latter less so. Organisms of this group produce much volatile organic acid which is probably an important factor in the local necrosis, especially in producing a negative chemotaxis; it may also contribute to the acidosis of the disease.[19]

RIGOR MORTIS[20]

This topic may be appropriately considered in connection with cell death, since it is a characteristic change occurring after general death. All forms of muscle, striped, smooth, and cardiac, undergo this change, which is shown by a shortening and thickening of the muscle, which also becomes opaque and hard. Rigor mortis begins first in the heart muscle, according to Fuchs,[21] but it is generally observed first in the eyelids, then in the muscles of the jaw, from which point it proceeds downward, although the upper extremities may not become rigid before the lower. The time of onset is extremely variable, but the following general rules may be stated: All conditions that lead to excessive muscular metabolism, with its resulting increase in the acidity of the muscle fluids, will hasten the onset of rigor mortis. Acute fevers, strychnine poisoning, tetanus, etc., cause likewise a rapid onset of rigor, which may, indeed, appear almost simultaneously with death or even before the heart has stopped beating. When a healthy individual meets death without previous exertion, rigor does not usually appear for four or six hours, but will be hastened by heat and retarded by cold. Death from hemorrhage or asphyxia is followed by a slow development of the rigor. Under ordinary conditions rigor usually begins between the first and second hour after death and is complete in one or two more hours.[22]

The duration of rigor mortis also is influenced by many factors. In general, it may be said that the duration is in inverse relation to the rapidity of onset, and directly to the musculature of the individual. Therefore, in an emaciated individual dying with fever, rigor may appear

[16] Complete literature by Weinberg and Séguin, "La Gangrène gazeuse," Paris, 1918.

[17] Johns Hopkins Hosp. Bull., 1897 (8), 68.

[18] Jour. Path. Bact., 1919 (22), 270.

[19] Wright and Fleming, Lancet, 1918 (i), 205.

[20] Review on chemistry of muscle and rigor mortis by Fürth, Handbuch d. Biochem., 1923 (4), 297.

[21] Zeit. f. Heilk., 1900 (21, Path. Abt.),; Oberzimmer and Wacker, Virchow's Arch., 1922 (236), 225.

[22] Rigor mortis may develop in the dead fetus while in the womb, but it generally disappears within five or six hours. Literature by Wolff, Arch. f. Gyn., 1903 (68), 549; Das, Brit. Jour. of Obstet. 1903 (4), 545.

and disappear again within two or three hours, or, indeed, escape observation altogether. The body of a muscular man dying from accident or hemorrhage may, on the other hand, show rigor for two or three weeks if kept in a cold place. Once the rigor has been broken by force, it does not again return.

Rigor mortis may be produced even before death, through poisons (monobromacetic acid, quinine), and its occurrence, even postmortem, does not necessarily mean that the muscle is dead, for if the part is transfused with a salt solution the rigor may be removed, and the muscle will then be found to react to stimuli. This indicates that the chemical changes of rigor mortis are not very profound.[23]

The chemistry of the changes involved in rigor mortis has been a much-contested problem. Two chief doctrines have been supported: one that rigor is not essentially different from ordinary muscular contraction except in degree,[24] and perhaps due to a loss of inhibition to contraction. The other looks upon it as a coagulation similar to the coagulation of the blood, and this idea, it may be said, has had the most general acceptance. Brücke in 1842 supported this view, and in 1859 Kühne extracted from muscle a plasma which coagulated like ordinary blood plasma. The protein which formed the clot is called *myosin*, and its coagulable antecedent, *myosinogen*.

This experiment has been since repeatedly verified and amplified, especially by Fürth and by Halliburton,[25] who have separated more definitely the proteins concerned in coagulation, and found them to be globulins. There seem to be two: one, coagulating at 47°, called *paramyosinogen* (Halliburton), constitutes but about one-fifth of the total clotting globulin, and passes readily into the insoluble clot, *myosin;* the other, which coagulates at 56°, constitutes the remaining four-fifths, is called *myosinogen* (Halliburton), or *myogen* (Fürth), and before becoming changed into myosin it passes through a soluble stage called *soluble myogen-fibrin*,[26] which is coagulated at the remarkably low temperature of 40°.

By analogy with fibrin-formation we should expect this clotting also to be brought about by an enzyme, but this has not been proved. Calcium is of influence, favoring coagulation greatly, but its presence is not absolutely essential (Fürth). Of particular importance is the acid reaction of the dead muscle. The proteins of muscle plasma are least soluble at *p*H 6.3 to 6.6, which is the reaction of tissues going into rigor (Collip).[27] Normal muscle is amphoteric when at rest, but when active the reaction becomes more and more acid, as it also does when the circulation is shut off, and hence acidity increases greatly after death. The acidity is due chiefly to lactic acid (although the neutral phosphates may become converted into acid phosphates in the presence of the lactic acid,

[23] See Mangold, Pflüger's Arch., 1903 (96), 498.
[24] See Mangold, Naturwissensch., 1922 (10), 895.
[25] "Chemistry of Muscle and Nerve," 1904.
[26] Corroborated by Benson, Jour. Biol. Chem., 1925 (63), Proc. p. lxxii.
[27] Jour. Biol. Chem., 1922 (50), xlv.

and thus seem to contribute to the acidity), and may increase in twenty-four hours after death by from 6.7 to 12.8 c.c. of n/10 acid for each 100 grams of muscle (Fürth[28]). The same author found that although the amount of acid might become in time sufficient to cause coagulation of the muscle proteins by itself, yet actually rigor mortis appears before the acidity has reached any such degree. Verzar[29] says that with vital stains it can be shown that in normal contraction no precipitation occurs, but it does take place in rigor mortis. Meigs[30] advanced the hypothesis that the rigor is due to the swelling of the muscle colloids under the influence of acids, a view which is accepted by Fürth and Lenk.[31] When sufficient acid is formed in the muscle the swelling may be so great that the structure of the muscle cell is destroyed entirely, and it goes into the condition of "waxy degeneration."[32] This readily explains why the time of appearance of rigor is so modified by the amount of muscle metabolism before death. It is, indeed, possible to produce rigor in living animals by transfusing a limb with slightly acid salt solution,[33] and in strychnine-poisoning the muscular spasm may pass imperceptibly into rigor mortis.

Rigor also occurs in non-striated muscle, and in leiomyomas, Hirsch[34] found it to be accompanied by an increased acidity which develops rapidly to about *p*H 6; and paralleled by a decrease in carbohydrates, presumably changed in part to lactic acid.

It has been suggested that the disappearance of rigor mortis depends upon beginning autolysis of the clot by the intracellular proteases of the muscle, which act best in an acid medium, but proteoses and peptones cannot be found in such muscle. Fürth holds that the re-solution of the rigor is caused by coagulation of the proteins,[35] thus reducing their hydrophilic tendency. Weber,[36] however, believes that the disappearance of rigor depends on actual liquifaction of the solidified colloids by the action of the lactic acid.

"Waxy" degeneration of muscles, although usually resulting from the action of toxic substances, is entirely different from cloudy swelling, in that the cytoplasm has become homogeneous and not granular. This is undoubtedly due to the increased accumulation of acid which takes place in muscles when they suffer from a defective oxygen supply, for I have found it possible to produce the typical appearance of Zenker's waxy degeneration by letting weak solutions of lactic or other acids act

[28] Hofmeister's Beitr., 1903 (3), 543; see also Fletcher and Hopkins, Jour. of Physiol., 1907 (35), 247; Wacker, Biochem. Zeit., 1916 (75), 101.

[29] Biochem. Zeit., 1918 (90), 63.

[30] Amer. Jour. Physiol., 1910 (26), 191.

[31] Biochem. Zeit., 1911 (33), 341; Wien. klin. Woch., 1911 (24), 1079.

[32] Wells, Jour. Exper. Med., 1909 (11), 1. Corroborated by Stemmler, Virchow's Arch., 1914 (216), 57.

[33] The hardness of a limb from which the blood-supply has been shut off by thrombosis or embolism, and also much of the cramp-like pain, is probably due to rigor mortis in the muscles caused by acid formation under conditions of suboxidation.

[34] Jour. Biol. Chem., 1921 (45), 297.

[35] See also Lentz, Zeit. angew. Chem., 1912 (25), 1513; and Schwarz, Biochem. Zeit., 1912 (37), 35.

[36] Arch. ges. Physiol., 1921 (187), 165; 1921 (191), 186.

on muscle fibers. Even excessive stimulation of muscles was found to be sufficient to cause waxy degeneration, the acid being formed faster than it can be removed.[32]

Muscles showing the "reaction of degeneration" have been analyzed by Rumpf and Schumm,[37] who found a great increase in the fatty matter, which was about fifteen times the normal amount. The muscle, deducting the fat, showed a loss of solid matter and an increase of water; sodium and calcium were increased, potassium decreased. There is also a great relative increase in the proportion of phosphorus bound to protein in muscles which have atrophied after nerve section, because of the persistence of nuclear and loss of non-nuclear elements (Grund[38]), but there is little change in the proportion of mono- and di-amino nitrogen.[39] The creatine content decreases steadily after the reaction of degeneration is first well established.[40]

ATROPHY

The chemical changes of simple atrophy have not, so far as I can find, been definitely studied. It is to be presumed, in view of the structural changes, that analysis of atrophied tissues would show a relatively high nucleic acid and collagen content. It is known that in atrophy the cell lipoids are not much altered, while the simpler fats may be increased in parenchymatous organs. In fatty tissues, of course, the fat is greatly reduced, its place being partly taken by serum (serous atrophy of fat). In the heart muscle, especially, but also to a less extent in the liver and kidney, during atrophy there is an increased pigmentation (brown atrophy) apparently consisting of lipochromes or lipofuscins; but it is to be doubted that this represents so much an actual increase in pigment as a relative increase through loss of other cellular constituents. In atrophied hearts the proportion of water is increased and the coagulable protein decreased,[41] but the carnosin content is unchanged.[42] An increase in the cholesterol content of the liver and adrenal cells may be observed in the atrophy of starvation.[43]

Atrophied tissues also tend to undergo a marked compensatory invasion by fatty areolar tissue if located in contact with such tissue; *e. g.*, atrophy of muscles after nerve section,[37] specific muscular dystrophies, and atrophy of the pancreas. In the muscle tissue of salmon migrating to the spawning grounds occurs one of the most marked examples of atrophy, and Greene[44] has found that at least 30 per cent. of the protein lost from the muscles may be considered as stored protein, since it can be lost without injury to the muscle.

Starvation, of course, produces typical atrophic changes in the tissues,[45] and the general effects on metabolism have been especially

[37] Deut. Zeit. f. Nervenheilk., 1901 (20), 445.
[38] Arch. exp. Path., 1912 (67), 393.
[39] Wakeman, Jour. Biol. Chem., 1908 (4), 137.
[40] Cathcart *et al.*, Jour. Physiol., 1918 (52), 70.
[41] Domagk, Zeit. klin. Med., 1924 (98), 171.
[42] Bubanovic, Biochem. Zeit., 1918 (92), 125.
[43] Okuneff, Beitr. Path. Anat., 1922 (71), 98.
[44] Jour. Biol. Chem., 1919 (39), 435.
[45] An extensive review on the effects of starvation are given in "Fasting and Undernutrition," Sergius Morgulis, Dutton, 1923; see also Lusk, Physiol. Reviews, 1921 (1), 523.

fully worked out by Benedict.[46] The structural changes in parenchymatous cells are described[47] as of two types; first granular changes and vacuolization of the cytoplasm, resembling the effects of osmotic pressure alterations; second and later, lysis of cytoplasm with also some involvement of the nuclei, after the order of autolytic changes. The cell walls may also become indistinct, so that the cells resemble a syncytium.[48] In the atrophied muscle after nerve section, Wakeman[39] found a decrease in solids, and a lowered proportion of diamino acids, and with rapid muscular atrophy there may be excessive excretion of creatine or creatinine.[49] The catalase content remains constant.[50] The per cent. of fat and the absolute fat content both increase, the water content on a fat-free basis and the water-soluble materials increase, while the per cent. of total nitrogen remains constant.[51] The increase in fat is more marked after nerve section than after tenotomy. Extensive destruction of muscle, as in muscular dystrophies, may lead to creatinuria,[52] with decreased creatinine and incomplete destruction of ingested creatine, associated with hypoglucemia. The rate of atrophy of muscle is much more rapid after nerve section than of autolytic disintegration of muscle *in vitro*, the contractile portions being especially labile.[53]

Morse has studied, by experimental methods, the question of the mechanism involved in atrophy, using especially the involuting tail of the tadpole as his test object.[54] He believes that autolysis is the primary factor, probably induced by acidity that results from vascular occlusion. The involution of the puerperal uterus, whether it can properly be called atrophy or not, seems to be the result of heightened autolysis, the products of which are excreted quantitatively in the urine.[55] Bradley[56] calls attention to the fact that atrophy occurs commonly under conditions of reduced blood supply, which implies partial asphyxia and a resulting tendency to local excess of H-ions, which would favor autolysis. Conversely, hypertrophy is observed with abundant blood supply which tends to keep the reaction of the tissues so low in H-ions that autolysis is held at a minimum.

CLOUDY SWELLING[57]

The characteristic appearance of organs the seat of cloudy swelling, which is frequently likened to a "scalded" appearance, suggests that the change consists in a coagulation of the cell proteins, which idea is supported

[46] Carnegie Inst. Publ., 1915, No. 203.
[47] Cesa-Bianchi, Frankf. Zeit. Path. 1909 (3), 723.
[48] Morgulis, Howe and Hawk, Biol. Bull., 1915 (28), 397.
[49] Meyer, Deut. Arch. klin. Med., 1920 (134), 219; Gibson and Martin, Jour. Biol. Chem., 1921 (49), 319.
[50] Guenther and Morgulis, Amer. Jour. Physiol., 1922 (59), 475.
[51] Audova, Skand. Arch. Physiol., 1923 (44), 1.
[52] Gibson *et al.*, Arch. Int. Med., 1922 (29), 82.
[53] Chen, Meek and Bradley, Jour. Biol. Chem., 1924 (59), 151; (61), 807.
[54] Biol. Bull., 1918 (34), 149.
[55] Slemons, Bull. Johns Hopkins Hosp., 1914 (25), 195.
[56] Full review in Physiol. Revs., 1922 (3), 415.
[57] Review of general features by Landsteiner, Ziegler's Beitr., 1903 (33), 237.

by the similarity of the microscopic changes observed in the cells with the earliest microscopic changes observed in cells after heating gently to about their maximum thermal point. On the other hand, the granules in cloudy swelling are generally described as being soluble in dilute acetic acid and dilute KOH, which indicates that they are not the result of ordinary heat coagulation. If we bear in mind, however, that cloudy swelling probably does not represent one single change, it may be possible to arrive at some understanding of the chemical changes that occur in the process. Albrecht[58] considers, with good reason, that we may have a granular appearance of cells which is simply an exaggeration of the normal granular structure, and, although it may be observed in tissues moderately affected by toxins, or in starvation, or in transitory anemia, the change is still to be looked upon as little more than physiological in response to stimuli and overwork.[59] Such a "cloudy swelling" may also occur in cells in the beginning of autolysis, or simply under the influence of salt solution. If the injury is greater, however, as in profound sepsis, or extreme local anemia, the granules become coarser, less soluble in acetic acid and KOH, and droplets resembling "myelin" make their appearance. If the injury is still more severe, true coagulation of the granules occurs, and they become insoluble, the fatty droplets become more prominent, and the cell reaches a condition that may with propriety be termed necrosis or fatty degeneration, or both. There is no very sharp line separating necrosis and cloudy swelling, especially if we consider only the changes in the cytoplasm.

A careful discussion of the character and characteristics of this process is given by Bell,[60] who concludes that the term cloudy swelling is sound only as a gross description, since microscopically the cells may be found to show albuminous granules, or fatty metamorphosis, or simple edema.

In the earliest stages the granules are perhaps due, in some cases, to simple aggregation of the colloids, without the development of a true coagulation, and so the granules are still soluble. Possibly bacterial toxins may also cause soluble precipitates, but this does not appear to have been established. Halliburton has shown that temperatures that may be reached in high fevers can cause turbidity in solutions of cell proteins, and hence heat precipitation may be partly responsible for the turbidity of cells in cloudy swelling, but it is doubtful if the granules thus formed would be soluble in acetic acid. When present, the granules are of unknown nature—they are not identical with Altmann's granules, although Aschoff and Ernst[61] both consider that many of them are derived from the mitochondria. An enormous number of granules may be present in the renal cells without demonstrable impairment of function.[62] They may disappear during acute infections, and they bear no constant relation to fatty changes.

[58] Verh. Deut. Path. Gesell., 1903 (6), 63.
[59] See also Davidman and Dolley, Jour. Med. Res., 1921 (42), 515.
[60] Jour. Amer. Med. Assoc., 1913 (61), 455.
[61] Verh. Deut. Path. Gesellsch., 1914 (17), 43 and 103.
[62] Shannon, Jour. Lab. Clin. Med., 1916 (1), 541.

We may speak with more assurance concerning the swelling of the cell, and attribute it to an edema of the cell contents, it having been shown that in cloudy swelling the water content of the organs is increased.[63] This might be produced by a rise in osmotic pressure due to abnormally rapid splitting of proteins with incomplete oxidation of the substances formed, which results in formation of many crystalloid molecules with high total osmotic pressure, from a smaller number of colloid molecules with almost no osmotic pressure. It has frequently been shown that the cell-walls do not lose their semipermeable character until the death of the cell occurs; hence in cloudy swelling water diffuses in much more rapidly than the crystalloids can diffuse out, causing a hydropic swelling. This hypothesis is supported by the observations of Demel,[64] who found that by modifying the osmotic conditions of the cells, particularly epithelial cells, he could closely reproduce many of the characteristic features of parenchymatous degeneration. It is possible, also, that too high concentration of crystalloids within the cells may be a factor in the precipitation of the cell colloids. In view of the fact that in the earliest stages of autolysis, histologic and microscopic changes closely resembling those of cloudy swelling are pronounced, and that organs the seat of cloudy swelling notoriously undergo autolysis with extreme rapidity after death,[65] we may also consider that this process is possibly in part responsible for the change of ordinary *intra vitam* cloudy swelling. The appearance of fine granules of lipoid substance[66] (myelin or "protagon") in cells during autolysis and during cloudy swelling is in support of this idea, and chemical analysis of organs showing cloudy swelling gives definite evidence of autolytic decomposition of the proteins and an increase in the water content.[67] Presumably this increase in water is the cause of the lowered specific gravity of organs exhibiting parenchymatous degeneration.[68] Landsteiner, through his studies of cloudy swelling in human material, also came to the conclusion that autolysis is an important element in its production. However, the chief change observed by Domagk[41] in chemical composition of human hearts showing cloudy swelling was an increase in the coagulable protein of the tissue extract, and a similar or even greater increase in the coagulable protein was observed with cloudy swelling of the liver by G. Hoppe-Seyler.[69]

Martin H. Fischer[70] applies the principles of colloidal cnemistry to the problem and concludes that the changes of cloudy swelling may be ascribed to acids developed in the cell. It is of significance that Chambers[71] has found that even slight mechanical injury of isolated cells

[63] Schwenkenbecher and Ingaki, Arch. exp. Path. u. Pharm., 1906 (55), 203.
[64] Lo Sperimentale, 1905; Cent. f. Path., 1905 (16), 613.
[65] See Medigreceanu, Jour. Exp. Med., 1914 (19), 309.
[66] Orgler, Virchow's Arch., 1904 (176), 413; Hess and Saxl, *ibid.*, 1910 (202), 149.
[67] Verh. Deut. Path. Gesell., 1903 (6), 76.
[68] See Olsho, Arch. Int. Med., 1908 (2), 171.
[69] Zeit. physiol. Chem., 1921 (116), 67.
[70] "Oedema and Nephritis," New York, 1915, p. 455; also Zeit. Chem. u. Indust. Colloide, 1911 (8), 159.
[71] Amer. Jour. Physiol., 1917 (43), 1.

under the microscope produces a demonstrable acidity in the protoplasm. Electro-negative proteins in the cell are precipitated by weak concentrations of acids, forming the granules in the cells, which can be dissolved again by a stronger concentration of acid as in the characteristic clearing of granular cells by acetic acid. The swelling is explainable by the increased affinity for water of the cell proteins under the influence of acids. This theory is supported by good experimental evidence and has much in its favor, the chief question being whether the blood cannot, under ordinary conditions of circulation, furnish sufficient neutralizing salts to prevent enough acidification in the cells to cause cloudy swelling.

CHAPTER XVIII

RETROGRESSIVE CHANGES (Continued)

Fatty, Amyloid, Hyaline, Colloid, and Glycogenic Infiltration

FATTY METAMORPHOSIS

In 1847, in the first number of his Archives, Virchow divided the forms of fatty changes that may occur in pathological conditions into two groups—"infiltration" and "degeneration"—a division that has since become classical. By infiltration he indicated the excessive accumulation of fat in the cells in the form of large droplets, without destruction of the nucleus or irreparable damage to the cells, and by the use of the term infiltration he implied his belief that the fat entered the cell from without. When the fat remained in the form of fine droplets and the cell became much disintegrated, Virchow considered that the fat was derived from the breaking down of the cell proteins, and hence the process was considered to be a fatty degeneration of the protoplasm. Since that time scarcely any other subject in pathology has been more warmly discussed than that of the origin of the fat in fatty degeneration, and an appalling amount of literature has accumulated concerning the questions involved. It will be impossible to give more than the essential facts that have been developed, referring the reader for the full details of the discussion and evidence to the numerous compilations of literature, particularly those of Rosenfeld[1] and to the original articles cited in the text.

Physiological Formation of Fat

Concerning the normal formation of fat we may summarize the evidence as follows:

(1) A large proportion of the fat of the body comes from the fat taken in the food, as also does the fat of the milk. This can be shown, as Rosenfeld particularly demonstrated, by starving an animal until it is as free from fat as possible, then feeding with a large amount of some fat that is of a type different from that normally found in the animal; the new fat that is then laid up in the fat depots of the animal will partake of the characters of the fat given in the food. In case the animal is lactating, the milk-

[1] "Fat Formation," Ergebnisse der Physiol., Abt. 1, 1902 (1), 651; *ibid.*, 1903 (2), 50. Also see discussion in the Verh. Deut. Path. Gesell., 1904 (6), 37–108, and the review by Leathes in his "Problems in Animal Metabolism," 1906, pp. 71–121, and "The Fats," Monographs on Biochemistry, London, 1910; von Fürth, "Chemistry of Metabolism," Amer. Transl., New York, 1916; Terroine, Ann. Sciences Naturelles, Zool., 1921 (4, Ser. 10), Nos. 1–6. Fat transport in the animal body is reviewed by Bloor, Physiol. Reviews, 1922 (2), 92. Concerning theories of rôle of lipase in fat metabolism see Chap. iii. Other reviews of literature on pathological fat formation by Christian, Johns Hopkins Hosp. Bull., 1905 (16), 1; Löhlein, Virchow's Arch., 1905 (180), 1; Pratt, Johns Hopkins Hosp. Bull., 1904 (15), 301 (particular reference to heart); Wohlgemuth, Handbuch d. Biochem., 1909, III (1), 150; Magnus-Levy and Meyer, *ibid.*, 1910, IV (1), 445; Dietrich, Ergebnisse der Pathol., 1909, XIII (2), 283. Later references of importance cited in the text.

fat will also resemble the fat of the food. As a matter of fact, the body fat is not of constant composition, even in the same individual; it varies greatly with age, having much less olein in infancy than in later years, varying somewhat in composition in the different fat depots in the same body, and apparently being more or less modified by diet.

(2) Fat may also be formed from carbohydrates. According to Rosenfeld, this fat differs from the fat formed on mixed diet in having less olein in proportion to the palmitin and stearin, and it is deposited particularly in the subcutaneous and mesenteric tissues rather than in the liver. Man does not seem to form fat readily from carbohydrates, but rather burns them to protect his proteins; on the other hand, swine and geese readily form fat from carbohydrates. As the fatty acid radicals of ordinary fat ($C_{18}H_{36}O_2$, $C_{16}H_{32}O_2$, $C_{18}H_{34}O_2$), are much larger than the carbohydrate radicals, a process of synthesis must be involved in the formation of fat from carbohydrates.[2]

(3) Proteins are a possible source of fat, but it has not been established that they are a common or an important source of fat in either physiological or pathological conditions, or, indeed, that they really ever do form fat. Upon this statement rests our present tendency to refute the long-cherished conception of fatty degeneration as a true degeneration of cell proteins into fat, as suggested by Virchow. This view was supported by the earlier work of Voit and his school, who believed that they had demonstrated that animals could form fat from protein food, and their work was for a long time accepted as correct. Later Pflüger and his pupils pointed out what seem to have been essential errors in these investigations, and, after much discussion and experimentation, the majority of physiologists now support the view advanced in the sentence opening this paragraph. Since proteins contain carbohydrate groups, and since fats can be formed from carbohydrates, the possibility of the formation of fats from the proteins in this indirect way cannot be denied. It is also possible that the nitrogen-containing groups may be split out of amino acids of the protein molecule, and that the non-nitrogenous residues can then be built up into fatty acid molecules as large as the molecules of stearic, palmitic, and oleic acids; but we have no proof that either of these processes occurs in the normal cell or in the cell that is undergoing degeneration. Atkinson and Lusk[3] have obtained evidence of some fat formation from meat fed to a dog, but this was only slight and obtained with difficulty.

Pathological Fat Accumulation

For a long time fatty degeneration was looked upon as one of the chief evidences that fat was formed directly from protein, for the cell protoplasm seemed, morphologically, to be changed directly into fat in this process. Additional support was also claimed from the supposed increase in fat in the ripening of cheese;[4] from the formation of abundant fat by maggots living in fat-poor blood or fibrin;[5] and by the apparent conversion of proteins into fatty acids and soaps in the postmortem change, *adipocere*. But it has now been well established that there is no true conversion of protein into fat in the fatty degeneration produced experimentally by poisoning with phosphorus, etc.,[6] and the other supposed instances of fat-formation above cited have been discredited by various methods which it will not serve our purpose to discuss here, beyond

[2] This, Magnus-Levy suggests, may be accomplished through lactic acid which is formed from sugar, and then, after reduction to an aldehyde, several of these molecules are combined into the higher fatty acid. See Leathes, *loc. cit.*,[1] p. 82. Kinttoff (Ber., 1923 (56), 439) believes that in yeasts fat is synthesized from glucose by the following steps: glucose → pyroracemic acid → acetaldehyde → aldol-glycerol esters.

[3] Proc. Natl. Acad. Sci., 1919 (5), 246.

[4] Even the increase of fat in ripening cheese is doubtful (Nierenstein, Proc. Royal Soc., B., 1911 (83), 301; Kondo, Biochem. Zeit., 1914 (59), 113).

[5] Mishikata (Jap. Jour. Biochem., 1922 (1), 261) maintains that maggots do develop fat from fibrin.

[6] See Taylor, Jour. Exp. Med., 1899 (4), 399; Shibata, Biochem. Zeit., 1911 (37), 315.

mentioning that one of the chief sources of error lies in the fact that many fungi and bacteria[7] can form fat from protein.

It having been rendered probable that fat was not formed by disintegration of the protein of the degenerating cells, it remained to determine what the source of the fat observed in the cells under pathological conditions might be, and this part of the problem has been largely cleared up by Rosenfeld. This investigator proceeded as follows: Animals were starved until they were extremely poor in fat, then fed upon easily identified foreign fats, such as mutton tallow (which has a high melting-point and can combine with little iodine) or linseed oil (which has a low melting-point and can combine with much iodine). The animals under these conditions laid up in their fat depots, including the liver as well as the subcutaneous tissues, large quantities of these foreign fats. By starving again for a few days the foreign fat was removed from the liver, leaving still a large amount in the other storehouses, and the animals were then poisoned with phosphorus or other poisons that cause a typical fatty degeneration of the liver and other viscera. When the fat was extracted from the fatty liver of these animals, it was found that the new fat that had appeared in the liver during the process was not normal dog fat (which it should have been if formed by degeneration of the cell proteins), but was, in part, of the same type as the foregin fat which the animals had deposited in their subcutaneous tissues and other fat storehouses. Furthermore, it was found that animals starved to an extremely low fat content do not develop the typical fatty liver of phosphorus-poisoning, a fact which Lebedeff had already noted in a case of phosphorus-poisoning in an emaciated patient.[8] Of similar significance is the fact that in fatty human livers the iodine number, normally high, falls as the amount of fat increases until it is approximately that of adipose connective tissue.[9] Therefore, it seems evident that *the fat accumulating in the liver during fatty degeneration is not derived, as Virchow thought, through a transformation of cell proteins into fat, but rather is an infiltrated fat brought in the blood from the fat deposits of the body to the disintegrating organ.* This work has since been corroborated and extended by many observers,[10] and its correctness can now hardly be questioned.[11] "Fatty

[7] See Beebe and Buxton, Amer. Jour. of Physiol., 1905 (12), 466; Slosse, Arch. Internat. Physiol., 1904 (1), 348; Walker, Jour. Physiol., 1922 (56), xliv.

[8] Salvioli and Sachetto (Frankf. Zeit. Path., 1922 (28), 111, 131) report that phosphorus poisoning causes fatty infiltration of the liver of dogs that have been starved to the maximum degree.

[9] Leathes, Lancet, Feb. 27, 1909; Hartley and Mavrogordato, Jour. Path. and Bact., 1908 (12), 371; Jackson and Pearce, Jour. Exp. Med., 1907 (9), 578.

[10] By a study of the iodine number of the tissue fats, Imrie (Jour. Path. and Bact., 1922 (25), 26) obtained evidence that apparently all the fat formed above that normally present in the liver (about 3 per cent. with an iodine number of 135) is fat such as is found in adipose tissue (iodine value about 65), but apparently in the heart only a little of the added fat comes from fat depots, and none at all in the fatty kidney. Corroborated for the fatty liver of tuberculosis by Goldberg (Amer. Rev. Tuberc., 1923 (8), 245).

[11] Schwalbe (Verh. der Deut. Path. Gesell., 1903 (6), 71) claims that in a similar way iodine compounds of fat can be demonstrated to be transported into the fatty organs. His analyses were merely qualitative, and by quantitative determinations I was unable to corroborate his conclusions (Zeit. f. physiol. Chem., 1905 (45), 412).

degeneration," therefore, at least in some cases, differs from "fatty infiltration" chiefly in the fact that in the former the process is associated with serious injury to the cell, caused by the action of toxins or loss of nutrition, while in the latter the cell is not seriously injured and is capable of returning to its normal condition whenever the fat is removed.[12]

Fatty "Degeneration" without Infiltration.—By showing that new fat in fatty livers is infiltrated fat, Rosenfeld did not entirely clear up the subject, for, in the course of his analyses of organs that were micro- or macro-scopically the seat of fatty degeneration, he found that there is not always any correspondence between the amount of fat that seems to be present, as determined by microscopic methods, and the amount that chemical analysis shows to be present. This is particularly true of the kidney. Thus, the amount of fat and lipoids, or *lipins*, present in normal kidneys (dog) was found to vary between 18.5 per cent. and 29.12 per cent. of the dry weight, the average being 21.8 per cent.; whereas, after producing a typical "fatty degeneration" by means of phosphorus and other poisons, the lipin content was still found to be between 16.9 per cent. and 22.6 per cent.[13] In all instances the amount of lipins in kidneys showing typical fatty degeneration under the microscope was found equal to or less than the normal amount—it was never increased. The same conditions were found to obtain in human kidneys that showed fatty metamorphosis. Microscopic examination of specimens stained with the specific fat stains,[14] therefore, gives no indication of the amount of fat contained in a degenerated kidney. A pathologic kidney containing 16 per cent. of lipins (18 per cent. is about the average amount in normal human kidneys) may show extreme "fatty degeneration" under the microscope, whereas another kidney may contain as much as 23 per cent. of lipins, yet not show any fat whatever by staining methods.[15]

[12] A striking proof of the lack of injury associated with fatty infiltration is shown by the fatty infiltration frequently seen in the liver, especially of alcoholics, in which it may be difficult to find, microscopically, any cell cytoplasm because of the fat, the tissue looking like fatty areolar tissue; and yet there may be no clinical evidence whatever that the liver function has been impaired by the process.

[13] Concerning the normal intracellular fats see introductory chapter.

[14] Fat-staining involves several principles of interest in this connection. (See reviews by Bullard, Jour. Med. Res., 1912 (27), 55; and Escher, Corrbl. Schweizer Aerzte, 1919 (49), 1609.) Osmic acid (OsO_4), the longest used for this purpose, is reduced to OsO_2 by oleic acid, imparting a black or dark-brown color to the fat; but it does not stain saturated fatty acids, such as palmitic or stearic acid. Thus, Christian found in pneumonic exudates fat that stained by other methods but not by osmic acid, apparently because it contained no oleic acid (Jour. Med. Research, 1903 (10), 109). Sudan III and scarlet R are two synthetic dyes which stain fat in a purely physical way, entering and remaining in the fat-droplets because they are much more soluble in fat than they are in water or alcohol. (Fully discussed by Michaelis (who introduced scarlet R) in Virchow's Arch., 1901 (164), 263.) These stains have the advantage of staining all sorts of fats and not staining other substances that may reduce osmic acid. Fatty acids and soaps may be stained with copper acetate, which forms a green copper salt, and thus be distinguished from fats (Benda, Virchow's Arch., 1900 (161), 194). J. Lorrain Smith (Jour. Path. and Bact., 1907 (12), 1) has introduced as a fat dye, Nile blue sulfate, which forms a blue salt with free fatty acids, while neutral fats are stained red by the oxazone base.

[15] However, Foulerton (Jour. Path. and Bact., 1921 (24), 257) has observed much increase in the quantity of fat in the kidneys in arsenobenzol poisoning.

The explanation of this remarkable discrepancy is as follows: Every tissue and organ seems to contain a greater or less amount of lipins, varying from 5 per cent. to 20 per cent. of the total dry weight of the organ in the case of most of the important tissues, yet this is usually held in such a form that it cannot be stained by any stains available for the purpose. Thus in the kidneys, as before remarked, we may have as much as 23 per cent. of lipins present and yet be unable to stain any of it by ordinary methods. The greater part of this seems to be essential to the cell, for it cannot be removed by the most extreme starvation; *e. g.*, the liver of the most emaciated dogs may contain 10 per cent. to 20 per cent. of fatty substances. Furthermore, the same resistance is shown by part of the fat to extraction with ether. A certain proportion of the fat can be extracted readily in twenty-four hours or less by ether, but after this time no more can be made to leave the tissues. Apparently the rest of the fat is held in a combination that is insoluble in ether, and a large proportion of this fixed material is not simple fat, but lecithin, cholesterol, and compounds of these lipoids. It has also been demonstrated that fatty acids can combine with amino-acids to form compounds (lipo-peptids) very similar in their properties to these "masked" fats.[16] By digesting the tissue for a short time by pepsin, however, the fixed lipins become freed, so that they can then be readily dissolved out in ether. We see, therefore, that much of the fat of normal cells is so firmly combined that it cannot be dissolved in ether, and under normal conditions all, or nearly all, of it cannot be stained. (This applies particularly to the parenchymatous organs; the fat of the areolar tissue is all readily extracted—Taylor.) By the use of Ciaccio's method for microscopic demonstration of intracellular lipoids, Bell[17] has been able to demonstrate in those cells that are fat-free by ordinary methods, sufficient lipoidal material to account for the normal "invisible fat," which is probably identical with the "liposomes." But when pathological changes in the cells result in decomposition of the cell protein through autolysis, or cause physical changes in the colloids that hold the lipins emulsionized, part of this normally invisible fat is set free, and, becoming visible, "*phanerosis*,"[18] producing the so-called "fatty degeneration." This explains the observations of Rosenfeld, cited above, that kidneys may show much fat to the naked eye and microscopically, when they actually contain even less than normal amounts of fat. Taylor[19] advanced this explanation, and supported it experimentally by showing that during fatty degeneration this protected fat actually is liberated, some two-thirds becoming ether-soluble in an experiment performed with phosphorus-poisoned frogs. Mansfeld[20] also found that in animals poisoned

[16] Bondi, Biochem. Zeit., 1909 (17), 543.
[17] Internat. Monats. Anat. u. Physiol., 1911 (28), 297; Jour. Med. Res., 1911 (24), 539.
[18] Klemperer, Deut. med. Woch., 1909 (35), 89.
[19] Jour. Med. Research, 1903 (9), 59.
[20] Pflüger's Arch., 1909 (129), 63.

with phosphorus, the proportion of fat which is present in a form free from protein union in both blood and viscera, is increased, while the firmly bound fat is decreased. As further support may be mentioned the fact that organs undergoing experimental autolysis show microscopically an apparently typical fatty degeneration, although analyses show that no actual increase in fat occurs.[21] Also Schnapp[22] found that after temporary ligation of the renal artery the fatty degeneration that results is accompanied by a decrease in the total lipin content of the kidney, the more labile lipins especially being removed.

Relation of Anatomical to Chemical Changes.—From the facts brought out in these various experiments we must consider that the anatomically established condition of "fatty degeneration" represents either or both of two conditions: (1) It may result from an increase in the normal quantity of fat in an organ undergoing parenchymatous degeneration, through an infiltration of fat from the outside; this is particularly true of the fatty degeneration of the liver, presumably because the liver normally receives the relatively saturated body fats to work them over into the more labile desaturated fats. (2) There may be no increase in the total amount of fat, but the invisible fat becomes visible through autolysis or hydration changes in the cell proteins. Thus, Bainbridge and Leathes[23] found that after ligation of the hepatic artery there is a marked fatty degeneration of the liver, without an increase in the amount of fat according to analysis. (3) Finally, of course, both factors may occur together. Of these various forms, in only the first and third would the chemist consider the organ "fatty," although from a morphological standpoint the second form is entitled to rank as a true "fatty degeneration," and the form that will occur seems not to depend upon the cause of the cell injury, but rather upon the organ under consideration. In a study of the relation of the morphological to the chemical changes Rosenfeld[24] arrived at the following results:

Normal human hearts contain, on an average, 15.4 per cent. of lipins; the hearts showing fatty degeneration[25] contain 20.7 per cent. on an average.[26] The pancreas, which normally contains 15.8–17.4 per cent., also contains an increased amount when showing fatty degeneration. The liver, however, takes on by far the greatest amount of fat after

[21] Dietrich, Arb. path. Inst. Tübingen, 1906 (5), H. 3; Hess and Saxl, Virchow's Arch., 1910 (202), 149; Ohta, Biochem. Zeit., 1910 (29), 1; Shibata, *ibid.*, 1911 (31). 321. The significance of the increase of lipins observed in perfused kidneys by Gross and Vorpahl is made doubtful by the article of Underhill and Hendrix, Jour. Biol, Chem., 1915 (22), 471, and by Munk and Rother, Deut. Arch. klin. Med., 1922 (140), 137; Goldberg, Ziegler's Beitr., 1924 (73), 1; See reply by Gross, *ibid.*, 1923 (142), 145.

[22] Zeit. physiol. Chem., 1924 (133), 62.

[23] Biochem. Jour., 1906 (2), 25.

[24] Berl. klin. Woch., 1904 (41), 587.

[25] Normal hearts often exhibit much microscopically visible fat (Master, Arch. Int. Med., 1923 (31), 221), which is readily mistaken for fatty degeneration.

[26] The amount of phospho-lipins in the heart is usually nearly constant, but alimentary fat may accumulate in the myocardium under certain conditions. See Wegelin, Berl. klin. Woch., 1913 (50), 2125; Bullard, Amer. Jour. Anat., 1916 (19), 1.

"steatogenetic" poisons,[27] and the microscopic picture usually gives a very good approximation of the amount of lipins it contains.[28] Apparently in these organs any excessive fat above the normal is observable microscopically, although the normal lipin content is not, and only in these three organs could Rosenfeld find an actual increase in fat after poisoning with phosphorus, etc. It would seem, on the other hand, that there is not often a real increase in the fat content of the "fatty" kidney.[29] Normal spleen contains 14.2 per cent. of lipins, and lung 17.3 per cent., but in both, "fatty degeneration" results in a lowering of this quantity. Degenerations in the nervous tissue, which Virchow considered the best evidence of the conversion of protoplasm into fat, also show a marked *decrease* in lipins, and voluntary muscle shows no increase in the normal quantity after poisoning. In general, these experiments support the contention of Taylor concerning the disclosure of the invisible fat through autolysis.[30] An explanation of many of the discrepancies lies in the newer studies on

The Relation of the Lipoids to Fatty Metamorphosis.[31]—In 1902 Kaiserling and Orgler described under the non-committal name of "myelin" certain intracellular droplets that may be found in the cells of the normal adrenal cortex, in amyloid kidneys, pneumonic exudates, tumor cells, retrogressive thymus tissue, corpus luteum, and bronchial

[27] In fatty livers in phosphorus-poisoning the amount of fat may reach 75 per cent. of the dry weight. Accompanying the fat increase are increase in water and a relative or absolute decrease in proteins, probably due to cell autolysis. (See G. Hoppe-Seyler, Zeit. physiol. Chem., 1921 (116), 67). In acute yellow atrophy a similar decrease in protein occurs, but without an increase in fat. (See v. Starck, Deut. Arch. klin. Med., 1884 (35), 481.)

[28] See Helly (Beitr. path. Anat., 1914 (60), 1) who examined 100 human livers which showed all variations in microscopic fat content, and chemically from 7.36 to 74.43 per cent. of lipins (dry weight). He found that there was usually a good correspondence between microscopic appearance and analytic results, altho some marked and unexplained discrepancies were observed. Generally the fat content was from 10 to 30 per cent. of the dry weight, with 19 to 21 per cent. the most common figures. When there is much fat present in the liver the fat content of the bile is increased (Le Count and Long, Jour. Exp. Med., 1914 (19), 234).

[29] This is contradicted by Landsteiner and Mucha (Cent. f. Path., 1904 (15), 752) and by Löhlein (Virchow's Arch., 1905 (180), 1) and Rosenthal (Deut. Arch. klin. Med., 1903 (78), 94), but is supported by Orgler (*ibid.*, 1904 (176), 413), and Dietrich, Verh. Deut. Path. Gesell., 1907 (11), 10. See also the later studies by Rosenfeld on the effects of various steatogenetic poisons on different organs, in Arch. f. Exp. Path. u. Pharm., 1906 (55), 179 and 344. It is probable that the truth lies between the opposing views, namely, the kidney may under some conditions take up fat from the blood, but it does so to a much less extent than the liver, and it may sometimes show marked fatty change anatomically without corresponding increase chemically.

[30] Pieces of tissue implanted into animals may show a peripheral fatty metamorphosis or infiltration, yet show upon analysis a decreased fat content (Dietrich, Verh. Deut. Path. Gesellsch., 1905 (9), 212).

[31] Literature by Leathes, "The Fats," London, 1910; Bang, Ergebnisse der Physiol., 1909 (8), 463, also, "Chemie u. Biochem. d. Lipoide," Bergmann, Wiesbaden, 1911; Kawamura, Virchow's Arch., 1912, (207), 469, also "Die Cholesterinesterverfettung," Fischer, Jena, 1911; Aschoff, Ziegler's Beitr., 1909 (47), 1, also Festschr. f. Unna, 1911 p. 23; Schultz, Ergebnisse d. Pathol., 1909 (XIII_2), 253; Hanes, Bull. Johns Hopkins Hosp., 1912 (23), 77; Anitschkow and Chalatow, Cent. f. Pathol., 1913 (24), 1. Jastrowitz (Zeit. exp. Path., 1914 (15), 116) gives many useful figures on the composition of the lipins in fatty organs.

secretions, and which differ from fat in being doubly refractile (anisotropic) when viewed through Nicol prisms, and in staining but slightly gray with osmic acid, although taking up other fat stains well.

As explained in Chapter I the myelins are probably mixtures of lipins, cholesterol-esters being prominent, and in many conditions in which fat-like vacuoles are prominent in cells, leading to the diagnosis of fatty degeneration, these substances are responsible, presumably having been liberated from combination with the cell proteins in some cases, in others actually being increased in the cell. This condition, which Aschoff refers to as a cholesterol-ester fatty metamorphosis, is especially seen in the parenchyma cells derived from the urogenital anlage—that is, the adrenal cortex, kidney, testicle, and corpus luteum. Aschoff states that doubly refractile droplets can be formed by lecithin and phosphatids generally, oleates, cholesterol esters, or cholesterol when dissolved in phosphatids, fats, or fatty acids, as well as by cholesterol esters dissolved in fats. Of these the most important quantitatively is the cholesterol ester group,[32] and the analyses of Windaus have shown that in pathological processes the increase is much greater in the cholesterol esters than in the free cholesterol. Cholesterol compounds stain differently from neutral fats, being more yellow than red with sudan III, and grayish rather than black with osmic acid. Pathologically the anisotropic droplets are also found especially in the above-named tissues, but also in tissues the site of chronic inflammation, including the mucosa of the gall bladder, where they may be of importance in the formation of cholesterol concretions. They are also found in the alveolar epithelium in pulmonary inflammation, in atheromatous patches in arteries, in many tumors, in most cells,[33] including even the adipose tissues themselves,[34] and occasionally in varied pathological tissues,[35] especially in xanthomas. Myelin deposits are found in the epithelium of the "large white kidneys,"[36] and myelin droplets are frequently found in the urine whenever there is tubular degeneration.[37] In Gaucher's disease there is also a remarkable lipoid accumulation in the foamy phagocytic cells.[38] According to Munk[39] true lipoid degeneration always means a serious injury to the cell, but there seem to be many exceptions to this. Indeed, according to Anitschkow and Chalatow (*loc. cit.*) the feeding of foods rich in cholesterol may cause the appearance in the liver of great quantities of anisotropic droplets, lipoid deposits in the aorta, enlargement of the

[32] See also Verse, Ziegler's Beitr., 1911 (52), 1.

[33] Ciaccio, Cent. f. Path., 1913 (24), 50.

[34] Cramer, Jour. Physiol., 1917 (51), xi.

[35] Pathological decrease in lipoids may also be observed, especially in the adrenal cortex, usually under the influence of toxic agents; *e. g.*, Hirsch found a marked decrease in delirium tremens (Jour. Amer. Med. Assoc., 1914 (63), 2186).

[36] Review by McNee, Jour. Path. Bact., 1922 (25), 425.

[37] Stengel and Austin, Trans. Assoc. Amer. Phys., 1914 (29), 312; Gross, Deut. Arch. klin. Med., 1920 (133), 9.

[38] See Wahl and Richardson, Arch. Int. Med., 1916 (17), 238; Mandelbaum and Downey, Folia Hematol., 1916 (20), 139.

[39] Virchow's Arch., 1908 (194), 527.

adrenal cortex, and the presence in practically all tissues of semifluid, doubly refracting, crystalline structures (*cholesterol steatosis*).[40]

In cells undergoing autolysis the fat-like "myelin" droplets which appear, differ from the above in not being anisotropic, but are undoubtedly closely related to them in composition. These "myelin" droplets are also found in cells showing cloudy swelling, presumably representing cell lipoids set free through changes in the cell proteins. They are characterized by staining with osmic acid but not by sudan III, which shows them not to be simple fats nor yet lipoids, but they are undoubtedly precursors of true fatty degeneration;[41] they probably consist chiefly of lecithin, with more or less free fatty acids and relatively little cholesterol (Aschoff).

It is possible to distinguish the lipoids of cells, whether normal or pathological, from the neutral fats by means of Ciaccio's method.[42] This consists in a preliminary treatment with bichromate, which renders the lipoids insoluble; the tissues can then be hardened and imbedded by the usual methods which remove the unchromated fats, leaving the lipoids stainable by sudan III. By this method Bell[43] has been able to stain the lipoids in the normal kidney and other tissues, in sufficient amount to account for all the so-called "masked fat," which thus seems to be, as also indicated by chemical evidence, largely lipoidal.

Jastrowitz[31] has studied the relation of lipoids to fats in the fatty changes produced by various means, and finds that in severe fatty changes with much transported fats there may be little change in the lipoids; with blood poisons which cause little increase in total fats, the lipoid content of both blood and organs may be high; usually the phosphatid content is unchanged or slightly increased, but it may be decreased. The proportion of cholesterol to neutral fats is usually within normal limits in tissues showing fatty changes.[44] The mitochondria seem to be compounds of phospholipins with proteins, and these agglutinate and form fatlike droplets in phosphorus poisoning;[45] presumably they play an important rôle in fatty metamorphosis. Cells in tissue cultures, however, may take up fat droplets from the surrounding medium (*i. e.*, fatty infiltration), without any association with or changes in the mitochondria.[46]

Summary.—We must conclude, therefore, that fatty degeneration of an organ means, in the case of the liver, myocardium, and pancreas, an infiltration of neutral fat from outside into cells which have been degenerated by the action of poisons or other injurious influences, plus a certain amount of apparent increase in fat because of the setting free of previously invisible fats and lipoids normally present in the affected cells. In the kidney, spleen, and muscles an increase of fat seldom occurs from

[40] See also Anitschkow, Deut. med. Woch., 1913 (39), 741; Wesselkin, Virchow's Arch., 1913 (212), 225; Rubinstein, Compt. Rend. Soc. Biol., 1917 (80), 191.
[41] Hess and Saxl, Virchow's Arch., 1910 (202), 149.
[42] Cent. f. Path., 1909 (20), 771; Arch. f. Zellf., 1910 (5), 235.
[43] Jour. Med. Res., 1911 (24), 539.
[44] Czyhlarz and Fuchs, Biochem. Zeit., 1914 (63), 131.
[45] Scott, Amer. Jour. Anat., 1916 (20), 237.
[46] M. R. Lewis, Science, 1918 (48), 398.

these causes, but the cells may show a marked fatty metamorphosis through the setting free of the invisible intracellular fat and lipoids by autolytic or physico-chemical changes. In the adrenal, kidney, and often in other tissues, the fatty material present in the cells is characterized by being doubly refractile, and then consists chiefly of cholesterol esters, together with greater or less quantities of phosphatids, fatty acids, soaps, and neutral fats.

Pathogenesis of Fatty Metamorphosis

Nevertheless, the old anatomical distinction of infiltration and degeneration still remains, provided we do not hold to the original idea that the term degeneration implies that the cell protein has been converted into fat; for we must recognize that under some conditions the cells may take up great quantities of fat without suffering any appreciable degenerative changes, whereas in other instances the appearance of fat is associated with marked and complete disintegration of both nucleus and cytoplasm. Furthermore, we have yet to explain why, under some conditions, the fat is removed from the fat depots to be stored up in the liver or other organs. By applying the commonly accepted ideas concerning fat metabolism, a satisfactory explanation seems to be possible. Fat is always utilized and transported in the form of its two constituents, fatty acid (or soaps) and glycerol, which are diffusible and soluble. It enters and leaves the cells in this condition, being split or combined, as may be necessary to produce equilibrium, by the action of lipase, which is present within the cells and in the blood and lymph. Under normal conditions there is little free visible fat in the cells of the parenchymatous organs, because it is largely used up through oxidation of the glycerol and fatty acids by the action of the intracellular oxidases. Where there is abundant lipase and but little oxidative activity, as is the case in the areolar fat tissue, fat accumulates in large amounts. When, for any reason, the oxidative power of the parenchymatous organs is reduced, fat accumulates in them as it does in the fat depots normally, and we find an excess of fat in the parenchymatous cells; thus, in pulmonary tuberculosis, severe or protracted anemias, etc., a great accumulation of fat occurs, particularly in the liver, where normally active oxidative processes continually balance the action of the abundant lipase of the liver-cells. The liver being normally concerned in the preparation of fat for metabolism, it is also perfectly possible to have an accumulation of fat in the normal liver merely as a result of increased function, and hence fatty changes may be purely physiological in this organ.[47]

If the fat accumulates in cells that are structurally normal or nearly so, the fat-droplets fuse together under the pressure of the cytoplasm, and we get the picture of a typical fatty infiltration; indeed, the only tissues in which we find this typical infiltration are the liver and the fatty areolar tissue, in both of which the process is presumably physiological in character even if not always physiological in degree. If the cells are much

[47] See Coope and Mottram, Jour. of Physiol., 1914 (49), 23; Helly, Beitr. path. Anat., 1914 (60), 1.

disintegrated through the action of the poison,—*e. g.*, phosphorus, bacterial toxins, etc.,—the accumulating fat-droplets are not crowded into one large droplet, but lie free in the granular débris of the disintegrating cell, constituting the typical appearance of fatty degeneration. Fatty degeneration is usually brought about by poisons, while abnormal fatty infiltration depends usually upon decreased oxidation, due to lack of either oxygen or hemoglobin in the blood. If the anemia is extreme, however, the cells degenerate, and then we find a true fatty degeneration caused by lack of oxygen.[48] Thus, in an anemic infarct, fat accumulates about the periphery of the dead area,[49] probably because fatty acids and glycerol diffuse in slowly from the surrounding parts where circulation still goes on, and are built up into fat by the cell lipase, for in anemic areas the intracellular oxidases cannot destroy these substances as they normally do, because of lack of oxygen. The accumulation of fat in dead areas depends, therefore, on the fact that the constituents of fat can diffuse into the dead tissue, whereas the oxygen, being held in the corpuscles, cannot enter the anemic area.[50] It is also possible that where fat is set free by autolysis of dead tissue, or when for any cause free fat or lipoid material is present in the vicinity of living cells, it may be phagocyted or in some way infiltrate the cells, causing a fatty metamorphosis by absorption (Dietrich).

It is to be supposed that poisons also cause fatty degeneration in a similar way—by interfering with oxidation. We have much evidence that in phosphorus, chloroform, and other poisoning associated with fatty degeneration of the liver, oxidation is impaired.[51] If we imagine for a moment, a cell in which oxidation is checked by any means, we shall have in this cell the lipase and the proteolytic enzymes not balanced, as they normally are by the action of the oxidases, and hence the processes of cell autolysis and of the accumulation of fat by the lipase will go on uncontrolled. The result will be a disintegrated cell containing many fat-droplets, *i. e.*, fatty degeneration.[52] In cloudy swelling there also appear

[48] Mohr (Zeit. exp. Path., 1906 (2), 434), denies that oxidation is decreased in anemia; and in a man with but about half the normal lung area the metabolism was not found altered to any extent by Carpenter and Benedict, Amer. Jour. Physiol., 1909 (23), 412.

[49] Fischler, Cent. f. Path., 1902 (13), 417.

[50] See Griesser, Ziegler's Beitr., 1911 (51), 115.

[51] See Welsch, Arch. int. de pharm. et therap., 1905 (14), 211.

[52] Interference with oxidation does not necessarily imply destruction of the oxidases. As yet we know practically nothing concerning the oxidases of the cells in disease, and the above hypothesis has yet to be demonstrated. Duccheschi and Almagia (Arch. Ital. Biol., 1903 (39), 29) found the normal amount of lipase in phosphorus-livers, but also observed no decrease in ability to oxidize salicylic aldehyde, which, however, does not prove a normal power to oxidize fats. Gierke's observation (Ziegler's Beitr., 1905 (37), 502) that glycogen and fat accumulate under identical conditions might be cited as indicating decreased oxidative power. Wells (Jour. Exper. Med., 1910 (12), 607) found that the power of liver tissue to oxidize purines was not decreased by the maximum degree of fatty degeneration, but Waldvogel (Deut. Arch. klin. Med., 1907 (89), 342) found that obese persons can burn fatty acids which arise in metabolism less readily than normal; and Quinan (Jour. Med. Res., 1915 (32), 73) found the ester-splitting lipolytic enzymes of the liver much reduced in the liver of chloroform necrosis, but the relation of these esterases to true lipases is not known.

droplets stained with osmic acid but not by sudan III, which Hess and Saxl[53] have shown to result from intravitam cell autolysis, and to be a precursor of true fatty degeneration.

Observations on cells in tissue cultures indicate that fatty changes of all types may occur independently of the circulation. Lambert[54] states that the amount of fat in the culture cells is roughly proportional to the amount in the culture medium, and cells rich in fat may move actively and undergo normal mitosis. Lewis, however, observed fatty changes in cells growing in fat-free media, and made the especially interesting observation that cells grown in 2.5–3 per cent. alcohol will show a rich fat accumulation. Also, an accumulation of fats and lipoids in cells grown in the presence of such steatogenetic poisons as phosphorus and *Oleum pulegii* has been observed by others,[55] which indicates that free cells behave the same under the influence of such poisons as the cells of the fixed tissues.

The process of unmasking the masked fats is explained by M. H. Fischer[56] on a physical basis, as follows: The fats of the cells are distributed as an emulsion in a hydration compound of water with hydrophilic colloids, notably proteins and soaps. Such an emulsion breaks down whenever the hydrophilic colloid is either dehydrated or diluted beyond certain ranges. As the usual conditions that cause fatty degeneration, such as poisoning with phosphorus, arsenic, etc., or local circulatory disturbances with local acidosis, all tend to dehydrate some of the cell colloids and to dilute others, it would seem probable that the appearance of the fat droplets in the cells is the result of such changes in the colloids that previously held them in an emulsion too fine to exhibit visible fat particles. The relation of cloudy swelling to fatty degeneration is readily explained on this basis, as follows: When a local acid intoxication of a cell occurs, some of the proteins will swell and others will precipitate, resulting respectively in the swelling and cloudiness of the cells characteristic of cloudy swelling; but at the same time the emulsifying capacity of these proteins will be impaired, permitting the coalescence of the fat droplets and the resulting picture will be that of fatty degeneration.

Summary.—Fatty metamorphosis involves changes of two kinds. First, infiltration of fat, which occurs when the oxidative power of the cells is decreased, so that fat is not destroyed, but is accumulated from the blood under the influence of the lipase of the cells; if there is not any serious injury to the cells, the histological changes consist in the accumulation of one or a few large droplets of fat in each cell, constituting the condition known anatomically as "fatty infiltration." This occurs, pathologically, chiefly in the liver. A similar condition may result from ingestion of more fats than the tissues can burn, in which case they

[53] Virchow's Arch., 1910 (202), 149.
[54] Trans. Assoc. Amer. Phys., 1913 (9), 93; Jour. Exp. Med., 1914 (19), 398.
[55] Krontowski and Poteff, Beitr. path. Anat., 1914 (58), 407.
[56] Fischer and Hooker, Science, 1916 (43), 468; Fischer, Fats and Fatty Degeneration, Wiley, New York, 1917.

accumulate not only in the adipose tissue but also in the accessory depots, of which the liver is chief. If at the same time the cytoplasm is disintegrated through autolytic changes, the fat-droplets do not fuse, but remain as small, more or less discrete, fat granules among the granules of cell débris, constituting the microscopic picture of "fatty degeneration;" this condition occurs particularly in the heart and liver.

Second, each cell contains a large amount of fat and lipoids (5–25 per cent. of its dry weight), which is so combined that it cannot be detected microscopically; this may be liberated during the autolytic processes and colloidal changes of cell disintegration and become visible, constituting a macroscopical and microscopical degeneration, but without any actual increase in fat—this condition occurs particularly in the kidney and nervous system. Third, a combination of both of the above processes—infiltration of fat and liberation of masked intracellular fat—may occur simultaneously in an organ.[57] Fourth, in certain cells, especially in the kidney, adrenal, ovary, and some tumors, there may be a great increase in the lipoids of the cell, "lipoidal degeneration," and especially of cholesterol esters and free cholesterol, part of which is infiltrated and part set free from combination in the cytoplasm.

PROCESSES RELATED TO FATTY METAMORPHOSIS

ADIPOCERE

The apparent transformation of the substance of dead bodies into a wax-like material was for a long time looked upon as evidence of a transformation of protein into fat, but in the light of more recent investigations this view can hardly be held. Adipocere is the product of a process that occurs particularly in bodies buried in very wet places or lying in water, and results in an apparent replacement of the muscles and other soft parts (but not the glandular organs) by a mass consisting of a mixture of fatty acids in crystalline and amorphous form, and soaps, particularly ammonium, magnesium, and calcium salts of palmitic and stearic acid (the oleic acid largely disappearing during the process). Analysis of samples of adipocere by Ruttan[58] gave the following figures:

[57] The above conception of the processes involved in fatty metamorphosis is more fully discussed by the writer in other publications (Jour. Amer. Med. Assoc., 1902 (38), 220; *ibid.*, 1906 (46), 341). Ribbert (Deut. med. Woch., 1903 (29), 793) has also advanced a similar explanation for the morphological differences between fatty "degeneration" and "infiltration," *i. e.*, that the degenerative changes are independent of fatty accumulation.

[58] Jour. Biol. Chem., 1917 (29), 319; Trans. Roy. Soc., Can., 1916 (10), 169.

COMPOSITION OF HUMAN AND PIG'S ADIPOCERE

	I Pigs (mature)	II Human hard	III Human soft
Ether soluble, per cent	94.1	82.9	75.8
Specific gravity at 100° C	0.8436	0.8397	0.8410
Refractive index 65° C	1.436	1.437	1.439
Melting point	60–63°	52–54°	50–51°
Acid value	201.7	207.3	203.8
Saponification value	207.0	211.0	212.2
Iodine value	6.04	9.65	12.52
Acetyl value	34.75	11.8	
Mean molecular weight	271.0	266.0	264.0
Saturated fat acids, per cent	70.82	71.78	59.2
Unsaturated fat acids	5.24	8.87	11.6
Hydroxy fat acids	14.80	8.24	7.8
Stearin and palmitin	1.21	0.91	0.90
Olein	0.16	0.15	0.83
Unsaponified matter	0.87	0.69	0.75
Calcium soaps	4.41	6.76	12.3
Protein	0.665	1.93	4.14
Ammonia	0.035	.....	0.054
Ash	0.574	1.99	2.25

Ammonium and other soluble soaps were absent.[59] The hydroxy-stearic acids, which are so characteristic of adipocere, are formed from the oleic acid of the original triolein.[60] Cholesterol has also been found in adipocere.[61]

The resulting material is absolutely resistant to putrefaction, and hence remains intact for many years. This replacement of the soft parts is, however, only apparent, for the total weight of a body in this condition is much lighter than that of the original body; indeed, one is always surprised at the light weight on lifting such a specimen. Adipocere occurs almost exclusively in fat bodies, and it seems probable that all the soaps and fatty acids found are *formed from the original fats of the corpse.*[62] These gradually flow into the places left by the disintegrating muscle, etc., a process that occurs readily in cadavers, according to Zillner,[63] or the infiltration may be acomplished through diffusion of the ammonium soaps formed during the decomposition. As the subcutaneous fat is hardened by the formation of soaps, and the bones remain to hold the parts in position, the general form of the body is preserved, creating the impression that its entire substance has been converted into adipocere, when the total mass may actually weigh but twenty pounds or so, and,

[59] Two analyses by Goy and Wende (Biochem. Zeit., 1922 (131), 8) gave similar results to those of Ruttan, the bulk of the material consisting of free fatty acids.

[60] Analyses of "bog butter" (butyrellite) by Ruttan and Howe (Jour. Biol. Chem., Proc., 1920 (41), 26), showed a somewhat similar composition. These authors say that the presence of theta- and iota-hydroxy stearic acids is characteristic of all "fossil fats."

[61] Van Itallie and Steenhauer, Pharm. Weekblad, 1917 (54), 121.

[62] Fatty changes in the viscera may favor their transformation into adipocere (Müller, Vierteljahrs. gericht. Med., 1915 (50), 251).

[63] Vierteljahrsch. f. gericht. Med., 1885 (42), 1.

according to Zillner's estimate, not more than one-tenth of the muscle substance is replaced by adipocere. This false impression is probably responsible for much of the mistaken idea concerning the conversion of tissue proteins into fatty acids. Thus, Schmidt[64] found that in early Egyptian mummies 60 per cent. of the weight of the lungs and 30 per cent. of the spleen consisted of fatty acids, and fell into the usual error of considering this conclusive evidence of transformation of proteins into fat.

Numerous attempts have been made to prove that muscle could be thus converted into fatty acids and soaps, but although success has been claimed by a few, the results are not entirely convincing.[65] Bacteria can convert proteins into fats, beyond a doubt, and they may do so to some slight extent in adipocere formation, but probably this factor is not important.

In the light of our present conception of fat metabolism it is probable that the process of adipocere formation occurs as follows: The fatty acids of the fat tissue are combined by the ammonia formed during putrefaction, removing these fatty acids from the normal balance of fat and fatty acids in the fat tissue; as a result, the lipase of the fat tissue continues to split the fat, and more fatty acids are produced, which likewise go to form soaps. This continues until practically all the neutral fat has been decomposed, the glycerol diffusing rapidly away. The soluble soaps, which the bacteria do not attack, diffuse into the softened muscle tissue, which they gradually replace in part. In the meantime, from the more soluble ammonium soaps, calcium and magnesium soaps are being slowly formed, according to the usual rule of double decomposition (that the least soluble salt will be formed under such conditions); or else, if an acid reaction develops, free fatty acids are precipitated. The oleic acid seems to be converted into the higher fatty acids (Salkowski).[66] It is also possible that the saponification is due to the gradual action of the alkaline fluids produced in decomposition of the tissues, or to the alkalinity of the water in which the body lies. Possibly bacteria may be responsible for this decomposition of the fats rather than the body lipase, for Eijkman[67] has observed that certain bacteria growing in fat-containing agar produce calcium, ammonium, and sodium soaps, simulating adipocere.[68]

Zillner[63] gives the following scheme of the changes that take place in a cadaver undergoing adipocere formation: (1) Migration of fluid contents of the body (imbibition of blood and transudation)—one to four weeks. (2) Decomposition of superficial epidermis, then of corium—first two months. (3) Decomposition of muscle and gland parenchyma, until only the inorganic part of the bones and the connective[69] and elastic

[64] Zeit. allg. Physiol., 1907 (7), 369.

[65] See Rosenfeld, Ergeb. der Physiol., Abt. 1, 1902 (1), 659.

[66] Festschr. f. Virchow, 1891, p. 23; corroborated by Schütze.[68]

[67] Cent. f. Bakt., 1901 (29), 847.

[68] See also Cevidalli, Vierteljahrschr. gerichtl. Med., 1906 (32), 219; and Schütze, Arch. Hyg., 1912 (76), 116.

[69] Florence (Bull. Soc. chim. biol., 1919 (1), 185) found the nitrogenous residue to be free from tyrosine and tryptophan, suggesting that it is collagen.

tissues remain—three to twelve months. (4) Migration of neutral fat, crystallization and partial saponification of the higher fatty acids in the panniculus; transformation of the blood pigment into crystalline form—four to twelve or more months.[70]

Lipemia

Normally the blood contains a considerable amount of fats and lipoids, varying somewhat, but not greatly, with the diet or with most diseases.[71] The older literature gave figures varying widely, but analyses by more modern methods[72] give figures for the ether-soluble constituents of the normal plasma (before breakfast) ranging ordinarily from 0.57 to 0.82 per cent., of which cholesterol and phosphatid[73] are about equal (0.2 to 0.3 per cent.) with very little neutral fat (0.1 to 0.2 per cent.).

There is a nearly constant ratio between the fatty acids and the unsaponifiable lipoids (mostly cholesterol), also between the saturated and unsaturated fatty acids.[74] Highly unsaturated fatty acids are found, presumably desaturated preliminary to utilization. The proportion of cholesterol and lecithin is normally a constant, and these two lipoids seem to be functionally antagonistic, excess of either leading to pathological conditions. With an excess of lecithin in the diet, hemolysis may occur from the excess of lecithin in the blood.[75]

In various diseases, exclusive of diabetes, the total lipin content was found by Bloor to be about normal, but the proportion of the different lipins varied somewhat. After taking fat-rich food, however, there may be a considerable excess of the food fats in the serum, and it is, therefore, extremely difficult to say just when the amount of fat in the blood is large enough to be considered as a lipemia, especially since after every fatty meal there is enough fat in the blood to make it turbid.[76] B. Fisher,[77] states that we may speak of a pathological lipemia when we have a distinctly cloudy blood or serum, which is clarified by shaking with ether through the dissolving out of fat which can then be separated from the ether. We may, however, sometimes find turbid plasma with normal lipin content and clear plasma with hyperlipemia (Gray). Earlier writers described, incorrectly, lipemia in many conditions, but recent writers mention it chiefly as occurring in alcoholism,[78] nephrosis and dia-

[70] *Sclerema neonatorum* is caused by hardening of the subcutaneous fat, perhaps because of a low proportion of oleic acid. (Beyer, Verh. Deut. Path. Gesell., 1908 (12), 305.) C. S. Smith, however, found normal oleic acid but a high figure for free fatty acids. Others have described high melting points for the fat, believing the condition to be merely an exaggeration of the normally high proportion of palmitic and stearic acids of infant fat tissues (Smith, Jour. Cut. Dis., 1918 (36), 436).

[71] Cowie and Hoag, Jour. Amer. Med. Assoc., 1921 (77), 1493.

[72] Bloor, Jour. Biol. Chem., 1916 (25), 577.

[73] Concerning blood lecithin see Feigl, Biochem. Zeit., 1918 (90), 361.

[74] Bloor, Jour. Biol. Chem., 1923 (56), 711.

[75] See Brinkman and van Dam, Biochem. Zeit., 1920 (108), 35.

[76] Neisser and Brauning, Zeit. exp. Path. u. Ther., 1907 (4), 747.

[77] Virchow's Arch., 1903 (172), 30. Résumé and complete literature.

[78] Also occurs in experimental alcoholism (Feigl, Biochem. Zeit., 1918 (92), 282; Bang, *ibid.*, (90), 383).

betes. By far the greatest amounts of fat are observed in the last condition, and diabetic lipemia is always accompanied by an acidosis, although acidosis often occurs without lipemia. Experimental pancreatic diabetes may be accompanied by lipemia.[79] Increases in the blood lipoids, not usually of sufficient magnitude to cause a distinct lipemia, may be found in nephritis (Bloor), cirrhosis, tabes and paralysis (Feigl),[80] and jaundice (Buerger).[81] Muggia[82] says that in advanced phthisis there is a lipemia which may be responsible for the fatty liver often seen in this condition.

Neisser and Derlin[83] found 19.7 per cent. of fat in the blood of a patient with diabetic coma (after death 24.4 per cent. was found) whose urine contained 0.8 per cent. of fat, and through analysis of this and other material came to the conclusion that the fat comes directly from the chyle; *i. e.*, that it is food fat, not body fat. Others have reported that without a high fat diet the lipemia is not marked even in severe diabetes.[84] Fischer found an average of 18.129 per cent. in his case, including at least 0.478 per cent. of cholesterol, with no lipuria and very small amounts of fatty acids; of the fat, about 67.5 per cent. was olein. Ringer[85] has found 14.4 per cent. of lipins, including 2.14 per cent. of cholesterol. As high as 27 per cent. of fat has been reported in the blood,[86] but in 1,062 analyses of diabetic blood, Gray[87] found but four with over 6 per cent. fat, the maximum being 9.55.

In many cases the increase is chiefly in the lipoids, *lipoidemia*,[88] and in acidosis there is said to be an especial increase in cholesterol (Adler).[89] With the high lipemia of diabetes, local deposits of cholesterol esters in the form of "xanthomas" are often found,[90] Major[91] having collected records of 74 authentic cases in the literature.

Study of a large number of diabetic bloods by Gray[92] gave the following results: Normal lipin values are seldom found, the most marked increases being in the total glycerides, next the total fatty acids, then the cholesterol, and least the phospholipins. Increase of both cholesterol and glycerides seems to be pathognomonic of chronic diabetic lipemia, as in alimentary lipemia the increase is in the fatty acids. A high blood lipin

[79] Seo, Arch. exp. Path. u. Pharm., 1909 (61), 1; Allen, Jour. Metabol. Res., 1922 (2), 219.
[80] Biochem Zeit., 1918 (88), 53; (90), 1.
[81] Münch. med. Woch., 1922 (69), 103.
[82] Riforma Med., 1922 (38), 73.
[83] Zeit. klin. Med., 1904 (51), 428.
[84] Wishart, (Jour. Metabol. Res., 1922 (2), 199) gives many analytical results. Marsh and Waller (Arch. Int. Med., 1923 (31), 63) question the dependence of diabetic lipema on the diet.
[85] Proc. Soc. Exp. Biol. Med., 1917 (15), 40.
[86] Frugoni and Marchetti, Berl. klin. Woch., 1908 (45), 1844.
[87] Amer. Jour. Med. Sci., 1924 (168), 35.
[88] See Weil, Münch. med. Woch., 1912 (59), 2096.
[89] Berl. klin. Woch., 1910 (47), 1323.
[90] See Fahr. Cent. allg. Path., 1920 (30), 609.
[91] Major, Johns Hopkins Hosp. Bull., 1924 (35), 27.
[92] Boston Med. Surg. Jour., 1917 (178), 16; see also Feigl, Biochem. Zeit., 1918 (90), 173.

figure seems to exclude a renal diabetes. The greater the duration of the diabetes the lower the lipins, and high figures give a bad prognosis, being usually associated with acidosis. Hyperglucemia and hyperlipemia do not run parallel,[93] but the lipemia has been observed to clear up under insulin treatment (Major). The changes concern chiefly the plasma. Coexistent nephritis does not modify the blood lipin figures. When the lipemia is accompanied by icterus the fats may clear up and a clear serum is present, despite a high fat content.[94] Lipemic blood has marked adsorptive properties which may modify its physiological actions.[95]

In general, the amount of blood lipins increases with the severity of the disease,[96] the averages in a large series of analyses being as follows: Normal, 0.59 per cent.; mild diabetes, 0.83; moderate, 0.91; severe, 1.41. Strikingly high lipin content is, however, not common, 90 per cent. of diabetics showing figures under 1.5 gm. per 100 cc. However, 78 per cent. had over 0.67 per cent. which is the maximum normal figure (Gray),[87] and most of the diabetics in Gray's series who lived two years or more had a lipemia under one per cent.; with 2 per cent. of fat few patients survived two years.

It is an important question whether, with high quantities of fat in the blood, fat embolism may result, for it is possible that at least some of the cases of diabetic coma are due to such fat embolism in the cerebral vessels. Ebstein[97] considers this a possible, but not a common occurrence, because the droplets are too small to cause occlusion of the vessels unless they combine to form large droplets. Fischer doubts if the droplets ever fuse together enough to cause embolism, supporting his contention both by experiments and clinical records, but cases have been reported as fat embolism from diabetic lipemia.[98] The occurrence of lipemia retinalis[99] does not seem to depend on the presence of any definite amount of fat in the blood.[1]

The cause of lipemia has not yet been satisfactorily determined. In alcoholism it is commonly ascribed to a failure to burn fat, because of the presence of the more readily oxidized alcohol, and the common coexistence of diabetes and lipemia suggests for both a common cause; *i. e.*, lack of oxidation of fat and sugar. In corroboration may be cited the occurrence of lipemia in other conditions associated with defective oxidation; *i. e.*, pneumonia, anemia,[2] phosphorus-poisoning. As we are still unfamiliar with the essential factors and steps in the oxidation of fat, it would be mere speculation to attempt to explain further the reason for the failure of destruction of the fat. The origin of the fat in lipemia is likewise

[93] Corroborated by Bang, Biochem. Zeit., 1919 (94), 359.
[94] Feigl and Querner, Zeit. exp. Med., 1919 (9), 153.
[95] Eichholtz, Biochem. Zeit., 1922 (128), 310.
[96] See Jour. Amer. Med. Assoc., 1917 (69), 375.
[97] Virchow's Arch., 1899 (155), 571.
[98] Hedren, Svenska Läk. Handl., 1916 (42), 933.
[99] Bibliography by Gray and Root, Jour. Amer. Med. Assoc., 1923 (80), 995.
[1] McCann, Johns Hopkins Hosp. Bull., 1923 (34), 302.
[2] See Boggs and Morris (Jour. Exper. Med., 1909 (11), 553), who produced lipemia by repeatedly bleeding rabbits.

undetermined. Ebstein considers that it arises partly from the food, partly from fatty degeneration of the cells of the blood, the vessel-walls, and the viscera. Neisser and Derlin consider it as merely food fat coming from the chyle and accumulated in the blood. Fischer believes that it is largely derived from the fat depots, and that because of loss of the lipolytic power of the blood it cannot be rendered diffusible, and hence it cannot enter the tissues where it is normally consumed. Sakai[3] also found a low lipase content in the blood and suggests that fat entering the blood is unable to leave it because of defective lipolysis. Klemperer and Umber hold that it comes from disintegration of tissue cells, but are unable to determine the cells concerned. Ervin[4] attributes diabetic lipemia to the glycogen deficiency of the cells, assuming that glycogen acts as a protective colloid which holds the intracellular fats in emulsion.

Bloor's studies[5] support strongly the view that the fats come from the food, for he found lipemia only in diabetics receiving fat in their food, and under fasting an existing lipemia disappears. The increase involves all the lipins, but neutral fat is increased most and lecithin least, but when lipemia disappears the cholesterol and lecithin may remain above normal. However, other observers have reported that an existing lipemia may improve on a high fat diet. Verse[6] says that a lasting lipemia can be produced by feeding rabbits mixed cholesterol and oil, but not with either of these alone. In severe diabetes without lipemia the lipins are all much increased in the plasma, but with the relative proportions about as in normal individuals, although with a tendency for the fats to accumulate in excess. The facts that fat oxidation depends upon carbohydrate oxidation, and that in diabetics excessive fat feeding is usual, are probably significant in the causation of diabetic lipemia. (See also cholesterolemia.)

OBESITY[7]

The popular idea that some persons become fat irrespective of how much or how little they eat, is justified by modern studies of metabolism, which are reviewed by Strouse.[8] It is found that such victims of "constitutional obesity" may carry on an active life without losing weight on a diet furnishing few more calories than theory demands for their basal metabolism, and much less than they theoretically consume in their activity. Presumably underweight in otherwise healthy persons represents the opposite extreme of the same lack of correspondence between caloric intake, output, and storage. Neither with excessive over- or under-weight is the basal metabolism, as observed in a large series of people, constantly different from normal, and hence obesity cannot be attributed to a particularly low basal metabolism. However, the specific dynamic action of protein was much less than normal in obese people and correspondingly higher in thin people, which may account for their respective fatness and leanness, especially as the obese also often show a lessened specific dynamic action of carbohydrate. Presumably abnormal function of the endocrine glands underlies

[3] Biochem. Zeit., 1914 (62), 387.
[4] Jour. Lab. Clin. Med., 1919 (5), 146.
[5] Jour. Biol. Chem., 1916 (26), 417; 1917 (31), 575; 1921 (49), 201.
[6] Münch. med. Woch., 1916 (33), 1074.
[7] Review and bibliography by Tileston, "Endocrinology and Metabolism," 1924 (4), 29.
[8] Strouse, Dye, Wang and Saunders, Arch. Int. Med., 1924 (34), 267, 275, 573.

this metabolic abnormality.[9] During fasting the obese may develop an acidosis as do people who are not obese, and the degree of this acidosis is not related to the degree of obesity.[10]

Pathological Occurrence of Fatty Acids

Fatty acids occasionally occur free in pathological processes. The best example of this is fat necrosis (*q. v.*)., where crystals of fatty acids appear in the necrotic fat-cells, arising through splitting of fat, and later becoming combined with calcium from the blood. Similar crystals, consisting of a mixture of palmitic and stearic acids, frequently called *margarin* or *margaric acid* crystals, may be found in decomposed pus, in sputum from bronchiectatic cavities and from gangrene of the lungs, in gangrenous tissue, and in atheromatous areas. According to Schwartz and Kayser,[11] the free fatty acids, at least in pulmonary gangrene, arise from lipolysis by bacterial action rather than by the lipase of the tissues. Eichhorst found crystals of fatty acids in the neighborhood of acute patches of sclerosis in the central nervous system in *multiple sclerosis*, and McCarthy[12] found them in a spinal cord undergoing secondary degeneration from compression. Whipple[13] describes a case with deposits of fatty acids and neutral fat in the wall of the intestine and the mesenteric glands, while soaps and fatty acids are said to be present in excess in chronic appendicitis.[14] Appendiceal concretions consist largely of soaps.[15] Soaps and fatty acids, especially oleic acid and oleates, are highly toxic, and their profound hemolytic power has been thought of importance in pathological conditions, especially bothriocephalus anemia.[16] The fatal dose of sodium oleate for rabbits is 0.15 gm. per kilo (Leathes). The salts of higher fatty acids above capric are hemolytic, while those from caproic down are not, nonoic acid salts being the turning point (Shimazono).[17] The toxicity of soaps may be related to their marked power to inhibit proteolytic enzymes.[18]

The fatty acids may be stained green by copper acetate, according to Benda's method, and if then treated with hematoxylin, they turn black.[19] With Nile blue sulfate they stain blue, forming a blue salt, while the neutral fats are stained red by the oxazone base (J. L. Smith). Fischler and Gross[20] state that fatty acids are present in atheromatous areas and about the margin of anemic infarcts, but are not recognizable by this method in such fatty degenerations as pneumonic exudates, caseation, etc. Klotz[21]

[9] There is also a reduction in relative blood and plasma volumes in obesity (See Brown and Keith, Arch. Int. Med., 1924 (33), 217).

[10] Folin and Denis, Jour. Biol. Chem., 1915 (21), 183.

[11] Zeit. klin. Med., 1905 (56), 111.

[12] Univ. of Penn. Med. Bull., 1903 (16), 141.

[13] Bull. Johns Hopkins Hosp., 1907 (18), 382.

[14] Anthony, Jour. Med. Res., 1911 (20), 359.

[15] Maver and Wells, Arch. of Surg., 1921 (3), 439.

[16] Faust, Suppl. Bd., Schmiedeberg's Arch., 1908, p. 171.

[17] Z. Immunität. Ref., 1911 (4), 656.

[18] Jobling and Petersen, Jour. Exp. Med., 1914 (19), 251.

[19] Fischler, Cent. f. Path., 1904 (15), 913.

[20] Ziegler's Beitr., 1905 (7th suppl.), 343.

[21] Jour. Exp. Med., 1905 (7), 633.

considers that calcium soaps are formed as the first step in pathological calcification, according to microchemical evidence; but a chemical investigation of the same question did not give the writer positive results.[22] In fatty cells, especially in the liver, crystals are often found and misinterpreted as fatty acids although really crystals of neutral fats.[23] However, Sammartino[24] found that the liver lipins do contain free fatty acids in not inconsiderable amounts, which he attributes to absorption from the intestines.

Pathological Occurrence of Cholesterol[25]

Since all cells contain cholesterol,[26] when tissue disintegration occurs it is accumulated as one of the least soluble products. The origin of the normal cell cholesterol is unknown, but that which is liberated by normal disintegration of cells seems to be retained and worked over,[27] and there is evidence of its synthesis in the animal body.[28] It is not destroyed during autolysis.[29] The adrenal cortex is often particularly rich in cholesterol, but the amount varies greatly (from 1.18 to 82.8 gm. per kilo),[30] so that these glands are considered by many to be the regulators of cholesterol metabolism. Cholesterol has sometimes been considered, but without convincing proof, to be a product of protein decomposition; if this is true, then the cholesterol found in disintegrating tissues may be formed from the cell proteins during their decomposition.[31] Feeding of excessive amounts of cholesterol leads to extensive deposition of cholesterol or cholesterol esters, often with serious effects, especially in the kidneys (Chuma).[40] (See Cholesterolemia, p. 467.)

Xanthomatous masses[32] of various kinds all seem to be composed of deposits of cholesterol esters as a sequel of protracted hypercholesterolemia,[33] which deposits lead to proliferative and phagocytic reactions in the fixed tissues.[34] In diabetes the nodules of xanthoma diabeticorum may be absorbed when the hypercholesterolemia is reduced by insulin treatment.[35]

[22] Wells, Jour. Med. Research, 1906 (14), 491.
[23] Smith and White, Jour. Path. and Bact., 1907 (12), 126.
[24] Biochem. Zeit., 1922 (132), 343.
[25] Concerning the chemistry of cholesterol see introductory chapter. Review on tissue cholesterol given by Fex, Biochem. Zeit., 1920 (104), 82.
[26] See Dorée, Biochem. Jour., 1909 (4), 72.
[27] Ellis and Gardner, Proc. Royal Soc. London, 1912 (84), 461.
[28] Gamble and Blackfan, Jour. Biol. Chem., 1920 (42), 401; Randles and Knudson, Science, 1924 (60), 530; Beumer and Lehmann, Zeit. ges. exp. Med., 1923 (37), 274.
[29] Corper, Jour. Biol. Chem., 1912 (11), 37; Shibata, Biochem. Zeit., 1911 (31), 321.
[30] Chauffard *et al.*, Ann. de Med., 1909 (4), 72.
[31] Of historical interest is Austin Flint's idea that cholesterol in the blood is an important factor in intoxications, especially in icterus (Amer. Jour. Med. Sci., 1862 (44), 29). All recent evidence is to the effect that cholesterol is not toxic.
[32] See F. Parkes Weber "Cutaneous Xanthoma, etc." London, 1924; Bloodgood *et al.*, Arch. of Surg., 1924 (8), 882.
[33] Burns, Arch. Derm. Syph., 1920 (2), 415; Mook and Weiss, *ibid.*, 1923 (8), 19.
[34] Literature given by Rosenbloom, Arch. Int. Med., 1913 (12), 395.
[35] Major, Johns Hopkins Hosp. Bull., 1924 (35), 27.

Windaus[36] found that normal aortas contain about 0.15 per cent. cholesterol, while in two atheromatous aortas he found 1.8 per cent. and 1.4 per cent., the increase being more in the cholesterol esters than in the free cholesterol. Cholesterol is also increased in the arterial walls in endarteritis obliterans.[37] Ameseder[38] found that 28.56 per cent. of the ether extract of atheromatous aortas was cholesterol. The view of Chauffard that arcus senilis, xanthelasma, and other ocular conditions depend on cholesterol deposition is not substantiated by Mawas,[39] but Verse[6] and Chuma[40] observed corneal opacity in rabbits fed cholesterol. Normal urine contains only traces of cholesterol (1.0 mg. per day), but 10–12 mg. may be found in diabetes or jaundice. Urinary cholesterol may be increased in chronic nephroses,[41] and myelin droplets are frequently found in the urine whenever there is tubular degeneration.[42] Extensive infiltration of the tubular epithelium with masses of cholesterol esters is a characteristic feature of the type of subacute parenchymatous renal "nephrosis" associated with edema.[43] In degenerative conditions of the central nervous system[44] demonstrable amounts of cholesterol may be present in the spinal fluid (Pighini),[45] and in an old pleural effusion as much as 3 to 4 per cent. of cholesterol has been found.[46]

Cholesterol in crystals is found under somewhat the same conditions as the fatty acids. The characteristic large flat plates of cholesterol may be seen in any tissue in which cells are undergoing slow destruction, and where absorption is poor. Therefore, they are found frequently in atheromatous patches in the blood-vessels, encapsulated caseous areas, old infarcts and hematomas, inspissated pus-collections, dermoid cysts, hydrocele fluids, etc.; especially large amounts occur in the cholesteatomatous tumors of the ear and cranial cavity. Rarely they are present in the urine, especially with renal lithiasis.[47] Apparently cholesterol crystals may be slowly removed, the chief factor probably being the giant-cells that are often found surrounding them,[48] and the large "foamy" endothelial cells that take up especially the uncrystallized cholesterol. In general, cholesterol deposits behave as inert foreign bodies. In liquids the crystals form glistening scales; in fresh tissues they may be recognized by their solubility in ether, choloroform, hot alcohol, etc., and by their

[36] Zeit. physiol. Chem., 1910 (67), 174.
[37] Labbé *et al.*, Compt. Rend. Soc. Biol., 1923 (88), 1131.
[38] Zeit. physiol. Chem., 1911 (70), 458.
[39] Monatsbl. f. Augenheilk., 1912 (13), 604.
[40] Virchow's Arch., 1923 (242), 275.
[41] Grunke, Biochem. Zeit., 1922 (132), 543.
[42] Stengel and Austin, Trans. Assoc. Amer. Phys., 1914 (29), 312; Gross, Deut. Arch. klin. Med., 1920 (133), 9.
[43] See Dyke, Quart. Jour. Med., 1924 (69), 77.
[44] Southard has described cholesterol concretions up to 2 cm. diameter in the brain and cord. (Jour. Amer. Med. Assoc., 1905 (45), 1731.)
[45] Riforma Med., 1909 (25), 67; Boltz, Amer. Jour. Psych., 1923 (3), 111.
[46] Ruppert, Münch. med. Woch., 1908 (55), 510; Zunz, Hedstrom, and others (See Chap. XVI).
[47] Dorner, Münch. med. Woch., 1922 (69), 661.
[48] See LeCount, Jour. Med. Res., 1902 (7), 166; Corper, Jour. Exp. Med., 1915 (21), 179; Stewart, Jour. Path. and Bact., 1915 (19), 305.

color reactions. In histological specimens prepared by the usual methods the cholesterol is dissolved out, but the resulting clear-cut clefts are quite characteristic. In fresh specimens in which cholesterol crystals are present, on treatment with five parts concentrated sulfuric acid and one of water, the edges of the crystals become carmine red, then violet. Concentrated sulfuric acid plus a trace of iodine colors the crystals in sequence, violet, blue, green, and red. Hirschsohn[49] recommends a reaction with a 90 per cent. solution of trichloracetic acid in HCl, which gives red, then violet, then blue The results of microchemical examination are said not to agree at all quantitatively with analytic results.[50]

Cholesterolemia.[51]—Normal blood contains 0.16 to 0.17 per cent. of cholesterol,[52] of which about 55 per cent. is in the corpuscles, but in pathological conditions the amount in the plasma varies greatly (Bacmeister and Henes).[53] Cholesterol-rich diet causes a slight increase,[54] but a more marked increase is said to be obtained in pregnancy,[55] tubular nephritis (or nephrosis),[56] early arteriosclerosis (arteritis obliterans),[57] hypertension,[58] obesity, diabetes, and obstructive but not in hemolytic jaundice.[59] Acute febrile diseases usually show a lowered cholesterol,[60] the amount is unchanged in tuberculosis, but chronic infections often increase the blood cholesterol.[61] High figures have also been observed in alcoholism, senile dementia and dementia precox.[62] In nephritis the amount of cholesterol bears no relation to the albuminuria or to albuminuric retinitis,[63] and in uremia it may be low. Stapp[64] describes marked cholesterolemia as accompanying severe parenchymatous nephritis (nephrosis), but not chronic interstitial types, and Gross[65] found myelin droplets in the urine to be evidence of tubular injury. Kollert and

[49] Pharm. Centralhalle, 1902 (43), 357.

[50] Thaysen, Cent. allg. Pathol., 1915 (26), 433.

[51] Bibliography by Dewey Arch. Int. Med., 1916 (17), 757; Myers, Jour. Lab. Clin. Med., 1920 (5), 776.

[52] According to Currie (Brit. Jour. Exp. Path., 1924 (5), 1923), there is a marked seasonal variation in blood cholesterol with high summer and low winter figures.

[53] Deut. med. Woch., 1913 (39), 544.

[54] See Luden, Jour. Biol. Chem., 1916 (27), 257.

[55] Benda, Arch. Gyn., 1923 (116), 506; Pribram, *ibid.*, 1923 (119), 57. The blood of the fetus corresponds closely to that of the mother in respect to free cholesterol but contains no cholesterol esters. (Slemons and Curtis, Amer. Jour. Obst., 1917 (75), 569.) Hence the fetal blood contains but about half as much total cholesterol as the maternal blood (György, Klin. Woch., 1924 (3), 483).

[56] Strauss and Schubardt, Zent. inn. Med., 1922 (43), 425).

[57] Heitz and Labbé, Compt. Rend. Soc. Biol., 1922 (87), 1024.

[58] Pribram and Klein, Med. Klin., 1924 (20), 572.

[59] Rothschild and Felsen, Arch. Int. Med., 1919 (24), 520; see also McMaster, Jour. Exp. Med., 1924 (40), 25.

[60] See Leupold and Bogendörfer, Deut. Arch. klin. Med., 1922 (140), 28; Kipp (Jour. Biol. Chem., 1920 (44), 215) found an increase in pneumonia during convalescence.

[61] Marie, Ann. Inst. Pasteur, 1923 (37), 921; 1924 (38), 945.

[62] Parhon, Encéphale, 1925 (20), 48.

[63] Gaudissart, Presse Méd., 1921 (29), 893.

[64] Deut. Arch. klin. Med., 1918 (127), 439; corroborated by Epstein, Amer. Jour. Med. Sci., 1917 (154), 638; Hahn and Wolf, Zeit. klin. Med., 1921 (92), 393.

[65] Deut. Arch. klin. Med., 1920 (133), 9.

Finger[66] state that hypercholesterolemia up to 0.28 per cent. may be found, but only when the kidney is excreting lipoids, and believe that the cholesterol is at least partly responsible for albuminuric retinitis. The blood content has been reported as low in febrile cutaneous diseases, but high in afebrile cutaneous diseases associated with eosinophilia.[67] Sometimes high figures are observed in syphilis, but they bear no relation to the Wassermann reaction.[56] Denis[68] states, after examination of a large number of cases, that hypercholesterolemia was found only in diabetes, and that low cholesterol values are found in cachexia or prostration, but are not characteristic of any particular disease.[69] The hypercholesterolemia of diabetes improves under insulin therapy. In Japan low values have been observed in beriberi, high in hemiplegia.[70] High blood cholesterol has been found associated with low basal metabolism, and conversely.[71] There may be a temporary increase during convalescence from pneumonia.[72] Numerous investigators have described hypercholesterolemia in patients with gall-stones (*q. v.*), and attribute a causal relation thereto. In patients with xanthoma, which is seen especially in diabetes, the blood cholesterol is usually very high,[73] up to 1200 mg. per 100 cc. having been recorded. The importance of the cholesterol of the blood in hemolysis and protection therefrom has been discussed under that subject; in anemia there is usually hypocholesterolemia.

Experimental hypercholesterolemia in animals[40] leads to a deposition of cholesterol in various organs, especially the aorta,[74] kidneys and liver, accompanied by degeneration in the parenchymatous structures, and excretion of cholesterol in the urine and bile; gall-stones may be formed (Dewey). Sometimes lipoid-filled endothelial cells become so abundant in the spleen as to resemble Gaucher's disease (Anitschkow, McMeans).[75] Excessive cholesterol in the blood reduces phagocytic activity and antibody formation in experimental animals,[76] which may be because it is taken up particularly by the reticulo-endothelial cells. Sometimes, however, cholesterol seems to stimulate the immunological defense.[77] Robertson believes cholesterol to have an accelerative action on cancer growth, related to its hydroxyl radical,[78] but in cancer patients there

[66] Münch. med. Woch., 1918 (65), 816.

[67] Fischl, Wien. klin. Woch., 1914 (27), 982.

[68] Jour. Biol. Chem., 1917 (29), 93.

[69] In starvation the figures are low (See Rosenthal, Berl. klin. Woch., 1919 (56), 793).

[70] Bull. Naval Med. Assoc., Japan, Feb. 1919.

[71] Epstein and Lande, Arch. Int. Med., 1922 (30), 563.

[72] Kipp, Jour. Biol. Chem., 1920 (44), 215.

[73] Yanakawa and Kashiwabara, Tohoku, Jour. Exp. Med., 1922 (3), 317; also Burns, Mook and Weiss, Major.

[74] See Adler, Trans. Assoc. Amer. Phys., 1917 (32), 255.

[75] Jour. Med. Res., 1916 (33), 481. The material in the cells in Gaucher's disease was thought to be a protein-phosphatid compound by Mandelbaum and Downey, (Fol. Hematol., 1916 (20), 139), but the material obtained by extraction was identified as *kerasin* by Lieb and Madenovic (Zeit. physiol. Chem., 1924 (140), 305); see also Epstein, Biochem. Zeit., 1924 (145), 398.

[76] Dewey and Nuzum, Jour. Infect. Dis., 1914 (15), 472.

[77] See Beumer, Zeit. Kinderheilk., 1923 (35), 298; Hênes, Arch. Int. Med., 1920 (25), 411; Tunnicliffe, Jour. Infect. Dis., 1923 (33), 285.

[78] Jour. Cancer Res., 1918 (3), 75.

seems to be no cholesterolemia (Denis).[79] However, it has been found as a normal feature of senility.[80] Some authors look upon hypercholesterolemia as an etiological factor in arteriosclerosis with atheroma.[81]

The ratio of fatty acids to cholesterol, and of free cholesterol to cholesterol esters, is nearly constant in normal human blood,[82] the esters being about 33.5 per cent. in the blood and 58 per cent. in the plasma; in pregnancy and during fat absorption the proportion of cholesterol esters is high, in cancer and nephritis it is low.[83] The blood of the fetus contains free cholesterol but no cholesterol esters. The belief that the adrenal cortex has a regulatory function in cholesterol metabolism is supported by the observation of Joelson and Schorr[84] who found that hypercholesterolemia follows removal of a large part of the adrenal tissue.

AMYLOIDOSIS[85]

Virchow, in 1853, made the first study of the nature of the substance characteristic of "lardaceous" degeneration, and considered it to be a sort of animal cellulose, because it often became blue if treated with iodine followed by sulfuric acid. To this resemblance in staining reaction we owe the unfortunate, misleading, but generally used, name amyloid.[86] It was but a few years (1859) before Friedreich and Kekulé showed that the substance in question was of protein nature; their methods were very crude, but the main fact was soon better substantiated by Kühne and Rudneff (1865). Krawkow,[87] in 1897 through his amplification of Oddi's[88] observation that amyloid organs contain *chondroitin-sulfuric acid*, concluded that amyloid is a compound of protein with this acid, similar to nucleoprotein, which is a compound of nucleic acid and protein. This work had received general acceptance, until a later paper by Hanssen[89] reported a study of amyloid material isolated in pure condition from sago spleens by mechanical means, which contained *no* chondroitin-sulfuric acid, although the amyloid organs taken *in toto* do contain an excess of sulfur as sulfate. Mayeda[90] also reported that a

[79] Luden reports an increase (Jour. Lab. Clin. Med., 1916 (1), 662).

[80] Parhon, Compt. Rend. Soc. Biol., 1923 (88), 231.

[81] See Verse, Deut. med. Woch., 1925 (51), 49.

[82] See Thannhauser, Deut. Arch. klin. Med., 1923 (14), 290; Bloor, Jour. Biol. Chem., 1923 (56), 711.

[83] Bloor and Knudson, Jour. Biol. Chem., 1917 (29); 7; 1917 (32), 337; Currie, Brit. Jour. Exp. Path., 1924 (5), 293.

[84] Arch. Int. Med., 1924 (34), 841; review on relation of adrenals to cholesterol.

[85] General literature to 1893, see Wichmann, Ziegler's Beitr., 1893 (13), 487; also Lubarsch, Ergeb. allg. Path., 1897 (4), 449; discussion in the Verh. Deut. Path. Gesellsch., 1904 (7), 2–51; Davidsohn, Virchow's Arch., 1908 (192), 226, and Ergebnisse allg. Path., 1908 (12), 424.

[86] In view of the fact that this substance is chemically related to chondrin, and that it also closely resembles this substance physically, it has seemed to the writer that the name "*chondroid*" would be much more appropriate than any of the many more or less misleading and inappropriate titles that are at present in use. The very multiplicity of these terms, however, prohibits any attempt to introduce still another. A particularly unfortunate source of confusion exists in the use of the name amyloid for a vegetable substance, formed by the action of acids upon cellulose.

[87] Arch. exp. Path. u. Pharm., 1897 (40), 196.

[88] *Ibid.*, 1894 (33), 377.

[89] Biochem. Zeit., 1908 (13), 185.

[90] Zeit. physiol. Chem., 1909 (58), 475.

preparation of amyloid which he had made did not yield sulfuric acid.

These two observations have obtained important support from the study by Eppinger[91] of a case in which the "amyloid" was deposited in tumor-like masses in the left lobe of the liver, the rest of the liver showing the usual picture of diffuse amyloid deposition, the spleen exhibiting the typical sago spleen, and traces of amyloid being found in the intestine and heart. No cause for the amyloidosis could be found, but the deposits gave the characteristic color reactions. Masses of amyloid material, weighing 11 gms. when dry, could be isolated from the liver practically free from tissue elements, and this unusually favorable material was analyzed. It contained no sulfur whatever, and no phosphorus or carbohydrate. The following amino acids were found: Tyrosine, 12.34; tryptophan, 4.23; arginine, 14.67; lysine, 4.34 per cent.; histidine and cystine were absent; purines, 1 per cent. By the Van Slyke method the following figures were obtained: Ammonia-N, 13.08; melanin-N, 4.41; mono-amino-N, 45.25; non-amino-N, 15.8 per cent.

In view of the similarity of the results obtained by Hanssen and Eppinger, the long-standing and generally accepted idea that amyloid is a compound protein with chondroitin sulfuric acid has become very doubtful, and it is to be hoped that these observations will soon be repeated.

Krawkow's Views on the Chemistry of Amyloid.—Krawkow separated amyloid from nucleoprotein, to which it is most closely related, by dissolving both substances from the minced amyloid organs with ammonia, precipitating with acid, and then taking up the amyloid with Ba $(OH)_2$ solution, in which the nucleoprotein does not dissolve. Amyloid thus isolated is a nearly white powder, which is easily soluble in alkalies, but slightly in acids, and is very resistant to pepsin digestion. The elementary composition was found by Krawkow to be approximately as follows: C = 49–50 per cent.; H = 6.65–7 per cent.; N = 13.8–14 per cent.; S = 2.65–2.9 per cent.; P in traces only. He considered that amyloid differs from normal chondroitinsulfuric acid compounds, such as cartilage, in that in the latter the acid radical is in a loose combination with the protein, while in amyloid the combination is a very firm one, perhaps in the nature of an ester. The occurrence of the typical amyloid reaction in what appears otherwise to be normal cartilage, occasionally observed in senile tissues, was thought to be due to the transformation of loosely bound into firmly bound chondroitin-sulfuric acid, amyloid being not essentially a pathological product, but rather a slightly modified normal constituent of the body.

Chondroitin-sulfuric acid, which has been studied especially by Mörner and by Schmiedeberg,[92] has the formula $C_{18}H_{27}NSO_{17}$, according to the latter, and yields on cleavage *chondroitin* and sulfuric aicd, as follows.

$$C_{18}H_{27}NSO_{17} + H_2O = C_{18}H_{27}NO_{14} + H_2SO_4$$

Kondo,[93] however, gives it an empirical formula of $C_{15}H_{27}NSO_{16}$, there being apparently two equivalents of the base for each SO_4 group. Levene and La Forge[92] have demonstrated that chondroitin-sulfuric acid consists of sulfuric acid, acetic acid, chondrosamine which is an isomer of glucosamine, and glucuronic acid. It unites with histones and forms a precipitate.[94] Chondroitin is a gummy substance which in turn may be split into acetic acid and a reducing substance, *chondrosin*. Chondroitin-sulfuric acid is the characteristic component of cartilage, but it is also found

[91] Biochem. Zeit., 1921 (127), 101.

[92] Mörner, Skand. Arch. Physiol., 1889 (1), 210; Zeit. physiol. Chem., 1895 (20), 357, and 1897 (23), 311; Schmiedeberg, Arch. exp. Path. u. Pharm., 1891 (28), 358; 1920 (87), 1. See also Levene and La Forge, Jour. Biol. Chem., 1913 (15), 69 and 155; 1914 (18), 123.

[93] Biochem. Zeit., 1910 (26), 116.

[94] Pons, Arch. internat. physiol., 1909 (8), 393.

in the walls of the aorta and other elastic structures (Krawkow). It has also been found in a uterine fibroma and in bone tissue by Krawkow, but could not be found in the parenchymatous organs, normal and pathological, or in chitinous structures. Mörner has found it in a chondroma.

Neuberg[95] has studied especially the protein constituent of the amyloid compound and he corroborated Krawkow's finding of a substance of apparently similar composition in the normal aorta. This is characterized by a high proportion of diamino-nitrogen,[96] as compared with most proteins, as shown in the following table giving the percentage of the total N contained in each of the three forms, amid-nitrogen (ammonia), monamino-acids, and diamino-acids:

TABLE I

	Monamino-acid nitrogen	Diamino-acid nitrogen	Amid nitrogen
Liver amyloid	43.2	51.2	4.9
Spleen amyloid	30.6	57.0	11.2
Aorta "amyloid"	54.9	36.0	8.8
Gelatin	62.5	35.8	1.6
Casein	76.0	11.1	13.4

The variations in the composition of the different amyloids, as shown in the above table, indicate that the protein group may vary in different organs in different cases, and also indicate that the "amyloid-like" substance of normal vessels is not the same as the pathological substance. Corresponding variations were found in the apportionment of the sulfur between that which is in the form of oxidized and the unoxidized sulfur. The proportion of the different amino-acids in the protein constituent of amyloid as found by Neuberg is strikingly like that of thymus histone, and entirely dissimilar to the apparently closely related elastin, as shown by Table II.

TABLE II

	Cleavage products (in percentages)		
	Amyloid	Elastin	Thymus histon
Glycine	0.8	25.8	0.5
Leucine	22.2	45.0	11.8
Glutaminic acid	3.8	0.7	3.7
Tyrosine	4.0	0.3	5.2
a-Proline	3.1	1.7	1.5
Arginine	13.9	0.3	14.5
Lysine	11.6	....	7.7

[95] Verh. Deut. Path. Gesell., 1904 (7), 19.

[96] Corroborated by Jackson and Pearce (Jour. Exp. Med., 1907 (9), 520), but not by Mayeda (Zeit. physiol., Chem., 1909 (58), 469), who found histidine, which Neuberg had missed, and a lower arginine and lysine content than histone requires. Eppinger however, found no histidine and a much higher tyrosine content.

This carries out the resemblance of amyloid to nucleoproteins, and likewise, Neuberg found amyloid very slowly digested by pepsin, and much better by trypsin, although less rapidly than simple protein; it is also destroyed by autolytic enzymes, for amyloid tissues readily undergo autolysis.[97] Neuberg considers, from the above results, that amyloid is probably a transformation-product of the tissue protein similar to the transformation of simple proteins into protamines that occurs in the testicle of spawning salmon as they go up the streams, as shown by Miescher's classical studies. However, his results are so different from those of Eppinger that doubt arises as to the purity of his material, for isolation of amyloid is a difficult matter.

Raubitschek[98] found that isolated amyloid, when used for immune reactions, behaved like a specific protein, different from the normal proteins of the animal from whence it came and apparently biologically the same in different species. This observation awaits confirmation. An important consideration is that amyloid deposition occurs under similar conditions in all sorts of animals, including birds; it is very often found in the livers of antitoxin horses, and mice are especially prone to a severe amyloidosis after relatively slight and brief infectious processes.[99] In Miss Slye's mice, both in those with and without cancer, I have often observed amyloidosis of a degree far beyond that ever described in human bodies. The readiness with which mice develop amyloidosis when not subject to any experimental procedure throws doubt on results obtained with them in the experimental production of amyloid.[1]

Staining Properties.—The classical reaction for amyloid is its staining a reddish brown when treated with iodine (best as Lugol's solution) in the fresh state. Such stained specimens, if afterward treated with dilute sulfuric acid, usually become blue or greenish, but may merely turn a deeper brown. Occasionally old compact amyloid may stain bluish or green with iodine alone. The iodine reaction disappears in specimens that have been kept for some time in preserving fluids, or in tissues that have become alkaline, and is generally less persistent than the metachromatic staining by methyl-violet or methyl-green, which color the amyloid red. Occasionally an otherwise typical amyloid will fail to react to iodine, but will stain well with methyl-violet. All these variations may occur in different specimens from the same body, and the blue iodine-sulfuric acid reaction is usually given well only by splenic amyloid. These variations probably depend upon the age and stage of development of the amyloid, or upon secondary alterations, and are perhaps related to Neuberg's observations on the difference in composition of amyloid of different origins. Sato[2] suggests that the iodine-sulfuric acid reaction depends on admixture of old amyloid with cholesterol. Krawkow studied these reactions with pure, isolated amyloid, and found evidence that the iodine reaction depends upon the physical properties of the amyloid, while the methyl-violet stain is a chemical reaction, and hence the iodine reaction is much the more readily altered or lost. As Dickinson[3] says, amyloid stains with iodine simply as if it absorbed the iodine more than does the surrounding

[97] Concerning the absorption of amyloid see Dantchokow, Virchow's Archiv., 1907 (187), 1. However, Lucke has observed that in an infarcted sago spleen the amyloid had resisted autolysis. Proc. Phila. Path. Soc., 1921 (42), 19.

[98] Verh. Deut. Path. Gesell., 1910 (14), 273.

[99] See Finzi, Lo Speriment., 1911 (65), 483; Davidsohn, Virchow's Arch., 1908 (192), 226.

[1] See, for example, Kuczynski (Klin. Woch., 1923 (2), 727; 2193) who reports that amyloid is produced in mice merely by overfeeding of proteins.

[2] Sci. Reports. Govt. Inst. Inf. Dis., Tokyo, 1922 (1), 393.

[3] Allbutt's System, vol. 3, p. 225.

tissue. Krawkow believed that the methyl-violet reaction is due to the dye forming a compound with the chondroitin-sulfuric acid, for he found that these substances unite with one another to form a rose-red precipitate. Hanssen, however, holds that the dyes react with the protein, the iodine with some other unknown labile substance. Schmidt found that implanted pieces of amyloid lost their iodine reaction as they underwent digestion, while the methyl-violet reaction was still very distinct.[4] It is evident, therefore, that iodine is not by itself a specific stain for amyloid, especially as glycogen gives a similar reaction,[5] while true amyloid may not react.

Leupold[6] summarizes his investigations as follows: Amyloid is a complex of different substances which are differentiated by micro-chemical reactions. The protein ground substance of the amyloid is refractory to the typical amyloid reactions. The group which is responsible for the methyl-violet reaction is intimately combined with this protein substance and is separated from it only by the action of alkali. The groups which give respectively the iodine and the iodine-sulfuric acid reactions are closely related to each other. Nevertheless the iodine-sulfuric acid reaction is a completely independent one and is not a modification of the iodine reaction. The occurrence of different colors in the iodine-sulfuric acid reaction depends upon different degrees of oxidation. Amyloid is an emulsion colloid in the gel state. After oxidation with potassium permanganate it is soluble in ammonia, NaOH and $Ba(OH)_2$. Conjugated sulfuric acid plays an important part in the production of amyloid in the organism. The existence of large amounts of conjugated sulfuric acid produces amyloid which gives the iodine reaction. The methyl-violet reaction also depends on the presence of conjugated sulfuric acid; however, for its production there must probably occur a reduction in the amyloid protein. The group which gives the iodine-sulfuric acid reaction occurs through decomposition and perhaps does not depend upon the sulfuric acid.

Congo red is taken up selectively by amyloid, not only in sections but also in the living subject, Bennhold[7] finding this fact capable of diagnostic application in cases of general amyloidosis, especially with liver involvement. Karczag[8] believes that the specific staining of amyloid with dyes depends on its strongly electronegative charge as compared with collagen, while hyalin shows still less of the negative charge than collagen. As in tissue disintegration the tendency is always towards a lowering of the charge, these observations suggest that amyloid is derived from substances of much stronger negative character, while hyaline may come from collagen or elastic fibers.

The Origin of Amyloid

This question has not been at all cleared up as yet by the advances made in our knowledge of the chemistry of amyloid substance. The view that chondroitin-sulfuric acid is a characteristic constituent suggested that this body may be liberated in considerable amount during the destructive processes to which amyloidosis is usually secondary; this idea is further supported by the fact that amyloidosis occurs particularly after chronic suppuration in bone and lungs, both of which tissues, according to Krawkow, contain chondroitin-sulfuric acid. This idea was not substantiated, however, by the experiments made by Oddi and by Kettner,[9] who fed and injected into animals large quantities of the sodium salt of chondroitin-sulfuric acid without producing amyloid changes. Unpublished experiments of the writer with the same material, as well as

[4] Litten (Verh. Deut. Path. Gesell., 1904 (7), 47) states that thionin and kresyl-violet are the most specific stains for amyloid, which they color blue; whereas methyl-violet stains red not only amyloid but also mucin, mast cell granules, and the ground substance of cartilage. V. Gieson's stain usually colors amyloid pale yellow, and hyalin red.

[5] See Wichmann, Ziegler's Beitr., 1893 (13), 487.

[6] Beitr. path. Anat., 1918 (64), 347.

[7] Deut. Arch. klin. Med., 1923 (142), 32.

[8] Zeit. ges. exp. Med., 1924 (41), 71.

[9] Arch. exp. Path. u. Pharm., 1902 (47), 178.

with ground-up cartilage and with mucin, were equally unsuccessful.[10] Likewise mice injected by Strada[11] with the nucleoprotein of pus, the so-called *pyin*, or with chondroitin-sulfuric acid, did not develop amyloidosis. Oestreich[12] injected cancer patients with chondroitin-sulfuric acid for therapeutic purposes, but no amyloidosis resulted. As it is possible to cause amyloidosis experimentally in animals, especially chickens and rabbits, by causing protracted suppuration or chronic intoxication with bacterial filtrates, these negative results speak strongly against the idea of a transportation of chondroitin-sulfuric acid, but do not determine it finally. They may also, with propriety, be used in support of the observation of Hanssen that amyloid does not contain chondroitin-sulfuric acid. Leupold[6] advances the following hypothesis: In chronic suppuration a soluble protein circulates in the blood which stimulates the formation of "defensive ferments." This protein substance, under certain conditions, is deposited in organs where large amounts of sulfuric acid occur. For the development of amyloid there are necessary three factors: A preformed protein, an increased amount of conjugated sulfuric acid, and an inefficiency of the amyloid-filled organ to eliminate the increased amount of conjugated sulfuric acid.

Amyloid is never deposited in the cells themselves,[13] and it seems to be now generally considered that the amyloid material is infiltrated in the form of a soluble modification or precursor and that it is not manufactured in the organ where it is found. It is an interesting fact that a practically identical substance is formed in all tissues and in all species of animals, even when the cause is quite different. Whether the precursors are brought to the organ in solution, or in leucocytes, is unknown—probably the former. Pollitzer[14] states that in various infections, especially coccus infections, chondroitinsulfuric acid is excreted in the urine; this and the fact that amyloidosis of the kidneys is associated with chondroituria[15] is in favor of the view that chondroitin sulfuric acid plays a role in amyloidosis, as also the observation of an increase in this acid in the blood of patients with amyloid (Dresel).[16] It may also be recalled that Hanssen found some increase in the sulfur content of amyloid organs. The presence of glucothionic acid in pus[17] is of similar significance.

There is usually much difficulty in producing amyloid experimentally, except in mice, for in only a certain proportion of cases are the experiments positive (in but about one-third of Davidsohn's[18] 100 trials, and many other experimenters have been much less successful).[19] Dav-

[10] Sato[2] was equally unsuccessful with ground cartilage.
[11] Biochem. Zeit., 1909 (16), 195.
[12] Zeit. Krebsforsch., 1911 (11), 44.
[13] See Ebert, Virchow's Arch., 1914 (216), 77.
[14] Deut. med. Woch., 1912 (38), 1538.
[15] Dietl, Beit. klin. Tuberk., 1922 (51), 18.
[16] Klin. Woch., 1923 (2), 2344.
[17] Mandel and Levene, Biochem, Zeit., 1907 (4), 78.
[18] Verh. Deut. Path. Gesell., 1904 (7), 39.
[19] See Tarchetti, Deut. Arch. klin. Med., 1903 (75), 526. Hirose, Bull. Johns Hop. Hosp., 1918 (29), 40.

idsohn, failing always to get amyloid experimentally after the spleen had been removed,[20] suggests that this organ (in which amyloid is usually earliest and most abundantly observed) produces an enzyme, which causes a precipitation of amyloid in the tissues from a soluble precursor brought in the blood from the site of cell destruction. Schmidt[21] considers it probable that some enzymatic action causes a precipitation or coagulation of the substance in the tissue-spaces or lymph-vessels. The hypothesis that amyloid is formed from disintegrating red corpuscles is probably incorrect. Amyloidosis is produced by the most varied species of bacteria and by their toxins, although the staphylococcus is usually most effective in experimental work.[22] Neither is suppuration absolutely essential, for injection of toxins alone (*e. g.*, in preparing diphtheria antitoxin[23]) without suppuration, may produce amyloidosis, as also frequently does syphilis without suppuration and, less often, many other non-suppurative conditions (*e. g.*, tumors). Wago[24] reports finding a widespread "amyloid-like" degeneration in rabbits immunized with sterile pancreatic extracts.

Local amyloid accumulations are of some interest in considering the genesis of the usual generalized form. They occur particularly as small tumors in the larynx, bronchi, nasal septum, and eyelids; as all these tissues are normally rich in chondroitin-sulfuric acid, it has been suggested that the amyloid arises from a local overproduction of chondroitin-sulfuric acid, which becomes bound with proteins *in situ*. This makes it seem more probable, in spite of the lack of positive experimental evidence, that general amyloidosis is due to liberation of excessive quantities of chondroitin-sulfuric acid in the sites of tissue destruction.

Another form of local amyloid is seen particularly in the regional lymph-nodes of suppurating areas; *e. g.*, the lumbar nodes in vertebral caries, the axillary glands in shoulder-joint suppuration. This local amyloidosis is undoubtedly due simply to the fact that these nodes receive first, and in largest amounts, the cause, whatever it may be, of the amyloid production.[25] Less readily explained are cases of extensive amyloidosis limited to the heart,[26] or of metastasizing amyloid tumors.[27] "Amyloid casts" are described as occurring in the urine, but as the amyloid is deposited only in interstitial elements, the existence of such casts is doubtful, and Saleeby[28] could find no casts giving amyloid reactions in fifty amyloid kidneys.

Corpora amylacea will be found discussed under "Concretions" (Chap. XIX).

HYALINE DEGENERATION [29]

Much confusion concerning this condition may be avoided if we appreciate that the term hyaline indicates a certain physical condition, which

[20] Not corroborated by Sato.[2]

[21] Verh. Deut. Path. Gesell., 1904 (7), 2.

[22] In a series of experiments directed to ascertain, if possible, which constituent of pus might be the cause of amyloid formation, I was unable to secure amyloid by protracted intoxication of rabbits by Witte's "peptone," which consists chiefly of proteoses (Trans. Chicago Path. Soc., 1903 (5), 240).

[23] See Lewis, Jour. Med. Research, 1906 (15), 449.

[24] Arch. Int. Med., 1919 (23), 251.

[25] Quite unexplained is the cause of the rarely observed localization of amyloid in the wall of the urinary bladder. See Lucksch (Verh. Deut. path. Gesell., 1904 (7), 34). Concretions giving the amyloid reactions have been found in the pelvis of the kidney. (Schmidt, Cent. f. Pathol., 1912 (23), 865. Miyauchi, *ibid.*, 1915 (26), 289.)

[26] See Hecht. Virchow's Arch., 1910 (202), 168.

[27] See Walpert, Virchow's Arch., 1920 (227), 173.

[28] Jour. Amer. Med. Assoc., 1925 (84), 344.

[29] General literature, see Lubarsch, Ergeb. and Path., 1897 (4), 449.

may be exhibited by many substances of widely different nature and origin. *There is no one chemical compound, "hyalin,"* which, accumulating in cells or tissues, produces a hyaline appearance. The limits of the application of the term "hyaline degeneration," even to histological findings, are not agreed upon, but in general it is used to apply to clear, homogeneous, pathological substances that possess a decided affinity for acid stains, such as eosin. Somewhat similar substances, usually of epithelial origin, which do not take either acid or basic stains strongly, are usually called "colloid." We may properly consider that pathological hyalin can be divided into two chief classes according to its origin; (1) connective-tissue hyaline; (2) epithelial hyalin.

Connective-tissue hyalin is characterized, like amyloid, by being deposited in or among the fibrillar substance of connective tissues, and not within the cells themselves, but there are undoubtedly several different sorts of chemical substances responsible for various forms of connective-tissue hyalin. One form is closely associated with amyloid, being found in organs showing amyloid degeneration, or in other tissues in the same body. In experimentally produced amyloidosis in animals it has been shown that such a hyaline substance may appear before the amyloid, which eventually replaces it; hence, it has been suggested that hyalin is a precursor of amyloid.[30] Such hyalin differs from true amyloid only in its failure to give the characteristic staining reaction of amyloid; in all other respects, *e. g.*, cause, location, termination, it is the same. As it has been shown (see preceding section) that the staining properties of amyloid are very inconstant, it is probable that the above-described variety of hyalin is merely an *incompletely developed*, or occasionally a *retrogressively altered* amyloid. However, it is probably not necessary, as some authors have thought, that amyloid should always pass through this hyaline stage in its formation.

Quite different, without doubt, is the form of hyalin observed in *scar tissue*. This variety develops almost constantly in any scar-tissue after the blood-supply has been reduced to a minimum through contraction, and is seen characteristically in the corpora fibrosa of the ovary, fibroid glomerules in chronic nephritis, thickened pleural, pericardial, and epi-splenitis scars, etc. Such hyaline substance occurs independent of the usual causes of amyloid, affects only abnormal fibrous tissue, never changes into amyloid, and is prone to undergo calcification—it surely has no close chemical relation to the form of hyalin that does become amyloid. Presumably, it is similar in nature to the collagen of normal fibrous tissue intercellular substance, which has undergone physical rather than chemical changes into a homogeneous hyaline substance. For its physiological prototype it has the thick "collagenous" fibers of the subcutaneous connective tissue.

Probably of quite different origin is the hyalin that develops from elastic tissue, as seen best in the thick-walled, partly obliterated arteries of the senile spleen; and less characteristically in the early stages of

[30] See Lubarsch, Cent. f. Pathol., 1910 (21), 97.

arteriosclerosis, since here the preceding form of connective-tissue hyalin may also occur. Although arterial elastic tissue is related chemically to amyloid, these hyaline vessels do not develop the usual amyloid reaction, but retain more or less of the specific, elastic tissue stains. Presumably this form of hyalin is an increased and physically altered elastin.[31]

Epithelial hyalin occurs within the cells, and includes substances of presumably widely diverse chemical nature, from the keratin of squamous epithelium to the small intracellular hyaline granules of carcinoma and other degenerating cells (Russell's fuchsin bodies).[32] Fuchsin bodies are found also in plasma cells and, less often, in other cells, including granulation tissue; the fuchsin bodies of this class are believed by Brown[33] to be derived from red corpuscles, a view also held by Saltykow, but not accepted by all pathologists.[34] Extracellular substances of hyaline character, but of unknown composition, may also be produced by epithelium, *e. g.*, hyaline casts in the renal tubules. They differ from red cells especially in being Gram-positive.[35]

The composition of none of these forms of hyalin is known, except that by using microchemical methods Unna[36] has found evidence that keratohyalin consists of two elements, one of acid character, apparently derived from the chromatin, and a basic substance resembling the globulins.

Many other pathological materials of widely differing nature may, under certain conditions, assume a hyaline appearance; *e. g.*, fibrinous exudates and thrombi, degenerated muscle-fibers (Zenker's or "waxy" degeneration), tumor-cells (cylindroma), etc. In all of these the chemical nature of the parent substance or substances is probably much less altered than its physical appearance, but whether the change is related to the process of protein coagulation or not is unknown. Occasionally hyalin, both in epithelium and connective-tissue, takes on a crystalline structure (Freifeld).[37] These crystals are insoluble in fat solvents, strong acids, or alkalies and give no iron reaction. Goodpasture[37] believes that the hyalin of epithelial cells, plasma cells, and prostatic acini is identical.

COLLOID DEGENERATION

This term, also, has a very indefinite meaning, and is applied to many different conditions by various authors. Thus, v. Recklinghausen includes under this name amyloid, epithelial hyaline, and mucoid degeneration. Marchand includes hyaline connective-tissue degeneration, and, also, as do most other writers, the mucoid degeneration of carcinoma. Ziegler rightly protests against the inclusion of mucin under this heading,

[31] See Schmidt, Verh. Deut. path. Gesell., 1904 (7), 2.
[32] Literature, see Hekitoen, Progressive Med., 1899 (ii), 241.
[33] Jour. Exp. Med., 1910 (12), 533.
[34] See discussion, Verh. Deut. path. Gesell., 1908 (12), 265; Münter, Virchow's Arch., 1909 (198), 105.
[35] McConnell and Lang, Jour. Med. Res., 1921 (42), 99.
[36] Berl. klin. Woch., 1914 (51), 598.
[37] Ziegler's Beitr., 1912 (55), 168; also Goodpasture, Jour. Med. Res., 1917 (35), 259.

but includes the corpora amylacea. On account of the discovery by Baumann of the specific chemical nature of thyroid colloid it becomes particularly unfortunate that the term "colloid" has such a wide and uncertain application. It would seem that the safest view to take is that *the word colloid is merely morphologically and macroscopically descriptive* of certain products of cell activity or disintegration, which have nothing in common except the fact that they form a thick, glue-like or gelatinous, often yellowish or brownish substance. *There is no one definite substance colloid*, according to the usual usage of the word in pathological literature, but many different protein substances may assume the appearance to which the name "colloid" is given. Looking at the matter in this way, we must recognize as the usual "colloid" substances, the following chemical bodies:

Thyroid colloid, the physiological prototype of the group. This consists of a compound of globulin with an iodine-containing substance, thyroiodine, the compound protein being called by Oswald iodothyreoglobulin. It occurs pathologically only in cystic and similar changes in the thyroid or accessory thyroids. Being a specific product of the thyroid with definite physiological properties, it manifestly has only a morphological relation to the other forms of colloid found in degenerating tumors, etc. In cysts of the thyroid, and less often in tumors, there is occasionally found more dense "colloid" material of deeper color, the "caoutchouc colloid" of the Germans; this seems to result largely from transformation of red corpuscles in hemorrhagic cysts (Wiget).[38] (The nature of thyroid colloid is discussed more fully under "Diseases of the Thyroid," Chap. XXIV.)

Mucin, when secreted in closed cavities, as in tumors, where it becomes thickened by partial absorption of the water, may take on a "colloid" appearance while retaining its chemical and tinctorial characteristics. This is particularly observed in the "colloid" carcinomas which arise especially from the mucous membrane of the alimentary tract. This substance is, of course, quite specific both in its chemical nature and its origin from specialized epithelial cells, and the process should properly be considered as a "mucoid degeneration."

Pseudomucin, which differs from mucin in not being precipitated by acetic acid, is a common component of ovarian cysts, and when somewhat concentrated by absorption of water, forms "typical colloid." Because it is alkaline, this form of colloid tends to stain rather with the acid dyes (eosin, acid fuchsin, etc.), while true mucin stains with basic dyes. Several varieties of pseudomucin have been described by Pfannenstiel, and their properties will be considered more fully in the section on "Ovarian Tumors" (Chap. XXI). The clear, glassy, yellowish substance contained in small cavities of ovarian tumors, which is usually called "colloid," consists of nearly pure pseudo-mucin. All these substances yield a reducing substance on boiling with acids, which is a nitrogen-containing body, *glucosamine*.[39]

Simple proteins (*e. g.*, serum-globulin, serum-albumin, nucleo-albumin, etc.) may, when in solution in closed cavities, become concentrated through absorption of water until they produce the physical appearance of "colloid." Probably the colloid contents of dilated renal tubules, cavities in various mesoblastic tumors, etc., are produced in this way.

MUCOID DEGENERATION

Mucin, in its typical form, is a compound protein, consisting of a protein radical and a conjugated sulfuric acid which contains a nitrogenous sugar.[40] Hence, when boiled with acids, mucin yields a substance reducing Fehling's solution. Mucin is acid in reaction, probably because

[38] Virchow's Arch., 1906 (185), 416; von Sinner, *ibid.*, 1915 (219), 279.
[39] Zängerle, Münch. med. Woch., 1900 (47), 414.
[40] Review of mucin chemistry by Schmiedeberg, Arch. exp. Path. Pharm., 1920 (87), 1.

of the presence of the sulfuric acid and, therefore, is characterized microchemically by staining with basic dyes. It is readily dissolved in very weak alkaline solutions, is precipitated by acetic acid, and its physical properties when in solution are quite characteristic. The term mucin, however, probably covers a number of related but distinct bodies. Some, such as the *pseudomucins*, are readily distinguished by not being precipitated by acetic acid, and by being alkaline in reaction; others yield reducing substances without previous decomposition with acids (paramucin); while even among the "true" mucins certain differences in solubility exist. The studies of Levene[41] indicate that the non-protein radicals of mucins are of two sorts: One, chondroitin-sulfuric acid, contains the nitrogenous hexose, chondrosamine, and is found in cartilage, tendons, aorta and sclera; the other, mucoitin-sulfuric acid, has as its carbohydrate, chitosamine, and is found in gastric and umbilical cord mucin, vitreous humor, cornea and ovarian cysts.

In the mammalian body we find mucin occurring in two chief localities; (1) as a product of secretion of epithelial cells; (2) in the interstices of connective tissue, especially of tendons.[42] (The resemblance of synovial fluid to mucin is more physical than chemical.) There is also evidence that mucin or a related body constitutes the cement substance between all the body-cells. Corresponding to these two chief sources of mucin we find mucoid degeneration occurring as distinct processes in mucous membranes (or tissues derived therefrom) and in connective tissue.

Epithelial Mucin.—As epithelial mucin represents a distinct product of specialized cells, it is questionable if the ordinary application of the term degeneration in the sense of the conversion of cell-protoplasm into mucin, is correct. Certainly the mucin formation of catarrhal inflammation is merely an excess of a normal secretion, and the degenerative changes that may be present in the epithelial cells are produced by the cause of the inflammation, and are not dependent upon mucin formation. Even in the extreme example of mucoid degeneration seen in carcinomas derived from mucous membranes (the so-called "colloid cancers"), the epithelial degeneration is not necessarily to be interpreted as a conversion of cell-cytoplasm into mucin, but is largely due to the pressure of secreted mucin upon the cells within the confined spaces of the tumor. The mucin in these forms of mucoid degeneration is chemically the same as the normal mucin coming from the same source, but mixed with larger or smaller quantities of other proteins derived from cell degeneration or from vascular exudates, and we do not yet know certainly

[41] Jour. Biol. Chem., 1918 (36), 105. Schmiedeberg[40] says that the non-protein radical of mucin is "hyaloidin."

[42] Schade (Zeit. exp. Path., 1913 (14), 23) says that the long controversy concerning the intercellular substance of mammalian connective tissue is settled by the work of Lier (Ledermarkt-Collegium, Frankfurt, 1909, p. 321), who found it to be a mucin similar to that of tendon or umbilical cord. That there are some chemical similarities in the protein moiety of epithelial and tendon mucin is indicated by their immunological inter-reactions (Elliott, Jour. Infect. Dis., 1914 (15), 501).

the chemical character of the secretion of normal mucous membranes.[43] (The stringy, mucin-like substance seen in some purulent exudates is probably composed largely of nucleoproteins derived from the degenerating leucocytes, and is not true mucin.)

Connective-tissue Mucin.—Excessive formation of connective-tissue mucin is observed most characteristically in myxedema (*q. v.*), but may also occur in connective tissues that are poorly nourished or otherwise slightly injured; it is seen particularly in the connective tissues surrounding the epithelial elements in adenomas and carcinomas. In the walls of large blood vessels there is a mucoid connective tissue, rich in mucin, which may be increased in arterio-sclerosis (Björling).[44] Connective-tissue tumors (myxosarcoma, myxofibroma, or myxoma) may also show a great quantity of mucinous intercellular substance, but many of the so-called myxomas are in reality merely edematous fibromas or polypoid tumors, in which the resemblance to true myxoma is largely structural rather than chemical. This form of mucoid degeneration seems to be merely a reversion to the fetal type of connective tissue, which is characterized, as in the umbilical cord, by an excessive accumulation of a mucin-containing, fluid, intercellular substance, and a paucity of collagenous fibrillar structure. Apparently, when connective tissue reverts to an embryonal type, either from intrinsic causes (tumor formation), or when the nourishment is insufficient, or possibly when the normal stimulus to cell growth is absent (myxedema), the mucoid characteristics of fetal tissue reappear.

The presence of mucin in the tissues seems to cause no reaction, and its absorption causes no harm. Rabbits that I injected with large quantities of pure tendon mucin almost daily for two to four months, showed absolutely no deleterious effects, either locally or constitutionally. Some of the French authors[45] claim that mucin possesses a slight bactericidal power. On the other hand, Rettger[46] and others have found an apparently typical mucin produced by certain varieties of bacteria.

GLYCOGEN IN PATHOLOGICAL PROCESSES[47]

It seems probable that all, or nearly all, cells contain larger or smaller quantities of glycogen, but it may be insufficient in amount to be detected either microscopically or chemically. Glycogen seems to be formed within the cells from the sugar of the blood, through a process of dehydration and polymerization, and to be reconverted whenever necessary into sugar, by a reverse process of hydrolysis. It is quite possible that both of these processes represent merely the reversible action of an intracellular enzyme, but this has not been established. We do know, however, that

[43] See Lopez-Suarez, Biochem. Zeit., 1913 (56), 167.
[44] Virchow's Archiv., 1911 (205), 71.
[45] Arloing, Compt. Rend. Soc. Biol., 1902 (54), 306, and 1901 (53), 1117.
[46] Jour. Med. Research, 1903 (10), 101
[47] Bibliography by Gierke, Ziegler's Beitr., 1905 (37), 502, and Ergebnisse Pathol., 1907, XI (2), 871; Klestadt, *ibid.*, 1911, XV(2), 349.

soon after death the intracellular glycogen is rapidly converted into dextrose.[48]

Properties of Glycogen.—Glycogen is frequently called an "animal starch," having the same general composition as the starches $(C_6H_{10}O_5)x$, and apparently, like the starches, it represents a relatively insoluble resting stage of sugar in the course of metabolism. It is readily soluble in water, forming an opalescent, colloidal solution, and, therefore, has no effect on osmotic pressure, and it is not diffusible.[49] Because of its solubility and the rapidity with which postmortem change to dextrose occurs, specimens that are to be examined microscopically for glycogen must be hardened while very fresh in strong alcohol, in which glycogen is insoluble.[50] One of the most characteristic reactions is the port-wine color given by glycogen when treated with iodine; this reaction may be applied microscopically, solution of the glycogen being avoided by having the iodine dissolved in a solution of gum arabic or in glycerol. Salivary ptyalin rapidly converts glycogen into glucose, and this reaction may also be used microscopically to prove that suspected granules are glycogen. However, failure to find glycogen microchemically does not always mean its absence from a tissue.[51]

Physiological Occurrence

According to Gierke, the normal glycogen of cells resembles fat, in that part of it disappears during starvation, while the rest cannot be removed in this way and probably is something more than a reserve food-stuff. In distribution glycogen somewhat resembles fat, being abundant in the liver[52] and muscles, but Gierke considers that the microscopic evidence of the quantity of glycogen present in the cell agrees better with the results of actual chemical analysis than is the case with fat. Rusk,[53] however, finds only a general agreement, with marked exceptions. Neither iodine nor Best's carmine stain is absolutely specific for glycogen, but Gierke believes that we may safely consider a substance as glycogen when it is homogeneous, rather easily soluble in water and more so in saliva, gives the usual iodine reaction, and stains bright red with Best's carmine solution.[54] With these controls, the microscopic findings were found to agree closely with the results of direct chemical analysis, and glycogen was found microscopically visible in muscle, liver, lung, heart, uterus, and skin (but not in the brain,[55] where it may be demonstrated chemically in minute quantities).

Glycogen is commonly said to be especially abundant in fetal tissues, but it is not present in all fetal cells,[56] nor is it always most abundant in the most rapidly growing tissues, being absent in dividing cells (Sundberg).[56] Although both fat and glycogen are quite abundant in fetal muscle and liver tissues, the liver of early embryos does not contain either.[57] Invertebrates and the lower vertebrates have more than the higher forms. In mammalian adults the liver and muscle contain the most glycogen, cartilage standing next, and it is also present in squamous epithelium (particularly the middle layers), especially that of the vagina (Wiegmann), but not in slightly

[48] Literature concerning physiology of glycogen by Pflüger, Pflüger's Arch., 1903 (96), 398; and Cremer, Ergeb. der Physiol., 1902 (1, Abt. 1), 803.

[49] See Gatin-Gruzewska, Pflüger's Arch., 1904 (103), 282.

[50] According to Helman (Cent. f. inn. Med., 1902 (23), 1017), glycogen may be found in specimens preserved in alcohol as long as fifteen years.

[51] Bleibtreu and Kato, Pflüger's Arch., 1909 (127), 118.

[52] In the livers of two executed criminals Garnier (Comp. Rend. Soc. Biol., 1906 (60), 125) found respectively 4 per cent. and 2.79 per cent. of glycogen.

[53] Univ. of California Publ., Pathol., 1912 (2), 83.

[54] Concerning staining methods see Klestadt, *loc. cit.*[47]

[55] May be present in fetal nervous tissues. (Gage, Jour. Comp. Neurol., 1917 (27), 451.)

[56] See Glinke, Biol. Zeit., Moskau, 1911 (2), 1; Sundberg, Zeit. Anat. Entwichl., 1924 (73), 168.

[57] Adamoff (Zeit. f. Biol., 1905 (46), 288) contests the idea that the amount of glycogen is in direct relation to growth energy; see also Mendel and Leavenworth (Amer. Jour. Physiol., 1907 (20), 117), who found no particular abundance in the tissues of the fetal pig.

stratified (cornea), transitional, or cylindrical epithelium. Normal human kidneys do not seem to show glycogen, but it may be present in the kidneys of mice, rabbits, and cats. There is considerable in the heart muscle.[58] The amount in different skeletal muscles varies,[59] usually being especially abundant in the diaphragm. Glycogen is most abundant in the uterus at the time of child-birth, and is abundant in the placenta;[60] but it is also present in the uterus and tubes independent of pregnancy,[61] increasing in the epithelium in the premenstrual involution.[62]

After pancreas extirpation, Fichera[63] observed a disappearance of all visible glycogen, except a little in the cartilage and stratified epithelium; hence he considers the glycogen-content as a function of cell nourishment. Fat and glycogen often occur together, although one may be present without the other (Gierke). Presumably the failure to find glycogen in certain cells depends rather on a failure of technic than on a total absence of glycogen.

There has been some diversity of opinion as to whether glycogen occurs as granules in the living cell, or whether the granules are formed from a homogeneous substance by hardening fluids. In view of the clear-cut, definite spaces it may leave in cells when dissolved out, glycogen probably occurs as granules, especially when present in abnormally large quantities. Ervin[64] believes that glycogen, like fat, may exist within the cells so finely divided that it cannot be stained by glycogen stains. The studies of Arnold have shown that in many cells the glycogen takes on a definite structure in close relation to the plasmosomes. It has been suggested that the intra-epithelial hyaline bodies (Russell's fuchsin bodies) are glycogen, which idea is probably not correct. Habershon and others have suggested that eosinophile granules are either glycogen or related to it. The presence of glycogen in the cells seems to cause no injury to the cytoplasm, and if it again disappears, the cells become quite normal. Even the nuclei may contain granules of glycogen without evident permanent injury.

Pathological Occurrence

According to the results obtained by Fichera and Gierke, it seems probable that glycogen accumulation is produced under the same conditions as are fatty changes, *i. e.*, when oxidation is locally or generally impaired. Fat and glycogen are, therefore, often found together in the margins of infarcts and of tubercles, in passive congestion of the liver, and in heart muscle with fatty changes due to severe anemia. The glycogen, being more labile, seems to disappear early when the cells become necrotic, and hence glycogen is not present in older necrotic areas where the fat still persists. (This probably accounts for the frequently repeated statement that glycogen and fat do not occur together.) Ervin[64] believes that glycogen is important in holding intracellular fats emulsionized, and hence in its absence in diabetes the fats become visible as fatty degeneration—hence the inverse ratio of glycogen and fat. Whether the glycogen can be transformed into fat, perhaps forming an intermediary stage in a transformation of protein into fat, has not been determined, but there seems to be little doubt that it is infiltrated from outside the cell, and not formed directly from degenerated protein. It seems to be deposited only in cells that are still living, although it can become split up in dead cells. All cells, but especially muscle-cells and leucocytes, seem able to

[58] Berblinger, Ziegler's Beitr., 1912 (53), 155.
[59] Lipska-Mlodowski, Beitr. path. Anat., 1917 (64), 18.
[60] Decreasing in amount as pregnancy progresses, while the glycogen content of the fetal liver increases progressively (Clogne *et al.*, Gynec. et Obstét., 1924 (10), 23).
[61] McAllister, Jour. Obs. Gyn. Brit. Emp., 1913 (34), 91.
[62] Szüle, Magyar Orvosi Arch., 1924 (25), 448.
[63] Ziegler's Beitr., 1904 (36), 273, literature.
[64] Jour. Lab. Clin. Med., 1919 (5), 146.

lay up glycogen in visible amounts under certain conditions. In inflamed areas glycogen is found in both tissue-cells and leucocytes, but not in cells showing nuclear degeneration (Best, Gierke). In pneumonia the leucocytes of the exudate, and to a less extent the alveolar epithelium, contain glycogen as well as fat. In tubercles glycogen is found in the cells which contain bacilli, and it is generally present in the epitheloid cells, rarely in giant cells, not at all in lymphoid cells or in the necrotic elements (Devaux). Liver glycogen is altered most in poisoning, being reduced by phosphorus, arsenic, chloroform, $HgCl_2$, and many other poisons; the amount is reduced when death from any cause is slow, or when putrefaction has occurred, but it is increased in carbon monoxide poisoning (Massari).[65] In rabbits, at least, it is deposited in the liver first about the central vein, and in fasting animals it disappears first from the periphery.[66] It seems to have a marked protective effect in phosphorus poisoning.[67]

Glycogen in Tumors.—Glycogen has been observed frequently in tumors. Brault believed the quantity an index of rate of growth, on the principle that glycogen appears most abundantly in embryonal tissues, and therefore in tumors the amount of glycogen should agree with the degree to which the cells have gone back to the embryonic type. Lubarsch considered that only tissues normally containing glycogen give rise to glycogen-containing tumors. Gierke[47] could corroborate neither of these ideas, and considers that glycogen appears in tumors under exactly the same conditions in which it appears in other tissues; *i. e.*, when cell nutrition and oxidation are impaired. Apparently, however, *both the embryonic origin and local retrogressive changes determine the deposition of glycogen in tumors.* Glycogen is particularly abundant in squamous epithelium of epitheliomas that have gone on to hornification,[68] in testicular tumors, hypernephromas, parathyroid tumors (Langhans),[69] endotheliomas, chondromas, and myomas, and it also occurs in the connective tissues surrounding tumors. Of 1,544 tumors of all sorts examined by Lubarsch,[70] 447 (or 29 per cent.) contained glycogen visible microscopically; fibromas, osteomas, gliomas, and hemangiomas were always free from glycogen; and lipomas and lymphangiomas nearly always. Adenomas are almost equally free from glycogen (two positive in 260 specimens), while it was constant in teratomas, rhabdomyomas, hypernephromas, and chorioepitheliomas. Of the sarcomas 50.7 per cent., and 43.6 per cent. of the carcinomas, showed glycogen, most abundant in squamous-cell epitheliomas; columnar-cell carcinomas contain glycogen much less often, and it is always absent in "colloid cancers."

[65] Gaz. degli Ospedali, 1906 (27), 537.
[66] Ishimori, Biochem. Zeit., 1913 (48), 332.
[67] See Simonds, Arch. Int. Med., 1919 (23), 362.
[68] In mouse tumors Haaland found glycogen only in squamous cell carcinomas, and in the connective tissue surrounding other tumors (Jour. Path. and Bact., 1908 (12), 439).
[69] Virchow's Arch., 1907 (189), 138.
[70] Virchow's Arch., 1906 (183), 188.

Animal parasites, in common with other invertebrates, usually show abundant quantities of glycogen.[71] It has been found in protozoa, as well as in all varieties of intestinal worms. According to Barfurth, nematodes in glycogen-free animals may contain glycogen. The glycogen is found chiefly in the connective tissues of the intestinal parasites, but in some of the nematodes it occurs chiefly in the sexual organs and muscle-cells. The walls of the hydatid cysts contain much glycogen, which is, perhaps, related to the usual presence of sugar in their contents. If Habershon's contention is correct, that eosinophile granules are related to glycogen, we may have here an explanation of the occurrence of eosinophilia in infection with animal parasites. (See also "Animal Parasites." Chap. V.)

Glycogen in Leucocytes.—The occurrence of glycogen in the blood has aroused much interest, particularly in relation to its diagnostic value. Many leucocytes contain granules that stain with iodine and although it is possible that these are not all granules of glycogen, yet, for the most part, they probably represent this substance in excessive quantities. The granules are observed chiefly in the polymorphonuclear neutrophiles, but seldom in large and small mononuclear cells and eosinophiles.[72] Occasional granules are also found free (or perhaps contained in blood-platelets) in all blood, whether normal or pathological.[73] Hirschberg[74] states that normal animals of all species have leucocytes giving an iodine reaction for glycogen if proper technic is used, but which is not obtained by the ordinary iodine-gum solution method unless the glycogen is rendered abnormally insoluble by toxic injury; this is an explanation for the relationship of iodophilia and infections. According to Wolff-Eisner the leucocytes in myeloid leukemia contain no glycogen granules. It does not seem to be settled whether the glycogen is taken on by the leucocytes at the place of pathological lesion, or in the bone-marrow under the influence of circulating poisons, or both. Habershon states that from 1 to 16 per cent. of all leucocytes normally contain glycogen granules, and Wolff believes that the glycogen seen in leucocytes represents normal glycogen made insoluble through injury. This may explain why the leucocytes in an infected area may give iodine reactions when the leucocytes in the circulating blood do not.

Locke gives the occurrence of this abnormal iodine staining of the circulating leucocytes, (termed *iodophilia*), as follows: "Septic conditions of all kinds, including septicemia, abscesses, and local sepsis (except in the earliest stages), appendicitis accompanied by abscess formation or peritonitis, general peritonitis, empyema, pneumonia, pyonephrosis, salpingitis with severe inflammation or abscess formation, tonsillitis,

[71] Elaborate treatise on occurrence of glycogen in lower animals by Barfurth, Arch. mikros. Anat., 1885 (25), 269; also Busch, Arch. internat. physiol., 1905 (3), 49; Brault and Loeper, Jour. Phys. et Path. Gen., 1904 (6), 295 and 720.

[72] See Bond, Brit. Med. Jour., Feb. 3, 1917.

[73] Literature—Locke and Cabot, Jour. Med. Research, 1902 (7), 25; Reich, Beitr. klin. Chir., 1904 (42), 277; Küttner, Arch. klin. Chir., 1904 (73), 438; Habershon, Jour. Path. and Bact., 1906 (11), 95; Wolff, Zeit. klin. Med., 1904 (51), 407.

[74] Virchow's Arch., 1908 (194), 367.

gonorrheal arthritis, and hernia or acute intestinal obstruction where the bowel has become gangrenous, have invariably given a positive iodophilia, and by its absence all these cases can be ruled out in diagnosis. In other words, no septic condition of any severity can be present without a positive reaction. Furthermore, the disappearance of the glycogen granules in the leucocytes in from twenty-four to forty-eight hours following crisis with frank resolution in pneumonia, and the thorough drainage of pus in septic cases, is of considerable importance." Clinical experience, however, seems not to have accorded any constant significance to iodophilia.[75]

In **exudates,** glycogen is found in the leucocytes as long as they retain their vitality, but disappears soon after retrogressive changes begin; hence it is not usually present in old sterile pus. Loeper[76] made quantitative estimates of the glycogen in exudates, finding from 0.59–0.62 gram per liter in cellular pneumococcus pleural effusion, 0.25 gm. in cellular tuberculous effusion, but only traces in serous tuberculous effusion and in an old tuberculous pyothorax. A pneumonic lung contained 0.85 gm. of glycogen per kilo, and traces were found in pneumonic sputum and in the contents of tuberculous cavities. It is very abundant in tuberculous sputum, as much as 2 to 3 per cent. in advanced stages, but absent in bronchial catarrh; in pneumonia 0.05 per cent. was found, in putrid bronchitis 0.25 per cent. (Pozzilli). When glycogen solution (1 per cent.) is injected into the peritoneal cavity, the endothelial cells and invading leucocytes become loaded with glycogen granules.

Glycogenic Infiltration in Diabetes.—Although in diabetes the chief normal storehouses of glycogen, the liver and muscles, are either poor in or free from glycogen, yet in other tissues in diabetes large accumulations of glycogen are found, the granules frequently fusing in the cells into droplets larger than the nucleus. When dissolved out in ordinary microscopic preparations, the clear round space left is exactly like the space left by a fat-droplet, except that the margins show a tendency to take the basic stain for some unknown reason. In even the most extreme cases, however, the nucleus is well preserved, although it, too, may contain large masses of glycogen, in which case there is no glycogen in the cytoplasm.[77] Glycogen is found particularly in the epithelium of Henle's tubules,[78] in heart muscle, and in the leucocytes. Fütterer describes masses of glycogen in the cerebral capillaries, resembling an embolic process; it is also present in the tissues of the eye.[79] Experimental diabetes (pancreas extirpation, piqûre) produces a marked glycogenic infiltration.[80] We cannot yet change van Noorden's statement: "We lack the biological explanation as to why certain cells retain the capacity to store glycogen and even exert it more actively than before, whilst the proper organs for the storage of glycogen have lost it."

[75] See Bernicot, Jour. Path. and Bact., 1906 (11), 304.
[76] Arch. Méd. Exp., 1902 (14), 576.
[77] Askanazy and Hübschmann, Cent. f. Path., 1907 (18), 641.
[78] See Fahr, Cent. f. Path., 1911 (22), 945.
[79] Shimagawora, Klin. Monatsbl. Augenheilk., 1911 (12), 682.
[80] Huber and MacLeod, Amer. Jour. Physiol., 1917 (42), 619.

CHAPTER XIX

CALCIFICATION, CONCRETIONS, AND INCRUSTATIONS

CALCIFICATION[1]

PATHOLOGICAL calcification occurs in two forms: one is a precipitation of calcium in secretions and excretions of the body; the other is the deposition of calcium salts in the tissues themselves. The former, which includes not only concretions in general, but probably also the deposition of calcium salts in the cells and tubules of the kidney,[2] both in disease and in experimental calcification after certain poisonings, is readily enough explained in most instances by recognizable alterations in the composition of the secretions, which lead to simple chemical precipitations. With this form we shall deal in the subsequent consideration of concretions, but, in referring to calcification shall indicate only depositions from the blood directly into the tissues.

The Calcium of the Blood.[3]—Normally the calcium content of the blood is quite constant, about 9–11 mg. per 100 cc. serum, and the quantity is not modified by most diseases.[4] It is, however, reduced except in nephritis, eclampsia, tetany, jaundice, osteomalacia, diarrhœa, and in acute rickets, especially when associated with tetany. (See discussion elsewhere of these conditions.) Very high blood calcium figures have been observed in terminal acidosis.[5] Feeding of calcium will not appreciably raise the blood calcium unless it is subnormal, and even injected calcium is removed very quickly, for the blood is, normally, near its saturation point with calcium.[6] In the late stages of pregnancy the blood calcium is decreased.[7] About 60–70 per cent. of the serum calcium is in a diffusible condition,[8] the balance being bound to the colloids. According to Tisdall and Harris[9] the phosphate content is from 5 to 6 mg. per 100 cc. in children, but when ossification stops at about 20 years the figure drops sharply to 4 mg. or a little less. During repair of fractures in adults there is a return of the P figure to that of childhood.

[1] Literature and résumé: Pfaundler, Jahrb. f. Kinderheilk., 1904 (60), 123; Wells, Jour. Med. Research, 1906 (14), 491, and Arch. Int. Med., 1911 (7), 721; Hofmeister, Ergebnisse Physiol., 1910 (9), 429; Schultze, Ergebnisse Pathol., 1910, XIV (2), 706.

[2] See Wells, Holmes and Henry, Jour. Med. Research, 1911 (25), 373.

[3] A review on absorption and deposition of calcium is given by Maver and Wells (Amer. Rev. Tuberc., 1923 (7), 1). A review of German literature on blood calcium is given by Billigheimer, Klin. Woch., 1923 (2), 1033.

[4] See Halverson, Mohler and Bergeim, Jour. Biol. Chem., 1917 (32), 171; Jansen, Deut. Arch. klin. Med., 1924 (144), 14; Denis and Talbot, Amer. Jour. Dis. Chil., 1921 (21), 29; Koechig, Jour. Lab. Clin. Med., 1924 (9), 679.

[5] Genck and Blühdorn, Jahrb. f. Kinderheilk., 1923 (102), 83.

[6] See Sieburg and Kessler, Arch. exp. Path. Pharm., 1923 (96), 180.

[7] See Plass and Bogert, Amer. Jour. Obst. Gyn., 1923 (6), 427; Widdows, Biochem. Jour., 1923 (17), 34.

[8] von Meysenburg, Jour. Biol. Chem., 1921 (47), 529; see also R. F. Loeb, Jour. Gen. Physiol., 1924 (6), 453.

[9] Jour. Amer. Med. Assoc., 1922 (79), 884.

Composition of the Deposits in Calcification.[10]—The composition of the inorganic salts in calcified areas in the body seems to be practically the same, if not identical, whether the salts are laid down under normal conditions (ossification) or under pathological conditions. Study of several specimens of calcified tuberculous lesions by Maver and Wells[11] showed that the silica found in calcified pulmonary and lymph node lesions is not large in amount, from 0.3 to 3.3 per cent. of the total inorganic material, and is to be explained as inhaled dust. With the blood continually passing between the bones and the calcified areas, the composition of the two must inevitably become similar or identical. This may be shown by a table giving the proportion of inorganic salts found by analysis of normal bone, and the proportion found in calcified materials.[12]

	$Mg_3(PO_4)_2$	$CaCO_3$	$Ca_3(PO_4)_2$
Pathological Calcification			
Bovine tuberculosis	0.84	12.8	85.9
Bovine tuberculosis	0.9	12.1	85.4
Bovine tuberculosis	1.2	11.7	86.4
Bovine tuberculosis (softened gland)	1.5	7.6	90.6
Human tuberculosis	1.2	10.2	87.8
Calcified nodule in thyroid	0.85	13.4	85.4
Thrombus, human	1.1	11.9	86.5
Normal Ossification			
Human bone (Zalesky)	1.04	±12.8	83.8
Human bone (Carnot)	1.57	10.1	87.4
Human bone (Carnot)	1.75	9.2	87.8
Ox bone (Zalesky)	1.02	.....	86.1
Ox bone (Carnot)	1.53	11.9	85.7

Gassmann[13] attempts to explain the constancy of the proportion of calcium salts in bone by assuming that they exist in a definite compound $\left[Ca\begin{pmatrix}OPO_3Ca\\ >Ca\\ OPO_3Ca\end{pmatrix}_3\right]CO_3$. This being a closed ring compound it should possess great stability. Bessett[14] found that under the conditions

[10] MacCordick (Lancet, Oct. 18, 1913) has advanced the interesting hypothesis that calcific deposits during life exist mostly as soft masses, like unset mortar. Only when sufficient accumulation of CO_2 occurs, as after death, or in the center of large areas of low vitality, such as fibroids, do the deposits become hardened; *e. g.*, in a gangrenous leg the calcified vessels are stiff and brittle, while higher up in the living tissues they are soft and pliable. This would explain why we do not more often observe fractures of calcified arteries. As yet this hypothesis has not received the critical tests its importance deserves. If true it will explain the cases of extensive calcification of the pericardium in which the heart is so encased that function would seem impossible if the deposit were rigid during life. (See Trans. Chicago, Pathol. Society, 1911 (8), 109, for consideration of pericardial calcification.) However, Klotz (Jour. Med. Res., 1916 (34), 495) has questioned the correctness of MacCordick's views on the basis of the occasional occurrence of fractures of calcified arteries, but without experimental evidence contradicting MacCordick.

[11] Amer. Rev. Tuberc., 1922 (6), 649.

[12] Wells, *loc. cit.*;[1] see also Maver and Wells, Amer. Review Tuberc., 1922 (6), 649.

[13] Zeit. physiol. Chem., 1910 (70), 161.

[14] Jour. Chem. Soc., 1917 (111), 620.

existing in blood serum, calcium and phosphate are in equilibrium, with the solid phase consisting of hydroxyapatite $(Ca_3P_2O_8)_3 - Ca(OH)_2$, and suggests that this is the form in which calcium exists in bone, together with some calcium carbonate, the magnesium and other inorganic elements present being merely adsorbed. This assumption, however, does not agree with analyses in which the P_2O_5 and CO_2 are found to balance the Ca and Mg present, for the above formula would yield an excess of Ca. Furthermore, careful analyses of bone in various conditions do yield figures indicating the existence of a definite variability about a certain mean, which fact seems best explained by assuming that the proportions of the inorganic salts vary according to conditions of saturation, which fluctuate slightly with slight variations in the composition of the blood and the tissues.[15]

Iron may be present in pathological calcification, and, according to Gierke,[16] in the fetus the entire skeleton contains iron as far as it has calcified, most at the points of active ossification. This statement has been questioned by Hück and others, who believe that most of the iron demonstrable in normal ossification is the result of an artifact, for calcium deposits seem to have a great affinity for iron. Because of this, pathological calcium deposits take up iron from old hemorrhages in the vicinity, hence in many areas where there have been hemorrhages, especially in the vicinity of elastic tissue, there occur actual "calcium-iron" incrustations.[17] S. Ehrlich[18] states that elastic fibers in the vicinity of hemorrhages take up the iron-containing derivative of the blood-pigment, and this acts as a mordant for subsequent calcium deposition. Analysis of similar deposits in a syphilitic spleen by Gettler[19] showed the presence of large amounts of silicates as well as calcium and iron, with much less potassium than in normal spleen tissue. The presence of iron in normal ossification is supported by Sumita[20] and Eliasscheff.[21] In the so-called iron-lime lung Gigon[17] found but a trace of calcium and much sodium and potassium. On the other hand, wherever iron is deposited, especially if in elastic and stroma elements, calcium is often found associated abundantly with it,[22] indicating the probability that both are adsorbed by the tissue matrix because of physical conditions rather than by chemical fixation.

Structure of Calcified Areas.—In calcification there is not the same uniform infiltration of the ground substance with lime salts that occurs in bone, yet the calcified area is possessed of a ground substance of organic material which does not dissolve in weak acids that remove the salts. There is, however, no definite ratio between the lime salts and this albuminoid matrix. At first the salts occur in granules, which may

[15] See Loll, Biochem. Zeit., 1923 (135), 493.
[16] Virchow's Arch., 1902 (167), 318.
[17] See Gigon, Ziegler's Beitr., 1912 (55), 46; Sprunt, Jour. Exp. Med., 1911 (14), 59; Klotz, Johns Hopkins Hosp. Bull., 1916 (27), 363.
[18] Cent. f. Pathol., 1906 (17), 177.
[19] Symmers, Gettler, Johnson, Surg. Gyn. Obst., 1919, (28), 58.
[20] Virchow's Arch., 1910 (200), 220.
[21] Ziegler's Beitr., 1911 (50), 143.
[22] See Schupisser, Virchow's Arch. 1922 (239), 320.

become fused to a greater or less degree. It has been thought by some that the deposition occurs in the form of "*calcospherites.*"

These are small calcareous bodies, usually of concentric structure, which were first described by Rainey (1857). They appear to occur widely distributed in normal tissues, both animal and plant, and seem to be the result of the formation of insoluble calcium salts in the presence of colloidal substances, just as urinary and other concretions are formed about an organic nucleus. If calcium chloride and soluble carbonates are allowed to combine very slowly to form calcium carbonate in a solution of egg-albumen, these or indistinguishable bodies are formed, which on being dissolved are found to possess an organic stroma that exhibits a marked affinity for any pigmentary substance that may be present. Apparently, when the proper concentration exists, the salts in crystallizing hold between the crystals the albuminous substances by which they are surrounded. Dastre and Morat believe that the substratum is lecithin which others have found occupying a similar place in prostatic concretions. Watt[23] found experimentally that the presence of lecithin particularly favors the production of spherules, and that these artificial spherites may undergo a spontaneous rearrangement with crystallization. Calcospherites have been found in tumors, in cystic cavities, and in bodies with beginning decomposition. It may be mentioned in passing that Littlejohn[24] observed the abundant formation of calcium phosphate crystals in bodies that had been immersed for some time in sea water. Oliver has found calcospherites in the tissues of a cancer of the breast. Pettit[25] found calcospherites in a sarcoma of the maxilla, presenting insensible transitions into the substance of the osseous tissue, and he suggests the possibility that the calcospherite formation may be related to the formation of bone. It seems, however, that they are probably more closely related to the formation of the shells of invertebrates, which are largely composed of carbonates in crystalline structure with an organic substance between them, and very little phosphate indeed.

Relation of Calcification to Ossification.[26]—In normal ossification we have to deal with the accumulation of lime salts within the stroma or cells of a tissue that has usually undergone certain preparatory changes in the way of formation of a more or less homogeneous ground substance, but has not suffered a total loss of vitality, although vitality is possibly decreased. Pathological calcification is similar, in so far as we have to deal with deposition of quite the same salts in tissues that have suffered either total or partial loss of vitality, and which very frequently indeed are hyaline. What appear to be essential differences are these: (1) In calcification the lime salts always remain in clumps and masses, often fusing to greater or less degree, but never with the diffuse even permeation of tissue seen in ossification. (2) All the cells within a calcified area, if not dead at the beginning of the process, eventually disappear for the most part, and we have, sooner or later, a perfectly inert mass, practically a foreign body, instead of a specialized tissue as in ossification. (3) Ossification is accomplished only in varieties of connective tissue, but calcification may involve any sort of cell or tissue provided it is degenerated sufficiently. Furthermore, any area of calcification is likely to be replaced by bone, no matter what tissue may be involved; apparently the presence of calcium salt deposits in any part of the body can stimulate the connective tissues to form bone,[27] but in the absence of calcium salts even the cells which are normally osteogenic will not form bone.

Occurrence of Pathological Calcification

As far as we know, calcification seldom occurs in normal tissue, except in the formation of bone. Often the infiltrated tissue is completely dead, as in infarcts, organic foreign bodies, caseous areas, and particularly in old inspissated collections of pus. It may be said that any area of dead tissue that is not infected, and that is so large or so situated that it cannot be absorbed, will probably become infiltrated with lime salts. Most

[23] Biol. Bull., 1923 (44), 280.

[24] Edinburgh Med. Jour., 1903 (13), 127.

[25] Arch. d. Anat. Micros., 1897 (1), 107.

[26] The proportions of organic material in bone at different ages is given by Radasch, Anat. Record, 1921 (21), 153.

[27] See Nicholson (Jour. Path. Bact., 1917 (21), 287) concerning heterologous ossification; also Asami and Dock, Jour. Exp. Med., 1920 (32), 745.

frequently calcified, next to totally necrotic tissues, are masses of scar-tissue that have become hyaline subsequent to the shutting off of circulation in the scar by contraction of the tissue about the vessels. Elastic tissue also seems prone to an early calcification, and it is not uncommon to see the elastic laminæ of small arteries calcified in an apparently selective manner. A peculiar form of calcification is that frequently found in ganglion-cells of the brain which have become degenerated or necrotic, particularly in the vicinity of old hemorrhages; the cells become infiltrated with lime salts until a complete cast of the cell, with dendrites and axis-cylinder well impregnated, is formed. The calcification of renal epithelium obtained experimentally by temporary ligation of the renal vessels or by the administration of certain poisons, is more closely related to the formation of ordinary urinary concretions than to tissue calcification, the calcium being present as the phosphate only.[28] Calcification of epithelial cells does occur, however, and seems to be preceded by hyaline changes, in which hyaline substance the calcium is later deposited, as in epithelial pearls, for example.

Metastatic Calcification.—What is perhaps the only exception to the rule that some form of tissue degeneration is required before calcification occurs, is the "*metastatic calcification*" of Virchow.[29] In conditions with much destruction of bone, as osteomalacia, caries, osteosarcoma, multiple myeloma,[30] etc., deposits of lime salts have been found distributed diffusely in various organs, particularly in the lungs and stomach. As much as 13.38 per cent. of the dry weight of the lung and 12.15 per cent. of the kidney have been found as CaO in such a case.[31] As there is no evidence that these organs have been the site of any diffuse tissue necrobiosis before the calcification occurred, it seems probable that the deposits have been made in practically or quite normal organs, because of oversaturation of the tissue fluids by calcium salts. The fact that the lung and stomach, and also to a less degree the kidney, are picked out, suggests that the calcification is related to the fact that in these same organs we have the excretion of acids into their cavities, which leaves the fluids in the substance of the organs correspondingly alkaline,[32] and an increase in the alkalinity of the fluids makes the calcium salts decidedly less soluble. In the stomach the calcium deposits are limited to the interglandular tissue about the upper portion of the glands of the fundus, exactly corresponding to the parietal cells which are supposed to secrete the acid. Presumably, under normal conditions, the amount of calcium in the blood is too slight to be thrown down in this way, but

[28] Wells, Holmes and Henry.[2] M. B. Schmidt (Cent. allg. Path., 1920 (30), 497) finds that injury to the colon epithelium by mercury and other poisons, whereby its capacity to excrete calcium is reduced, is an important factor in renal calcification.

[29] Virchow's Arch., 1855 (8), 103; review by Kockel, Deut. Arch. klin. Med., 1899 (64), 332. Bibliography and review by Wells, Arch. Int. Med., 1915 (15), 574.

[30] See Meyer and Cajori, Arch. Int. Med., 1924 (33), 581.

[31] Virchow's Arch., 1909 (197), 112.

[32] Stieglitz (Arch. Int. Med., 1924 (33), 483) has shown that when the kidney is excreting acid urine the secretory epithelial cells are alkaline and when the urine is alkaline these cells are acid.

when oversaturated because of calcium absorption from the skeleton,[33] precipitation occurs in the parts of the body where the alkalinity of the blood or tissue fluids is greatest, or the CO_2 concentration least. There also occurs a true metastatic calcification in the large arteries, pulmonary veins, and beneath the endocardium of the left side of the heart; that is, always in the places where the blood contains the least CO_2. This fact supports the hypothesis that the CO_2 is an important factor in the solution of calcium salts in the blood, and that when there is an oversaturation with calcium it is deposited where the CO_2 is least abundant. When the amount of calcium in the blood is increased by injecting or feeding calcium salts, depositions of calcium salts may take place in injured tissues,[34] or even in normal tissues, as in Tanaka's experiments.[35]

Extensive calcification sometimes occurs in the lungs without any evident bone disintegration, nor yet nephritis which has been thought at times to lead to enough calcium retention to account for metastatic calcification (Harbitz).[36] Rabl[37] suggests, and supports with experimental evidence, the theory that the metastatic calcification sometimes seen without destructive bone lesions, as in the so-called calcium gout,[41] may depend on renal lesions with decreased elimination of phosphoric acid. The retained H_3PO_4 is buffered by calcium withdrawn from the bones, so that the blood comes to contain an abnormally high calcium content. Whenever the *p*H of the blood is raised, as by taking of food containing an abundance of bases, the excess calcium is deposited in the tissues.

The condition described as "marble bones" by Albers-Schönberg,[38] is apparently closely related to metastatic calcification. In this disease in addition to excessive calcification of the bones with increased fragility, extensive calcification has been found in the lungs, arteries, and tendons. The etiology is unknown, cases having occurred without evident renal lesions, and even in infants.

In these cases of metastatic calcification of unknown etiology it is necessary to take into consideration the parathyroids, since Collip[39] has found that hypercalcemia may result from administration of parathyroid hormone, and Hubbard and Wentworth[40] have observed metastatic calcification and hypercalcemia in a man with parathyroid hyperplasia.

Some have attempted to include the calcification of the vessels and other tissues in old age in the metastatic calcifications, ascribing the

[33] Hellwig (Zeit. Biol., 1921 (73), 281) calls attention to the fact that NaCl has a solvent effect on calcium phosphate, which is prevented by the presence of an excess of calcium, and suggests that the actual absorption of bone which takes place when the blood calcium is low is accomplished by the NaCl.

[34] See Thayer and Hazen, Jour. Exp. Med., 1907 (9), 1.

[35] Biochem. Zeit., 1911 (35), 113; (38), 285; see also Katase, Beitr. path. Anat., 1914 (57), 516.

[36] Norsk Mag. Laeg., 1917 (78), 1129.

[37] Klin. Woch., 1923 (2), 202; Virchow's Arch., 1923 (245), 542.

[38] Full review by Schulze, Arch. klin. Chir., 1921 (118), 410; see also Alexander, Amer. Jour. Roentgenology, 1923 (10), 280.

[39] Jour. Biol. Chem., 1925 (63), 439.

[40] Proc. Soc. Exp. Biol. Med., 1921 (18), 307.

origin of the salts to the senile absorption of bone, but senile calcification is probably dependent rather upon the extensive hyaline degeneration of the connective tissues that occurs in the senile scleroses,[41] a change which seems to be physical rather than chemical.[42]

Chemistry of the Process of Calcification

In analyzing the etiological factors in the production of pathological calcification for the purpose of determining the chemical changes that occur in the process, we have the following facts upon which to base the consideration:

(1) The calcium salts must come from the blood, where they are held in solution or in suspension by the proteins, either as the carbonate and phosphate themselves, or as calcium-ion-protein compounds, or perhaps both. This suspension or solution is an unstable condition, possible only because of the extremely small proportion of calcium in the plasma (about 1:10,000), and, therefore, capable of being overthrown by increased alkalinity of the blood, changes in the proteins or CO_2 content, or changes in the quantity or composition of the calcium salts. It is suggested by the work of Barillé, that the calcium of the blood exists as a soluble complex double salt, tribasic calcium-carbon-phosphate ($P_2O_8Ca_2H_2$: $2CO_2$, $(CO_3H)_2Ca$), this compound being possible because of an excess of CO_2.

(2) Retrogressive changes in the tissues are a *sine qua non* except in metastatic calcification. Hyaline degeneration, the chemical nature of which is not understood, is a very favorable condition, as also is necrosis when absorption is deficient.

(3) In the areas that are to become calcified the circulation is very feeble, the blood plasma seeping through the tissue as through any dead foreign substance of similar structure, without the presence of red corpuscles to permit of oxidative changes.

We may, therefore, imagine that the deposition of calcium salts in such areas of tissue degeneration depends upon one or more of the following conditions:

(1) Increased alkalinity or decreased CO_2 in the degenerating tissues, causing precipitation of the inorganic salts in the fluids seeping slowly through them.

(2) Utilization of the protein of the fluids by the starved tissues so completely, because of its slow passage through them, that the calcium cannot be held longer in solution.

[41] Under the name of "calcium gout," M. B. Schmidt has described a case with generalized deposition of calcium in other tissues than those usually affected in metastatic calcification (Deut. med. Woch., 1913 (39), 59). A few cases of extensive subcutaneous calcification of unknown etiology have been described, but their relation to metastatic calcification is doubtful, as they seem to be localized deposits. (See Mosbacher, Deut. Arch. klin. Med., 1918 (128), 107; Ducasse, Arch. Derm. Syph., 1923 (7), 373.)

[42] See analyses of elastin from calcified and normal aortas by Ameseder, Zeit. physiol. Chem., 1913 (85), 324.

(3) The formation within the degenerated area of a substance or substances having a special affinity for calcium.

(4) Production of a physical condition favoring the local absorption of salts, the least soluble salts accumulating in excess.

The first of these conditions seems to come into play especially in metastatic calcification, already discussed. We have no evidence that in degenerating tissues, much less in normal ossification, an alkaline reaction is developed; on the contrary, an acid reaction is more usual. But, as explained below, decrease in the CO_2 content in calcifying tissues, especially when combined with other changes, may be of importance.

Lichtwitz[43] especially has laid emphasis on the possible part played by changes in the proteins in inducing calcification. He advances the idea that precipitation of the colloids in the degenerated area, as in caseation, decreases the amount of crystalloids that can be held in solution, wherefore the least soluble salts, those of calcium, are precipitated; by laws of osmotic pressure more calcium in solution will then enter to establish equilibrium, be precipitated, and make way for more calcium, until the amount of deposit prevents further osmotic diffusion. Although suggestive in regard to pathological calcification, and probably of importance in the formation of concretions, this conception is difficult to apply to normal ossification; also in pathological calcification one would expect precipitation of calcium to occur in the outermost surface of the degenerated area, soon leading to a shell of inorganic material which would limit the deposition.

The possibility of the formation of calcium-binding substances within the degenerated area has always seemed the most attractive, and has received the most attention by investigators. Of the special substances that might be present in such areas that would have a high affinity for calcium, *phosphoric acid* usually receives first consideration, since it is as phosphate that most of the calcium is bound, and also since the possible sources of phosphoric acid in decomposed nucleoproteins and lecithin are so obvious. *Fatty acids* offer another possibility, especially in view of the fatty degeneration that so frequently precedes calcification. Proteins might also be formed with a particularly high affinity for calcium.

Formation of Calcium Soaps.—In favor of the possibility that the calcium is first bound as soaps are the following facts: Calcification occurs chiefly in places where fatty degeneration has occurred, such as tubercles, atheromatous vessels, etc. In fat necrosis, fatty acids are formed, which soon combine with calcium to form calcium soaps. Virchow observed calcification in the form of soaps in a lipoma, and Jaeckle[44] found that a calcifying lipoma contained 29.5 per cent. of its calcium in the form of calcium soaps. Klotz[45] obtained staining reactions in calcifying tissues that suggested the presence of soaps, which he also extracted by solvents, and he strongly urges, as the first step in the for-

[43] Deut. med. Woch., 1910 (36), 704.
[44] Zeit. physiol. Chem., 1902 (36), 53.
[45] Jour. Exper. Med., 1905 (7), 663; 1906 (8), 322.

mation of pathological calcified masses, that the calcium is first laid down as soaps, afterward undergoing a transformation into the less soluble phosphate and carbonate. Fischler and Gross[46] also obtained microchemical reactions for soaps in the margins of infarcts and in atheromatous areas, but not in caseous areas; they therefore consider that calcium-soap formation is an important step in the process of pathological calcification, but that it is not essential. The value and the interpretation of the histological evidence of the participation of calcium soaps is, however, open to question.

On the other hand, studying large quantities of material chemically, I have found at most doubtful traces of calcium soaps in calcifying matter, even in the earliest stages, and also very small amounts of other soaps or fatty acids.[47] It was also found that *when calcium soaps are implanted in the tissues they do not become transformed into calcium phosphate and carbonate*, but are largely absorbed and removed. Therefore, I am obliged to question the view that calcium soaps constitute an essential or usual step in calcification, although not doubting that under certain conditions (*e. g.*, calcifying lipomas, fat necrosis) this may occur. In calcification at all stages the proportion of calcium carbonate and phosphate was found quite constant, and exactly the same as in normal bone, namely, in the proportion expressed by the formula $3Ca_3(PO_4)_2$-$:CaCO_3$, which Hoppe-Seyler advanced to express the composition of the salts of bone. Hence it seems probable that there are no essential differences between the processes of ossification and pathological calcification,[48] and there seems to be as yet no reason for assuming that in the former calcium soaps constitute an essential step in the process. Recent studies of rickets (*q. v.*) and also of the repair of fractures,[49] have shown conclusively that for bone formation the blood must contain a proper amount of both Ca and P, indicating again that the calcium is deposited as phosphates and not as a soap or bound to some other binding substance.

Phosphoric Acid in Calcification.[50]—It has generally been assumed that in normal ossification the calcium is combined by phosphoric acid, which is derived from the cartilage cells, possibly through autolysis of the nucleoproteins or some similar process.[51] Grandis and Mainini,[52] by using microchemical methods, thought they had found evidence that the phosphorus of ossifying cartilage is converted from an organic combination into an inorganic form which then takes up calcium from the

[46] Ziegler's Beitr., 1905 (7th suppl.), 339.

[47] See review in Arch. Int. Med., 1911 (7), 721.

[48] Dyes that stain the bones when fed to living animals (madder) also stain pathological calcific deposits (Macklin, Anat. Record, 1917 (11), 387).

[49] See Peterson, Bull. Johns Hopkins Hosp., 1924 (35), 378.

[50] Hilda Kincaid (Pharm. Jour. 1914 (93), 350) points out the interesting fact that the framework of the lower animals is mostly $CaCO_3$, the phosphate increasing with the evolutionary scale, to become practically constant in the vertebrates.

[51] Hanes, who observed that the phosphatids disappear from the liver of the developing chick, suggests this as a source of the phosphoric acid required for ossification (Jour. Exper. Med., 1912 (16), 512).

[52] Arch. per la sci. Med. Torino, 1900 (24), 67.

blood. The methods used have been questioned, and Pacchioni,[53] from his studies, was inclined to the opinion that the calcium entered the cartilage already combined as phosphate. Wells implanted into the abdominal cavity of rabbits various tissues that had been killed and sterilized by boiling, and found that tissues rich in nucleoproteins showed no tendency to take up calcium in greater amounts than did tissues poor in nucleoproteins, which result speaks against the idea that phosphoric acid derived from nucleic acid combines the calcium. On the other hand, implanted dead cartilage soon became thoroughly impregnated with calcium salts, which seemed to be deposited in the same proportion as to carbonate and phosphate as in bone. Robinson[54] has advanced evidence that the blood contains hexophosphoric esters which may be split by an enzyme present especially in those parts of bone which are ossifying, thus liberating phosphoric acid which might serve to bind calcium. This theory, however, fails to account for calcification of dead tissue, and for the selective absorption of calcium by cartilage even when this has been boiled, as in my implantation experiments.[55]

Physical Adsorption of Calcium Salts.—As there could be no question of "vital activity" on the part of this boiled cartilage, it seems most probable that there exists in cartilage a specific absorption affinity for calcium salts,[56] similar to the absorption affinity that Hofmeister[57] observed exhibited by other organic colloids (gelatin disks) toward various crystalline substances in solution. It is of significance that the substances in which calcium is deposited are, in most instances, of similar physical character, being homogeneous and often hyaline, although of the most varied chemical composition; in other words, *they agree much more in physical than in chemical structure.* Also we find that hyaline tissues with an affinity for calcium often exhibit a similar affinity for other substances, such as pigment and iron, *e. g.* iron-lime incrustations, cartilage pigmentation in ochronosis (*q. v.*).[58] Hofmeister advances the hypothesis that when the cartilage or other matrix becomes saturated with calcium salts, any decrease in CO_2 content of the solution will lead to a precipitation of calcium salts, thus restoring to the cartilage its power of absorbing more calcium salts whenever the fluid comes to it with a higher degree of saturation with calcium salts and CO_2. In support of Hofmeister's idea is the fact that a similar fluctuating effect, produced by alternately varying the reactions by feeding acids and bases, was found by Rabl[37] to lead to metastatic calcification. This hypothesis is also in harmony with Barille's observation that when the amount of CO_2 is reduced the complex carbon-phosphate of calcium precipitates a mixture of carbonate and phosphate, in the same proportions as found in bones and calcific deposits generally. The fact that this ratio (10 to 15 per cent.

[53] Jahrb. f. Kinderheilk., 1902 (56), 327.

[54] Biochem. Jour., 1923 (17), 286; 1924 (18), 740.

[55] Freudenberg and György (Biochem. Zeit., 1924 (147), 191) have found that cartilages suspended in solutions will selectively bind Ca but not PO_4.

[56] Corroborated by Watt, loc. cit.[23]

[57] Arch. exper. Path. u. Pharm., 1891 (28), 210.

[58] See Sprunt, Jour. Exp. Med., 1911 (14), 59.

$CaCO_3$ and 85 to 90 per cent. $Ca_2(PO_3)_4$), is found in all stages of calcification,[59] is entirely in favor of the above hypothesis, and opposed to the idea that any special chemical precipitant formed in the calcifying area is responsible for the deposition of calcium, or that the calcium exists in any absolutely constant chemical combination.[60]

Taken all in all, the evidence seems in favor of the view that normal ossification and pathological calcification (except possibly the calcification of fat necrosis and other åreas of necrotic fat tissue) depend more upon physico-chemical factors and variations in CO_2 concentration than upon the presence of chemical precipitants in the tissues. This view is supported by the observation of Macklin[61] that calcifying and ossifying tissues become stained alike with madder fed during their formation, through the deposition of stained calcium salts from the blood.

The physico-chemical influences that lead to deposition of relatively insoluble substances as surface membranes or as structural forms come into wide play in nature, and especially with calcium salts, because of their common occurrence in approximate saturation in fluids of unstable character and with an abundance of colloidal constituents. Mathews[62] describes clearly how calcium deposition may occur under these conditions, in the following words:

"Willard Gibbs pointed out that any substance dissolved in a liquid so that the surface tension of the solution was lower than that of the pure solvent would concen-

[59] Hermann (Arch. klin. Chir., 1924 (130), 284) maintains that in young callus there is relatively little P as compared to Ca, the ratio being but 0.2 to 1 instead of 0.6 to 1 as in mature bone, the latter ratio being reached only when the callus has become replaced by true bone.

[60] Freudenberg and György (See Biochem. Zeit., 1923 (142), 407; 1924 (147), 191) have made extensive studies of the adsorption of calcium by cartilage and other tissues *in vitro*, and believe that the products of tissue metabolism have an inhibiting effect on this adsorption, hence when metabolism ceases in a tissue, calcium begins to accumulate. They picture calcification as occurring through the following steps: First, a reversible combination of calcium with tissue proteins, which, in the lack of tissue metabolism to reverse the reaction, becomes permanent until a phosphate—carbonate—protein-calcium complex is formed. The phosphate predominates because the acid character of the protein liberates most of the CO_2. Gradually the calcium—phosphate—carbonate is split from the protein fraction, which now binds more calcium and thus continues the calcification. This theory of calcium-binding function of proteins as a chemical rather than a purely physical adsorption, has met with vigorous criticism. (See Rabl, Virchow's Arch., 1923 (245), 542; Liesegang, Biochem. Zeit., 1923 (145), 96.) It fails to explain why any and all dead or hyalinized proteins or even non-protein materials such as gauze sponges and linen sutures, readily bind calcium.

Watt (Biol. Bulletin, 1923 (44), 280) has confirmed the older observations that in the early stage of bone formation the calcium deposit is in the form of minute granules which quickly coalesce, so that in mature bone no granules are found. This he interprets as a secretion by the osteoblasts rather than as a mere precipitation; the absorption of bone being also attributed to the osteoblasts rather than to chemical solution by the blood or tissue fluids, the possibility of which he is inclined to doubt. However, the readiness with which changes in the composition of the blood actually do lead to absorption of bone calcium and its deposition in other tissues, as shown, for example, in the experiments of Rabl,[37] is apparently good evidence that simple chemical variations are adequate to account for the deposition and absorption of calcium in a proper physical matrix, without demanding a hypothetical secretory action of cells.

[61] Jour. Med. Res., 1917 (36), 493.

[62] Physiol. Reviews, 1921 (1), 575.

trate in the surface film, for by so doing it would reduce the intensity factor (the surface tension) of the surface energy. But it will be observed that while by so concentrating it lowers the intensity factor of the surface energy, it increases the intensity factor of the osmotic energy. Consequently if it remains in solution, it can only accumulate until the increase in the intensity factor of the osmotic energy, the osmotic pressure in other words, just equals the decrease in the intensity factor of the surface energy. In virtue of this fact, and also because of the extremely minute amount of liquid in the surface film, extremely little accumulation of substances is to be looked for in the surface film, particularly when the very small surface tension changes which are possible are considered. And it may be said at once that only very minute changes in concentration can be produced in this way, as long as the substance remains in solution. But if the solubility of the substance is not high, so that the limit of solubility is passed before the equilibrium of lowered surface tension and increased osmotic pressure is attained, there will be a continuous separation of the solid substance at the surface. If the substance thus separating is colloidal it will form a membrane on the surface, this membrane being composed either of the solid colloid itself, or having a gel structure; if, on the other hand, the substance is crystalline, such for example as calcium carbonate, or various dyes or other substance of low solubility, it may separate in crystals, and in particular in small spherocrystals. The formation of a scum on milk, when it is heated; the formation of membranes at the surface of protoplasm; the formation of a solid soap layer in a soap film or in a soap bubble are instances of accumulations of this kind. And no doubt the deposition of calcium salts of various kinds at the surfaces of animals may be due to a similar process of secretion. Protective membranes tending to separate the phases may thus be produced. By this very simple method an individual is rendered to some degree independent of its environment and a great step forward in securing freedom from that environment, in other words the first step forward in evolution of primitive protoplasm, was taken. In this way then shells, bones, spicules of minerals, nuclear membranes, ectoplasm and other membranes may be produced by the continued secretion into the surface layer of substances which lower the tension of that layer and which by their insolubility are prevented from reaching equilibrium."

Calcification as a Simple Precipitation.—Modern studies on calcium metabolism in rickets (*q. v.*) have added much evidence in favor of the views previously expressed in this chapter, that the deposition of calcium in bone and in pathological calcification does not depend on the existence of a specific calcium-binding substance in the tissues that are to be calcified, but upon the saturated condition of the calcium salts in the blood. Howland[63] and his associates have found that calcium, phosphoric acid and carbon dioxide exist together in the blood in a delicately balanced condition aided by the protective action of the plasma colloids. As Howland says,

"In the serum, tricalcium phosphate is subjected to the influence of carbonic acid which is present in considerable concentration. We may write the formula as follows:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows 2CaHPO_4 + Ca(HCO_3)_2 \rightleftarrows 2Ca^{++} + 2HPO_4^{--} + Ca^{++} + 2HCO_3^{-}.$$

If calcium phosphate is exposed to the action of carbonic acid an equilibrium is established between dicalcium phosphate and calcium bicarbonate and between these and calcium, HPO_4^{-} and HCO_3^{-} ions. When the CO_2 tension of this solution is reduced a reversal of the process takes place and tricalcium phosphate is precipitated again. The reduction of the CO_2 tension in a solution containing HCO_3 ions is equivalent to increasing the *p*H, *i. e.*, the alkalinity. There must be a solubility product constant for Ca and HPO_4 ions which has a definite value at a given *p*H and temperature. When this constant is exceeded precipitation must take place. When the calcium ion concentration is very low no precipitation can occur and when the HPO_4 ion concen-

[63] John Howland, Harvey Lectures, 1922–3 (18), 189; and Medicine, 1923 (2), 349.

tration is very low there will likewise be no precipitation because there is a definite physiological limit of increase of concentration for each ion in the serum. The calcium cannot increase indefinitely to compensate for a low phosphorus concentration and the phosphorus cannot increase indefinitely to compensate for a low calcium concentration."

It has been found that there is such a solubility-product constant in rickets, for if one multiplies the concentration of the calcium by the inorganic phosphorus concentration, each expressed in mgm. per 100 cc. of serum, a product is obtained, which with the normal child is between 50 and 60 and with the rat about 80. When the product is below 30 rickets is invariably present. When it is above 40, either demonstrable healing is taking place or there has never been any rickets. With products between 30 and 40 rickets is usually present.

If an artificial serum is prepared containing salts in the same proportion as in normal serum, it is found that when the *p*H is about 7.35 and the product of the concentrations of Ca and P is 40 or more, a precipitate always forms. The precipitate is composed chiefly of tricalcium phosphate with some carbonate. It makes no difference in the result or in the character of the precipitate if the calcium is 8 and the phosphorus 5 or the calcium 10 and the phosphorus 4 milligrams per 100 cc. When the product is less than the numbers mentioned, no precipitate forms. On the basis of these and other experimental observations, Howland says, "We may visualize calcification somewhat in this way. Serum contains calcium and inorganic phosphorus in solution in much higher concentration than water would hold them, on account of the high CO_2 tension. There is some evidence to show that part of the calcium is bound to protein. If it is assumed that this calcium-protein compound is poorly ionized this would be equivalent to reducing the total calcium concentration. Intercellular fluid of the tissues contains roughly the same amount of calcium and phosphorus, according to our determinations, as the serum, but the protein is much less, apparently about 1 per cent., as contrasted with the 7–9 per cent. in the serum. The CO_2 tension is relatively high on account of cellular activity. But supposing that the CO_2 tension is low because the tissue is inactive or dead, then conditions are favorable for precipitation. *Cartilage and the trabeculæ of bone are both inactive tissues and it is in them that one would expect the CO_2 tension low and it is in them that precipitation takes place.*

"This view of the factors that control calcification and of the mechanism of calcification, explains the normal process and the failure in rickets and tetany of children and the rickets of animals. It also explains calcification in dead or inactive tissues such as tuberculous lesions and in the fascial planes of the muscles in myositis ossificans, where the first change seems to be a hyaline transformation of the connective tissue. What is further of much importance, it explains the constancy of composition of the bones of all animal species in which the calcium and inorganic phosphorus concentrations of the serum so far as they have been studied do not differ greatly from those of man."

OSTEOMALACIA[64]

In this condition the quantity of inorganic salts in the bone is greatly decreased, while, at the same time, their place is taken in part by new-formed osteoid tissue; as a result, the proportion of the weight of the bone formed by inorganic salts is reduced to as low as 20 to 40 per cent., instead of being from 56 to 60 per cent., as in normal bone. This has suggested that the cause of the disease, may be a solution of the lime salts by some acid, but Levy[65] found that in osteomalacia the proportion of calcium carbonate and phosphate in the bones remains constant, as also does the proportion of calcium and phosphoric acid; if the decalcification occurred through solution by lactic or other acids, he argued, the carbonate should be decomposed first,[66] whereas the lime salts seem to be taken out as molecules of calcium carbonate-phosphate; *i. e.*, in the same proportion as they exist in the bone. On the other hand, it has been found in Pawlow's laboratory that dogs kept for long periods after a pancreatic fistula has been established, develop a condition resembling osteomalacia,[67] which would seem most reasonably explained as due to the constant loss of alkali in the pancreatic juice.[68] Furthermore, investigation of Levy's objection to the acid solution theory has led to the observation that when mixtures of calcium carbonate and phosphate are *in colloids* they are dissolved at equal rates.[69] That acidosis may deplete the bone calcium is shown by the observation of Rabl[70] that administration of NH_4Cl to children may soften the bones so that deformities may be corrected by manipulation.

Histologically, absorption seems to depend largely upon a direct eating out of bone tissue, both organic and inorganic substance, by osteoclasts (Cohnheim), followed by a formation of an uncalcified osteoid tissue. (Senile osteoporosis differs chiefly in that no new osteoid tissue is formed.) According to Dibbelt[71] when osteomalacia is experimentally induced in pregnant dogs and then recovery is allowed to take place, the decalcified bone substance present in the active stage does not become calcified, but is absorbed and replaced by new bone.

Studies of metabolism in osteomalacia have shown a loss of calcium by the body, especially in the urine, as shown by the following table given by Goldthwait *et al.*:[72]

[64] See also review by McCrudden, Arch. Int. Med., 1910 (5), 596; 1912 (9), 273; "Endocrinology and Metabolism," 1924 (4), 733.

[65] Zeit. physiol. Chem., 1894 (19), 239.

[66] Goto reports that in experimental HCl acidosis the bones lost 20 per cent. of their $CaCO_3$ without appreciable loss of phosphate (Jour. Biol. Chem., 1918 (36), 355).

[67] Babkin, Zeit. Stoffwechsel, 1910 (11), 561; Looser, Verh. Deut. Path. Gesell., 1907 (11), 291.

[68] However, defective fat assimilation may cause a loss of calcium carried out in the stools as calcium soaps (Telfer, Biochem. Jour., 1921 (15), 347).

[69] Kranz and Liesegang, Duet. Monat. Zahnheilk., 1914, p. 628.

[70] See Bernhardt, Zeit. klin. Med., 1924 (100), 735.

[71] Arbeit, Path. Inst. Tübingen, 1911 (7), 559.

[72] Goldthwait, Painter, Osgood and McCrudden, Amer. Jour. Physiol., 1905 (14), 389.

	Limbeck	Neumann	Goldthwait
CaO in urine (gm.)	1.773	.....	3.859
CaO in feces	3.834	.....	1.800
Total excreted	5.607	11.65	5.66
Total in food	2.965	11.26	4.56
Loss of CaO	2.965	0.39	1.10

McCrudden also found a considerable retention of nitrogen and sulfur, which may be retained in the new-formed osteoid tissue; magnesium[73] is also retained, probably being substituted for calcium in the bones. It is known that when magnesium and strontium are given to growing animals, they will partially replace the calcium in the bones,[74] while it is said by Etienne[75] that excessive feeding of calcium itself leads in time to decalcification of the bones. Zuntz[76] found the respiratory metabolism in osteomalacia within normal limits, but tending to be low; protein metabolism shows nothing striking, but there is a high excretion of phosphoric acid through the feces.

Castration of women with osteomalacia has been frequently, but not always, followed by improvement or recovery,[77] and Neumann, and also Goldthwait, have found that in these cases the calcium loss is replaced by a marked calcium retention after the operation. What the relation of the ovaries to calcium metabolism or to osteomalacia may be has not yet been ascertained.[78] Scharfe[79] and Bucura[80] both state that there are no characteristic or constant structural alterations in the ovaries in osteomalacia. McCrudden[81] found that the improvement in calcium metabolism observed after castration may be but temporary, and therefore believes that the primary cause of the disease does not lie in the ovaries. He is of the opinion that repeated drains on the calcium of the bones, incited most often by pregnancy, occasionally by tumors, sometimes by unknown causes, result in an excessive reaction to the stimuli, so that eventually the losses become too great to be made up; that is, osteomalacia is an exaggeration of a normal process resulting either from excessive stimulation of that process, or a failure to recover when the stimulus ceases. It is known that in pregnancy the blood calcium is reduced (Widdows).[7] The beneficial effects of castration are probably ascribable chiefly or solely to the prevention of pregnancy. Osteitis deformans seems to be a localized osteomalacia. The relation of the

[73] Corroborated by Cappezzuoli, Biochem. Zeit., 1909 (16), 355.
[74] See Lehnerdt, Zeit. exp. Med., 1913 (1), 175.
[75] Jour. Physiol. et Path., 1912 (14), 108.
[76] Arch. f. Gyn., 1913 (99), 145.
[77] Bibliography by Schnell, Zeit. Geb. u. Gyn., 1913 (75), 178.
[78] See Rittmann, Wien. Arch. inn. Med., 1924 (8), 261.
[79] Cent. f. Gyn., 1900 (24), 1216.
[80] Zeit. f. Heilk., 1907 (28), 209.
[81] Amer. Jour. of Physiol., 1906 (17), 211.

adrenals to osteomalacia advocated by Bossi,[82] is of questionable significance, and there is no definite evidence as to any relation of exophthalmic goiter[83] or the parathyroids,[84] although hyperplasia of the parathyroids has been described.[85]

Modern studies of nutrition, and especially the studies of Miles and Feng[86] on osteomalacia in China, bring this disease into the same catagory as rickets, for these authors found that it may be prevented or cured by the addition of fat-soluble vitamins to the diet. Often there is also a deficiency in calcium supply or utilization, and the blood shows a low calcium content, frequently associated with tetany, the calcium content increasing with administration of cod liver oil. The calcium figures varied from 5.2 to 7.4 mg., the phosphorus from 1.8 to 3.8 mg., per 100 cc., the former never being normal whereas the phosphorus occasionally was normal. The CO_2 combining power of the blood (47–57) did not indicate acidosis. There is usually a negative calcium and a positive phosphorus balance, but the calcium balance may become positive when cod liver oil is given. As with rickets, absence of exposure to sunlight seems to be a factor.[87]

Osteoporosis, or "war osteomalacia," was common during the war in countries where there was a food shortage, and in Vienna it was observed that recovery followed addition of cod liver oil and other substances according to their known antirachitic qualities.[88] "Osteomalacia" often occurs in caged animals[89] with diets deficient in fat-soluble vitamins.[90] It is not established, however, that all these forms of osteoporosis are identical with the osteomalacia of child-bearing women.[91]

Rickets[92]

Modern studies of nutrition have largely cleared up the problem of the etiology of rickets, establishing it definitely as a true deficiency disease, among which it is discussed (pp. 320–322). Here will be discussed only the chemical features of the disease itself.

As with osteomalacia, chemical studies of the bones in rickets have thrown little light upon the etiology or pathogenesis of this condition.

[82] Zent. f. Gyn., 1907 (31), 69 and 172.
[83] Tolot and Sarvonat, Rev. d. Méd., 1906 (26), 445.
[84] Erdheim, Cent. med. Wiss., 1908 (46), 163.
[85] Bauer, Frankfurter Zeit. Pathol., 1911 (7), 231; Strauch., *ibid.*, 1922 (28), 319.
[86] Jour. Exp. Med., 1925 (41), 137.
[87] See Hutchinson and Patel, Glasgow Med. Jour., 1921 (95), 241.
[88] Dalzell and Chick, Lancet, 1921, Oct. 25, 842; Hume and Nirenstein, ibid., 849.
[89] See "Diseases in Captive Wild Mammals and Birds," Herbert Fox, Phila., 1923, p. 349.
[90] White, Arch. Int. Med., 1922 (30), 620. Osteoporosis in horses is said to be cured by adding calcium to the diet (van Sageghen, Abst. in Bull. Agric. Intelligence, 1919 (10), 1162).
[91] Loll (Biochem. Zeit., 1923 (135), 492) finds in "war osteoporosis" bone ash a marked decrease in the P_2O_5 with a relative increase in calcium, these changes not being present in osteomalacia or rickets.
[92] Complete literature and full discussion by Park, Physiol. Reviews, 1923 (3), 106; Howland, Harvey Lectures, 1922–3 (18), 189.

As the following table (taken from Vierordt[93]) shows, there is a marked deficiency in the concentration of inorganic salts in the bones in rickets. The relative proportion of the different salts seems to be quite the same as in normal bone.

	Normal bone of a two months old child		Rachitic bones				
	Tibia	Ulna	Femur	Tibia	Humerus	Ribs	Vertebræ
Inorganic matter	65.32	64.07	20.60	33.64	18.88	37.19	32.29
Organic substance	34.68	35.93	79.40	66.36	81.12	62.91	67.71
Calcium phosphate	57.54	56.35	14.78	26.94	15.60		
Magnesium phosphate	1.03	1.00	0.80	0.81			
Calcium carbonate	6.02	6.07	3.00	4.88	2.66		
Soluble salts	0.73	1.65	1.02	1.08	0.62		
Collagen (or ossein)	33.86	34.92	72.20	60.14	81.22		
Fat	0.82	1.01	7.20	6.22			

More modern analyses[94] show a relative increase in magnesium, with a persistence of the normal ratio of calcium phosphate and carbonate.[95] As an essential difference from osteomalacia is the fact that there is a failure on the part of the osteoid tissues to calcify in rickets, whereas in osteomalacia absorption of calcified tissue takes place with subsequent substitution by osteoid tissue. Furthermore, in rickets the deficiency in calcium is said to be present only in the bones,[96] whereas in osteomalacia the soft tissues are also poor in lime salts. According to Schmorl[97] the first structural abnormality in rickets is a failure of small islands of cartilage in the zone of preparatory calcification to lay on calcium.

In view of the fact that rickets is not solely a disease of bone tissue, but that all the various important viscera, as well as the muscles and tendons, show pathological changes, it seems most reasonable that rickets should be looked upon as a *constitutional* disease, in which the bone changes are prominent chiefly because the disease occurs at a time when the bone tissue is most actively forming and when the other organs are relatively quite completely developed. Stöltzner[98] looked upon the failure of calcification as depending upon an abnormality in the calcified

[93] Nothnagel's System, vol. 7, part ii, p. 21.

[94] Gassmann, Zeit. physiol. Chem., 1910 (70), 161; Telfer, Quart. Jour. Med., 1922 (16), 63.

[95] The bones and muscles in *Barlow's disease* show quite the same deficiency in calcium as in rickets (Bahrdt and Edelstein, Zeit. Kinderheilk., 1913 (9), 415).

[96] There is a decrease in the calcium of the muscles according to Aschenheim and Kaumheimer (Monatschr. f. Kinderheil., 1911 (10), 435).

[97] Verhandl. Deut. Path. Gesell., 1905 (9), 248.

[98] Jahrb. f. Kinderheilk., 1899 (50), 268.

bone tissue itself.[99] He found evidence of a preliminary alteration in normal osteoid tissue which prepares it to take the salts out of the blood, and Pfaundler[1] supported this view, suggesting that this preparatory change in the osteoid tissue may depend upon autolysis, which is perhaps deficient in rickets.[2]

Metabolism in Rickets.—The blood in rickets ordinarily shows a decrease in inorganic phosphorus. Normally the serum contains 10–11 mg. of calcium per 100 c.c. and about 5 mg. of inorganic phosphorus, but during the active period of rickets the phosphorus is regularly reduced, even to 1 mg. per 100 c.c., although the calcium remains normal or but slightly reduced in amount (Howland and Kramer).[3] When the rickets is associated with tetany, however, the calcium in the blood is reduced as low as 3.5 mg. per 100 cc., while the phosphorus figure may remain normal. In these cases with tetany, administration of calcium by mouth causes a rise in the blood calcium, and the symptoms of tetany are removed because they depend on the hypocalcemia. Furthermore, it has been found (*a*) that the concentration of the inorganic phosphorus and calcium of the serum cannot be made to exceed the concentrations regarded as normal; (*b*) that when the concentration of either calcium or inorganic phosphorus in the serum is low, it may be increased by increasing the amount of the respective element in the diet; (*c*) that when the inorganic phosphate of the serum is low it may be increased by starvation, by the addition of phosphorus to the diet in organic or inorganic form, or addition of various fats (the fish oils and butter fats), and by exposure to ultraviolet rays.

Park considers that most of the published metabolism studies in rickets are inconclusive because they were carried out without full knowledge of the underlying principles now available. Presumably there occur negative balances of Ca and P at times, but usually there is merely a diminished retention, and according to Telfer[4] the loss of P is greater than of Ca. An important fact to consider is that the normal infant excretes about 0.200 gram of calcium oxide daily, regardless of the intake or weight. For an adequate calcium retention to occur, therefore, it is necessary that considerably more than that amount be supplied in the food.

How the deficiency in radiant energy or of antirachitic vitamin lead to the observed chemical changes in the blood, and the structural changes in the bone, is still unsolved. Park says there are several possible explanations for the diminution in the retention of calcium and phos-

[99] How phosphorus causes growing bones to lay on increased calcium is an unsolved problem, but a striking fact. (See Phemister, Jour. Amer. Med. Assoc., 1918 (70), 1737.)

[1] Jahr. f. Kinderheilk., 1904 (60), 123.

[2] Concerning the chemical changes of *osteogenesis imperfecta* (congenital fragility of bones), see Schabad, Zeit. Kinderheilk., 1914 (11), 230. No significant changes in blood phosphorus or in metabolism were found by Flagstad *et al.*, Minn. Med., 1924 (7), 800.

[3] See also Anderson, Brit. Jour. Childrens' Dis., 1924 (21), 107.

[4] Quart. Jour. Med., 1922 (16), 45 and 63.

phorus in rickets. One is that the calcium and the phosphorus are not absorbed from the intestinal tract. On this supposition it is likely that either the digestive functions are disturbed in some peculiar way, or else the combination of food substances is such that insoluble products incapable of absorption are formed in the intestines, for example, tri-calcium phosphate or calcium soaps. Another possibility is that the calcium and phosphorus are absorbed, but, on account of abnormal conditions in the internal metabolism, are not allowed to accumulate in the blood; they are either driven out of the body or are used to carry out some other substance. Under this latter supposition it is necessary to suppose the primary disturbance to be in the internal metabolism. In either case it seems altogether probable that the deposition of lime salts in the bones is determined chiefly, if not entirely, by the products of the concentration of the calcium and phosphate ions in the circulating fluid as Howland and Kramer have indicated. When the concentrations of calcium and phosphate in the blood increase sufficiently, the deposition of lime salts apparently occurs. It seems easier to explain the action of radiant energy and also of cod liver oil in rickets on the ground that the metabolism of the whole body is altered, rather than on the basis of a peculiar composition of the food or a local disturbance in the digestive system.

Recent studies from Howland's laboratory[5] have shown that infants with active rickets fail to retain calcium and phosphorus, although an adequate amount is present in the diet. Ultraviolet irradiation causes large amounts of these elements to be retained in the body. Increased amounts of calcium and phosphorus are found in the urine after ultraviolet irradiation, indicating an increased absorption from the intestine. From these facts it is argued that the defective absorption from the intestine, found in active rickets, is the cause of the low concentrations of calcium and phosphorus found in the serum, and is the ultimate cause of the defective calcification of the bones. It was found that excessive amounts of calcium in the diet tend to increase the total absorption and retention of calcium, but tend to impair phosphorus retention. Excessive amounts of phosphorus in the diet exercise an unfavorable influence on the calcium metabolism, and are accompanied by an increase in the calcium lost in the feces. The retention of one element in the intestine by an excessive amount of the other in the diet is best explained by the formation of insoluble phosphates of calcium which cannot be absorbed. The factor above all that favors solution of calcium phosphate is acidity, and foods that are acid-forming evidently improve calcium retention; alkalies or base-forming foods seem to have a contrary effect. The absorption of calcium and phosphorus from the intestine depends on the concentration of calcium and phosphate ions, and the presence of soluble undissociated phosphates of calcium. Such physicochemical factors as alter these concentrations of salts or ions or their activities may greatly influence the absorption of these elements.

[5] Orr *et al.*, Amer. Jour. Dis. Chil., 1923 (26), 362; 1924 (28), 574.

CONCRETIONS

All pathological concretions appear to be laid down according to a definite law. There must first be a *nucleus* of some substance different from the substance that is to be deposited, and which is most frequently a mass of desquamated cells, but may consist of clumped bacteria, masses of mucus, precipitated proteins, or a foreign body of almost any sort. Upon this nucleus substances crystallize out of solution, much as cane-sugar crystallizes on a string to form rock candy, but with the important exception that among the crystals is usually deposited more or less mucin or other organic substance, which forms a framework in which the crystals lie, and which remains, if the crystals are dissolved out, as a more or less perfect skeleton of the concretion. In no case would the concretion form were it not that the solution is overcharged with some substance, but not infrequently it is the presence of the nucleus that leads to the precipitation of the substance; *i. e.*, the nucleus may play either a primary or a secondary role. With few exceptions, the dissolved substance is deposited in crystalline form, although the crystalline structure may in time partly disappear through condensation or through filling of the interstices with some other material. Even so structureless a substance as amyloid may, when forming concretions, appear in a crystalline form (Ophüls). The structure of a concretion depends upon two factors: The crystals tend to be deposited at right angles to the surface, and thus give a *radiating* structure; but the rate of deposition is usually irregular, and during the periods of quiescence the surface tends to become covered with mucin or other organic substances, hence we also get a *concentric, laminated* structure. Frequently both of these lines of formation are easily discerned, but either one or the other may become obscured.

Concretions consist, therefore, of mixtures of colloids and crystalloids deposited from solutions of the same character, and hence the application of the principles of colloidal chemistry throws much light on the conditions of their formation.[6] Colloidal solutions hold in solution greater quantities of crystalloids than simple solutions, for the reason that at the surface of each colloidal particle there is a zone in which the crystalloids are more concentrated than elsewhere, thus permitting more crystalloids to be dissolved in the solvent between the colloidal particles. On the other hand, the concentration of the crystalloids on the surface of the colloidal particles causes the colloids to serve as the starting point of precipitation whenever the crystalloids are in excess. When the crystalloid goes out of solution, therefore, it will form crystals or precipitates which are intimately associated with the colloids, as we see when uric acid crystallizes out of urine, taking with it the colloidal pigments by which it is adsorbed. Or, if the colloids are precipitated, the solvent power of the solution is reduced, and the crystalloids will deposit in intimate relation to the colloids. As Schade pointed out, if a colloid precipitates in

[6] See Schade, Münch. med. Woch., 1909 (56), 3; 1911 (58), 723; Zeit. exp. Path., 1910 (8), 92; also Lichtwitz, Ergeb. inn. Med., 1914 (13), 1; also his monograph "Ueber die Bildung der Harn- und Gallensteine," Springer, Berlin, 1914.

an irreversible form (*e. g.*, fibrin), the concretion will be permanent, as with ordinary concretions, but if the colloid precipitate is reversible the mass may be dissolved again, as with the precipitate of urates in the tubules of the infant's kidney.

Biliary Calculi[7]

As may be judged from the above statements, concretions are never composed of one substance in a pure form, but usually consist of a mixture of the constituents of the fluid in which they are developed. This is particularly true of gall-stones, which contain in greater or less quantities several or all of the constituents of the bile. While cholesterol forms the chief part of nearly all biliary concretions, and is present in greater or less amounts in all, calcium salts of the bile-pigments are always present; usually inorganic salts of calcium (carbonate and phosphate) are also present, as well as small amounts of fats,[8] soaps, lecithin, mucus, and other products,[9] and occasionally traces of copper,[10] iron,[11] and manganese.[12] The quantity of bile salts, the chief constituent of the bile is usually extremely minute, apparently only so much as may percolate into the crevices of the concretion. However many stones there may be in a gall-bladder, they usually are all of approximately the same composition and structure. In gall-stones from the domestic animals the proportion of inorganic salts is usually much higher than it is in man.

Naunyn[16] has classified gall-stones according to their composition, as follows:

1. **"Pure" Cholesterol Stones.**—The purity is only relative, since even the purest always contain some pigment as well as a stroma and a nucleus; but the amount of cholesterol may reach 98 per cent., and is usually over 90 per cent. Crystalline structure is usually well marked, while stratification is slight. The color varies from nearly pure white to yellow, or even brown on the surface.

2. **Laminated Cholesterol Stones.**—These consist of about 75–90 per cent. of cholesterol, and differ from the preceding form in containing more pigment which is deposited in layers alternating with the white layers of cholesterol. The pigment here, as in all other gall-stones, consists always of the calcium salts of the pigments—not of pure bilirubin and biliverdin themselves. Considerable calcium carbonate is also usually present, particularly in the green layers of biliverdin-calcium.

3. **Common Gall-bladder Stones.**—The composition of this form is but little different from the above, the chief difference being in the structure. They present externally a firmer crust, usually distinctly laminated; in the center is a softer pigmented nucleus which frequently shows a central cavity containing fluid. Such calculi are not distinctly crystalline in structure, and are small, seldom larger than a cherry.

[7] Bibliography by Bacmeister Ergeb. inn. Med. 1913 (11), 1. An interesting discussion of the theories of gall-stone formation is given by Aschoff, "Lectures on Pathology," Hoeber, 1924.

[8] Salkowski (Zeit. physiol. Chem., 1916 (98), 25; 1917 (100), 259) found palmitic acids but no neutral fat.

[9] Fischer and Rose found about 0.1 gm. *carotin* in 1280 gms. gall stones from cattle. (Zeit. physiol. Chem., 1913 (88), 331.)

[10] See Mizokuchi, Cent. f. Pathol., 1912 (23), 337.

[11] Gonnermann (Zeit. physiol. Chem., 1920 (111), 32) has published analyses of the ash of several gall stones, unfortunately without consideration of the types of stones, and finds Si and Al constantly present, sometimes in surprisingly large amounts, iron seldom being present in appreciable amounts although always present in traces.

[12] Gall-stones have been found enclosing droplets of mercury. (Naunyn, Frerichs.)

4. **Mixed Bilirubin-calcium Calculi.**—These generally occur singly, but sometimes in groups of three or four, and are of large size. Although the chief constituent is bilirubin-calcium, there is always much cholesterol, often over 25 per cent. Copper and traces of iron may also be present. Their structure is laminated, with sometimes a crystalline cholesterol nucleus.

5. **"Pure" Bilirubin-calcium Calculi.**—In addition to the chief constituent, *biliverdin-calcium*, the related pigments *bilifuscin* and *bilihumin*[13] are practically always present. *Bilihumin* is at times the chief ingredient, and may form over half of the substance; *bilicyanin* is rarely present. There is always some cholesterol, but sometimes only traces. These calculi are small, from the size of a grain of sand to that of a pea, and they occur in two distinct forms. One form is of wax-like consistence; the other is harder, steel gray or black in color, with a metallic luster. Pure bilirubin and biliverdin, not combined with calcium, are practically never present in concretions.

6. **Rarer Forms.**—(*a*) *Amorphous and incompletely crystalline cholesterol gravel.* Cholesterol externally giving a pearly luster; pigment in the center.

(*b*) *Calcareous Stones.*—Consist chiefly of a mixture of calcium carbonate and bilirubin-calcium. Calcium carbonate may occur either as a superficial crust, or as small masses within an ordinary calculus; calcium sulphate and phosphate occur rarely in traces. Stones consisting mainly of calcium carbonate are extremely rare in man, but more frequent in cattle and other herbivora, in which all forms of concretions contain much calcium, either combined with pigment or as carbonate and phosphate. A calcium oxalate gall-stone has also been described.[14]

(*c*) *Concretions with included bodies, and conglomerate stones.*

(*d*) *Casts of Bile-ducts.*—Occur particularly in cattle, and consist chiefly of bilirubin-calcium. Rarely and imperfectly formed in man.

Aschoff and Bacmeister differ somewhat from Naunyn as to the composition of gall-stones, which they classify as follows:

1. Pure cholesterol stones.
2. Stratified cholesterol-calcium stones.
3. Cholesterol-pigment-calcium stones.
4. Composite stones, composed of cholesterol and a mantle of cholesterol and calcium.
5. Bilirubin-calcium stones, usually found in the bile passages of the liver.
6. The very rare calcium carbonate stones.

To these Torinoumi[15] adds

7. Pure pigment stones, and
8. Composite stones consisting of a central pigment stone with a mantle of cholesterol-pigment-calcium.

Formation of Gall-stones.—Until recently our views concerning the chemistry and pathology of the formation of gall-stones were dominated by the observations and conclusions of Naunyn[16] and his pupils.

[13] *Biliverdin* differs from *bilirubin* in containing one more atom of oxygen in the molecule, and it is easily formed from bilirubin—even exposure to air will slowly bring about the oxidation. *Bilifuscin* is a still more oxidized derivative—so much so that it does not give Gmelin's reaction (with $HNO_3 + HNO_2$) for bile-pigments. *Bilihumin* represents the most oxidized of these products, is brown in color, and is the chief constituent of the residue left after treating gall-stones with ether, alcohol, and chloroform to dissolve out the cholesterol.

[14] Montlaur, Bull. sci. pharmacol., Vol. 18, p. 19.

[15] Mitt. Grenz. Med. Chir., 1924 (37), 385.

[16] An English translation of this classic work, by A. E. Garrod, has been published by the Sydenham Society, 1896, vol. 158. More recently Naunyn has published a further exposition of his views, "Die Entstehung u. d. Aufbau d. Gallensteine," Jena, 1923; also Mitt. Grenz. Med. Chir., 1923 (36), 1; 1924 (37), 545.

Former observers, having learned that bile normally contains cholesterol (Hammarsten found from 0.06–0.16 per cent. in human gall-bladder bile),[17] sought the cause of gall-stones in either an increased elimination of cholesterol by the liver, or a decrease in the power of the bile to hold the cholesterol in solution. Thus Frerichs, finding that the presence of large amounts of bile salts and an alkaline reaction favored the solution of cholesterol, imagined that a diminution of either bile salts or alkalinity led to the precipitation of the cholesterol. Naunyn and his pupils, however, finding that the amount of cholesterol present in the bile did not depend upon the amount taken in the food or the amount present in the blood, and that it did not vary in disease, except when gall-stones were present, concluded that the cholesterol of the bile is neither a product of general metabolism nor a specific secretion-product of the liver. Finding that pus and the secretions from inflamed mucous membranes (bronchitis) contained as much or more cholesterol as normal bile, they concluded that the chief source of cholesterol in gall-stone formation was the degenerating and desquamated epithelial cells of the gall-bladder and bile tracts. This idea was supported by the large amount of cholesterol found in the contents of gall-bladders shut off from the common duct, and by the formation of gall-stones in such isolated gall-bladders. Some further evidence has since been brought forward in favor of this same view,[18] but others, finding no abundance of cholesterol in the wall of the gall-bladder have not accepted this origin.[19] The great rapidity with which gall-stones may form in the human gall bladder is indeed difficult to explain if the cholesterol must all come from degenerating cells.[20]

On the basis of Naunyn's hypothesis the ordinary steps in the formation of a cholesterol concretion are as follows: Some injury to the mucous membrane of the bile tracts is the starting-point; this injury is usually produced by infection, the colon and typhoid bacilli being the most common organisms in this process.[21] Through the degeneration of the

[17] Nathan (Virchow's Arch., 1920 (228), 51) found fistula bile to contain 0.045 to 0.055 per cent. of cholesterol.

[18] Thus Wakeman (quoted by Herter, Trans. Congress Amer. Physicians, 1903 (6), 158) was able to cause an increase in the cholesterol of the bile in the gall-bladder of dogs by injecting, into it, $HgCl_2$, phenol, or ricin. At first the cholesterol seems to be contained largely in the degenerating desquamated cells. Also the interesting case of a cholesterol calculus in a pyosalpinx, described by Thies (Arb. Path. Inst. Tübingen, 1908 (6), 422), shows the possibility of an inflammatory origin for such concretions, and independent of bile.

[19] Aschoff, Münch. med. Woch., 1906 (53), 1847 and 1913 (60), 1753; Aschoff and Bacmeister, "Cholelithiasis," Gustav Fischer, Jena, 1909; Laroche and Flandin Compt. Rend. Soc. Biol., 1912 (72), 660.

[20] See Cameron (Jour. Amer. Med. Assoc., 1923 (81), 1671), who observed that 38 gall-stones, weighing dry 2.77 gm., formed in a human gall-bladder in less than 86 days.

[21] See Cushing (Johns Hopkins Hosp. Bull., 1899 (10), 166), who produced gall-stones experimentally by injecting typhoid bacilli into the circulation after injuring the gall-bladder. Literature on the relation of bacteria to gall-stones given by Funke, Proc. Path. Soc., Philadelphia, 1908 (11), 17; also see Rosenow who finds that streptococci are often responsible (Jour. Infect. Dis., 1916 (19), 527). Grieg noted the frequent occurrence of gall-stones in rabbits immunized with cholera vibrios (India Jour. Med. Res., 1916 (3), 397). Agrifoglio found that, of several methods tried, only injection of attenuated typhoid bacilli into the gall-bladder produced stones (Clin. Med. Ital., 1924 (55), 89).

epithelial cells an excess of cholesterol is formed, while at the same time the desquamated cells and clumped bacteria offer suitable nuclei upon which the cholesterol begins to crystallize out. Apparently after the calculi have reached a certain size they cause sufficient mechanical injury to keep up the cell degeneration and cholesterol formation, even after the infection has subsided. A certain amount of infection and inflammation is a favoring condition, however, for Harley and Barratt[22] found that fragments of cholesterol calculi introduced aseptically into the gall-bladders of dogs were slowly dissolved and disappeared, but this was prevented by infecting the gall-bladder with *B. coli*.[23] According to Naunyn's investigations, it is not an alteration in the composition of the bile, as formed in the liver, which causes the precipitation of cholesterol, but rather the presence of the nidus, and the production of large quantities of cholesterol in immediate proximity to this nidus, that determines the formation of a concretion. In case the bile stagnates in the gall-bladder, the cholesterol that is being constantly formed by the normal disintegration of surface epithelium accumulates, until, even without infection, there forms a sediment of soft yellowish and brownish masses, consisting chiefly of cholesterol and bilirubin-calcium. From this material calculi may eventually form, and by their irritation lead to further formation of cholesterol and increased growth.[24] But bacteriological studies indicate that generally an infectious influence is present in cholelithiasis, and bacilli may be found alive in gall-stones for remarkably long periods.

Recent applications of colloidal chemistry add much to our understanding of gall-stone formation. Thus, Lichtwitz points out that the colloids of normal bile, all of which are electro-negative, may be precipitated by positive serum colloids coming from the blood when the gall-bladder is inflamed; hence we get a precipitate of cholesterol, bilirubin, and proteins. When the colloids are thus thrown down the solvent power of the bile for the alkali earths it contains is decreased, and so calcium or magnesium are added to the mixture. Cholesterol is in solution in the bile as an emulsion colloid, and when stagnation of the bile leads to absorption or disintegration of the cholates and fats which keep it in solution, the droplets become confluent, and then crystallization takes place (Schade) with formation of spheroliths, and eventually a crystalline cholesterol calculus. If even the slightest pressure is brought to bear on the myelin-like masses before they crystallize, however, they will be pressed into scales, and the common laminated structure results; hence crystalline calculi are single,[25] while multiple gall-stones are laminated, with perhaps partial crystallization between the lamellæ. Also when the

[22] Jour. of Physiol., 1903 (29), 341; see also Hansemann, Virch. Arch. 1913 (212) 139.

[23] Fragments of gall-stones left in the human gall-bladder have also been known to dissolve (See Hedinger, Schweiz. med. Woch., 1921 (51), 1037). Anne Rosin (Zeit. physiol. Chem., 1923 (124), 282) found that salts of desoxycholic acid have a greater solvent action on cholesterol stones than salts of the other bile acids.

[24] Concerning the structure of gall-stones see Ribbert, Virchow's Arch., 1915; (220), 20.

[25] Aschoff, loc. cit.[7]

gall-stones result from inflammation, and there is much serum colloid present, the stones are lamellated because these colloids deposit in that form (*e. g.*, corpora amylacea and other protein concretions). These considerations explain the formation of gall-stones in the gall-bladder from either inflammation, or stagnation without inflammation.

Aschoff and Bacmeister[26] hold that the usual series of events in the formation of gall-stones is, first, the formation of a pure cholesterol stone without inflammatory cause, because of actual increased excretion of cholesterol by the liver, the result of cholesterolemia or because of resorption of solvent substances from stagnating bile; these primary cholesterol stones then cause inflammation and occlusion, leading to the formation of the common mixed stones. Bacmeister ascribes more importance to calcium than do most other investigators, in which he is supported by Rosenbloom,[27] while Kuru[28] states that fibrin is usually present. Rosenbloom reports a small series in which concretions composed chiefly of calcium were found in all cases with a history of infection, while in cases without infection the stones were cholesterol. Failure of the gall-bladder wall to establish a natural degree of acidity, perhaps because of inflammatory exudation, may be responsible for these calcium deposits.[29]

More recent studies of the cholesterol content of the blood and bile also have reacted against the concept that all the cholesterol of gall-stones comes from the wall of the bile tract through inflammatory changes. It has been found that patients with gall-stones often show a hypercholesterolemia;[30] that pregnancy, which seems to be apre disposing cause of cholelithiasis, is accompanied by hypercholesterolemia;[31] that in races subject to cholelithiasis there is more cholesterol in the diet and in the blood than in those races that seldom have gall-stones (DeLangen); that with hypercholesterolemia there is an increased output of cholesterol in the bile,[32] and that experimental hypercholesterolemia may lead to the formation of gall-stones without evident infection of the bile tracts (Dewey[33]).

As far as the existing evidence permits one to draw conclusions, it would seem probable that *both local and systemic conditions are of importance in gall-stone formation.* Gall-stones may perhaps be produced from cholesterol derived from the inflamed bile tract walls, independent of the amount of cholesterol present in the bile; but presumably in some cases they may derive part if not all the cholesterol from the bile. In either event, a hypercholesterolemia (*q. v.*) will favor their formation,

[26] Ziegler's Beitr., 1908 (44), 528.
[27] Jour. Amer. Med. Assoc., 1917 (69), 1765.
[28] Virchow's Arch., 1912 (210), 433.
[29] See Drury, McMaster and Rous, Jour. Med. Res., 1924 (39), 403.
[30] Henes, Surg. Gyn. and Obst., 1916 (23), 91; Moynihan, Brit. Med. Jour., 1925 (1), 393. Not corroborated by Campbell, Quart. Jour. Med., 1924 (18), 123.
[31] Benda, Arch. Gynäk., 1923 (116), 506.
[32] See McMaster, Jour. Exp. Med., 1924 (40), 25.
[33] Arch. Int. Med., 1916 (17), 757; see also Aoyama, Deut. Zeit. Chir., 1914 (132), 234. Not confirmed by Engel and Cserna, Wien. klin. Woch., 1925 (38), 123.

and hence any given condition of injury to the gall bladder will more often give rise to concretions in persons with a high cholesterol content in the blood[34]. Changes in the bile itself may be produced by disease of the liver that will alter bile composition in such a way that its capacity to sustain cholesterol in solution or suspension will be lowered,[35] and this factor also cannot be dismissed as without importance.

Suggestive observations on the effect of the bile salts in maintaining cholesterol in solution have been reported by Oliver,[36] who collected human bile at operations and permitted it to stand in sealed tubes. He found that in all specimens shown chemically to be deficient in bile salts the cholesterol deposited slowly, sometimes in concentric layers alternating with pigment as in gall stones; no such deposits formed in bile containing normal or excessive amounts of bile salts. Bile from inflamed gall bladders with stones has been found generally poor in solids and pigment but of very inconstant composition.[37]

Transient thickening of the bile, such as may occur in any febrile disease, may also very possibly initiate precipitation and stone formation. More and more this last factor is receiving consideration, together with hypercholesterolemia, as of importance in producing cholelithiasis. Rovsing,[38] quoting Boysen's analysis of 200 autopsy cases of cholelithiasis, which showed that all recent deposits and the centers of older concretions consisted of calcium-pigment, especially emphasizes this transitory concentration of bile. The demonstration by Rous and McMaster[39] that the gall bladder concentrates the bile which enters it, as much as ten times in 24 hours, undoubtedly offers an explanation of the formation of gall stones that removes many of the problems that formerly existed. The mere passage of the bile through the gall bladder may concentrate it two to four times. The upper part of the cystic duct has the same property, but elsewhere the ducts secrete the clear fluid seen in "white bile," and have no power to concentrate the bile. Boyd[40] finds evidence that the gall bladder mucosa absorbs cholesterol, and hence failure of this function might also play an important role, and papillary masses saturated with cholesterol may serve as the nucleus for concretions.

Pigment Concretions.—It was formerly supposed that the calcium-pigment concretions were produced by the presence of excessive calcium in the bile, derived particu-

[34] This relation of hypercholesterolemia and infection to cholelithiasis is supported by the extensive observations of Rothschild and Wilensky (Amer. Jour. Med. Sci., 1918 (156), 239, 404, 564; Arch. Int. Med., 1919 (24,) 520), who find some types of cases accompanied by cholesterol increase, which is missing in many cases of inflammatory cholelithiasis. (See also Reimann and Magown, Surg. Gynec. and Obst., 1918 (26), 282; Fasiani, Arch. Sci. Med., 1918 (41), 144; Oliver).[36] Babarczy (Klin. Woch., 1922 (1), 1828) found hypercholesterolemia only for about one week after the attack.

[35] See D'Amato, Biochem. Zeit., 1915 (69), 353.

[36] Jour. Lab. Clin. Med., 1923 (8), 242.

[37] Fitz and Aldrich, Jour. Amer. Med. Assoc., 1922 (79), 2129.

[38] Hospitalstidende, 1915 (58), 249.

[39] Jour. Exp. Med., 1921 (34), 47.

[40] Brit. Jour. Surg., 1923 (10), 327; also Torinoumi, Ziegler's Beitr., 1924 (72), 456.

arly from lime-laden drinking-water, but it has been demonstrated that increase of calcium in the food does not cause an increase in the amount in the bile. The calcium content of the blood is so nearly constant that little change can be expected in the bile calcium from blood changes.[41] On concentrating bile, which contains both bilirubin and calcium, the free bilirubin separates out and not the calcium compound of bilirubin; and also, Naunyn found that the bile salts prevent precipitation of calcium-bilirubin, even when calcium salts are added in considerable amounts. Apparently it is the presence of positively charged protein substances that leads to the precipitation of this electro-negative substance from bile, and hence the formation of pigment calculi is also favored or initiated by inflammation of the bile tracts, particularly as most of the calcium salts seem to come from the mucous membrane;[42] later as we have seen, these pigment concretions often become covered with cholesterol derived from the injured epithelium, and the common mixed calculi are then formed. In view of the fact that much of the pigment in these calculi is composed of the oxidation products of bilirubin, especially *bilihumin*, it is possible that oxidation processes in the stagnating bile are important causes of the precipitation; Naunyn suggests that bacteria may be the cause of the oxidation. Pigment calculi are particularly important as the starting-point of the larger mixed calculi. Aufrecht[43] indeed, holds that gall-stone formation usually begins with particles of pigment that are expelled from the liver cells as such, and ordinarily are discharged into the intestine; if they make their way back into the gall-bladder they form the nuclei of concretions. It is possible, Naunyn believes, for the pigment to be later gradually replaced by cholestrol.

Urinary Calculi[44]

These differ from the bile concretions in two important respects: First, there is no evidence that any considerable part of their constituents may come from the walls of the cavities that contain them; they are usually deposited on account of an over-saturation of the urine, or on account of a change in composition of the urine, which renders them insoluble. Second, the composition of urinary calculi is usually less mixed than that of biliary calculi, although it is seldom, if ever, pure. Thus, Finsterer found but six concretions composed of only one substance, in a collection of 114 calculi. As with the bile, the chief constituent of the urine (urea) is so soluble that it never forms concretions, but only the less soluble minor constituents are thrown down. For the formation of calculi, however, it is not sufficient to have merely an excess of a substance in the urine, for we may have deposition of urates, phosphates, or uric acid in simple crystalline form without the formation of calculi.[45] A *nucleus* of some sort is present as well as a *binding substance*,[46] which is often mucus derived from the walls of the passages, although the center of the concretion most often consists of uric acid or urates.[47] As emphasized especially by the observation of Rosenow and Meisser,[48] infection

[41] See Dittrich, Zeit. ges. exp. Med., 1924 (41), 355.

[42] This commonly-held view is denied by Lichtwitz and Bock (Deut. med. Woch., 1915 (41), 1215), who found the calcium content of bile from fistulas to be from 65–84 mg. per liter, and in bladder bile to vary from 85 to 325 mg., but not according to the presence or absence of inflammation.

[43] Deut. Arch. klin. Med., 1919 (128), 242.

[44] General Bibliography given by Finsterer, Deut. Zeit. klin. Chir., 1906 (80), 414; and Lichtwitz.[6]

[45] The crystallography of urinary concretions is discussed by H. Nakano, Jap. Jour. Biochem., 1923 (2), 437.

[46] Hippocrates appreciated the existence and importance of the mucoid binding substance in urinary concretions (Schepelmann, Berl. klin. Woch., 1911 (48), 525).

[47] See Nakajima, Zeit. f. Urol., 1922 (16), 155.

[48] Jour. Amer. Med. Assoc., 1922 (78), 266; Arch. Int. Med., 1923 (31), 807.

undoubtedly plays an important role,[49] presumably in furnishing the nucleus and the binding substance.

Although the amount of colloidal material in urine is relatively small, yet it undoubtedly plays an important part in maintaining in solution the less soluble crystalloids, which are especially the urates and calcium oxalate.[50] Normal urine contains no colloids which form irreversible gels, and hence ordinary deposits can be readily dissolved, but in inflammatory conditions there appears fibrinogen which readily forms the irreversible fibrin, and conditions thus become favorable for the formation of concretions of any crystalloid with which the urine may be saturated or over-saturated at the time (Schade). Possibly other colloids may play a similar rôle. Aschoff and Kleinschmidt[51a] hold that most urinary calculi begin as primary calculi, formed independent of inflammation from excess of the main constituent (uric acid, oxalates, xanthine, but chiefly ammonium urate); this calculus forms the crystalline nucleus of the laminated secondary deposits of other substances, chiefly uric acid, oxalates, and phosphates, all being deposited without inflammation. The inflammatory formations consist chiefly of ammoniomagnesium phosphate and ammonium urate, usually deposited on a foreign body or a primary calculus. The extensive study of the microscopic structure of urinary calculi by Shattock,[51] shows also that a nucleus of cells or other organic material is, at least in uric acid calculi, extremely rare, the center being almost always a primary crystalline deposit from a supersaturated solution.

Calculi formed because of changes in the urinary composition independent of evident infection are often called "primary," in contradistinction to those arising from changes in composition brought about by infection and ammoniacal decomposition. Because of the injury produced by a primary calculus, infection frequently results, and then the primary calculus may become the nucleus of a secondary calculus; indeed, on account of the change of reaction, the crystalloids of the primary calculus may be dissolved out, and their place taken by the secondary deposit (*metamorphosed calculi*). Practically all calculi contain more or less phosphate, especially in the outer part, because of secondary infection with alkalinization.[52] Traces of many other elements may be present, Si and Al being sometimes found, especially in the urinary concretions of domestic animals.[53]

In structure, urinary calculi usually show both radiating and concentric lines of formation, and when the chief constituents are dissolved

[49] See Paul, Jour. Urol., 1923 (9), 345.

[50] See Keyser (Arch. of Surg., 1923 (6), 525) who produced calculi experimentally by feeding oxamid to animals, but considers a colloid protective mechanism responsible for the failure of normal urinary constituents to produce calculi ordinarily.

[51a] "Die Harnsteine," Berlin, 1911.

[51] Proc. Roy. Soc. Med., Path. Sec., 1911 (4), 110.

[52] Iwano, Jour. Biochem., Japan, 1923 (3), 67.

[53] See Gonnermann, Zeit. physiol. Chem., 1920 (111), 32; Schlicht, Pharm. Ztg., 1922 (67), 316.

away an organic framework remains. They are generally classified according to their prominent component, as follows:

Uric-acid Calculi.—Uric water is but slightly soluble, only one part dissolving in 39,480 of pure water at 18°, and it is even less soluble in the presence of acids.[54] The presence of sodium diphosphate in the solution makes it much more soluble, and various organic bodies also favor its solution, among them being the urinary pigments.[55] As can be seen, the maintenance of uric acid in solution is by a small margin, even in normal conditions; hence the mere cooling of the urine frequently suffices to cause an abundant deposition of uric acid combined with pigment, as the familiar "brick-dust" deposit. The formation of uric-acid calculi is, therefore, not only a question of the amount of uric acid in the urine, but depends even more upon the amount of the substances that hold it in solution, and as both these factors are subject to wide variations under both physiological and pathological conditions, uric acid and urates are common in urinary concretions.

The older literature indicates that the most common calculus is of this nature, but a number of recent analyses indicate that the importance of uric acid and urates has been overestimated. On the contrary, this material rarely forms a considerable part of the calculi, but is usually present in greater or less amount in most or all urinary calculi (Kahn).[56] It is probable, however, that uric acid is important as furnishing the primary nucleus of calculi of preponderatingly calcareous or mixed composition. Apparently there are marked differences in the prevailing composition of calculi in different countries; in China, for example, Pfister[57] found eleven of twelve calculi composed of uric acid.

Uric acid is eliminated combined chiefly with sodium, potassium, and ammonium; according to some authors, as a biurate, according to others, as a quadriurate. If the urine is excessively acid, it contains much acid phosphates, which withdraw part of the bases from the uric acid, and this, when free, crystallizes out if in excess. Hence the formation of uric-acid concretions is favored by high acidity of the urine, by concentration of the urine, or by an increased elimination of the uric acid. The last may result from excessive nuclein-rich food, or from excessive catabolism of the tissue nucleoproteins (*e. g.*, leucocytosis from inflammatory diseases or leukemia), which conditions are also usually associated with an increased urinary acidity. In chronic myelogenous leukemia the occurrence of renal calculi is so frequent as to suggest that, in this case at least, supersaturation of the urine with a poorly soluble substance may be adequate to lead to stone formation, perhaps independent of other factors.

[54] Concerning solubility of uric acid in urine see Haskins, Jour. Biol. Chem., 1916 (26), 205.

[55] According to Schade (Zeit. klin. Med., 1922 (93), 1) when uric acid comes out of a saturated solution there is an intermediary step in which it is colloidal, and this colloidal stage is important in permitting the existence of supersaturated solutions.

[56] Arch. Int. Med., 1913 (11), 92; review of literature. Rosenbloom, (Jour. Amer. Med. Assoc., 1915 (65), 161) found but two uric acid stones of twenty-six analyzed.

[57] Zeit. Urol., 1913 (7), 945.

(The chemistry of uric acid is discussed more fully in the chapter on Gout, Chap. XXV.)

Uric-acid calculi are formed chiefly in the pelvis of the kidney, but many pass into the bladder. They are quite hard, and yellow or reddish-yellow in color, because of the presence of *urochrome* and *urobilin*, the former of which seems to be chemically combined and the latter but physically, since it can be washed out with water. *Urærythrin* or *uromelanin* (a decomposition product of urochrome) may also be present. Not infrequently calcium oxalate is present, sometimes in considerable quantities. Other urinary constituents may be present in small amounts. In case the calculus enters the urinary bladder it may set up irritation leading to infection; the urine then becoming alkaline, calcium and ammonio-magnesium phosphate will be deposited upon the surface, and the uric acid will be more or less dissolved out and replaced by the phosphates (metamorphosis).

Urate calculi occur chiefly in new-born or young infants, and rarely in adults. In the young they are related to, and may originate in, the deposits of urates in the pyramids of the kidney (the so-called urate or uric-acid "infarcts"), which have been supposed to result from the decomposition of the nucleoproteins of the nucleated fetal red corpuscles. (See Uric Acid, Chap. XXV.) The concretions are composed chiefly of either ammonium or sodium urate, but potassium and even calcium and magnesium urate may be admixed. Their genesis in the young probably depends upon injury to epithelium by the excessive urates of the "infarcts," which affords a suitable nucleus for their start; their growth depends chiefly upon the concentration of the infant's urine. In adults they may arise secondary to an ammoniacal decomposition of the urine. Urate concretions are not common; they are generally rather soft, and often much colored by pigments.

Calcium oxalate calculi are, according to many observers,[56] the most common urinary concretions.[58] Often they show admixtures of urates or uric acid, which latter frequently constitutes the nucleus, and when urinary infection occurs they may in turn serve as the nucleus to phosphatic deposits. On account of the hardness and roughness of these stones they frequently cause bleeding, which may result in their being very dark in color and containing blood-pigment. They are usually first formed in the pelvis of the kidney, and arise chiefly in persons excreting excessive quantities of oxalic acid. Normally but about 0.02–0.05 gram of oxalic acid is eliminated daily in the urine, apparently all as calcium oxalate, which is kept in solution by the acid phosphates. The amount may be increased by certain foods rich in oxalates, particularly rhubarb, grapes, spinach, etc.; also probably by gastric fermentation.[59] Oxalic acid may possibly be formed from uric

[58] Concerning their structure see Fowler, Johns Hopkins Hospital Reports, 1906 (13), 507.

[59] Baldwin, Jour. Exp. Med., 1900 (5), 27.

acid, and perhaps also from the carbohydrate group of proteins,[60] and it is possible that abnormally large amounts arise from these sources under pathological conditions. During bacterial decomposition of the urine oxalic acid may be formed from uric acid (Austin).[61]

Phosphate calculi are formed as a result of decomposition of the urine, with formation of ammonia from the urea.[62] In the ammoniacal solution thus formed the magnesium is precipitated as NH_4MgPO_4, the calcium as $Ca_3(PO_4)_2$, and calcium oxalate and ammonium urate are also thrown down, so that the concretions consist of a mixture of these substances, the magnesium salt being the most abundant. In none does one substance occur in a pure state. Pigments of various kinds, and more or less mucus or other organic constituents of the framework are also present. Phosphate calculi are the typical "secondary" concretions, and they are formed usually in the bladder as a consequence of cystitis, but may be formed in the renal pelvis or in the urethra. In some cases the salts are precipitated in such large quantities that they form great masses of a sediment which does not aggregate into concretions. Occasionally stones consisting principally of $Ca_3(PO_4)_2$ or $CaHPO_4$ are formed, but these are rarities. As the calcium taken in the food is chiefly eliminated in the feces, the amount in the urine does not vary directly with the amount in the food, and the formation of phosphatic concretions is always a matter of urinary reaction and not of diet.[63] As these stones fuse to a black, enamel-like mass under the blow-pipe, they have been called "fusible calculi."

Calcium carbonate calculi are formed frequently in herbivora, but they are very rare in the urinary passages of man, although occurring elsewhere in the body not infrequently. Occasionally these are soft and chalky, but if well crystallized, they are the hardest of concretions.

Cystine calculi[64] are rare[65] but very interesting formations. Cystine $\begin{matrix} S\text{-}CH(NH_2)\text{-}COOH \\ | \\ S\text{-}CH(NH_2)\text{-}COOH \end{matrix}$ is important as the sulfur-containing portion of the protein molecule. Under normal conditions all the cystine taken in food is completely oxidized and none (or uncertain traces) appears in the urine. In certain individuals the urine contains considerable quantities of cystine constantly (*cystinuria*, see Chap. XXIII), and occasionally in these cases soft concretions of nearly pure cystine are formed in the urinary passages. Cystine calculi may reach the size of a hen's egg,

[60] See Austin, Boston Med. and Surg. Journal, 1901 (145), 181. Contradicted by Wegrzynowski, Zeit. physiol. Chem., 1913 (83), 112.

[61] Jour. Med. Research, 1906 (15), 314

[62] Under the name "struvit stone," Pommer (Verh. deut. Path. Gesell., 1905 (9), 28) describes a urinary calculus composed of very pure ammonio-magnesium phospate, forming the hard, rhombic crystals known to mineralogists as "struvit." This is an example of a phosphate stone formed independent of ammoniacal decomposition, a rare occurrence.

[63] Osborne (Jour. Amer. Med. Assoc., 1917 (69), 32) has observed numerous cases of formation of phosphate calculi in the urinary bladder of rats kept on diets deficient in fat soluble vitamins. The reason for this association of diet and concretions is not known; possibly the dietary deficiency causes lessened resistance to urinary infection.

[64] Literature concerning cystine, see Friedmann, Ergeb. der Physiol., 1902 (i), 15; Marriott and Wolf, Am. Jour. Med. Sci., 1906 (131), 197.

[65] Mörner found 47 cases of cystine calculi among 5292 cases of urinary calculi (Upsala Läker. Forhl., 1920 (25), 265).

are crystalline in structure, and in the urine of such patients the characteristic hexagonal crystals may usually be found. The cystine of calculi is identical with that from proteins and may be associated with tyrosine.[66] Since cystine is soluble in alkalies, the concretions may be prevented or redissolved by maintaining an alkaline urine and limitation of protein diet.[67] Sodium bicarbonate actually decreases the amount of cystine excreted in the urine.[68]

Xanthine Calculi.—Xanthine is the most abundant of the purine bases normally present in urine, but the total amount is extremely small. Like uric acid, it fluctuates in amount according to the amount of destruction of nucleoproteins, either of the food or of the tissues. Concretions consisting chiefly of xanthine, which is often mixed with uric acid, are extremely rare, but a few isolated specimens having been described. Rosenbloom could collect but six cases in the literature, adding one himself.[69]

Indigo calculi, derived from the indican of the urine through oxidation, have also been described a few times.

Urostealith calculi, composed of fatty matter, have been occasionally observed.[70] Although some of the concretions described under this head have really represented foreign bodies introduced through the urethra (*e. g.*, Kruckenberg's concretion of paraffin from a bougie),[71] yet true fat concretions do occur. The origin of the fat in these stealiths is unknown; possibly it comes from degenerated epithelium. Horbaczewski[72] analyzed such a specimen which had the following percentage composition:

Water	2.5
Inorganic matter	0.8
Organic matter (chiefly protein)	11.7
Fatty acids	51.5
Neutral fat	33.5
Cholesterol	traces

The fatty acids consisted of stearic, palmitic, and probably myristic acid.

Cholesterol calculi have been found in the urinary bladder in a few instances, the cause being unknown. Horbaczewski[72] describes one weighing 25.4 grams, found in a patient who had previously had cystine calculi; it contained 95.87 per cent. of cholesterol and but 0.55 per cent. of inorganic material. Gall-stones have been known to enter the urinary bladder through a fistula between the gall-bladder and urinary bladder.[73]

Fibrin "calculi," formed from blood-clots, often more or less impregnated with urinary salts, have occasionally been observed. Other proteins may also form similar calculi.[74]

General Properties of Urinary Concretions.[75]—The hardness depends partly upon the chemical composition of the calculus, but more upon the rate and condition of formation (Rowlands, Kahn). Under comparable conditions it is said that those composed of amorphous phosphates are the softest; next come those with some admixture of crystalline phosphates. Urate concretions are harder than these, but are still softer than uric acid and crystalline phosphate calculi. Oxalates are usually the hardest, except for the rare crystallized calcium carbonate stones. Cystine and amorphous concretions can be scratched with the

[66] Abderhalden, Zeit. physiol. Chem., 1907 (51), 391; 1919 (104), 129.
[67] See Crowell, Surg. Gyn. Obst., 1924 (38), 87; Jour. Urol., 1924 (11), 545.
[68] Looney *et al.*, Jour. Biol. Chem., 1923 (57), 515.
[69] N. Y. Med. Jour., Jan. 16, 1915.
[70] See Kropp, Deut. med. Woch., 1923 (49), 982.
[71] See also Turner, Jour. Urol., 1924 (11), 582.
[72] Zeit. physiol. Chem., 1894 (18), 335.
[73] See Finsterer, Deut. Zeit. klin. Chir., 1906 (80), 426.
[74] See Morawitz and Adrian, Mitt. Grenz. Med. u. Chir., 1907 (17), 579.
[75] Systems for procedure in determining the nature of urinary calculi are given by Hammarsten (Text-book of Physiol. Chem.) and by Smith (Reference Hand-book of Med. Sci.).

finger-nail, while even the hardest varieties of calculi can be scratched with a wire nail. Genersich[76] gives the following degrees of hardness for different calculi: Cholesterol, 1.5–1.6; ammonium urate, 2.5; soft phosphate (Mg) 2.6; hard phosphate (Ca), 2.75; uric-acid stones (also salivary and prostatic calculi, atheromatous patches, and phleboliths), 2.9; calcium oxalate (also rhinoliths and lung stones), 3.3–3.5; calcium carbonate stones of herbivora, 4.5. But the hardness or gross appearance of a urinary calculus give little or no indication of its chemical composition, and, as a matter of fact, all urinary concretions are of more or less mixed composition (Iwano).

The **rate of growth** also varies according to composition, but is, of course, much modified by other factors. Oxalate and urate stones grow most slowly, phosphate stones most rapidly. A urate stone has been known to increase by about two ounces during seven and one half years, while a catheter fragment or other foreign body may become covered with a crust several millimeters thick in a few weeks.[77]

Spontaneous disintegration of urinary concretions is limited almost solely to calculi composed entirely or largely of uric acid. Out of 121 cases collected by Englisch,[78] in all but 7 this was the case, these being composed of calcium and magnesium phosphate (5), or calcium phosphate or carbonate (1 each). The disintegration is brought about through solution of the binding substance and mechanical shattering of the stone into fragments. This occurs but rarely, Bastos[79] estimating that perhaps one calculus in ten thousand undergoes disintegration. Cystine calculi, however, may disintegrate when the urine remains persistently alkaline.

Corpora Amylacea[80]

In the case of these widely-spread concentric bodies we find the name misleading, for the bodies are not a form of animal starch, as was suggested by their laminated structure and iodine reaction, nor are they so closely related to amyloid material as the name implies. Different authors disagree decidedly concerning the staining reactions of these bodies, but it may be said that the reactions are extremely inconstant. Sometimes the corpora are stained bluish or green with iodine, sometimes brown, often little at all; occasionally they react partly with methyl-violet, but more often they do not; sometimes portions of one body react one way, while the remainder behaves differently. Seldom if ever do the ordinary concretions of the prostate give all the amyloid reactions characteristically, but the corpora amylacea of the lungs are much more likely to do so (Stumpf).[81] It seems improbable that these bodies, which occur in the prostate of every adult, can be the same as the amyloid, which is seldom observed except as the result of serious processes of tissue destruction. According to their structure they obey the usual laws of

[76] Virchow's Arch., 1893 (131), 185.
[77] Zuckerkandl, Nothnagel's System, vol. 19, pt. 2, p. 229.
[78] Arch. klin. Chir., 1905 (76), 961 (elaborate review).
[79] Folia Urol., 1913 (8), 81.
[80] General literature, Posner, Zeit. klin. Med., 1889 (16), 144; Lubarsch, Ergeb. allg. Pathol., 1894 (I_2) 180; Ophüls, Jour. Exp. Med., 1900 (5), 111; Nunokawa, Virchow's Arch., 1909 (196), 221; Brutt, *ibid.*, 1912 (207), 412.
[81] Virchow's Arch., 1910 (202), 134.

the formation of concretions, having a central nucleus and a structural framework of different composition from the chief substance. It seems most probable that they should be interpreted as simple concretions of protein nature,[82] which form under certain conditions when a nucleus of some sort (usually pigment, degenerated cells, or inorganic crystals) exists in a stagnating, protein-rich fluid. At times the resulting concretion may be of such a physical nature that it absorbs iodine readily (just as they often show a marked absorption-affinity for pigments), and occasionally it may react metachromatically with methyl-violet, possibly because of the presence of chondroitin-sulfuric acid derived from the mucin of the cavities where the concretions form, but perhaps for some other unknown reasons. Occasionally pure amyloid may form typically concentric (or even crystalline) bodies in the tissues, as in Ophül's case, but this is the exception. It seems probable that corpora amylacea are usually protein concretions,[83] and neither amyloid nor animal starch. Those formed in the central nervous system may be of myelin or neuroglia origin.[84]

The small amount of material available prevents an accurate analysis of the corpora amylacea; it is known that they are very insoluble in water, acids, alkalies, etc., behaving like coagulated protein in this respect. Even hot concentrated nitric acid will not dissolve them, according to Posner. This author considers lecithin and cholesterol to be important constituents, and by Ciaccio's staining method lipoids can be found in prostatic corpora amylacea.[85] However, it is said by Björling[86] that the ordinary hyaline and granular corpora do not contain fats or lipoids, but that a certain class of "lipoid" prostatic concretions contain many granules of this nature. The corpora amylacea of the lateral ventricles seem to consist chiefly of calcium salts deposited in a concentric arrangement through the medium of an organic basis. Posner considers that the presence of lecithin in prostatic corpora prevents their calcification, although this change occasionally does occur.

Other Less Common Concretions

Pancreatic Calculi.[87]—The cause of the formation of stones in the pancreatic duct is not definitely known, but apparently infection is the most important factor, since simple experimental stasis will not cause their formation.[88] The calculi consist usually of a mixture of calcium phosphate and carbonate, associated with more or less organic matter, frequently including cholesterol, but all the usual products of proteolysis may be present because of the presence of trypsin. Occasionally the cal-

[82] Protein crystals have been found in the thyroid follicles and in renal tubules (Rehsteiner, Cent. allg. Path., 1923 (33), 449).

[83] Ramsden's observations (Proc. Royal Soc., 1903 (72), 156) on the precipitation of proteins by the action of surface contact may have some bearing on the formation of such protein concretions.

[84] See Lafora, Virchow's Arch., 1911 (205), 295.

[85] Posner, Zeit. f. Urologie, 1911 (5), 722.

[86] *Ibid.*, 1912 (6), 30.

[87] Literature by Scheunert and Bergholz, Zeit. physiol. Chem., 1907 (52), 338. Möckel, Frankf. Zeit. Path., 1920 (24), 78.

[88] See Lazarus, Zeit. klin. Med., 1904 (51), 530. Literature.

culi consist chiefly of calcium carbonate, which may be almost pure.[89] Shattock[90] has observed a pancreatic concretion composed of calcium oxalate. Sodium phosphate and chloride, magnesium phosphate, and proteins have also been found in these concretions. Taylor[91] describes a pancreatic concretion containing, according to the analyst, chiefly silicate (!), a finding difficult to understand or accept.

Baldoni[92] found, on analysis of a stone weighing 3.1 grams, the following percentage composition:

Water	3.44
Ash	12.67
Proteins	3.49
Free fatty acids	13.39
Neutral fatty acids	12.40
Pigments and soap	40.91
Cholesterol	7.69
Undetermined	6.01

Usually, however, pancreas stones consist chiefly of inorganic substances. Johnson and Wollaston report analyses of two stones, one containing 72.30 per cent. calcium phosphate and but 8.80 per cent. organic matter; the other 91.65 per cent. calcium carbonate, 4.15 per cent. magnesium carbonate, and but 3 per cent. organic matter. Legrand[93] found only 0.7 per cent. organic matter in another concretion which contained 93.1 per cent. calcium carbonate. Pancreatic juice being strongly alkaline, can hold but a small quantity of calcium salts in solution (normally but 0.22 part per thousand—C. Schmidt); presumably the little normally present is held in the form of a colloidal suspension by the proteins. Possibly when stasis occurs, digestion of the proteins leads to the precipitation of the calcium salts, or more probably, the excessive calcium is largely derived from the exudate from the inflamed ducts, as seems to be the case with the calcium of biliary calculi.

Salivary Calculi.[94]—These have a similar composition, in the main, to the concretions of the pancreatic duct, except that they generally contain more organic matter, resembling in this respect the "tartar" of the teeth.[95] Bessanez found in one 81.3 per cent. of calcium carbonate and 4.1 per cent. of calcium phosphate, whereas in another the carbonate was but 2 per cent. and the phosphate 75 per cent. Potties has described a calculus with a central portion composed chiefly of uric acid and a peripheral portion containing 69 per cent. of calcium phosphate and 20.1 per cent. of calcium carbonate. Harlay[96] found in one specimen 15.9 per cent. organic matter, 75.3 per cent. calcium phosphate, 6.1 per cent. calcium carbonate. Hopkins[97] has reported one composed of calcium oxalate. Irvine's[98] specimen was of phosphates chiefly, while Prouzerque[99] gives as the composition in three cases, calcium phosphate, 55–68; calcium carbonate, 6–7.35; organic matter, 21–75 per cent. Roberg believes that bacteria alone do not usually cause salivary calculi to form, but that a foreign body entering the duct is the chief factor. Increased alkalinity may also favor precipitation of calcium from the saliva. In Roberg's case of sialolithiasis the saliva was of normal composition.

The deposit which forms on the teeth, and which dentists also call salivary calculus, is composed, according to an analysis by Schehevetsky,[1] of 67 parts $Ca_3(PO_4)_2$, 8 of $CaCO_3$, 1.5 of CaF, and 22 part per 100 of water and organic matter. Black believes that the deposit is first in the form of spherules of calcium salts combined with globulin.

[89] Rosenthal, Arch. f. Verdauungskr., 1914 (20), 619.
[90] Brit. Med., Jour. 1896 (i), 1034.
[91] Lancet, Dec. 18, 1909.
[92] Schmidt's Jahrb., 1900 (268), 210.
[93] Jour. Pharm. et Chim., 1901 (14), 21.
[94] Literature, see Roberg, Annals of Surgery, 1904 (39), 669.
[95] Particles of gold have been found in a salivary calculus by Maurin (Repert. pharm., 1919 (30), 257), presumably derived from fillings.
[96] Jour. Pharm. et. Chim., 1903 (18), 11.
[97] Boston Med. Surg. Jour., 1921 (185) 378.
[98] Canadian Med. Assoc. Jour., 1924 (14), 392.
[99] Arch. de Méd. et pharm. mil., 1911 (58), 125.
[1] Quoted by G. V. Black, "Special Dental Pathology," Chicago, 1915.

Intestinal Concretions.—These always have a nucleus of some indigestible foreign substance, most often hair, but sometimes cellulose structures or solid indigestible particles, including gall-stones, fruit-stones, bone, etc. The bulk of the concretions is usually made up chiefly of ammonio-magnesium phosphate, with some calcium phosphate, carbonate, and sulfate, protein matter, and occasionally calcium and magnesium soaps. Two intestinal concretions analyzed by Schuberg[2] had the following percentage composition when dried:

Ammonio-magnesium phosphate	57.1	63.9
Calcium phosphate	15.7	23.8
Calcium carbonate		4.6
Calcium sulfate	3.0	0.7
Alcohol-ether extract	1.9	0.8
Other organic substances	21.5	6.0

In countries where oatmeal is largely eaten, intestinal concretions are not infrequent; they contain calcium and magnesium phosphate, about 70 per cent.; oatmeal bran, 15–18 per cent.; soaps and fats, about 10 per cent. (Hammarsten). Enteroliths containing albumin in abundance, attributed to eating bran bread[3]; and of bismuth[4] from "bismuth meals" have been reported. Choleic acid is occasionally a conspicuous constituent.[5] Occasionally concretions consisting largely of fats and soaps are found, and after taking large doses of olive oil masses of solidified oil may be passed that are readily mistaken for softened gall-stones, for the removal of which the oil is usually given. The "fecal stones" found in appendices often show the structure of calculi, and, unlike other enteroliths, consist less of ammonio-magnesium phosphate than of calcium salts[6], soaps may be important constituents.[7] A series of such appendiceal concretions were reported by Maver and Wells[8] to contain about one-half their weight of fat-soluble materials, chiefly soaps and koprosterol, about one-fourth inorganic, chiefly calcium phosphate. A pure cholesterol stone has been found in an appendix.[9]

Bezoar stones are intestinal concretions probably coming from *Capraægagrus* and *Antelope dorcas*. One variety consists chiefly of *lithofellic acid*, $C_{20}H_{36}O_4$, which is related to cholalic acid, and gives an aromatic odor when heated. The other variety ("false bezoars") does not give the aromatic odor, and consists chiefly of *ellagic acid*, $C_{14}H_6O_8$, a derivative of gallic acid, and, therefore, probably derived from the tannin of the food of the antelopes. They often have foreign bodies as the nucleus.

Intestinal "sand" occurs as (1) "false sand," consisting of particles of indigestible food, such as the sclerenchymatous particles in the flesh of pears and bananas;[10] and (2) true sand, consisting largely of inorganic material, and formed, according to Duckworth and Garrod,[11] in the upper part of the large intestine. Analyses of specimens by Garrod showed the following composition:

Water	12.4	calcium oxide	54.98
Organic material	26.29	phosphorus pentoxide	42.35
Inorganic material	61.31 containing	carbon dioxide	2.20
		traces of Mg, Fe, etc	0.47

Analyses by other observers have given similar results, the absence of the large proportion of magnesium found in larger concretions being striking. Williams[12] found that about half the calcium was in the form of soaps of palmitic and stearic acids. The color is usually brown, due chiefly to urobilin, unaltered bile-pigments being scanty.

[2] Virchow's Arch., 1882 (90), 73.
[3] Gonnermann, Biochem. Zeit., 1918 (88), 403.
[4] Mülleder, Zent. f. Chir., 1923 (50), 384.
[5] Raper, Biochem. Jour., 1921 (15), 49; Mörner, Zeit. physiol. Chem., 1923 (130), 24.
[6] Harlav, Jour. pharm. et chim., 1910 (2), 433.
[7] Williams, Biochem. Jour., 1907 (2), 395.
[8] Arch. Surg., 1921 (3), 439.
[9] Navarro, Anales Fac. Med. Montevideo, 1922 (7), 307.
[10] Myer and Cook, Amer. Jour. Med. Sci., 1909 (137), 383.
[11] Lancet, 1902 (i), 653. Full résumé and literature.
[12] Brit. Med. Jour., July 27, 1907.

Preputial concretions sometimes form beneath a prepuce that cannot be retracted, through deposition of urinary salts on and in the accumulated smegma.[13] The composition is, therefore, very mixed, and consists of an organic base containing much cholesterol, fats, and soaps, incrusted with inorganic substances, of which ammonio-magnesium phosphate and calcium phosphate are usually the most abundant.

Prostatic concretions originate in the corpora amylacea through growth accretion of inorganic salts, until they may reach considerable size. Stern[14] gives the following results of analysis of such a prostatic stone: Water, 8.0; organic matter, 15.8; lime, 37.64; magnesia, 2.38; soda, 1.76; potash, 0.5; phosphoric acid, 33.77; iron, trace. Another specimen analyzed by Macallum[15] had the following composition: $Ca_3(PO_4)_2$, 30.39; $Mg_3(PO_4)_2$, 20.48; organic, 46.49; the high Mg content indicates that this concretion was largely deposited from the urine.

Lung Stones.[16]—These may be formed in the bronchi, through accretion about an inorganic nucleus, similar to the formation of calculi in other epithelial-lined passages; or they may consist of calcified areas of lung tissue or peribronchial glands, which have been sequestrated through suppuration and have entered the bronchi. In the latter case, the calculi present the usual composition of pathological calcified areas. That the expectorated stones frequently represent calcified tubercles is shown by Stern[16] and by Bürgi,[16] who demonstrated tubercle bacilli in decalcified lung stones. The following percentage figures are taken from Ott:[17]

Calcium phosphate	52.0	72.8
Magnesium phosphate		1.0
Magnesium carbonate	2.0	
Calcium carbonate	13.0	6.0
Fat and cholesterol	24.0	7.0
Other organic substances	4.0	10.0

Scherer,[18] and Maver and Wells,[19] have also found that these concretions have much the same composition as calcified lesions generally, *i. e.*, Ca phosphate and carbonate in about the same ratio as in bone; silica when present represents inhaled dust.[20]

Rhinoliths[21] are formed about nasal secretions, blood-clots, and most frequently about foreign bodies. They therefore contain much organic substance in addition to the inorganic salts deposited upon them.[22] Berlioz,[23] gives the following table from the analysis of four specimens:

Weight of specimens, grams	1 3.75	2 1.34	3 0.63	4 0.95
Water	5.80	5.10	4.00	6.90
Organic matter	16.60	18.20	16.00	18.10
Calcium phosphate	62.02	60.61	61.40	47.63
Magnesium phosphate	5.08	6.28	3.93	6.68
Calcium carbonate	10.50	9.81	14.67	20.69
Traces of iron	Doubtful.	Distinct.	Doubtful.	Distinct.

[13] See Zeller, Arch. klin. Chir., 1890 (41), 240.

[14] Amer. Jour. Med. Sci., 1903 (126), 281.

[15] Reported by MacKenzie and Seng, Canad. Med. Assoc. Jour., 1924 (14), 912.

[16] Literature. Poulalion, Thesis, Paris, 1891; Stern, Deut. med. Woch., 1904, (30), 1414. Bürgi, Deut. med. Woch., 1906 (32), 798; Gerhartz and Strigel, Beitr. z. klin. Tuberc., 1908 (10), 33.

[17] "Chem. Path. der Tuberc.," 1903, p. 92.

[18] Beit. klin. Tuberc., 1921 (49), 17.

[19] Amer. Rev. Tuberc., 1922 (6), 649.

[20] See also Alpers, Arch. Pharm., 1923 (261), 172.

[21] Literature, Scheppegrell, Jour. Amer. Med. Assoc., 1896 (26), 874; Gerber, Deut. med. Woch., 1892 (18), 1165.

[22] One analyzed by Debucquet, (Jour. pharm. chim., 1922 (25), (305) contained 79.5 per cent. $Ca_3(PO_4)_2$, 10.7 per cent. $CaCO_3$, insoluble residue 0.65 per cent.

[23] Jour. Pharm. et. Chim., 1891 (23), 447.

Tonsillar concretions consist chiefly of carbonate and phosphate of calcium deposited upon the inspissated secretions and desquamated cells of the tonsillar crypts.[24] According to some authors, leptothrix threads frequently form the nucleus of the concretions.

Tartar or "salivary calculus," shows a variable composition because of the amount of organic material present. Prinz[25] gives as the average of five analyses of human material, calcium phosphate, 63.42; calcium carbonate, 9.14; magnesium phosphate, 1.31; organic 15.16 per cent. Presumably the tartar is formed by precipitation of calcium in the secretions of the salivary and buccal glands because of loss of the CO_2 which keeps it in solution.

Cutaneous concretions are occasionally observed, located chiefly in the subcutaneous tissue, often occurring multiple. The origin is possibly in dilated sebaceous glands with retained secretions. Unna considers that calcium soaps are formed as a first step, but an analysis of such material by Harley[26] showed 87.2 per cent. of ash, 12.8 per cent. organic matter, 0.9 per cent. of fat; calcium phosphate constituted 65.2 per cent., and calcium carbonate 16.4 per cent. Gascard[27] found in similar material 23.4 per cent. organic matter, and of the inorganic matter, 91.1 per cent. was calcium phosphate, and 8.9 per cent. calcium carbonate.

Gouty deposits observed in the subcutaneous tissues, as well as along the tendons, articular cartilages, etc., consist usually of nearly pure biurate of sodium and potassium. Ebstein and Sprague[28] found the composition of such material to be as follows:

Uric acid	59.70
Tissue organic matter	27.88
Sodium oxide	9.30
Potassium oxide	2.95
Calcium oxide	0.17
MgO, Fe, P_2O_5, S	traces

After a time, however, calcium salts may be deposited, and Dunin[29] has observed deposits resembling gouty tophi that were merely calcium salts.

PNEUMONOKONIOSIS[30]

In a number of cases of the different forms of this condition quantitative analyses have been made, which may be briefly discussed as follows: Not only does the lung of every adult contain considerable amounts of coal-pigment stored up in the connective tissues (and also in the peribronchial glands), but also, which is perhaps less generally appreciated, considerable quantities of silicates are also present (chalicosis) from inhaled dust. Woskressensky[31] found silicates in all of 54 lungs examined, except two from infants. The lungs of individuals whose occupations do not expose them especially to dust inhalation contain increasing amounts of silicates in direct proportion to age; the silicates constitute then from 3.5 to 10 per cent. of the total ash of the lungs. There is always a larger proportion of silicates in the peribronchial glands than in the lungs, constituting from 6 to 36 per cent. of the

[24] McCarthy, Brit. Med. Jour., Oct. 28, 1911.
[25] Dental Cosmos, 1921 (63), 231, full review.
[26] Jour. Pharm. et Chim., 1908 (18), 9.
[27] *Ibid.*, 1900 (12), 262.
[28] Virchow's Arch., 1891 (125), 207.
[29] Mitt. Grenzgeb. Med. u. Chir., 1905 (14), 451; also Kahn, Arch. Int. Med., 1913 (11), 92; and M. B. Schmidt, Deut. med. Woch., 1913 (39), 59.
[30] Reviewed by Landis, Jour. Indust. Hyg., 1919 (1), 117; Wells, "Chemistry of Tuberculosis," Baltimore, 1923, p. 156.
[31] Cent. f. Path., 1898 (9), 296.

ash, corresponding with Arnold's observation that in gold-beaters the glands contain more metal than the lungs. In stone-workers Schmidt found a higher proportion of SiO_2 in the lungs than in the glands. In normal adults the amount of coal-pigment is greater than the amount of silicates; in children the reverse is the case.

Thorel[32] reports that the lungs of a worker in soapstone contained 3.25 per cent. of ash, including 2.43 per cent. of soapstone.

In *siderosis* iron has been found in the lungs in proportions varying from 0.5 per cent. to 7.9 per cent. of the dry weight, the last amount having been found by Langguth[33] in the lungs of an iron miner, which contained also 11.92 per cent. of SiO_2.

An analysis of a lung from a knife-grinder is reported by Hodenpyl,[34] which gave the following results: Total weight of dried and powdered lung, 48.1009 grams; total solids, 44.7986; ether-soluble substance, 14.6017. Composition of the ether-soluble substance: free fatty acids, 7.498; neutral fats, 4.044; cholesterol, 3.037. Proteins, 15.4759; charcoal (total carbon less protein carbon), 7.198; ash, 4.2903. The composition of the ash (in grams) was as follows: K_2O, 0.2167; Na_2O, 0.3523; CaO, 0.0965; Fe_2O_3, 0.0879; Al_2O_3, 1.4628; SO_3, 0.0704; P_2O_5, 0.9565; SiO_2, 1.2043. The amount of emery, represented by the oxides of aluminum and silicon made up more than one-half of the ash, and the iron constituted about one-fourth. The man had worked at the trade of knife-grinder for about fifteen years.

McCrae[35] has analyzed the lungs of six gold mine workers, in South Africa, finding from 9 to 21.7 grams of ash per lung, of which 29 to 48 per cent. was silica; aluminum was also high, and an increased P_2O_5 content was ascribed to the accompanying fibrosis. Klotz[36] found from 1.2 to 5.3 grams of free carbon in each lung, of dwellers of Pittsburg, as contrasted with 0.145 and 0.405 grams found in the lungs of residents of Ann Arbor. Hirsch[37] analyzed four average Chicago lungs, finding in grams per lung:

	I	II	III	IV
Carbon	2.72	0.71	1.20	0.19
Silica	0.18	0.28	0.69	0.04
Calcium oxide	0.45	0.12	0.02	0.05

It is remarkable how little damage a large amount of coal dust may cause, and the harder, sharp edged stone dusts are much more injurious. Böhme[38] says that coal dust does not cause induration of the lungs unless it is over 1.4 per cent. of the fresh weight, whereas half this amount of

[32] Ziegler's Beitr., 1896 (20), 85.
[33] Deut. Arch. klin. Med., 1895 (55), 255.
[34] Medical Record, 1899 (56), 942.
[35] "The Ash of Silicotic Lungs," John McCrae, Johannesburg, 1914.
[36] Amer. Jour. Publ. Health, 1914 (4), 887. General review on anthracosis.
[37] Jour. Amer. Med. Assoc., 1916 (66), 950.
[38] Klin. Woch., 1924 (3), 1909.

stone dust causes marked fibrosis. He gives the following analytical results:

	H_2O	Dry wt.	Ash	Coal dust fraction	Sand
Normal lung	83.88	16.12	0.78	0.10	0.02
Miners' lungs not showing pneumonoconiosis	83.92	16.08	1.10	0.30	0.08
	78.7	21.3	1.70	1.81	0.37
Lungs of miners who died with a severe pneumonoconiosis:					
Severely diseased portion	77.78	22.22	2.05	1.67	0.73
	78.37	21.53	2.76	2.35	1.36
	73.90	26.10	4.23	7.72	2.28
Mildly diseased portion	80.5	19.5	1.11	0.50	0.21
	82.15	17.85	1.47	0.54	0.22
	82.65	17.35	1.56	not detd.	3.35

CHAPTER XX

PATHOLOGICAL PIGMENTATION[1]

MELANIN[2]

Melanin occurs normally as the coloring-matter of hair, of the choroid of the eye, of the skin, in the pigment matter of many lower animals, and most strikingly as a defensive substance in the "ink" ejected by squids to render themselves invisible in the water. Pathologically, melanin occurs chiefly as the result of an excessive production of this pigment by cells normally forming it, as in freckles, melanotic tumors and perhaps in Addison's disease. Cells that do not normally form melanin probably do not acquire this power in pathological conditions. Pathological failure to form melanin is also observed, as in skin formed in the healing of wounds and after syphilitic lesions; or in *albinism*, in which the failure to form melanin may be attributed to hereditary influences.[3] Occasionally in domestic animals, especially in calves, a congenital melanosis is observed involving many parts of the body.[4] A melanin or some similar pigment may be found in nerve cells (*e. g., substantia nigra*), and Dolley[5] believes it to be a result of nuclear metabolism under conditions of depression. The function of melanin is evidently that of protection from light rays,[6] and Young[7] has found that isolated melanin from human skin absorbs violet and ultra-violet rays. Probably this protection is responsible, at least in part, for the relative infrequency of skin cancers in the colored races.[8]

Melanin seems always to be produced through metabolic activity of specialized cells, and old theories concerning its origin from hemoglobin have been abandoned.

Composition of Melanin.—The elementary composition of different specimens of melanin examined by various observers has been found to vary greatly. This

[1] Literature by Oberndorfer, Ergebnisse Pathol., 1921 ($19_{(2)}$)47.

[2] Literature and résumé given by Fürth, Handb. d. Biochem., 2nd Ed., 1924, Bd. 1, p. 944; Wien. med. Woch., 1920 (70), 229.

[3] See Magnus, Norsk Mag. Lægevid., 1922 (83), 509. Gortner holds that dominant whites are due to the presence of antioxidase, while regressive whites have neither the power to form pigments nor to inhibit their formation (Amer. Naturalist, 1910 (44), 497).

[4] See Caspar, Ergebnisse allg. Path., 1896 (III_2), 772; Jaeger, Virchow's Arch., 1911 (204), 430; Schnackers, Frankf. Zeit. Path., 1909 (3), 447.

[5] Science, 1919 (50), 190.

[6] See Weidenreich, Zeit. Morph. u. Anthropœ., 1912 (Sonderh. II), 59.

[7] Biochem. Jour., 1914 (8), 460.

[8] However, Hanawa found white areas in skin less affected by chemical irritants and infections than dark areas. (Dermatol. Zeit., 1913 (20), 761.) This is not in agreement with most observers who have found pigmented skin more resistant. (See Hanzlik and Tarr, Jour. Pharm., 1919 (14), 221.)

probably depends on three factors: First, it is extremely difficult to obtain melanin in a pure condition; second, the process of purification requires the action of strong acids and alkalies, which undoubtedly modify the composition of the melanin; thirdly melanin is probably not a single substance of definite composition, but includes several related but different bodies. The values found vary for carbon from 48.95 to 60.02 per cent.; for hydrogen from 3.05 to 7.57 per cent.; for nitrogen, 8.1 to 13.77 per cent.[9] Hofmeister gives, as a characteristic of melanins, that their elementary molecular composition is always nearly in the proportions N:H:C = 1:5:5.

Gortner's[10] studies led him to accept the general principle that melanin is formed through the action of an oxidase on an oxidizable chromogen, but that in keratinous structures there exist at least two types of melanins, one, a "melanoprotein," soluble in dilute acids and existing dissolved in the keratins; the other, insoluble in dilute acids, exists as pigment granules and is of unknown nature. Those whose studies of melanin formation have been made with the microscope, state that the nucleus is active in the process,[11] and some find the melanin so closely related to the lipoids that they consider it a lipochrome.[12]

A particularly prominent constituent of some melanins is sulfur, which has been found in as high proportion as 10 per cent. in melanin from sarcomas, and even 12 per cent. in sepia from the squid; in melanin from hair the sulfur is usually about 2–4 per cent.; but in choroid melanin, and in some other forms, sulfur seems to be absent. The proportions of sulfur obtained from the same specimen purified by different methods show wide variations, and hence Fürth considers that neither the sulfur nor the iron are indispensable constituents of the melanin.[13] Probably the melanin molecule contains atom-complexes that have a tendency to bind certain sulfur and iron compounds (*e. g.*, cystine or hematin derivatives). Brahn[14] found that melanin is very similar in elementary composition to melanoidin from keratin, and believes it to be 3, 4-dihydroxyphenylcysteine. The studies of Salkowski and his students go to show that sulfur is indeed a true constituent of melanin, and that there are several melanins.[14a]

There is much reason to believe that the melanin is derived from certain groups of the protein molecule that seem readily to form colored compounds. The aromatic compounds of the protein molecule, such as tyrosine, phenylalanine, and tryptophan, readily condense with elimination of water and absorption of oxygen, to produce dark-colored substances. When proteins are heated in strong hydrochloric acid, we obtain a dark-brown material, which closely resembles the melanins both in elementary composition and in general properties, so that it is referred to as "artificial melanin" or "melanoid substance." These substances, like the natural melanins, when decomposed by fusing with caustic potash, yield skatole, indole, and pyrrole derivatives, which are undoubtedly derived from the tyrosine and tryptophan of the protein molecule. Therefore, it seems probable that both the melanoid substances and the true melanins are formed from the chromogen groups of the protein molecule through processes of condensation, elimination of water, and the taking up of oxygen.[15]

In the sepia sacs of the cuttle-fish, in meal-worms which form a melanin-like pigment, and in plants that produce the black Japanese lacquer, have been found *oxidizing enzymes* that have the property of producing black pigment by their action upon tyrosine and other aromatic compounds. Neuberg[16] found that extracts of a melanosarcoma of the adrenal could produce pigment from epinephrine and β oxyphenylethylamine, but not from tyrosine. The ink sacs of the squid contain an enzyme forming a pigment from epinephrine, apparently through oxidation and condensation. Winternitz[17] found in the hog uvea and in the black cutaneous tissue

[9] See Heinlein, Biochem. Zeit., 1924 (154), 24.

[10] Biochem. Bulletin, 1911 (1), 207; résumé.

[11] Staffel, Verh. Deut. Path. Ges., 1907 (11), 136; Schultz, Jour. Med. Res., 1912 (26), 65.

[12] Dyson, Jour. Path. and Bact., 1911 (15), 298; Kreibich, Wien. klin. Woch., 1911 (24), 117.

[13] Salkowski says that melanin contains phosphorus (Virchow's Arch. 1922 (240), 353).

[14] Virchow's Arch., 1920 (227), 137.

[14a] Reviewed by Herold, Med. Klin., 1922 (18), 184.

[15] See Herzmark and von Fürth, Biochem. Zeit., 1913 (49), 130.

[16] Zeit. f. Krebsforsch., 1909 (8), 195.

[17] Arch. Dermatol., 1918 (126), 252.

of the horse, an enzyme causing darkening of tyrosine. These enzymes may, therefore, possibly be responsible for the production of melanin in animal tissues, by causing oxidative changes in the chromogen groups of the protein molecule that are liberated by autolysis (see "Tyrosinase").[18] Fürth urges strongly the view that both normal and pathological melanin formation depend upon the action of the tyrosinase or allied enzymes in conjunction with autolytic enzymes; the latter split free from the chromogen groups of the protein molecule, which are then oxidized by the tyrosinase, undergo condensation, and take up sulfur-and iron-holding groups and also other organic compounds, the entire complex forming the melanin. In support of this, Hartwell[19] reports that the more tyrosine and tryptophan there is in the diet of rats the darker their coats become.

Bruno Bloch[20] has found that the occurrence of melanin in the skin corresponds to the location of cells with the capacity of oxidizing 3.4-dioxyphenylalanine, which is closely related in structure to epinephrine, and which he believes may be the usual antecedent of melanin. He has found this oxidizing property exhibited by the dark patches in variegated animals, but not by the white areas; the pigmented ocular structures do not oxidize this substance.[21] However, Lignac[22] found that skin long preserved in alcohol and formalin, or boiled for ten minutes, shows development of pigment when exposed to quartz light, wherefore he questions the significance of specific enzymes in the production of melanin. He believes the antecedent of melanin to be a dioxybenzene derivative, and that the melanin is formed by polymerization of quinoidines, these processes being accomplished by the action of light itself. Lemmel[23] also found the so-called "dopa" reaction of Bloch more active in boiled than in fresh tissues, and questions its enzymatic nature. Others have found that injection of pyrrole produces in animals a melanuria resembling that seen in persons with melanosarcoma[24] and therefore maintain that melanins are merely simple cyclic complexes of the pyrrole nucleus.[25]

Properties of Melanin.—When isolated in a pure condition, melanin is a dark-brown substance of amorphous structure, no matter how black the material from which it is derived may be.[26] It is quite insoluble in all ordinary reagents except alkalies, in which some melanins dissolve easily, and some with difficulty. Strong boiling hydrochloric acid scarcely affects melanins. By the action of sunlight or oxidizing agents on melanin-containing sections the pigment can be bleached out. The chief decomposition-products formed on fusing with alkalies are indole, skatole, and "melanic acid;" no cystine, leucine, tyrosine, or other amino-acids can be isolated. Most authors, therefore, consider the melanins as heterocyclic compounds standing in some relation to the indole nucleus.

If melanin is injected subcutaneously into animals (rabbits and guinea-pigs), there appears in the urine a substance which turns dark

[18] Postmortem incubation of skin at 56° C. causes pigment to increase (Neubürger, Munch. med. Woch., 1920 (67), 74).

[19] Biochem. Jour., 1923 (17), 547.

[20] Bloch and Ryhiner, Zeit. exp. Med., 1917 (5), 179; Zeit. physiol. Chem., 1917 (100), 226.

[21] Rondoni (Sperimentale, 1920 (74), 157) also found that black rabbit skin oxidizes pyrrole more than white skin. The absence of "dopa" oxidation in unpigmented tissues is attributed to their greater acidity, by Przibram (Arch. Entwickl. Organ., 1921 (48), 140).

[22] Virchow's Arch., 1923 (240), 383.

[23] Cent. allg. Path., 1921 (32), 89.

[24] See Angeli and Pierone, Atti accad. Lincei, 1921 (30), 241.

[25] Saccardi, Biochem. Zeit., 1922 (132), 443.

[26] Spiegler (Hofmeister's Beitr., 1903 (4), 40) isolated from white wool a white chromogen, closely related to melanin chemically, but Gortner (Amer. Naturalist, 1910 (44), 497) believes this to be a decomposition product of keratin, unrelated to melanin.

brown after the urine has stood for some time (Kobert, Helman). The pigment is apparently reduced, particularly by the liver, to a colorless melanogen, which is eliminated in the urine. The same process occurs when melanin is produced in excess and enters the blood, as in the case of melanosarcoma, a colorless melanogen being formed which is excreted in the urine, constituting "melanuria." Occasionally the urine is dark when first passed, because of the presence of melanin, but usually it must be subjected to oxidizing agencies (bromine water, nitric acid, hypochlorites, etc.), or exposed to air to bring out the brown color. Helman[27] says that true melanogen may be considered to be present in urine: (1) If the careful addition of ferric chloride causes the development of a black precipitate. (2) If this precipitate dissolves in sodium carbonate, forming a black solution. (3) If from this solution mineral acids precipitate a black or brownish-black powder. All three reactions must be obtained, for substances other than melanin may give the first two. Expecially to be distinguished are alkaptonuria, chronic intoxication with phenols, and some cases of extreme indicanuria.[28] In support of the view that tryptophan is the mother substance of melanin is the fact that feeding tryptophan to melanurics increases the melanin excretion (Eppinger).[37]

The coloring power of melanin is very great, for urine containing but 0.1 per cent. of melanin has the color of dark beer (Hensen and Nölke), and the entire skin of a negro contains only about 1 gram of melanin (Abel and Davis).[29] Excessive quantities of melanin may be in part deposited in the lymph-nodes and skin, causing diffuse pigmentation; it may be deposited in the endothelium lining the blood-vessels. Kobert injected melanin into albino rabbits, but did not succeed in getting any deposition in the choroid or skin. Helman[27] found some evidence of toxicity when large doses of melanin dissolved in sodium carbonate were injected into animals, but this is possibly due to the alkali rather than to the melanin.

Melanotic Tumors.[30]—Tumor melanin does not differ from melanin produced by normal cells in any essential respect. Usually it contains much sulfur, even as much as 10 per cent., yet Helman in eight specimens found but four that contained both sulfur and iron, in three only sulfur, in one only iron and no sulfur; therefore, tumor melanins show the same variations in composition as do normal melanins. Iron is frequently found microscopically in the pigment in melanosarcoma, but this is chiefly due to admixture of blood-pigment coming from extrav-

[27] Cent. f. inn. Med., 1902 (23), 1017; Arch. internat. Pharmakodynam., 1903 (12), 271.

[28] Melanuria fully discussed by Feigl and Querner, Deut. Arch. klin. Med., 1917 (123), 107.

[29] Jour. Exp. Med., 1896 (1), 361.

[30] Under the title *Acanthosis Nigricans* (see Pollitzer, Jour. Amer. Med. Assoc., 1909 (53), 1369) is included a group of cases of widespread cutaneous pigmentation with papillary hypertrophy, commonly associated with cancer, most often abdominal. While ascribed to action of the sympathetic nervous system injured by the cancer, this explanation is far from satisfactory, and the possibility of metabolic pigmentary disturbance must be considered.

asations of blood. The peculiar fact that melanosarcoma is very common in white or gray horses, but very seldom occurs in dark-coated horses, has not been explained. The frequent occurrence of melanuria and melanemia in patients with melanosarcoma is not due to any peculiar property of sarcoma melanin, but to the enormous quantity of melanin that is produced by the tumor and set free in the degenerating portions. Thus, while Abel and Davis[29] estimate that there is only about 1 gram of melanin in the entire skin of a negro, Nencki and Berdez have obtained from a sarcomatous liver 300 grams of melanin, and estimate that in this case the entire body contained 500 grams. Helman[27] states that the melanin may constitute 7.3 per cent. by weight of the fresh substance of some melanosarcomas.

Melanuria is not ordinarily observed unless there is extensive development of the tumors, and is seen in only about 20 per cent. of cases of melanosarcoma, chiefly when the liver is extensively involved. Formerly the excretion of true melanin or melanogen was supposed to be diagnostic of the presence of melanosarcoma, but melanuria has now been observed in persons without these tumors and with no other evident source of the pigment.[31] It has been accompanied with evidence of increased non-protein N and low chloride content in the blood, and in several cases there was intestinal obstruction, from whence the precursors of the pigment may have come through protein disintegration.

As mentioned above, Neuberg found that a melanotic sarcoma of the adrenal produced pigment from epinephrine and from β-oxyphenylethylamine, but he failed to get positive results with melanosarcomas of the eye[32] and from the horse. Alsberg[33] succeeded in finding in melanosarcoma from the liver an enzyme oxidizing pyrocatechin, and Jager[34] found that horse melanosarcoma extracts will oxidize epinephrine to a pigment. Brahns[35] states that melanin, like epinephrine, causes vasoconstriction. The "dopa reaction" of Bloch,[20] which depends on the presence of specific oxidizing enzymes in the cells, may be exhibited by the connective tissues quite generally throughout the body in some cases of melanosarcoma.[36]

Eppinger[37] found that the urine of a patient with melanosarcoma gave intense reactions for indole and tryptophan, and that when tryptophan was fed to a patient there was a great increase in the melanuria. He therefore concludes that the power of the body to destroy the pyrrole ring is reduced, and instead it undergoes reduction, methylation, and union with sulfuric acid, to form a sulfate of methylpyrrolidine-hydroxycarbonic acid (CH_3-$C_5H_9N_2O_4$). Abderhalden[38] also found a relation to tryptophan, for in the urine of a melanuric was present a substance rich

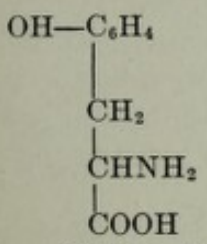
[31] Peters, Arch. Int. Med., 1923 (32), 709; Haden and Orr, Johns Hop. Hosp. Bull., 1924 (35), 58; Churchman, Proc. Soc. Exp. Biol. Med., 1924 (22), 135.
[32] See also Mawas, Compt. Rend. Soc., Biol., 1923 (88), 332.
[33] Jour. Med. Res., 1907 (16), 117.
[34] Virchow's Arch., 1909 (198), 62.
[35] Virchows Arch., 1920 (227), 137.
[36] Matsunaga, Frankf. Zeit. Path., 1919 (22), 69.
[37] Biochem. Zeit., 1910 (28), 181.
[38] Zeit. physiol. Chem., 1912 (78), 159.

in tryptophan. Primavera[39] found the urine in a case of melanosarcoma to contain free tyrosine, fluctuating in amount with the pigment.

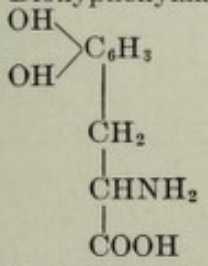
Addison's disease is associated with the deposition of a pigment[40] in the skin that is generally considered to be a melanin, differing from that produced normally in the skin only in quantity and not in origin or composition.[41] No satisfactory explanation of the relation of the adrenal to this pigmentation seems yet to have been made, although it is natural to assume that when the function of the adrenal is destroyed, substances accumulate in the blood that have a stimulating effect on the pigment-forming cells. Abnormal protein catabolism, with excessive accumulation of the chromogenic constituents of the protein molecule, has been suggested, as also have alterations in the influence of the sympathetic nervous system upon the chromophore cells, for nerve lesions (*e. g.*, neurofibroma) often are accompanied by pathological pigmentation of the skin.[42]

It is significant that the active constituent of the adrenal medulla, the epinephrine, is an aromatic derivative closely related to tyrosine, since the production of pigment by the action of oxidizing enzymes upon such substances is well known, and Saccardi[43] has found that partial oxidation of epinephrine produces a black substance with the characteristics of the pyrrols. Furthermore, Neuberg has described a melanotic adrenal tumor which produced pigment by oxidizing epinephrine. On this basis the pigmentation of Addison's disease would seem to be the result of an abnormal accumulation or distribution of aromatic compounds, because of their failure to be converted into epinephrine. In support of this hypothesis is the observation of Meirowsky that the human skin contains an enzyme capable of oxidizing epinephrine to a pigment, and that pieces of skin kept warm will develop a postmortem pigmentation, and this is supported by Könegstein[44] who found that the pigmentation was greater in animals deprived of their adrenals or given injections of epinephrine. Bloch[20] believes that the pigmentation results from the precursor of epinephrine, 3.4-dioxyphenylalanine, which is oxidized in the epidermal cells to melanin. The relation of these substances is shown by their structural formulæ:

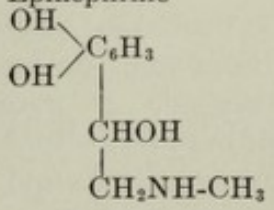
Tyrosine



Dioxyphenylalanine



Epinephrine



[39] Giorn. Int. Scienze Med., 1908 (29), 978.

[40] Eiselt, Zeit. klin. Med., 1910 (69), 393; full discussion on the Pigment of Addison's disease.

[41] Concerning histogenesis of the pigment see Pförringer, Cent. f. Path., 1900 (11), 1.

[42] See résumé by Schmidt, Ergeb. der Pathol., 1896 (Bd. 3, Abt. 1), 551.

[43] Biochem. Zeit., 1922 (132), 439.

[44] Wien. klin. Woch., 1910 (23), 616.

As exact chemical studies of the pigment in Addison's disease have not been made, we have no positive proof that it is a melanin, hence any speculation as to the cause of its formation is premature. Carbone[45] and Eiselt[40] reported isolating from the urine in Addison's disease a pigment that contains much sulfur, considered to be similar to or identical with the melanogen of melanuria.

Ochronosis[46] is a condition characterized by a black pigmentation of the cartilages, first described by Virchow in 1866. In 1922 Oppenheimer and Kline[46] reported a case and found forty others in the literature up to that time. Virchow suspected that the condition was due to a permeation of cartilage by hematin derivatives, but Hansemann, finding a case associated with melanuria, considered that the pigment is probably of metabolic origin. Hecker and Wolf studied the urine of a similar case, and concluded that the pigment must be melanin. Albrecht,[47] however, suggested a relation of ochronosis to *alkaptonuria*, having found homogentisic acid in the urine of a case reported by him (see "Alkaptonuria"). Osler's two patients were brothers with alkaptonuria, the evidence of ochronosis consisting of discoloration of the cartilages of the ears. Langstein[48] examined a specimen of urine preserved from Hansemann's case, and found no evidence of alkaptonuria.[49]

Pick[50] summarizes the results of his study of a case and of the literature, as follows: Ochronosis is a definite form of melanotic pigmentation, the pigment of ochronosis being in most of the cases very closely related to melanin.[50a] The pigment, or its chromogen, circulating freely in the blood, is imbibed not only by cartilage, but also by loose connective tissue, voluntary and involuntary muscle-cells, and epithelial cells, without any decrease in vitality of these cells being observable; however, degenerated tissues show the greatest amount of pigmentation. The diffuse pigment may become granular after a time; it is iron-free, but under certain circumstances may contain fat. *This melanin arises from the aromatic nucleus of the protein molecule* (tyrosine, phenylalanine), and the related hydroxylized products, *under the influence of tyrosinase.* In some cases the constant absorption of minute quantities of phenol from surgical dressings seems to have been the cause of the condition. Besides this formation of pigment from such "exogenous" aromatic substances, however, it is probable that in alkaptonuria the "endogenous" aromatic substance (homogentisic acid) present may be converted into pigment by the tyrosinase. In many of the cases of ochronosis the pigment or a precursor may be excreted in the urine, which then undergoes spontaneous darkening when exposed to the air. The kidneys may also become pig-

[45] Giorno R. Acad. med. di Torino, 1896.

[46] See Adler, Zeit. f. Krebsforsch., 1911 (11), 1; Poulsen, Ziegler's Beitr., 1910 (48), 346; Oppenheimer and Kline, Arch. Int. Med., 1922 (29), 732.

[47] Zeit. f. Heilk., Path. Abt., 1902 (23), 366.

[48] Hofmeister's Beitr., 1908 (4), 145.

[49] Also see Langstein, Berl. klin. Woch., 1906 (43), 597.

[50] Berl. klin. Wochenschr., 1906 (43), 478.

[50a] See Kleinschmidt (Frankf. Zeit. Path., 1922 (28) 73), who gives full review of anatomical findings and reports the 34th recorded case.

mented and granular masses of pigment may be present in the renal tubules.

Poulsen[51] states that of the 32 known cases of ochronosis (in 1911) in 17 there was alkaptonuria, in 8 carbolic acid dressings had been used for long periods, and in the remaining 7 cases the cause was not determined. These facts are conclusive evidence of the origin of ochronotic pigment from aromatic radicals, whether these radicals are converted into true melanin or not. The localization of the pigment is explained by the demonstration by Gross and Allard,[52] that cartilage has a greater affinity than other tissues for homogentisic acid. Ochronosis can be produced experimentally with homogentisic acid, and often is associated with an arthritis.[53] There are, however, numerous cases of alkaptonuria without ochronosis. The ochronosis described in lower animals is not the same as human ochronosis, affecting the bones rather than the cartilages,[54] and being more properly designated by the name *osteohemochromatosis* (Schmey),[55] or, according to Oberndorfer, *osteohematoporphyria*.

Malarial pigmentation[56] according to Ewing,[57] might have any one of the following origins:

(1) Pigment elaborated by the intracellular parasite. (2) Hematoidin derived from the remnants of infected red cells. (3) Hematoidin or altered hemoglobin deposited in granular or crystalline form from red cells dissolved in the plasma. (4) Bilirubin or urobilin granules or crystals.

Of these, the pigment formed by the parasites has been considered by many as a true melanin, but this cannot be considered as established, especially as Ewing found it to have the same relation to solvents as do the blood-pigments. Carbone and also Brown[58] consider the malarial pigment to originate from hematin, with which it agrees in solubility, spectroscopic properties, and in containing iron.[59]

Pigmentation of the Colon.[60]—Sometimes the mucosa of the entire colon is found deeply pigmented, with a material of unknown character, but resembling in many respects a melanin. The cause of the condition is unknown. Abderhalden[61] has found pigments that seemed to be derived from tryptophan, while Niklas[62] attributes the coloration to tyrosinase activity.

Pigmentation of the oral mucosa. with a pigment resembling melanin, has been described especially in pernicious anemia. It does not seem to be related to the adrenal.[63]

Lipochrome and Lipofuscin

In normal plant and animal tissues occur pigments that are either fats or compounds of fat, or substances highly soluble in fats. In animals

[51] Münch. med. Woch., 1912 (59), 364.
[52] Arch. exp. Path. u. Pharm., 1908 (59), 384.
[53] Gross, Deut. Arch. klin. Med., 1919 (128), 249.
[54] See Ingier, Ziegler's Beitr., 1911 (51), 199.
[55] Frankfurter Zeit. Pathol., 1913 (12), 218; also Teutschlaender, Virchow's Arch., 1914 (217), 393.
[56] See Lignac, Cent. allg. Path., 1924 (35), 129.
[57] Jour. Exp. Med., 1902 (6), 119.
[58] Jour. Exper. Med., 1911 (13), 290.
[59] Concerning iron in malarial pigment see Mayer, Virchow's Arch, 1922 (240), 117.
[60] Full review by McFarland, Jour. Amer. Med. Assoc., 1917 (69), 1946.
[61] Zeit. physiol. Chem., 1913 (85), 92.
[62] Münch. med. Woch., 1914 (61), 1332. See also Hattori, Mitt. med. Gesellsch., Tokio, 1916 (30), No. 6.
[63] See Weber, Quart. Jour. Med., 1919 (12), 404.

they occur normally in the corpus luteum, in the epithelium of the adrenals,[64] seminal vesicles, testicles and epididymis; in ganglion-cells, especially in the sympathetic nervous tissue; in the Kupffer cells of the liver and in fat tissue. Pathologically, such pigments are found particularly in the muscle-cells in brown atrophy of the heart, and less abundantly in the epithelium of atrophied livers and kidneys (Lubarsch[65] and Sehrt[66]). All are characterized by staining by such fat stains as sudan III and scarlet R, and usually, but not constantly, by osmic acid; they are dissolved by the usual fat solvents. It is questionable if all pigments that stain for fat should be considered as true lipochromes, however, for their other reactions are variable; and Borst would distinguish these pathological pigments from the true lipochromes by calling them *lipofuscins* including under this term the brown "waste pigments," which Hueck[67] believes to be formed from disintegrated lipoids or fatty acids. Many pigmentary substances are probably soluble in fats, and in this way the lipofuscins are formed.[68] In the renal epithelium is found a pigment resembling the lipofuscins, increasing with age and not related to the urinary pigments.[69]

It has been shown by Escher[70] that the pigment of the corpus luteum is identical with the *carotin* of carrots and the same is true of the adrenal cortex. Apparently carotin and xanthophyll (a crystalline pigment from green plants) are the chief pigments of milk fats, egg yolk, and probably of body and blood fats.[71] In the body lipins these pigments accumulate throughout life because of their great solubility in lipins, which explains the high color of the fat of old persons. Carotin seems to be almost or quite devoid of toxicity,[72] and in persons eating carrots in large quantities there may be enough pigment present in the blood (*carotinemia*) to produce skin pigmentation resembling jaundice.[73] Related to this is *aurantiasis cutis*, which is carotin pigmentation of the skin from excessive consumption of oranges.[74] Currie[75] believes that animal fats also contain endogenous pigments not derived from plant lipochromes, and describes "*adipochrome*" as an unsaturated aliphatic hydrocarbon manufactured directly from the fat itself and increased in some diseases,

[64] See Findlay (Jour. Path. Bact., 1920 (23), 482), who finds carotin, xanthophyll and perhaps true melanin in adrenals.

[65] Cent. f. Pathol., 1902 (13), 881.

[66] Virchow's Arch., 1904 (177), 248. See also Mayer *et al.*, Jour. physiol. et path. gén., 1914 (16), 581.

[67] Ziegler's Beitr., 1912 (54), 68.

[68] Ciaccio (Biochem. Zeit., 1915 (69), 313) agrees with Hueck, and finds it possible to distinguish between pigments from phosphatids, which stain poorly with sudan III, and those from free fatty acids which stain deeply with this dye.

[69] Schreyer, Frankf. Zeit. Pathol., 1914 (15), 333.

[70] Zeit. physiol. Chem., 1913 (83), 198.

[71] Full review in "Carotinoid and Related Pigments," L. S. Palmer, New York, Chem. Catalog Co., 1922.

[72] Wells and Hedenburg, Jour. Biol. Chem., 1916 (27), 213.

[73] Hess and Myers, Jour. Amer. Med. Assoc., 1919 (73), 1743; 1920 (74), 32; Head and Johnson, Arch. Int. Med., 1921 (28), 268.

[74] Miyake, Arch. Derm. Syph., 1924 (147), 184.

[75] Biochem. Jour., 1924 (18), 235.

especially in the fat tissue near tumors. However, it has been found that in animals receiving no plant lipochromes, the tissue fat may become practically pigment-free, which throws doubt on any endogenous lipochrome formation.

The work of Palmer[71] indicates that carotin and xanthophyll are much more widely distributed than was formerly appreciated. Animals with colored fats owe the color to these plant pigments, which are also present in the blood of these same animals, but not in the blood of animals with colorless fats (swine, rabbits, dogs, sheep, goats), and the so-called lipofuscin of the ganglion cells has been shown to be carotin.[76] Palmer found that carotin is the pigment of milk fat, body fat, and corpus luteum of the cow, while xanthophyll with some carotin colors the egg yolk, body fat, and blood serum of the fowl. Chickens deprived of these pigments from the time of hatching have no pigment in their fat or egg yolk although the fowls are healthy and their colorless eggs are fertile.[77] This work makes doubtful the existence of other fat-soluble intracellular pigments in man, such as lipofuscin, and Dolley states that cardiac muscle pigment, ordinarily called lipofuscin, consists of a mixture of melanin and carotinoids.[78] Schmidtmann[79] also finds this pigment to be physically and chemically similar to melanin, probably absorbed or dissolved by lipins.[80]

Xanthosis diabetica[81] also seems to depend on an excess of lipochromes in the blood, probably partly endogenous from mobilization of tissue fats and chiefly exogenous from the abundance of green vegetables in the diet. Hypercholesterolemia is usually present. The pigment is sometimes carotin and sometimes xanthophyll.[74]

Staining of Lipochromes.—It has been said that typical plant lipochromes, as also the pigments of *Staphylococcus pyogenes aureus* and *citreus*, are colored blue by concentrated sulfuric acid with formation of small blue crystals of *lipocyanin*. With iodin-potassium-iodide solution they are colored green. Lipochrome of frog-fat stains blue with this solution (Neumann);[82] lipochrome of the corpus luteum (called lutein) occasionally gives a faint blue with sulfuric acid or Lugol's solution (Sehrt); but the fat-holding pigments of the other tissues mentioned above do not give either of these reactions. Possibly these last are not true lipochromes, therefore, but rather pigments chemically or physically combined with fat. Cotte[83] believes that the true lipochromes of plants and animals have a cholesterol base, but the presence of glycerol in plant and bacterial lipochromes can be demonstrated by the acrolein test; possibly, therefore, both cholesterol and neutral fats are present. Melanins and pigments derived from hemoglobin do not stain with sudan III and are not soluble in ether, etc., and hence can be readily distinguished from the fatty pigments. Probably, however, it is not the carotin itself that stains, but only the fats in which the pigment is dissolved (Palmer)[71] or associated, fat-soluble materials.

Chloroma.[84]—The pigment that causes the peculiar green color characteristic of these malignant growths, was considered by Chiari, Huber and others as a fatty

[76] Dolley and Guthrie, Jour. Med. Res., 1919 (40), 295.
[77] Jour. Biol. Chem., 1919 (39), 299; 1921 (46), 559.
[78] Dolley and Guthrie, Jour. Med. Res., 1921 (42), 289.
[79] Virchow's Arch., 1920 (227), 141; 1922 (239), 488.
[80] See Kutschera, Wien. klin. Woch., 1922 (35), 554; Lubarsch, Virchow's Arch., 1922 (239), 491.
[81] Bürger and Reinhart, Zeit. exp. Med., 1918 (7), 119.
[82] Virchow's Arch., 1902 (170), 363.
[83] Compt. Rend. Soc. Biol., 1903 (55), 812.

substance related to or identical with the lipochromes. It commonly fades on exposure to air, and also when in the usual preservative fluids, to which it does not impart its color. The color may be brought back after formaldehyde preservation by H_2O_2 or by weak alkalies (Burgess).[84] Ottenberg[85] has suggested that the green color may be due to eosinophiles which abound in chloromas, since in fresh preparations eosinophile granules have a faint greenish tinge. It contains no iron, is soluble in absolute alcohol and in ether, and is usually, but not always (v. Recklinghausen), stained black with osmic acid.[86] Treadgold states that as the green color is not present from the beginning it would seem that cellular degeneration must play a part. Possibly a degeneration of the granules of the myelocytes and myeloblasts, aided by the products of hemoglobin disintegration, is responsible.[87]

Kossel and Giese[88] say that the faded color of a chloroma may be restored by ammonium sulfide, which is a reaction for free iron, but as they found very little iron in the tumor (0.125 to 0.15 per cent.), they suggest that the green color depends on the presence of both free sulfur ions and a certain amount of iron in a reactive state. As the color can be restored to chloroma tissue by ammonium sulfide even after the tissue has been extracted with ether and boiling alcohol, the pigment cannot well be a lipochrome. These authors suggest that it is similar to the green iron sulfide pigmentation seen on the outer surface of hard boiled egg yolk.

Chromophile cells may be considered in this connection. Kohn[89] has described certain cells with a decided affinity for chromic acid and its salts, found abundantly in the sympathetic nervous system, in the carotid gland, and in the medulla of the adrenal. They are also present in tumors derived from these organs. Extracts from such organs have a marked effect in raising blood pressure, and, according to Wiesel,[90] they are greatly involved in Addison's disease. The nature of the chromophile substance is unknown, but it can be fixed only by chromic acid or chromates; cells hardened by other means show merely spaces in the places occupied by this substance. It is generally believed to be the same as the epinephrine but it does not always seem to parallel in amount the quantity of epinephrine as determined chemically. Ogata[91] states that the chrome reaction depends on the reduction of chromic acid to chromium dioxide by epinephrine.

BLOOD PIGMENTS[92]

Red corpuscles behave much as do other non-nucleated fragments of cells, undergoing disintegration rapidly and constantly when under normal conditions,[93] as well as when subjected to various harmful influences (see "Hemolysis"), or when outside of the vessels in extravasations of blood. The processes and products of their disintegration are, therefore, much the same whether occurring under normal or pathological conditions. The hemoglobin molecule is large and complex, and from it are derived many substances of the nature of pigments; indeed, hemoglobin itself may appear free as a pigment.[94]

[84] Literature by Dock, Amer. Jour. Med. Sci., 1893 (106), 152; and Dock and Warthin, Med. News, 1904 (85), 971; Burgess, Jour. Med. Res., 1912 (27), 133.

[85] Amer. Jour. Med. Sci., 1909 (138), 565.

[86] The pigment of *xanthelasma multiplex* seems to be a fatty substance (Poensgen, Virchow's Arch., 1883 (91), 354). Probably it is merely carotin and related pigments dissolved in the lipoids.

[87] Quart. Jour. Med., 1908 (1), 239; Weber, Proc. Roy. Soc. Med., Clin. Med. Sec., 1916 (9), 7.

[88] Zeit. physiol. Chem., 1921 (114), 127.

[89] Prag. med. Woch., 1902 (27), 325.

[90] Zeit. f. Heilk., Path. Abt., 1903 (24), 257.

[91] Jour. Exp. Med., 1917 (25), 807.

[92] Literature by Whipple, Arch. Int. Med., 1922 (29), 711.

[93] See review on destruction of red corpuscles by Rous, Physiol. Reviews, 1923 (3), 75.

[94] Muscle tissue contains a respiratory pigment, myoglobin, similar to but not identical with hemoglobin (Günther, Virchow's Arch., 1921 (230), 146).

Hemoglobin is a compound protein, consisting of a protein group (*globin*) and a coloring-matter (*hematin* or *hemochromogen*).[95] The protein globin is of a basic nature, and seems allied to the histones; the hematin is, therefore, presumably acid.[96] Hemoglobin ordinarily does not crystallize readily, especially the hemoglobin of man, and it is doubtful if it ever does so in the living tissues, although possibly this may occur in the center of large hematomas. In bodies that have undergone postmortem decomposition, and occasionally in specimens kept for microscopic purposes, irregular orange yellow crystalline masses of hemoglobin may be found. This occurs particularly if the blood has been acted upon by hemolytic agents or has undergone putrefactive changes, and then is hardened in alcohol. The crystals are either oxyhemoglobin, or more often an isomeric or polymeric modification, *parahemoglobin* (Nencki). Hemoglobin also enters cells unchanged, imparting a diffuse yellowish color, and apparently it is non-toxic.[97] If present in the blood in large enough amounts it is excreted unchanged in the urine, but at least one-sixtieth of the total number of red corpuscles must be in solution at one time to produce hemoglobinuria; in man, at least 17 c.c. of laked corpuscles must be injected to accomplish this.[98] (See also Hemolysis, Chap. XI.)

Addis[99] has developed the following conception of the metabolism of hemoglobin. Free hemoglobin, liberated especially by the phagocytes of the spleen, is taken up by the other phagocytes, notably the Kupffer cells of the liver, which pass it on to the liver cells. The pigment moiety, *hematin*, is separated from the globin, and converted through removal of its iron into *bilirubin*. The bilirubin excreted into the intestine is there reduced to *urobilinogen*, which is in part reabsorbed and polymerized into *urobilin*, which in turn is possibly polymerized into a larger complex. In the liver this urobilin complex has restored to its pyrrol nuclei the original side chains, and then is used to form new hemoglobin molecules. Whipple[92] however, doubts that such a reconstruction of urobilin to hemoglobin takes place, but suggests that there may be some sort of pigment complex formed in the body which may build up to hemoglobin or break down into simpler excretory pigments according to the supply and demand.

Methemoglobin and Sulfhemoglobin.[1]—It has generally been supposed, since the early studies of Hoppe-Seyler and Araki, that hydrogen sulfide[2] reacts readily with hemoglobin, and that the greenish dis-

[95] Halliburton and Rosenheim recommend that the name "hemochromogen" be dropped in favor of "reduced hematin" (Biochem. Jour., 1919 (13), 195).

[96] Herzfeld and Klinger believe that the globin and the pigmentary substance, which they call "hemochrome," are merely united by adsorption, not chemically (Biochem. Zeit., 1919 (100), 64). Partos believes this "hemochrome" to be hematin (*ibid.*, 1922 (129), 89).

[97] Barratt and Yorke, Brit. Med. Jour., Jan. 31, 1914.

[98] Sellards and Minot, Jour. Med. Res., 1916 (34), 469.

[99] Arch. Int. Med., 1915 (15), 412.

[1] Mason and Conroy, Johns Hop. Hosp. Bull., 1921 (32), 391. Review of lit.

[2] Review of literature on H_2S by Mitchell and Davenport, U. S. Pub. Health Reports, No. 892.

coloration seen in post mortem decomposition is due to the presence of the resulting product, designated as sulfmethemoglobin. Later observations[3] have shown that this assumption is incorrect. It has been learned that H_2S unites with hemoglobin only with difficulty unless something is present to reduce the hemoglobin, when even traces of H_2S will react. Wallis explained the occurrence of sulfhemoglobinemia by the action of some strong reducing agent, such as hydroxylamines formed by bacterial action on proteins. Presumably under postmortem conditions such a reduction of the blood is readily accomplished. Since during life H_2S is constantly formed in greater or less amount, the capacity of the blood to oxidize it without union occurring with the hemoglobin, which Haggard has shown to be very marked, is an important mechanism of defense. If, however, through bacterial action suitable reducing substances are formed, or if they arise through taking such drugs as phenacetin and acetanilid, then sulfhemoglobinemia may result. Since the active substances come from the intestine the condition is called "enterogenous cyanosis," which term also covers *methemoglobinemia* produced by nitrites formed in the intestines.[4]

The latter condition is also present in poisoning by phenacetin,[5] aniline, and acetanilid, and related pigments appear in the blood in poisoning with chlorates and nitrobenzol. Pneumococci and *Streptococcus viridans*, as well as some other bacteria, may produce methemoglobin,[6] and Stadie[7] believes that in pneumonia there may be enough methemoglobin formed to cause a decrease in the oxygen capacity of the blood. However, living tissues seem to have the power to reduce methemoglobin to hemoglobin and hence to protect against methemoglobinemia.[8] In infections with *B. emphysematosus* Schumm found this pigment free in the blood.[9]

Van den Bergh[10] has found sulfhemoglobinemia in puerperal sepsis, and probably these pigments could be found in other conditions if sought, the diagnosis being made readily by spectroscopic examination of the blood. Sulfhemoglobin is found only in the red cells, whereas methemoglobin may be found also in the serum and urine. Van den Bergh states that there are three sorts of methemoglobinemia; namely, septic sulfhemoglobinemia with hemolysis, intraglobular methemoglobinemia, and intraglobular sulfhemoglobinemia. There must be, he says, at least 10 per cent. sulfmethemoglobin in the blood to be measured

[3] Reviewed by Haggard, Jour. Biol. Chem., 1921 (49), 519.

[4] West and Clarke, Lancet, Feb. 2, 1907; Davis, *ibid.*, Oct. 26, 1912; Gibson Quart. Jour. Med., 1907 (1), 29; Long and Spriggs, *ibid.*, 1918 (11), 102; Jamieson, *ibid.*, 1919 (12), 81.

[5] See Heubner, Arch. exp. Path., 1913 (72), 241.

[6] Cole, Jour. Exp. Med., 1914 (20), 363; Blake *ibid.*, 1916 (24), 315; Kämmerer, Arch. Exp. Path. Pharm., 1920 (88), 247; Schnabel, Zeit. Hyg. Infkr., 1921 (93), 175.

[7] Jour. Exp. Med., 1921 (33), 627.

[8] Neill, Jour. Exp. Med., 1925 (41), 299.

[9] According to Kämmerer[6] mesohematin has a specific bactericidal or inhibiting effect on gram-positive bacteria, but porphyrins and bilirubin are inactive.

[10] Nederl. Tijd. Geneesk., 1918 (1), 1774.

spectroscopically.[11] It is probable that not enough sulfide will be taken up from the bowel to produce this amount of sulfhemoglobinemia unless there is also some formation of methemoglobin through the action of such reducing agents as nitrites, etc. This is especially favored by a combination of phenacetin and sulfur administration as described by Snapper, or in the case of nitrobenzene and sulfur effect reported by Vogel[12] as the twenty-first case of sulfhemoglobinemia in the literature to 1924.

Hematin.—In the decomposition of hemoglobin the first step is the splitting of the globin (which does not form pigments) from the hematin or hemochromogen from which many pigments may be derived. The formula given for this substance by Nencki is $C_{32}H_{32}N_4FeO_4$ while Hoppe-Seyler proposed the formula $C_{34}H_{34}N_4FeO_5$, although it is not certain that the hematin of all animals is the same. It is found frequently as an amorphous, dark-brown or bluish-black substance, in large, old extravasations of blood,[13] but seldom in small hemorrhages. It is said to be present in the serum of most apparently normal fetuses and in infants during the first two weeks.[14] As a pathological pigment, hematin is by no means so frequently found as its derivatives. Schumm[15] observed a patient with chromium poisoning who showed for several days abundant hematin free in the blood. He has also found it in malaria, pernicious anemia, congenital hematoporphyria, and generally with acute toxic hemolysis, including patients infected with *B. emphysematosus*, when the hematin may be accompanied by methemoglobin without a corresponding urinary excretion of these pigments. Feigl found hematinemia in many cases of poisoning with the war gases.[16] Brown[17] found that solutions of hematin cause chills and fever, and suggests that this pigment may be at least partially responsible for the symptoms of malaria.[18]

Most writers state that hematin is gradually split into an iron-free pigment (*hematoidin*) and an iron-containing pigment (*hemosiderin*). This change may be represented by the following equation, according to Nencki and Sieber:[19]

$$\underset{\text{(hematin)}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O = \underset{\text{(hematoidin)}}{2C_{16}H_{18}N_2O_3} + Fe.$$

However, finding that the pigment in the malarial spleen is hematin, Brown[20] suggests that hematin cannot well be an intermediary product in

[11] Nederl. Tijd. Geneesk., 1922 (1), 2499.

[12] Amer. Jour. Med. Sci., 1924 (168), 89.

[13] The dark pigment found in formalin-preserved tissues, especially in the blood, seems to be related to hematin or methemoglobin, and is formed only if the blood has undergone more or less disintegration before preservation (Hueck, Beitr. path. Anat., 1912 (54), 68). It may be readily dissolved out in weak alkalies.

[14] Haselhorst and Papendieck, Klin. Woch., 1924 (3), 979.

[15] Zeit. physiol. Chem., 1912 (80), 1; 1913 (87), 171; 1916 (97), 32.

[16] Biochem. Zeit., 1919 (93), 119.

[17] Jour. Exper. Med., 1912 (15), 580; 1913 (18), 96.

[18] Disputed by Butterfield and Benedict, Proc. Soc. Exp. Biol., 1914 (11), 80.

[19] Arch. exp. Path. u. Pharm., 1888 (24) 440; Brugsch and Yoshimoto, Zeit., exp. Path., 1911 (8), 639.

[20] Jour. Exper. Med., 1911 (13), 290; 1911 (14), 612.

hemoglobin disintegration, since this malarial pigment persists a very long time in the tissues without change. He has made other observations that led him to conclude that hematin is not an intermediary substance between hemoglobin and hemosiderin, but that when once formed it is destroyed very slowly, by oxidation rather than hydrolysis. Injected into rabbits it produces vascular lesions in the kidneys[21] and in large doses causes a marked fall in blood pressure.[22]

Hematoidin may be found in old, large extravasations, as orange-colored or red rhombic plates, first described by Virchow. Sometimes, however, hematoidin occurs in the form of yellowish granular masses, and it may be associated with lipoids; it is also found in crystalline form in icterus (Dunzelt).[23]

It may also form round spheroliths with projecting needles, in which structures calcium may be found (Lignac),[24] as sometimes is the case with the rhombic plates. Hematoidin seems to be nearly or quite identical with the bile-pigment, bilirubin, and it is probably the source of this substance under normal conditions. When hematoidin is formed in excessive amounts, either through increased destruction of corpuscles in the vessels or in extravasations, the amount of bile-pigment is increased (see "Icterus"). Possibly some of the hematoidin becomes transformed directly into urobilin, and is then eliminated in the urine.

Although general agreement has not been reached as to the relation of hematoidin to bilirubin, their identity is made more probable by the chemical and crystallographic studies of H. Fischer and Reindel.[25] Whether bilirubin can be formed from hemoglobin outside the liver has long been a disputed point, but the experiments of Whipple and Hooper,[26] and of Mann and Magath,[27] together with the undoubted fact that hematoidin, the close relative of bilirubin and perhaps identical with it, can be formed in extravasations of blood, seems strong evidence for the possibility of exthrahepatic bilirubin formation.[28] Van den Bergh[29] has reported the finding of bilirubin in a subdural hematoma in a concentration as high as in normal bile.

Hematoporphyrin.[30]—There are several closely related pigments derived from hematin that are appropriately grouped under the designation of *porphyrins*, for they are not all identical with the pigments prepared artificially from hematin by Nencki and called by him *hematoporphyrin* and *mesoporphyrin*, the former apparently representing a reduction, the latter an oxidation product. The porphyrins found in the urine and feces are said to be different from each other and from those prepared by

[21] Arch. Int. Med., 1913 (12), 315.
[22] Brown and Loevenhart, Jour. Exp. Med., 1913 (18), 107.
[23] Cent. f. Path., 1909 (20), 966.
[24] Virchow's Arch., 1923 (243), 273.
[25] Zeit. physiol. Chem., 1923 (127), 316.
[26] Jour. Exp. Med., 1913 (17), 593.
[27] Amer. Jour. Physiol., 1924 (69), 391. Full review.
[28] See Aschoff, "Lectures on Pathology," Hoeber, New York, 1924; Klin. Woch., 1924 (3), 961.
[29] Der Galdenfarbstoff im Blut, Leiden, 1918.
[30] Literature and full review by Günther, Ergeb. allg. Path., 1922 (20), 608.

Nencki.[31] Physiologically, these pigments are of great interest, because of the close chemical relation they have been found to bear to chlorophyll,[32] with which hemoglobin is so closely related functionally. It is also interesting to consider that whereas carnivora obtain much hemoglobin in their food, herbivoria obtain much chlorophyll. Intestinal bacteria can form hematoporphyrin from hemoglobin.[33]

Pathologically, porphyrin is of interest as a urinary pigment, being found normally in the urine in traces, varying with the amount of hemoglobin in the food (Schumm), but present in considerable quantities in many diseases, such as rheumatism, tuberculosis, lead poisoning, various liver diseases, and, most strikingly, after the administration of sulfonal, veronal or trional. It is not demonstrable in the blood of normal persons or in most diseases.[34] An acute form of porphyrinuria has been described, usually in women, sometimes associated with ascending motor paralysis,[35] and sometimes with severe abdominal symptoms (Günther). Porphyrinuria is also sometimes observed in severe degenerative conditions of the liver.[36] Günther also describes a group of cases with increased excretion of hematoporphyrin, especially in the feces, characterized clinically by nervous irritability and a tendency to skin pigmentation. The etiology of both acute and chronic forms of "idiopathic hyperporphyrism" is unknown.

A congenital form of hematoporphyrinuria occurs, in which the blood contains free hematin, bilirubin, and a porphyrin (Schumm),[37] about 0.3–0.4 gm. being usually excreted daily in the urine. When in abundance it may color the urine a rich Burgundy red, and it is sometimes accompanied by a precursor, *uro-fuscin*. It is present in the bones of animals showing hemochromatosis and in the bones of persons[38] exhibiting the congenital form of "hematoporphyria" or "porphyrinuria" described by Günther, which is accompanied by severe skin lesions that are ascribed to the action of light upon the skin sensitized by the hematoporphyrin. Even the teeth may sometimes be stained pink,[39] and the porphyrin is present in the blood and in serous effusions.[40]

Hausmann[41] and others have studied extensively the photosensitizing action exhibited by hematoporphyrin and other porphyrins, and find evidence suggesting a relationship between hematoporphyria and "hydroa æstiva,"[42] and other conditions in which the skin is abnormally sensitive

[31] See H. Fischer and Zerweck, Zeit. physiol. Chem., 1924 (137), 176; Schumm, *ibid.*, 1924 (139), 219.
[32] For literature see Abderhalden, "Lehrbuch der physiol. Chemie," 1906.
[33] Kämmerer, Klin. Woch., 1924 (2), 1153.
[34] Schumm, Zeit. physiol. Chem., 1923 (126), 169; Papendieck, *ibid.*, 1924 (136), 293.
[35] Löffler, Corr.-bl. f. Schweizer Aerzte, 1919 (49), 1871.
[36] See Harbitz, Norsk. Mag. Laegevidens., 1924 (85), 422; Arch. Int. Med., 1924 (33), 632.
[37] Zeit. physiol. Chem., 1916 (98), 123; 1919 (105), 158.
[38] Hegler *et al.*, Deut. med. Woch., 1913 (39), 842.
[39] Mackey and Garrod, Quart. Jour. Med., 1922 (15), 319.
[40] Schumm, Zeit. physiol. Chem., 1924 (132), 62.
[41] Biochem. Zeit., 1910 (30), 276; 1914 (67), 309.
[42] Review by Senear and Fink, Arch. Derm. Syph., 1923 (7), 145.

to light. After injection of 0.2 gm. hematoporphyrin into his own veins Meyer-Betz[43] found himself so sensitized to light that exposure to the sun caused severe skin reactions during a period of weeks, and exposure to the Finsen light produced ulceration; but little hematoporphyrin escaped in the urine. Many other products of blood destruction tested on animals were without sensitizing effects. Methylation of the pyrrol groups only partially removes the activity of hematoporphyrin. Porphyrin obtained from urine and feces by Fischer also sensitized mice to light. Sufficient doses of hematoporphyrin may sensitize mice so that they become narcotized and die in a few minutes after exposure to intense light, a true "light stroke." Jobling and Arnold[44] have suggested that some pigment of this sort may be responsible for the skin lesions of pellagra. Presumably through its photodynamic effects, hematoporphyrin will prevent rickets in experimental animals.[45]

Hemosiderin[46] is relatively insoluble, and, therefore, is more slowly removed when formed in hemorrhages, and more abundantly deposited in the tissues when formed after excessive hemolysis. Hemosiderin generally appears in the form of brown or yellowish-brown granules, and not as crystals. After a time it is taken up and deposited to a large extent in the liver, spleen, bone-marrow, and kidney, either as hemosiderin or possibly as some other iron compound of similar nature. From these sites it seems to be later taken up to be utilized in the manufacture of new red corpuscles. Whenever there is hemosiderin deposition in the kidney, granules of the pigment may be found in the urine, free or in cells (Rous).[47]

In acute hemolytic anemia a third of the total iron of the blood may be deposited in the liver, spleen and kidneys within 24 hours.[48] In infarcts hemosiderin soon disappears (Schmidt),[49] presumably because dissolved by the acids formed during autolysis. According to Neumann, hemosiderin is produced only under the influence of living cells and in the presence of oxygen, while hematoidin arises independent of cellular activity,[50] but Brown[51] has found that hemosiderin can be formed during autolysis of the liver, especially when air is present, and therefore probably by an oxidizing enzyme. He suggests that in hemosiderin the pigment is still hematoidin, and that the formation of hemosiderin takes place in the nuclei, the hemosiderin being made directly from hemoglobin without the intervention of hematin. It may also be formed from the iron-con-

[43] Deut. Arch. klin. Med., 1913 (112), 476.
[44] Jour. Amer. Med. Assoc., 1923 (80), 365.
[45] van Leersum, Jour. Biol. Chem., 1924 (58), 835.
[46] See Neumann, Virchow's Arch., 1888 (111), 25; 1900 (161), 422; 1904 (177), 401; also Arnold, *ibid.*, 1900 (161), 284; Leupold, Beitr. path. Anat., 1914 (59), 501.
[47] Jour. Exp. Med., 1918 (28), 645. (See also Pernicious Anemia and Hemochromatosis.)
[48] Muir and Dunn, Jour. Path. and Bact., 1915 (19), 417.
[49] Verh. Deut. Path. Gesell., 1908 (12), 271.
[50] The accumulation of iron in the liver which follows poisoning with hemolytic agents, is not prevented or diminished by preliminary removal of the spleen (Meinertz, Zeit. exp. Path. u. Ther., 1906 (2), 602).
[51] Jour. Exper. Med., 1910 (12), 623.

taining protein of the cells during autolysis, independent of hemoglobin.[52] Milner[53] considers that, under similar conditions, an iron-containing pigment is also formed, which differs from hemosiderin in having the iron so combined that it cannot react with the usual reagents; this pigment may later change into hemosiderin. Up to the present time we do not know the chemical nature of hemosiderin, nor its exact fate in the body, but it is probably utilized in the manufacture of new hemoglobin, for it is known that the iron liberated when hematin is broken up in the body under experimental conditions is deposited and not eliminated (Morishima).[54] The ready solubility of hemosiderin in very weak acids, and the ease with which it is stained by agents reacting with free inorganic iron, indicate that it is not a very complex compound, nor firmly bound to larger aggregates. Strasser[55] holds that it is simply iron oxide, and Lignac[56] does not confirm Brown's idea that the coloring matter of hemosiderin is hematoidin.

All told the average human body contains about 3.2 grams of iron, of which 2.4 to 2.7 grams is in the blood. According to Meyer[57] iron is present in the body in three forms: 1. Not demonstrable by reagents because too firmly bound (hemoglobin). 2. Loosely bound iron, colored by $(NH_4)_2S$ acting for a long time (ferratin). 3. Salt-like compounds with proteins, and inorganic compounds, reacting at once with reagents.[58] *Ferratin* is the iron compound in the liver, containing 6 per cent. iron. He believes that *hemosiderin* probably is not a definite substance, but merely indicates compounds of the third class. Iron pigments may be transformed from one class to another, *e. g.*, in corpus luteum scars, whose age can be estimated, class three may be replaced by class two. We may find in the sputum and lungs "Herzfehlerzellen" that either do or do not stain with ferrocyanide. In *morbus maculosus*, Kunkel found the pigment of the internal organs to be pure iron oxide. Hueck also holds that hemosiderin is an inorganic iron compound, perhaps colloidal iron oxide, loosely bound to proteins and fats, and that it never forms an iron-free pigment, as has been stated. He believes that there is very little iron in the tissues in a firm union like hemoglobin, and that by proper technic some iron can be stained in every organ which contains chemically demonstrable iron. Hueck[13] says that the amount visible microscopically corresponds closely with the results of chemical analysis. Whenever there is in the liver over 50 mg. of iron per 100 grams of dry liver tissue it can be recognized in stained sections. Ischida[59] believes that an iron-containing pigment may be formed in striated muscles from the iron normally there, without requiring a hematogenous origin. The iron-con-

[52] Sprunt *et al.*, Jour. Exp. Med., 1912 (16), 607.
[53] Virchow's Arch., 1903 (174), 475.
[54] Arch. exp. Path. u. Pharm., 1898 (41), 291.
[55] Beitr. path. Anat., 1922 (70), 248.
[56] Cent. allg. Path., 1924 (35), 129.
[57] Ergeb. der Physiol., 1905 (5), 698; literature.
[58] A light green pigment, found in the spleen especially in infarcts, is believed to be iron phosphate by Kraus (Beitr. path. Anat., 1922 (70), 234).
[59] Virchow's Arch., 1912 (210), 67.

taining pigment frequently found in the bowel walls may at times be hemosiderin, but it may also be iron absorbed from the intestinal contents.[60]

Hemochromatosis.[61]—This name was given by von Recklinghausen to a condition in which the organs and tissues throughout the body are abundantly infiltrated with two pigments; one, iron-containing, identical with hemosiderin; the other seems to be the same as the hemofuscin described below. It is usually distinguished from general hemosiderosis in which only the iron pigment is deposited,[62] although there are numerous observers who believe that all the pigment in hemochromatosis contains iron, but in some of the pigment the iron is firmly bound and difficult of demonstration. The hemosiderin is found chiefly in the parenchyma cells of the glandular organs, especially the liver and pancreas, which organs usually show marked interstitial proliferation. The hemofuscin is found especially in the smooth muscle fibers of the gastro-intestinal tract, blood vessels, and genito-urinary tract. Under the heading of local hemochromatosis, von Recklinghausen grouped such conditions as brown atrophy of the heart and pigmentation of the intestinal wall, which probably are quite distinct from the generalized hemochromatosis, since the local form occurs as a physiological process in old age. Hess and Zurhelle found 38.7 gm. of iron in the liver in one case (the normal amount is 0.3 gm.), and Bernouille[63] found 18.3 gm. or 2.95 per cent. of the dry weight in the liver, 2.65 per cent. in the pancreas, and the same in the spleen. Anschütz found 14.69 per cent. in the lymph nodes, 7.62 per cent. in the liver, and 5 per cent. in the pancreas of a case. Muir and Dunn[64] obtained the following percentage figures: Liver, 6.43; pancreas, 2.49; spleen, 0.825; retroperitoneal glands, 11.64; kidneys, 0.406; adrenals, 0.121; heart, 0.714; skin, 0.188; small intestine, 0.14. (Other analytical results are given by Howard and Stevens.)[61]

Opie states the accepted conclusions concerning this disease as follows: (1) There is a distinct morbid entity, hemochromatosis, characterized by widespread deposition of an iron-containing pigment in certain cells, and an associated formation of iron-free pigments in a variety of localities in which pigment is found in moderate amount under physiological conditions. (2) With the pigment accumulation there occur degeneration and death of the containing cells and consequent interstitial inflammation, notably of the liver and pancreas, which become the seat of inflammatory changes accompanied by hypertrophy of the organ. (3) When chronic interstitial pancreatitis has reached a certain grade of intensity, diabetes ensues and is the terminal event in the disease.

[60] See Kawashima, Virchow's Arch., 1923 (247), 151.

[61] Literature given by Sprunt, Arch. Int. Med., 1911 (8), 75; Roth, Deut. Arch. klin. Med., 1915 (117), 224; Howard and Stevens, Arch. Int. Med., 1917 (20), 896; Eppinger and Ranzi, "Die hepato-lienalen Erkrankungen," Berlin, 1920.

[62] In lower animals occurs a form of hemochromatosis affecting especially the bones, and sometimes mistaken for ochronosis. (See Teutschlaender, Virchow's Arch., 1914 (217), 393.)

[63] Corr.-Bl. Schweiz. Aertze, 1911 (40), 610.

[64] Jour. Path. and Bact., 1914 (19), 226.

Diabetes occurs in the majority of the cases of generalized hemochromatosis (50 of 63 collected by Sprunt) and was called by Hanot, "bronzed diabetes," because of the coloration of the skin. It has been suggested that the pigmentation is due to decomposition of the blood-corpuscles in the diabetic blood, but the pigmentation and sclerotic changes precede the diabetes, which is secondary to the atrophic and sclerotic changes in the pancreas. It seems probable that both the pigment formation and the tissue changes depend upon some intoxication, the origin and nature of the toxic agent being entirely unknown. In many cases it has seemed possible that alcohol might have been the inciting cause. There is no evidence of abnormal blood destruction which might account for the pigmentation, and Meltzer and Parker have suggested that the difficulty lies in the inability of the tissues to get rid of the iron set free in normal catabolism. Metabolism studies have indicated that there is some retention of food iron, which may be interpreted as supporting but not proving this hypothesis.[65] Rous and Oliver,[66] finding that protracted hemolysis of foreign corpuscles in rabbits produces a typical mild hemochromatosis, suggest that the liver cirrhosis is primary and renders this organ unable to deal adequately with the blood pigments, which therefore accumulate in the organs and cause diffuse fibrosis.

Mallory[67] has advanced the hypothesis that hemochromatosis may be the result of a binding of the hemoglobin set free in normal hemolysis by copper derived from food or from copper dust inhaled and swallowed. This binding prevents the normal cycle of processes by which the hemoglobin is worked over, the copper-pigment compound being deposited in the tissues. It was found that rabbits given small amounts of copper for several months develop a pigmentary hepatic cirrhosis, and also that a large proportion of cases of hemochromatosis give a history of exposure to copper,[68] or of drinking distilled liquors which often contain copper from the stills. He believes that it requires at least ten years for this disease to become well developed.

Eppinger has suggested that hemochromatosis is a condition of hypofunction of the reticulo-endothelial system, whereby the capacity to prepare hemoglobin properly for reconstruction is impaired. This is to be placed opposite hereditary hemolytic icterus with its hyperactivity of the cells destroying hemoglobin, in support of which idea Frisch[69] advances the observation of familial hemochromatosis. Kühl[70] found in one case an excessive urobilin excretion at times, with intervals of blood regeneration; the corpuscles showed a normal resistance to hemolysis in hypotonic salt solution. No hematoporphyrin excretion was found in this case, although it has often been observed, at least in the late stages. It is

[65] See Howard and Stevens (*loc. cit.*[61]) and McClure, Arch. Int. Med., 1918 (22), 610.

[66] Trans. Assoc. Amer. Phys., 1918 (33), 132.

[67] Jour. Med. Res., 1921 (42), 461; Amer. Jour. Path., 1925 (1), 117. See also R. G. Mills, Jour. Amer. Med. Assoc., 1925 (84), 1326.

[68] E. S. Mills, Arch. Int. Med., 1924 (34), 292.

[69] Wien. Arch. inn. Med., 1922 (4), 149.

[70] Deut. Arch. klin. Med., 1924 (144), 331.

not strange that evidences of excessive or altered blood destruction should be missed, for in view of the number of years during which the disease is developing the amount of change present during any period of observation might well be unrecognizable.

Hemofuscin is the name given by von Recklinghausen to the brownish pigment found in involuntary muscle-fibers, particularly in the wall of the intestine. It does not give reactions for iron, and is insoluble in alcohol, ether, chloroform, or acids; therefore it is not a lipochrome. It is bleached by H_2O_2, and is often found associated with hemosiderin which is not bleached. Von Recklinghausen, and also Goebel,[71] ascribe this pigment to an alteration of hemoglobin which enters the cells in dissolved form, but Rosenfeld,[72] who has submitted the material to analysis after isolation, found 3.70 per cent. of sulfur, from which he considers that it is related to the melanins or melanoid substances. The substance is readily dissolved by alkalies, and contains no iron. It is probable that the name hemofuscin has been given to several different pigments, which resemble one another only in that they do not contain iron. Sträter[73] says that the name hemofuscin should not be used for the pigment of the involuntary muscles, as he finds evidence that this does not arise from hemoglobin and is probably a waste pigment, but true hemofuscin is found in epithelial and connective tissue cells. Mallory[67] found more or less hemofuscin in 11.6 per cent. of adults, but only after the forty-fifth year, always associated with at least traces of hemosiderin into which it is gradually changed. He believes it to be an intermediate between hemoglobin and hemosiderin with properties different from each, and says that chronic poisoning of animals, and presumably of man, with copper or zinc, leads to deposition of hemofuscin.

Pseudomelanosis.—When loosely bound iron is present in the tissues, and in the same tissues sulfides are produced through bacterial action, a discoloration with sulfide of iron will result, which is called *pseudomelanosis*, because the pigment resembles true melanin in its blackness. This is most frequently observed as a postmortem phenomenon in and about the abdominal cavity, and in the ordinary postmortem discoloration both the liberation of the iron from its firm organic combination, and the production of hydrogen sulfide, are the work of bacteria. Pseudomelanosis may occur *intravitam*, particularly in the margins of infected areas, and it may also be observed in the intestines, liver, and spleen, and about the peritoneum, in bodies examined immediately after death, before any evident postmortem decomposition has set in. This seems to depend upon the previous *intravitam* formation of hemosiderin, which is then combined with sulfur liberated from tissue proteins through bacterial action.[74]

ICTERUS[75]

Pigmentation of the tissues of the body in jaundice depends upon the presence in them of bile-pigments, which usually have been formed

[71] Virchow's Arch., 1894 (136), 482.
[72] Arch. exp. Path. u. Pharm., 1900 (45), 46.
[73] Virchow's Arch., 1914 (218), 1.
[74] Ernst, Virchow's Arch., 1898 (152), 418. Literature.
[75] Early literature by Stadelmann, "Der Icterus," Stuttgart, 1891; Minkowski, Ergebnisse der Pathol., 1895 (2), 679. Review on the composition of bile by Whipple, Physiol. Reviews, 1922 (2), 440. Later literature by McNee, Quart. Jour. Med., 1923 (16), 390.

in the liver and reabsorbed either into the lymph or blood (or both). However, a pigment that seems to be chemically identical with bilirubin (*hematoidin*) may be formed from hemoglobin liberated on the breaking up of red corpuscles, and possibly this may be produced in sufficient amounts outside of the liver to give rise to general icterus. Certainly the local greenish-yellow pigmentation occurring in the vicinity of extravasations of blood, due to hematoidin formation, may be looked upon as a "local jaundice." (This is discussed further under Hematoidin, p. 540.)

Bile-pigments.—*Bilirubin* is of a reddish-yellow color, and it is the chief pigment of human bile. Its formula is $C_{34}H_{38}N_4O_6$ or $C_{33}H_{36}N_4O_6$, and its relation to hematin, from which it is formed, is shown by the following formula, which expresses the manner in which blood pigment may be converted into bilirubin by the liver under normal conditions, and into hematoidin (its isomer) in the tissues and fluids of the body in pathological conditions:

$$\underset{\text{(hematin)}}{C_{34}H_{34}N_4O_5Fe} + 2H_2O = \underset{\substack{\text{(hematoidin or}\\ \text{bilirubin)}}}{C_{34}H_{38}N_4O_6} + FeO.$$

Bilirubin is not soluble in water, but dissolves in the body fluids as a soluble compound, "bilirubin alkali." It is very slightly soluble in ether, benzene, carbon disulfide, amyl-alcohol, fatty oils, and glycerol, but is more soluble in alcohol and in chloroform. Although very similar to the porphyrins, Küster[76] believes it to be a different substance.

Biliverdin, $C_{34}H_{38}N_4O_8$, as its formula indicates, is an oxidation product of bilirubin. Bilirubin in alkaline solutions will oxidize into biliverdin merely on exposure to the air, and the change from yellow to green of icteric specimens when placed in oxidizing solutions (*e. g.*, dichromate hardening fluids) is due to the formation of the green biliverdin. Biliverdin is the chief pigment of the bile of carnivora, but it is also present in varying amounts in human bile.

The various other biliary pigments, namely, *bilifuscin*, *biliprasin*, *choleprasin*,[77] *bilihumin*, and *bilicyanin*, are probably not normal constituents of bile, but are oxidation products of bilirubin, and are found chiefly in gall-stones (*q. v.*). A pigment similar to urobilin may be present in normal bile. The total amount of pigments present in bile is probably not far from one gram per liter; rather under than above this amount.

Etiology of Icterus.—Although hematoidin, which is isomeric if not identical with bilirubin, may be formed outside of the liver when red corpuscles are broken up in hemorrhagic extravasations, and possibly also when they are broken up within the vessels by hemolytic agents, yet it was formerly held that a true general icterus does not occur without the liver being implicated. This view rested on evidence of various sorts. First, the classical experiments of Minkowski and Naunyn,[78] which demonstrated that in geese the production of hemolysis by means of arseniuretted hydrogen leads to icterus, but if the livers of the geese have been previously removed, no icterus follows the poisoning.[79] Second, the repeated demonstration that in icterus produced by septic conditions, poisoning, etc., which was formerly looked upon as a "hematogenous" icterus, the urine contains bile salts as well as pigment, indicating an absorption of bile from the liver. Third, the finding of histological evi-

[76] Zeit. Angew. Chem., 1921 (34 Aufsatz), 246.
[77] See Küster, Zeit. physiol. Chem., 1906 (47), 294.
[78] Arch. f. exp. Pathol. u. Pharm., 1886 (21), 1.
[79] Recently corroborated by McNee and Prusik.[83]

dence that in so-called hematogenous icterus there occur occlusions or lesions of some sort in the bile capillaries, which might account for the reabsorption of the bile into the general circulation.[80] Therefore, it was believed that the pigments that produce the general discoloration of icterus are, at least for the most part, manufactured by the liver, whatever the cause of the reabsorption of the bile from the liver into the blood may be. That hemolytic agents cause icterus was explained by the fact that on account of the large amounts of free hemoglobin brought to the liver, excessive amounts of bile-pigments are formed, which render the bile so viscid that it blocks up the fine bile capillaries; on account of the low pressure at which bile is secreted, a slight obstruction of this kind is sufficient to stop entirely the outflow of bile, which then enters the capillaries of the liver and also, to a less extent, the lymphatics.[81] It is also possible that the hemolytic poisons injure the liver-cells so much that the minute intra- and intercellular bile capillaries become disorganized, and permit of escape of bile into the lymph-spaces and its absorption into the blood-vessels.[82] Swelling of the degenerated liver-cells may also be an important factor in the occlusion of the bile capillaries; swelling of the lining cells of the bile capillaries may also coëxist, and fibrin may occlude them in toxic or infectious icterus.

However, Whipple and Hooper,[83] obtained experimental evidence that after intravenous injection of hemoglobin into dogs with the liver excluded from the circulation, bile pigments appear in the urine and icterus is manifested in the fat tissues, from which observations it is concluded that the liver may not be the only place in which bile pigment can be formed from hemoglobin. This has been corroborated by Mann and Magath[27] who observed the formation of bilirubin in animals from which the liver had been removed, and by Ernst and Szappanyos[84] who observed the formation of bilirubin in blood perfused through the spleen.

Several authors have found bilirubin produced in hemorrhagic effusions located where the liver could have had no influence,[85] and Froin believes the yellow color of spinal fluid in xanthochromia (*q. v.*) to be due to local formation of bilirubin from hemorrhages. We also recognize types of hemolytic icterus in which the liver does not seem to be concerned, and with bile pigments present in the blood and urine unaccom-

[80] See Eppinger, Ziegler's Beitr., 1903 (33), 123; Gerhardt, Münch. med. Woch., 1905 (52), 889. Lang (Zeit. exp. Path. u. Ther., July, 1906 (3), 473) has demonstrated the presence of fibrinogen in the bile in phosphorus-poisoning, which perhaps accounts for the "bile thrombi" observed by Eppinger in toxic icterus.

[81] See Mendel and Underhill, Amer. Jour. Physiol., 1905 (14), 252; Whipple and King, Jour. Exp. Med., 1911 (13), 115.

[82] Sterling, Arch. exp. Path., 1911 (64), 468; Fiessinger, Jour. Physiol. et Pathol., 1910 (12), 958. Oertel (Arch. Int. Med., 1918 (21), 73) suggests that intracellular precipitation of bile pigment within liver cells altered by poisons may prevent its excretion into the bile canaliculi.

[83] Jour. Exper. Med., 1913 (17), 593 and 612. These results could not be confirmed by McNee and Prusik (Jour. Path. and Bact., 1924 (27), 95); and Rich (Bull. Johns Hopkins Hosp., 1923 (34), 321).

[84] Klin. Woch., 1922 (1), 614.

[85] Hooper and Whipple, Jour. Exp. Med., 1916 (23), 137.

panied by bile salts (dissociated icterus), so that the old dictum of the essential implication of the liver in icterus seems to be incorrect.[86]

Because of all these facts it is now believed by many that the bile pigments are produced, not by the liver cells, but by the reticulo-endothelial cells.[87] Presumably other reticulo-endothelial cells besides the Kupffer cells of the liver have the capacity to manufacture bilirubin. Undoubtedly, however, most of the bile pigment responsible for icterus in ordinary conditions comes from the liver, perhaps the greater part of that present even in hemolytic icterus, for the Kupffer cells seem to be especially active in the formation of bilirubin,[88] but the place of maximum formation of bilirubin may perhaps vary in different species. The relative importance of injury to the hepatic tissues as compared with simple bile stasis in producing icterus is shown by the observations of McMaster and Rous,[89] who found that three fourths and more of the bile ducts of the liver may be occluded without producing icterus.

Joannovics[90] gave in 1904, on the basis of the view then held on the origin of icterus from bile obstruction and icterus from hemolysis, the following chief differences: "Icterus due to hemolysis appears sooner than icterus from bile-duct occlusion, and reaches a much higher degree; the obstruction in hemolytic icterus, when present, is intra-acinous; in stasis it is chiefly inter-acinous; in hemolytic icterus there is a large splenic tumor due to accumulation of degenerated red cells in the spleen, where they become disintegrated preliminary to the formation of bile-pigment." It has been reported that if the spleen is removed, hemolytic agents may not cause icterus, because the corpuscles are not then prepared for pigment formation.[91] In obstructive icterus there is a cholesterolemia in proportion to the amount of icterus, which is not usually true of hemolytic icterus.[92]

More recent studies have shown that there are differences in the forms of bilirubin present in the serum in obstructive and in hemolytic jaundice (see McNee[75]). Van den Bergh[93] found that the bilirubin in the blood serum in the two conditions behaves differently in the diazo reaction, the "obstructive bilirubin" being more readily oxidized then the "hemolytic bilirubin." Other differences have been observed, generally attributed to physical differences in the state of the bilirubin in the plasma, the hemolytic bilirubin probably being in larger aggregates.[94] For example, in obstruc-

[86] See Lepehne, Beitr. path. Anat., 1917 (64), 55.

[87] See Aschoff, "Lectures on Pathology," 1924; Klin. Woch., 1924 (3), 961. Review on formation of bile pigment by Rich, Physiol. Reviews, 1925 (5), 182.

[88] Elek, Klin. Woch., 1924 (3), 143.

[89] Jour. Exp. Med., 1921 (33), 731.

[90] Zeit. f. Heilk., Path. Abt., 1904 (25), 25.

[91] The etiology of *icterus neonatorum* (when not obstructive) has not been ascertained, but a natural tendency towards icterus is said to exist in the new-born, their blood containing much more bile pigment then than later. It is apparently of hemolytic origin (Hellmuth, Monats. Geb. Gyn., 1921 (54), 341).

[92] Rothschild and Felsen, Arch. Int. Med., 1919 (21), 520; Rosenthal and Holzer, Deut. Arch. klin. Med., 1921 (135), 257.

[93] "Der Gallenfarbstoff im Blut," Leiden, 1918.

[94] See Andrewes, Brit. Jour. Exp. Path., 1924 (5), 213; Quart. Jour. Med., 1924 (18), 19.

tive jaundice the conjunctiva becomes more yellow and the bile pigment pours out into the urine, while in hemolytic jaundice the pigment less readily enters either the conjunctiva or the urine, suggesting that in the latter condition the pigment is bound in the blood, perhaps by the protein.[95] In view of the numerous observations based on van den Bergh's methods, it now seems probable that the bilirubin derived from hemolyzed red cells is formed in the reticulo-endothelial cells, especially but by no means solely in the Kupffer cells of the liver. From the Kupffer cells it is presumably passed to the epithelial cells of the liver, in which it undergoes some slight change before being excreted into the bile capillaries, so that it now gives the "direct" or immediate reaction, whereas bilirubin that has not passed through the liver epithelium gives only the "indirect" or delayed van den Bergh reaction. It seems that the old problem of hepatogenous versus hematogenous icterus has at last been settled and that it is now possible to distinguish between them.[96]

Toxicity of Bile.—In any event, we must appreciate that in icterus not only are abnormally large quantities of bile-pigment present in the blood, but also the other less conspicuous constituents of the bile. Whole bile of rabbits is fatal to rabbits in doses of 0.25 to 0.5 c.c. per kilo, by intraperitoneal injection, and about half as much intravenously (Bunting and Brown[97]). Death is the result of changes in the myocardium, where necrosis is produced, and severe degenerative changes are also found in the kidneys and liver; when the bile is injected into the peritoneum, pancreatitis and fat necrosis result. The relative toxicity of the bile-pigments and the bile salts is not as yet uniformly agreed upon.

Bile-pigments.—Bouchard[98] and others have claimed that the bile-pigments are far more toxic than the bile salts, which is contradicted by Rywosch and others. For rabbits, 0.1 gm. of pure bilirubin has been found to be a fatal dose by intravenous injection.[99] King and Stewart[1] state that the amount of pigment in a lethal dose of whole bile will cause death, but the bile salts present in the same quantity of bile will not cause recognizable effects; uncombined pigment is more toxic than its calcium or magnesium salts. Bile from which the pigment is removed has very little toxicity. They suggest that calcium is increased in the blood in icterus as a protection against the toxic effects of the pigments. The combining of the calcium with bile pigment, however, renders it unavailable for fibrin formation; this seems to be an important factor in the hemophilic tendency of icterus,[2] and Pettibone records a marked decrease in blood calcium in protracted jaundice.[3] The decrease in available calcium

[95] Blankenhorn, Arch. Int. Med., 1921 (27), 131.
[96] See McNee, Brit. Med. Jour., Sept. 24, 1924.
[97] Jour. Exper. Med., 1911 (14), 445.
[98] Literature and discussion by Stadelmann, Zeit. f. Biol., 1896 (34), 57.
[99] Fromholdt and Nersessoff, Biochem. Zeit., 1921 (125), 153.
[1] Jour. Exper. Med., 1909 (11), 673.
[2] See Lee and Vincent, Arch. Int. Med., 1915 (16), 59; Bowler and Walters, Ann. of Surg., 1924 (80), 545.
[3] Jour. Lab. Clin. Med., 1918 (3), 275.

may also be responsible for the bradycardia and some of the mental and nervous symptoms, as well as for osteoporosis seen in chronic icterus.

Bilirubin is normally present in the blood and is probably responsible for the yellow color of the plasma.[4] It is always present in excess in icterus of whatever degree.[5] Van der Bergh,[6] whose method for determining bilirubin is now widely used, gives as the normal bilirubin content of serum from one to two parts per million. There is a marked variation between different normal individuals, but for the same person the figures are nearly constant.[7] The threshold value for the blood seems to be about 1 part in 50,000; when this proportion is exceeded the pigment begins to be deposited in the skin and excreted by the kidneys (v. d. Bergh). However, Blankenhorn[8] believes that at times the bilirubin in the plasma is so bound that the kidneys do not excrete it, and yet it may be able to diffuse into the skin. Meulengracht[9] also says that ordinarily bilirubin leaves the blood for the tissues at a lower concentration than the threshold for excretion in the urine. As the above statements indicate, there may be a distinct excess of bilirubin in the blood before there is any observable jaundice,[10] but the total amount does not become very high because of excretion in the urine and deposition in the tissues. In arsenical jaundice a maximum figure of 175 parts per million was observed by Schamberg and Brown,[11] but jaundice was present with as little as 7 or 8 parts. Hyperbilirubinemia is present in most cases of cholecystitis even without visible jaundice, and often in pneumonia.[12] With reduced renal function the amount of pigment in the blood may be increased without hepatic disease. In pregnancy the bilirubin in the blood is constantly increased,[13] and newborn infants exhibit bilirubinemia.[14]

Bile salts are said to be toxic, generally producing depression of the central nervous system, with resulting coma and paralysis; they are also decidedly toxic to cells of all sorts, causing hemolysis and marked destruction of tissue-cells. Small quantities of bile salts stimulate the central end of the vagus, and large amounts influence the heart itself; hence in icterus we observe a slowing, and often an irregularity, of the pulse, and the blood pressure is lowered. Although there has been much dispute as to whether the chief effects of icterus upon the heart depend upon action of the bile salts upon the vagus, or upon the intracardiac ganglia, or upon the muscle itself,[15] yet Weintraud demonstrated that in some cases of icterus administration of atropine, which paralyzes the vagus,

[4] Blankenhorn, Arch. Int. Med., 1917 (19), 344; 1918 (21), 282.
[5] Feigl and Querner, Zeit. exp. Med., 1919 (9), 153.
[6] Presse Méd., 1921 (29), 441.
[7] Bauer and Spiegel, Deut. Arch. Klin. Med., 1919 (129), 17.
[8] Arch. Int. Med., 1918 (21), 282; Trans. Assoc. Amer. Phys., 1920 (35), 204.
[9] Ugeskrift f. Laeger., 1919 (81), 1785.
[10] See Bernheim, Jour. Amer. Med. Assoc., 1924 (82), 291.
[11] Jour. Amer. Med. Assoc., 1924 (82), 1911.
[12] Friedman and Strouse, Jour. Amer. Med., Assoc., 1924 (82), 1248.
[13] Herrmann and Kornfeld, Wien. klin. Woch., 1924 (37), 1215.
[14] Williamson, Surg. Gyn. Obst., 1923 (37), 57.
[15] See Minkowski, Ergeb. der Pathol., 1895 (2), 709.

stops the bradycardia, indicating the importance of the effects of the bile salts upon the vagus in causing this feature of cholemia. According to Meltzer and Salant,[16] bile also contains a tetanic element which disappears from stagnating bile; the bile salts contain this tetanizing agent in less amount than does the whole bile. But King[17] and others ascribe most of the effects of bile on the heart to the bile pigments, perhaps through abstraction of the calcium. Taurin given to man in 10 gm. and even larger doses by mouth, subcutaneously, and intravenously, produced no noticeable effects (Schmidt).[18]

It is commonly stated that bile salts are hemolytic, but Ponder[19] has found that serum has a marked inhibitory effect, and observed no hemolysis in a rabbit receiving bile salts intravenously. Whether a more protracted cholemia, or the presence of abnormally fragile corpuscles, may lead to hemolysis by bile salts, was not determined. Unfortunately we have no accurate method for quantitative determination of the amount of bile salts in the blood, normally, and the amounts present in severe hepatogenous icterus have been found to be only about 0.1 g. per liter.[20] Comparative estimations of the amount of bile salts have been made by observing their effect in lowering the surface tension of the blood and urine (Borchardt).[21]

The necroses observed in the liver in icterus, "*icteric necrosis*," are generally ascribed to the cytotoxic effects of the bile salts, although it is difficult always to exclude infection extending along the bile-ducts to the liver tissue. Possibly the power of bile salts to dissolve lipoids may be responsible for the cytotoxic effects[22] as well as for the hemolysis. The itching and irritation of the skin in icterus may be due to the effect of the bile-salts deposited in it, for pruritus is said to be absent in the pigmentary jaundice of congenital hemolytic icterus. There is also an increase in the cholesterol in the blood, which may be related to the "xanthomas" (*q. v.*) that form in chronic icterus.

In cholemia there may occur a latent lipemia, (*i. e.*, increased lipin content without turbidity), with hypercholesterolemia, in which the proportion of free to combined cholesterol is increased. Hemolytic icterus does not exhibit lipemia (Buerger),[23] and cholesterolemia is absent in icterus neonatorum and the icterus of pernicious anemia.[24]

The tendency to spontaneous hemorrhages frequently observed in icterus, probably depends upon injury to the capillary endothelium by the bile salts,[25] while the protracted, often uncontrollable, hemorrhage that may occur from wounds in icteric patients, is related to the slowed

[16] Jour. Exp. Med., 1906 (8), 128; review and literature concerning toxicity of bile.
[17] See King, Bigelow and Pearce, Jour. Exper. Med., 1912 (14), 159.
[18] Schmidt *et al.*, Jour. Biol. Chem., 1918 (33), 501.
[19] Brit. Jour. Exp. Path., 1921 (2), 289.
[20] Gilbert *et al.*, Compt. Rend. Soc. Biol., 1920 (83), 1602.
[21] Klin. Woch., 1923 (2), 541.
[22] Neufeld and Händel, Arb. kaiserl. Ges.-Amte, 1908 (28), 572.
[23] Münch. med. Woch., 1922 (69), 103.
[24] Rosenthal and Meier, Arch. exp. Path. Pharm., 1921 (91), 246.
[25] See Morawitz, Arch. exp. Path., 1907 (56), 115.

coagulation of the blood, an important factor in which is probably the binding of the plasma calcium by bile pigments.[26]

The bile salts themselves may delay coagulation by interfering with the conversion of fibrinogen into fibrin.[27] The cytotoxic effect of the bile salts is also shown by the albuminuria of icteric persons. Although bile itself is toxic to many bacteria, especially the pneumococcus,[22] yet in icterus the bactericidal power of the blood is lowered, and infections are prone to develop and to be severe; moreover, the growth of several species of bacteria is favored by bile.[28]

Croftan[29] summarizes the physiological effects of bile acids as follows: (1) A powerful cytolytic action, affecting both blood-corpuscles and tissue-cells. (2) A distinct cholagogue action. (3) In small doses they aid coagulation. (4) In large doses they retard coagulation. (5) Slow the heart action.[30] (6) In small doses they act as vasodilators; in large doses, as vasoconstrictors. (7) Reduce motor and sensory irritability. (8) Act on the higher cerebral centers, causing coma, stupor, and death. Sellards[31] found that injection of bile salts into guinea pigs causes ulceration and hemorrhage in the stomach.

It is difficult to decide how much of the profound intoxication that is sometimes present in icterus ("cholemia" and "icterus gravis") to ascribe to the reabsorbed bile, for frequently there is an accompanying infection, and even if there is no infection the impairment of liver function by the obstruction of bile outflow must also be reckoned with.[32] The liver is not only a destroyer of toxic substances absorbed from the alimentary canal, but it is also an important seat of nitrogenous metabolism, interference with which may lead to accumulation of many toxic nitrogenous substances in the blood.[33] The long duration of severe icterus in some cases of occlusion of the bile-ducts, with relatively slight evidences of intoxication, would seem to indicate, however, that on the whole the bile is not so much responsible for the intoxication observed in icterus as are the associated conditions. On the other hand, in not a few instances it has been observed that escape of large quantities of bile into the peritoneal cavity may be followed by symptoms similar to those of icterus gravis; in these cases only the bile can be held responsible for the intoxication.[34]

Dissociated Jaundice[35] is the existence of either bile salts or bile pigment separately in the blood. This may be produced either by the bile salts being excreted by the

[26] See Koechig, Jour. Lab. Clin. Med., 1924 (9), 679; Bowler and Walters, Ann. Surg., 1924 (180), 545.

[27] Haessler and Stebbins, Jour. Exp. Med., 1919 (29), 445.

[28] See Meyerstein, Cent. f. Bakt., 1907 (44), 434.

[29] New York Med. Jour., 1906 (83), 810; see also Faust, "Die tierische Gifte," Braunschweig, 1906, p. 29.

[30] See Berti, Gaz. degli Osped., 1916 (37), 1233.

[31] Arch. Int. Med., 1909 (4), 502.

[32] The supposed occurrence of leucine and tyrosine in the urine in icterus is probably dependent on the mistaken identification of bile pigment crystals (Dorner, Deut. med. Woch., 1922 (1), 1158).

[33] See Bickel, Exper. Untersuch. uber der Pathol. der Cholæmie, Wiesbaden 1900.

[34] See Ehrhardt, Arch. klin. Chir., 1901 (64), 314; Fibich, Wien. med. Woch., 1920 (70), 321.

[35] Hoover and Blankenhorn, Arch. Int. Med., 1916 (18), 289.

kidney, leaving only the less diffusible pigment in the blood, or by separate escape of oile salts from the liver into the blood. Also in true hemolytic icterus we may have bile pigments present in the blood and urine without bile salts, but this is said not to occur in obstructive icterus.[36]

Hereditary or "Congenital" Hemolytic Icterus.[37]—This term describes a condition characterized by a chronic, non-obstructive jaundice, without evident intoxication, with very definite hereditary features, and often not congenital but appearing after adolesence, hence it is properly called "hereditary icterus." A similar condition is also observed developing in adults, without recognized familial tendencies. The congenital form usually shows familial character and commonly is associated with other developmental defects, but isolated congenital cases are said to occur.[38] It is the result of active hemolysis, apparently taking place chiefly in the spleen, and leading to an icterus without evident participation of the liver. The cause of the hemolysis is entirely unknown, although there is a marked fragility of the erythrocytes, evidenced by reduction of their resistance to hypotonic solutions and often to other hemolytic agents. There is moderate anemia with marked reticulation of the erythrocytes, a color index of about one, and hemolysins cannot be demonstrated in the blood. A characteristic feature is the excretion of much urobilin in the stools and urine, and the blood contains bilirubin which is not excreted in the urine. The jaundice is usually unaccompanied by evidence of cholemia, icteric pruritus or hemophilia. The spleen is greatly enlarged and improvement generally follows splenectomy, but the exact relation of the spleen to the disease is not known.[39] After removal of the spleen the fragility of the corpuscles may remain unimproved, but their hemolysis is not rapid enough to cause icterus. The frequent occurrence of gall stones in this condition may be the result of hypercholesterolemia from hemolysis, but this has not been demonstrated.

Metabolism studies show loss of nitrogen, calcium, magnesium, cholesterol, and iron, and a much increased uric acid excretion.[40] These conditions may improve after splenectomy.[41] The spleen, kidney and liver may contain much or little iron, sometimes the amount being large enough in the liver to resemble hemochromatosis.

The Pigmentation in Icterus.—Living tissues have but a slight tendency to take up bile-pigments, much of the tissue-staining observed at autopsy being due to postmortem imbibition from the blood and lymph. Quincke[42] found that after subcutaneous injection of bilirubin only the connective tissue (both the cells and intercellular fibrils), becomes diffusely colored; later, it fades out of the cells, leaving only the fibrils stained. Muscle-cells, fat-cells, and vessel-walls take up the pigment only after death. If the jaundice continues for a long time, the subcutaneous deposits of

[36] Borchardt, Klin. Woch., 1922 (1), 988.

[37] Reviews by Tileston, Medicine, 1922 (1), 355; Meulengracht, "Der Chronische Hereditäre Hemolytische Icterus," Leipsic, 1922.

[38] See Gannslen *et al.*, Deut. Arch. klin. Med., 1925 (146), 1.

[39] See Pearce, Krumbhaar and Frazier, "The Spleen and Anemia," Phila., 1918.

[40] McKelvy and Rosenbloom, Arch. Int. Med., 1915 (15), 227; Denis, Arch. Int. Med., 1917 (20), 79.

[41] Goldschmidt, Pepper and Pearce, Arch. Int. Med., 1915 (16), 437.

[42] Virchow's Arch., 1884 (95), 125.

bilirubin may undergo a slow oxidation, the color changing to an olive or to a dirty grayish green. The pigment in the connective tissues is at first in solution, but may be deposited in a granular form after a considerable amount has accumulated. Bile pigments and bile salts may both be present in considerable amounts in the blood and not pass through the kidneys, and also they may fail to pass into the tissues; hence we may have cholemia without icterus or choluria, because of the firmness with which the pigments are bound in the plasma.[35]

The question whether in icterus the skin may be colored by other pigments than bilirubin, especially by its reduction product, urobilin, seems to have been decided negatively. Bile-pigment is probably not absorbed as such from the intestine in sufficient quantity to cause icterus. Such bile-pigment as enters the blood from the liver is excreted through the kidneys chiefly, but also in the sweat. Ordinarily, other secretions (milk, tears, saliva, sputum) are not colored in jaundice, but if the secretions are mixed with inflammatory exudations, they may then be colored (*e. g.*, pneumonic sputum). When the bile-pigment is resorbed from the skin, it may be in part transformed into urobilin, which appears in the urine in increased amounts during the period of recovery from jaundice. Part of the bile-pigment is probably eliminated by the liver after the cause of obstruction has been removed from the bile-passages.

Digestive Disturbances in Obstructive Icterus.[43]—In case the icterus depends upon the occlusion of the main bile-passages by stones, tumors, etc., the situation is complicated by the effects of the absence of this natural secretion in the intestinal canal. Carbohydrate and protein digestion seem to be but little affected, especially the former, but the proportion of the ingested fat that appears in the feces increases from the normal 7–11 per cent. to 60–80 per cent. The fatty acids carry out with them in the feces a large amount of calcium,[44] partly withdrawn from the blood, which may account in part for the osteoporosis observed at times both in animals and man when bile is prevented from entering the bowel. The products of bacterial decomposition of the undigested fat may lead to injury of the intestinal wall and disturbance of its function. Failure of absorption of fat also favors intestinal putrefaction by enveloping the protein substances so that they are not readily digested and absorbed. The relation of bile to intestinal putrefaction is still not exactly determined. Frequently, but by no means always, there is an increased intestinal putrefaction which may result in diarrhea and the appearance of excessive quantities of indican and phenol in the urine. The idea once held that the bile salts acted as intestinal antiseptics has not been established by experimental investigations; however, it is possible that through their function as natural cathartics, by stimulation of peristalsis, they prevent stagnation and putrefaction of proteins.

UROBILIN[45]

This pigment is said to be formed chiefly from bile pigments by the action of reducing bacteria in the intestine, which are supposed to change bilirubin into stercobilin, homologous with urobilinogen, but Whipple[46] says that it is not established that stercobilin is absorbed. He believes that the urobilin is formed in the liver and bile passages, and perhaps outside these structures in hemorrhagic extravasations. Furthermore, it has been reported that stercobilin is found in the feces and that urobilinuria may occur when there is complete obstruction of the common bile duct,[47] so that evidently the amount of urobilin excreted in the urine cannot be dependent solely on the amount of bile pigment entering the intestine. Adler[48] holds that there is no one place where urobilin is manufactured, but that although the bowels are the chief site of urobilin

[43] Concerning metabolism in icterus see Vannini, Zeit. klin. Med., 1912 (75), 136.
[44] Telfer, Biochem. Jour., 1921 (51), 347.
[45] Bibliography and review by Meyer-Betz, Ergeb. inn. Med., 1913 (12), 734; Wilbur and Addis, Arch. Int. Med., 1914 (13), 235.
[46] Arch. Int. Med., 1922 (29), 711.
[47] Brulé and Spilliaert, Ann. de Méd., 1921 (9), 377.
[48] Klin. Woch., 1922 (1), 2505.

formation, it isalso undoubtedly manufactured in the liver, and in all probability the reticulo-endothelial cells in all parts of the body, in conjunction with the liver cells, can produce urobilin. Nevertheless, the prevailing idea, which seems to have generally corresponded with clinical observations, has been that urobilin is chiefly of enterogenous origin. Bauman[49] recapitulates the evidence in support of this origin as follows: (1) The transformation of bilirubin into urobilin by bacteria *in vitro*. (2) The absence of urobilin in the stool in severe jaundice due to obstruction of the common bile duct. If, to such patients, bile be administered (by stomach tube) urobilin promptly appears in the stool. (3) Although bilirubin occurs in the stool of the new-born, urobilin is not present until the third day, when the bacterial flora of the intestine have had an opportunity to develop. (4) Diarrheal stools contain bilirubin but often no traces of urobilin. It is believed that increased peristalsis produces a rapid passage of bile through the intestine before the bacteria have had time to reduce bilirubin to urobilin.

It is excreted in the urine only as its chromogen, urobilinogen, but in the feces both urobilin and urobilinogen may be found; when exposed to air the chromogen oxidizes quickly to urobilin. Addis[50] believes that bilirubin is reduced to urobilinogen in the bowel and is then largely absorbed, to be at once oxidized and polymerized into urobilin, two molecules of urobilinogen uniting under the influence of oxygen to form one of urobilin. In the liver the urobilin is largely worked over to form new hemoglobin, and hence the functional capacity of the liver is indicated by the completeness with which it utilizes the urobilin, except in cases of excessive formation of urobilinogen as a result of hemolysis. The amount of urobilinogen in the urine will be found increased, therefore, in hemolytic icterus, and decreased in obstructive icterus. Adler[51] says that normally 20 to 25 mg. is excreted daily in the urine, somewhat increased by fasting or meat feeding, the largest amounts being found in severe degenerative diseases of the liver, such as acute yellow atrophy and cirrhosis, in which conditions as much as a gram of urobilin may be excreted daily. Severe infectious diseases, such as pneumonia, may cause an excretion up to 600 mg., which is greater than that usually seen in hemolytic icterus (300 to 400 mg.) whereas in pernicious anemia but about twice the normal amount is usually found. Pathological urobilinuria is characterized by excretion of 20 mg. or more in the morning urine, which often contains more urobilin than after eating, this being the reverse of the normal condition. Weltmann[52] notes the abundance of urobilin demonstrable in the blood in pneumonia and in cardiac decompensation, and the small amount in catarrhal jaundice as compared with cholelithiasis. According to Peters[53] there is normally about five times as much in the feces as in the urine, but in cirrhosis this relation is reversed.

[49] Arch. Int. Med., 1921 (28), 475.
[50] Arch. Int. Med., 1915 (15), 412.
[51] Deut. Arch. klin. Med., 1922 (140), 302.
[52] Wien. Arch. inn. Med., 1923 (6), 587.
[53] Ned. Tijd. Geneesk., 1919, p. 1602.

Exceptionally, urobilinogen may be formed from blood disintegrated in bloody effusions without evident participation of the liver, *e. g.*, urobilinogenuria from hemorrhagic ascites, hemolytic poisons, etc. With a normal liver, urobilinogenuria is found only when there is excessive hemolysis, otherwise urobilinogenuria occurs only with an injury to the liver parenchyma (Hildebrant). In general, except with hepatic lesions, the amount in the urine is an index of the amount of blood destruction.[54] There seems to be little if any retention by imperfectly functioning kidneys (Blankenhorn) and it can often be found in the urine when not demonstrable in the blood. Occlusion of the bile ducts stops an existing urobilinogenuria, which is usually present in cholelithiasis with patent ducts, by preventing the formation of urobilinogen in the intestine.[55] The amount of urobilin and urobilinogen excreted in the feces, seems to vary directly with the amount of hemolysis,[56] and the same is true for the duodenal contents.[57] The evidence of abnormal hemolysis is said to occur first in the stools, then in the duodenal contents, and lastly in the urine; the presence of even small amounts of urobilinogen in the urine being evidence of a probable pernicious anemia in the absence of signs of biliary and hepatic disease.[58] Bile normally contains but traces of urobilin, the amount being increased when through disease the liver has a reduced capacity to transform urobilin, when the liver is overwhelmed as in hemolysis, or when bacteria are present in the bile ducts to reduce the bilirubin (Winternitz).[59]

URINARY PIGMENTS[60]

In addition to bile pigments, melanin and other abnormal pigments, the urine has its own normal coloring matter, which is commonly referred to as

Urochrome.—Pelkan[61] found that in normal subjects a low protein diet reduces the daily urochrome excretion, and a high protein diet increases it. Since in proteins a substance was found giving the color tests for urochrome, he concluded that a large proportion of urinary urochrome must come from the food proteins. However, in starvation the excretion of urochrome continues, and as the highest values for urochromogen are often found in patients who have taken little or no protein food for some time (*e. g.*, tuberculous meningitis, typhoid fever), the endogenous origin of most of the urochromogen, at least, seems probable. There is probably not any definite substance which can be designated as the "nor-

[54] Dubin, Jour. Exp. Med., 1918 (28), 313.
[55] See Hansen, Ugeskr. f. Laeger, 1920 (82), 415.
[56] Robertson, Arch. Int. Med., 1915 (16), 1072; McCrudden, Bost. Med. Surg. Jour., 1917 (177), 907.
[57] Giffin, Sanford and Szlapka, Amer. Jour. Med. Sci., 1918 (155), 562.
[58] Hausmann and Howard, Jour. Amer. Med. Assoc., 1919 (73), 1262.
[59] Wien. Arch. inn. Med., 1923 (7), 201.
[60] See also "Chemistry of Tuberculosis," Wells, DeWitt and Long, 1924, pp. 327–338.
[61] Jour. Biol. Chem., 1920 (43), 237.

mal yellow pigment" or urochrome. Among the several unidentified substances which together are probably responsible for the yellow color is

Urochromogen, which is found in appreciable amounts only in pathological urines, especially in conditions associated with tissue destruction or wasting, *e. g.*, cachexia, cancer, tuberculosis, phosphorus poisoning, and other states in which increase in the residual-N fraction of the urine is observed. This urochromogen fraction, when oxidized with a weak solution of permanganate, gives a color resembling that of urochrome, but it is now doubted that urochrome is merely a more completely oxidized urochromogen. With further oxidation, urochromogen goes over into darker pigments, including *uromelanin* (Weiss).[62] Although a considerable part of the neutral sulfur of the urine comes down in the urochromogen fraction, it is by no means certain that this is a part of the urochromogen itself.

The urochromogen fraction contains the substance of the urine which, when treated with Ehrlich's diazo reagent followed by ammonia, gives the typical "diazo reaction." If the urine gives a positive Erhlich reaction, it will also give the permanganate test for unrochromogen. The nature of the substance giving the diazo reaction has not yet been determined, but it is probably a phenol or a phenol derivative.

The *Penzoldt-Pauly diazo reaction*, which is given by the normal urine even after removal of the coloring matter, when treated with Ehrlich's reagent followed by NaOH solution, is apparently entirely unrelated to the substance which is found in pathological urines and which gives the Ehrlich diazo reaction with ammonia. It probably depends on the presence of a derivative of histidine.

We have to deal here, therefore, with either three or four substances found in the proteic acid fraction of urine; two normal constituents of the urine, urochrome and the colorless, diazo-reacting body of the Penzoldt-Pauly test; and constituents found in appreciable quantities only as a sign of abnormal tissue breakdown, constituting the urochromogen fraction, which give the permanganate test, and the substance or substances giving the Ehrlich diazo-reaction, which is associated with the urochromogen, but which may be a different substance.

[62] Biochem. Zeit., 1922 (133), 331.

CHAPTER XXI

THE CHEMISTRY OF TUMORS[1]

CHEMICAL investigations of tumors have so far detected little that indicates any marked deviation of the chemical processes of tumor cells from those of normal cells of similar origin. Likewise, the chemical composition of tumor tissue resembles closely, on the whole, the composition of related normal tissues. It is hardly to be imagined that the course of chemical changes is greatly different in tumor cells from that in normal cells, in view of the abundant evidence that the metabolic products of tumor cells are identical with those of the cells from which they arose. Thus, metastatic growths of thyroid tissue will produce thyroiodin in any part of the body, liver carcinoma metastases produce bile, tumors from the choroid or from pigmented moles produce melanin, etc.[2] The capacity of tumor cells to produce complicated products of metabolic action specific for the parent cells from which they arose, as illustrated above, indicates beyond question that the course of their chemical activites is very much like that of normal cells. So, too, the composition of the cells is found to be similar indeed to that of the parent cells, both in regard to primary and secondary constituents. Thus, Bang found that sarcomas derived from lymph-glands contain the particular nucleo-proteins that are found normally only in lymph-nodes, hypernephromas contain much fat, lecithin, and cholesterol; squamous cell carcinomas develop great amounts of kerato-hyalin; carcinomas of mucous membranes may contain much mucin, etc.

Many have sought in cancer tissues a poison that might account for the cachexia characteristic of new-growths. Extracts have been obtained that were destructive to red corpuscles (hemolytic), and that were sometimes slightly toxic to animals, but the results have not seemed sufficiently striking to account for the appearance of cachexia. Because of the interference with circulation brought about in tumors by pressure of the growing tissues upon their blood-vessels, areas of necrosis frequently develop, and these, undergoing autolysis, yield substances that are hemolytic and toxic. Whether these are the cause of cancer cachexia, however, may be questioned; but they are sufficient to account for most of the experimental results as yet obtained. No substance has yet been isolated from or detected in malignant growths that is peculiar to them and not found in normal cells, and still less has any substance been detected in tissues that accounts in any way either for the occurrence of tumors or for the effects that they produce.

[1] Earlier literature given by Neuberg, Zeit. Krebsforsch., 1910 (10), 55; and Blumenthal, Ergebnisse Physiol., 1910 (10), 363.

[2] See Wells and Long, Zeit. Krebsforsch., 1913 (12), 598.

Tumor cells seem to depend upon much the same conditions as normal body cells for their growth, since anything that leads to wasting, malnutrition, or atrophy in the tissues of the host usually tends to impede the rate of growth of the tumor cells, in marked contrast to infectious diseases. Specific attempts to modify tumor growths by diets which stunt the animals because lacking certain amino-acids necessary for growth, have been partly successful,[3] but it is difficult to be sure that this effect depends on the specific absence of a definite substance rather than on general malnutrition.[4] Furthermore, the constituents of the hypophysis that stimulate somatic-tissue growth are also said to stimulate growth of tumor tissues.[6]

Chemical Stimulation of Cancer Growth.—Experimental studies have confirmed the clinical observation that cancer may result from the protracted stimulation of growth of tissue cells. This stimulant may be mechanical, or chemical, or radiant energy, and apparently often a mixture of forces; in fact, it is usually difficult or impossible to exclude chemical influences in inciting any tissue growth, since physical factors may always act indirectly through the formation of growth-stimulating substances from injured tissue elements. The relative importance of mechanical and chemical factors is suggested by the fact that the mechanical irritation of coal dust does not produce cancer, while soot and coal tar without mechanical irritation do produce cancer. The stimulation of cancer formation by organic chemicals was perhaps first clearly established with dye-workers cancer of the bladder,[7] although it is not yet agreed whether the inciting agent is aniline or more complex organic compounds, or perhaps even arsenic present in the chemicals, as suggested by Alice Hamilton.[8] Curschmann[9] found that beta-naphthylamine seems to be particularly concerned, Schwerin[10] also incriminates benzidin and fuchsin, while Nassauer believes aniline is alone responsible. In favor of the arsenical origin of these cancers is the well-known fact that chronic arsenic poisoning often leads to dermatoses which occasionally develop into carcinoma.

Cancer in workers in tar[11] and pitch is undoubtedly due to the stimulating effect of some constituent of these mixtures upon the epithelium, and Yamagiwa initiated an entire new line of cancer research by applying this fact to the experimental production of cancer by painting tar on the skin or injecting it into animals.[12] The extensive study which has since been made with tar cancer has not shown which of the constituents of the

[3] See Sweet, *et al.*, Jour. Biol. Chem., 1915, (21), 309.
[4] Rous, Jour. Exp. Med., 1914 (20), 433.
[6] Robertson and Burnett, Jour. Exp. Med., 1916 (23), 631.
[7] Literature reviewed by Nassauer, Frankf. Zeit. Path., 1920 (22), 353.
[8] Jour. Indust. Hygiene, 1921 (3), 16.
[9] Zent. f. Gewerbh., 1920 (8), 169.
[10] Zent. f. Gewerbh., 1920 (8), 64.
[11] History of tar cancer, chimney sweep's cancer, etc., given by Fibiger, Hospitalstidende, 1921 (64), 51.
[12] Review by Yamagiwa in Virchow's Arch., 1921 (233), 235. Also see Jour. Cancer Res., 1924 (8), 119.

tar is responsible, and indeed it would seem probable that in such a mixture as tar there might be many substances which, alone or combined, will stimulate cell proliferation. Bloch and Dreifuss[13] found cancer produced only by the fraction boiling at over 300°. Kennaway[14] corroborated this, obtaining an active fraction boiling at 490-550°, and finding that cancer is not produced by any of the pure substances isolated from this fraction, such as anthracene, but crude creosote oil[15] or anthracene oil is effective. Of the different tars, most active is gas works tar, then shale oil, then crude petroleum, while blast furnace tar is inactive. There is no evidence that pure paraffin or oils rich in paraffin produce cancer, and the term "paraffin cancer" would seem to be a misnomer (Kennaway). Gas works pitch produces more cases of tar cancer than all other related materials together, the briquette industry furnishing a particularly large proportion. All the acids and bases may be removed from tar without destroying the activity. Presumably some unidentified substance or substances present in the tar in small quantities, is the active agent.[16] The condensation products of isoprene will produce experimental cancer,[17] more rapidly than coal tar, which is of interest since isoprene contains only C and H.

These observations mark a distinct advance on the studies with scarlet-R initiated by B. Fischer,[18] for this dye and related substances produced merely epithelial overgrowth, but not true cancer, in the hands of most observers. Apparently under certain conditions scarlet-R may produce true tumors, at least Yamagiwa and other Japanese workers report success with injections of this dye into the mammary gland and uterus of animals. Stoeber has considered substances related to scarlet-R that might have this stimulating effect, and found naphthylaminol most active. In general, fat-soluble organic basic substances only were found to have this property, among them being indole, skatole, and pyridine.[19]

One general principle comes out of these and the tar-cancer studies, namely, that the active agents are fat-soluble substances which stimulate cell growth, and recall the observations of E. P. Wolf,[20] who found that substances specifically capable of stimulating inflammation, are fat-soluble. Another point of significance is that the same stimulus may produce either sarcoma or carcinoma, indicating the fundamental identity of these two forms of malignancy. With all these chemical cancrogenic substances the stimulation must be continued for a very long time, but growths may develop long after the application of the stimulant has

[13] Schweiz. med. Woch., 1921 (51), 1033.
[14] Jour. Indust. Hyg., 1924 (5), 462. Full review.
[15] See Cookson, Brit. Med. Jour., Mar. 1, 1924, p. 358.
[16] H. C. Ross ("Researches into Cell Reproduction and Cancer," London) has made extensive studies of the relation to cancer of substances which cause leucocytes to multiply, designating them as "auxetics." These seem to be present in the anthracene fractions of tar (Norris, Biochem. Jour., 1914 (8), 253).
[17] Kennaway, Jour. Path. Bact., 1924 (27), 233.
[18] Full review of chemistry and effects by Stoeber, Münch. med. Woch., 1910 (57), 739 and 947; Haga, Zeit. Krebsforsch., 1913 (12), 525.
[19] Münch. med. Woch., 1909 (56), 129; 1910 (57), 947.
[20] Jour. Exp. Med., 1923 (37), 511.

ceased.[21] Systemic changes, notably hypercholesterolemia[22] are generally found to have a stimulating effect on growth of both transplanted and spontaneous cancers in animals.[23]

A. CHEMISTRY OF TUMORS IN GENERAL

Numerous observations have been made concerning the chemistry of tumors, which, although they do not as yet throw any important light on the fundamental problems of tumor pathology, are of much interest. These may be briefly summarized as follows:

(1) **Proteins.**—Earlier studies showed that tumor growths contain the same sorts of proteins as do normal tissues, apparently in about the same proportions, and in spite of certain contradictory reports this statement seems to be correct.

In all probability the nucleoproteins of tumors share the specific characteristics of the nucleoproteins of the tissues from which they arise—at least this is the case with the nucleoproteins of lymphosarcoma, according to Bang.[24] This seems to have been confirmed by Beebe,[25] who found nucleo-histone only in lymphatic tissue, but the distinction between thymus and lymph-node nucleohistone is probably not so easily made as Bang intimates. Because of their richly cellular structure cancers may contain more nucleoprotein than the tissues from which they arise.[26] However, Wells and Long[27] found the proportion of purine nitrogen in tumors of several classes to be much lower than might be expected from the nuclear content as shown by the microscope; also, Satta[28] found unexpectedly low phosphorus figures and Yoshimoto[29] found no parallelism between the number of nuclei and the nuclein content. The purines present in tumor tissues are quite the same in nature and proportion as in normal tissues (Wells and Long), as also are the nucleoproteins. Oswald[30] found in the nucleoprotein of a secondary carcinoma of the liver, 1.10 to 1.28 per cent. phosphorus and 0.68 per cent. iron, both figures somewhat lower than commonly found for liver nucleoprotein.

Bergell and Dörpinghaus[31] have studied the nature of the proteins in tumors by determining the proportion of the various amino-acids that compose them. Because of the amount of material necessary for the ester method, they were obliged to use a mixture of various primary and secondary cancers and one sarcoma. The protein of this tumor mixture was characterized by the very high proportion of alanine,

[21] See Leitch, Brit. Med. Jour., 1922 (2), 1107.

[22] See Borst, Zeit. Krebsforsch., 1924 (21), 337; Yamagiwa et al., Jour. Cancer Res., 1924 (8), 127.

[23] Concerning the influence of digestion products of ductless glands (*optones*) on cancer growth see Engel, Zeit. Krebsforsch., 1923 (19), 339.

[24] Hofmeister's Beitr., 1903 (4), 368.

[25] Amer. Jour. Physiol., 1905 (13), 341.

[26] Petrey, Zeit. physiol. Chem., 1899 (27), 398.

[27] Zeit. f. Krebsfrsch., 1913 (12), 598.

[28] Arch. Ital. Biol., 1908 (49), 380.

[29] Biochem. Zeit., 1909 (22), 299.

[30] Zeit. physiol. Chem., 1916 (97), 264.

[31] Deut. med. Woch., 1905 (31), 1426.

glutaminic acid, phenylalanine and aspartic acid, there being from 5 to 10 per cent. of each. Leucine was very low, 5–10 per cent., as against 20 per cent., or higher, found in most normal tissues. Glycine and tyrosine were present in small quantities, and serine was probably also present. Neuberg[32] found in cancer protein 1.3 per cent. of tyrosine, 17 per cent. of leucine, scarcely 1 per cent. of glutaminic acid, and 4.92 per cent. of glycine. In five human tumors of different sorts, Kocher[33] found high figures for diamino-nitrogen; his averages were: Arginine, 12.42; histidine, 4.86; lysine, 11.23; total, 28.47 per cent. of the protein nitrogen. Drummond's[34] careful studies in this field have shown that the diamino-acid content of tumors is generally slightly higher than in corresponding normal tissues, probably varying directly with the amount of nuclear material, there being nothing found to indicate that the hexone bases are in any way responsible for increased growth. Strange, and as yet unexplained, variations in tryptophan content in various tumors were found by Fasal,[35] some having a very high tryptophan figure, while in others none could be found. Centanni[36] found that tryptophan and tyrosine inhibit, while skatole and indole stimulate carcinoma growth.

Certain authors have believed that the cancer cell has a specific chemistry,[37] but most of these analyses, including that of Abderhalden and Medigreceanu,[38] seem to indicate that cancer proteins have much the same composition as normal proteins. Furuhata[39] found no difference in the amino acid partition of the Rous chicken sarcoma and normal chicken tissues. Cramer and Pringle[40] find that there is less nitrogen in mouse cancers than in equal amounts of other mouse tissue, the decrease being in the coagulable nitrogen, incoagulable nitrogen being relatively increased. The water content of rapidly growing tissues, whether normal or cancerous, was found to be high. This corresponds with the analysis of Robin.[41] who found the water content high and nitrogen low in carcinomas of the liver, sulfur being especially low, and Chisholm[42] has found the proportion of nitrogen in several human tumors lower than in the somatic tissue. However, the lack of any marked specific individuality of cancer proteins when tested by immunological reactions, indicates a very close chemical agreement with normal tissue proteins.

On account of the amount of autolysis going on in tumors the products of protein splitting are usually present. Beebe[43] found in a number of tumors leucine, tyrosine, tryptophan, proteoses (biuret reaction), and in

[32] Arb. a. d. Path. Inst. zu Berlin, 1906, p. 593.
[33] Jour. Biol. Chem., 1915 (22), 295.
[34] Biochem. Jour., 1916 (10), 473.
[35] Biochem. Zeit., 1913 (55), 88.
[36] Tumori, 1913 (2).
[37] Blumenthal, Zeit. Krebsforsch., 1907 (6), 183.
[38] Zeit. physiol. Chem., 1910 (69), 66.
[39] Gann, 1921 (15), 27.
[40] Proc. Royal Soc., B., 1910 (82), 315; Jour. Physiol., 1916 (50), 322.
[41] Cent. Phys. Path. Stoffwechs., 1911 (6), 577; Bull. Acad. Méd. 1919 (81), 799 and 1920 (83), 178; Compt. Rend. Acad. Sci., 1919 (168), 1071.
[42] Jour. Pathol. and Bact., 1913 (17), 606.
[43] Amer. Jour. Physiol., 1904 (11), 139.

one glycine. Drummond[44] has found leucine, tyrosine, and creatinine commonly present in water extracts of malignant tumors. Because of the deficient circulation in the tumors, the amino-acids accumulate in the cancer tissues in sufficient amounts to be detected, and may be found even when no macroscopic evidences of degeneration are present. Possibly on account of this poor absorption no proteoses, peptones, or amino-acids could be found in the urine of cancer patients by Wolff;[45] but Ury and Lilienthal[46] found a positive reaction for albumose in the urine in about two-thirds of all carcinoma cases examined by them; however, it may be absent even in advanced stages. Lactic acid is also present in tumors, according to Fulci[47] and Saiki,[48] the latter finding 0.48 gm. of lactic acid per 100 gms. of cancer of the stomach. According to Clowes[49] cancer tissues are much more permeable to ions than are normal tissues.

(2) **Other Organic Constituents.**—These, in general, resemble the organic constituents of the tissue from which the tumor arises, for a structural resemblance to the parent tissue always exists, and as structural features depend largely on the proportion of the chemical components, a structural similarity fairly implies a chemical similarity. For example, adrenal and renal tissues contain much lecithin and cholesterol, and hypernephromas show a similar composition; the fat of a lipoma is, in its qualitative features, almost identical with the normal fat of the same individual; tumor melanin shows no characteristic chemical distinction from normal melanin, etc.

Glycogen has been particularly studied in tumors, especially because of the erroneous idea advanced by Brault that the quantity of glycogen is in direct proportion to the malignancy.[50] From a summary of all the evidence, it seems that two chief factors determine the presence and amount of glycogen in tumors. One is the embryonic origin of the tumors; thus tumors of cartilage, striated muscle, or of squamous epithelium, which tissues normally contain much glycogen, are likewise provided with an abundance of this material. Second, the occurrence of areas of impaired cell-nutrition favors the accumulation of glycogen in the degenerating tumor-cells just as it leads to a similar accumulation in all other tissues (Gierke).[51] The most extensive consideration of this topic is reported by Lubarsch,[52] who found glycogen microscopically demonstrable in 447 (or 29 per cent.) of 1544 tumors examined. It was present in only 3 out of 184 fibromas, osteomas, gliomas, hemangiomas, lipomas, and lymphangiomas, and in but 2 out of 260 adenomas from various parts of the body. It occurred in all teratomas, rhabdomyomas, hypernephro-

[44] Biochem. Jour., 1917 (11), 246.
[45] Zeit. f. Krebsforschung, 1905 (3), 95.
[46] Arch. f. Verdauungskr., 1905 (11), 72.
[47] Gaz. internaz. dimed., 1910, No. 24.
[48] Arch. méd. expér., 1911 (23), 376.
[49] Proc. Soc. Exp. Biol. Med., 1918 (15), 107; corroborated by Waterman, Biochem. Zeit., 1922 (19), 101.
[50] See Sokoloff and Cartotto, Compt. Rend. Soc. Biol., 1923 (89), 628.
[51] Ziegler's Beitr., 1905 (37), 502.
[52] Virchow's Arch., 1906 (183), 188.

mas, and syncytiomas. In 138 sarcomas glycogen was present in 70 (50.7 per cent.); of 415 carcinomas it was found in 181 (43.6 per cent.). In the squamous epithelial cancers 70 per cent. contained glycogen, while the mucoid or colloid cancers were always free from glycogen. The glycogen undoubtedly enters the cells from without, probably entering as sugar, and being converted into glycogen by intracellular enzymes. We have no reliable studies of the actual quantity of glycogen in various tumors, although Meillère[53] states that the microscopic and chemical examination of tumors give corresponding comparative results, which Gierke states is generally true with glycogen estimations.

Pentose.—Neuberg[54] reports finding, as a product of autolysis of a carcinoma of the liver, a pentose which was not produced by autolysis of either normal liver tissue or the primary growth in the stomach. Beebe[55] found that in carcinoma of the mammary gland the percentage of pentose (*xylose*) is somewhat higher than the amount in normal mammary glands (about 0.23 per cent.). Carcinoma in the liver did not show any constant excess of pentose above that of normal liver tissue (about 0.38 per cent.). A primary carcinoma of the liver showed quite the same pentose and phosphorus content as normal liver tissue. In general, no constant relation of pentose to origin, malignancy, or degeneration of tumors was observed.

Purines and Purine Enzymes.—The purines of both benign and malignant tumors have been studied by Wells and Long,[56] who found them the same as those in normal tissues, and in much the same relative proportions. The proportion of the total nitrogen of tumors which is constituted by the purine nitrogen is less than would be expected from the histological evidence of the amount of nuclear material contained in the tumors. Tumors also seem to contain much the same purine enzymes as the normal tissues. Thus, guanase seems universally present in tumors derived from human tissues, and adenase is missing, although autolyzing tumors can disintegrate their nucleic acid (nuclease) and change the adenine radicals of the nucleic acid into hypoxanthine, presumably by way of adenosine and inosine (Amberg and Jones). Secondary tumors growing in the human liver do not acquire the enzyme, xanthine-oxidase, which is a characteristic enzyme of this organ. The liver tissue between the cancer nodules seems to oxidizes purines less actively than normal liver tissue. Long[57] has also found similar conditions in tumors from sheep, pigs, and cattle, observing that primary carcinoma of the liver does not contain xanthine oxidase, a point of interest in view of the fact that in the development of mammals the xanthine oxidase does not appear until late. Water extracts from various tumors have been found to contain small amounts of free purines, chiefly adenine, guanine, and hypoxanthine (Drummond).[44]

[53] Compt. Rend. Soc. Biol., 1900 (52), 324.
[54] Berl. klin. Woch., 1904 (41), 1081; 1905 (42), 118.
[55] Amer. Jour. Physiol., 1905 (14), 231.
[56] Zeit. f. Krebsforsch., 1913 (12), 598.
[57] Jour. Exper. Med., 1913 (18), 512.

Lipins.—Tumor cells seem to contain much the same fats and lipoids as normal cells, and, as far as known, in much the same proportions as characterize the cells from which the tumors arose. Thus Wells[58] found that hypernephromas show the same high proportions of lecithin and cholesterol as he found in normal adrenal, and as are found in the renal cortex. Other malignant tumors have much less lipoids and fats (see Hypernephromas). A secondary carcinoma of liver cells, metastatic in the skull, was found by Prym[59] to show the same sort of fatty infiltration that is characteristic of fatty liver cells. On account of the poor blood supply of many tumors, fatty changes are usual, occurring under the same conditions and showing the same microscopic features as fatty degeneration in other tissues,[60] being more common in malignant than in benign tumors; especially abundant in squamous cell carcinomas, and scanty in sarcomas.

Crystals of cholesterol or cholesterol compounds are often present in tumors.[61] Dewey[62] found the chief lipoid in jaw tumors to be cholesterol, with more or less free fatty acids and soaps, according to microchemical determinations. Some authors consider the presence of cells foamy with cholesterol esters as significant of an endothelial origin.[62a]

Even lipoma fat shows no difference from normal fat.[63] Currie[64] found that the tissue fats of carcinoma patients have a higher iodine number than normal fats, the fats of sarcomas and inflammatory lesions being intermediate in this respect between carcinoma and normal fat. The fat close to the tumor has a higher iodine number than fats in other parts of the body. The high color observed in the fat tissue of persons dying of malignant disease[65] is common to wasting diseases and not characteristic of cancer; presumably it depends largely on a concentration of the fat-soluble pigments because of the withdrawal of the fats for nutrition. This may also account for the observation of Wacker[66] that the cholesterol of depot fat is much increased in carcinoma, as in other wasting diseases. This is seen most in mesenteric fat, with a decrease in cholesterol in the adrenals. A wax-like substance accompanies cholesterol in the depot fat, and is more abundant than cholesterol in a ratio of 2:1.

Murray[67] says that the lipoids of degenerating uterine fibroids are strongly hemolytic, which may account for the so-called "red degeneration" of these tumors. Freund and Kaminer[68] suggest that the fatty

[58] Jour. Med. Res., 1908 (17), 461.
[59] Frankf. Zeit. Path., 1912 (10), 170.
[60] See Haga, Berl. klin. Woch., 1912 (49), 342; Joannovics, Wien. klin. Woch., 1912 (25), 37.
[61] White, Jour. Path. and Bact., 1908 (13), 3.
[62] Jour. Cancer Res., 1919 (4), 263.
[62a] See Dyke, Jour. Path. and Bact., 1924 (27), 5.
[63] See Wells, Arch. Int. Med., 1912 (10), 297.
[64] Jour. Path. and Bact., 1922 (25), 213.
[65] See also Beatson, Lancet, 1922, Sept. 23, p. 655; Currie, Biochem. Jour., 1924 (18), 235.
[66] Zeit. physiol. Chem., 1912 (78), 349; 1912 (80), 383.
[67] Jour. Obst. Gyn. Brit. Emp., 1910 (17), 534.
[68] Wien. klin. Woch., 1912 (25), 1698.

acids of tissues are of importance in determining whether a tissue is a suitable soil for secondary growth, these substances being deficient in tissues where growths develop. Mitochondria, which seem to be closely related to the intracellular lipins, show no constant differences in cancer, benign tumors, and normal cells, except that sometimes in cancer they fix stains less firmly (Goodpasture).[69]

There has been some effort to correlate the cholesterol and lecithin contents of blood and tissues with the rate of cancer growth; apparently lecithin inhibits growth and cholesterol stimulates.[70] However, Bullock and Cramer[71] found much more cholesterol in a slowly growing mouse carcinoma than in a rapidly growing one, somewhat more phosphatid in the latter, much more phosphatid in a sarcoma than in the carcinoma, and cerebrosides only in the latter; in necrotic portions of tumors they found an increase in simple fats. These figures are based on too few observations to be interpreted as yet, but it may be recalled that as a rule old degenerated tissues show a high cholesterol content.

(3) **Inorganic Constituents.**—These have been studied by Clowes and Frisbie[72] under exceptionably favorable conditions, in that the age of the tumor could be accurately estimated, in the inoculable carcinoma of mice. They found that rapidly growing tumors contain a high percentage of potassium and little or no calcium, whereas in old, slowly growing, relatively necrobiotic tumors the relation is reversed, the potassium decreasing greatly while the calcium increases. Magnesium is present only in traces, while the proportion of sodium fluctuates much less, but is usually greater than either the potassium or calcium, although in very old tumors the latter may become excessive. The most rapid growth, however, seems to occur in tumors in which both calcium and potassium are present in the ratio of

$$\frac{K}{C_a} = \frac{2}{1} \text{ or } \frac{3}{2}$$

Waterman[73] has studied by microchemical methods the distribution of Ca and K in mouse skin during the development of cancers from tar application. He finds that in normal skin the calcium is almost solely in the connective tissues, the potassium in the epithelium. With the development of the malignant epithelial growth there is a transposition of these elements, potassium beginning to appear in the underlying connective tissue, while even more the calcium increases in the epithelial cells and decreases in the connective tissue. When areas of necrosis of epithelium appear, these become heavily infiltrated with calcium. If this sort of process is constant in tumor formation, which has not yet been shown, then the old figures giving a high K in early tumors and high

[69] Jour. Med. Res., 1918 (38), 213.

[70] See Robertson and Burnett, Jour. Exp. Med., 1913 (17), 344; 1916 (23), 631; Sweet *et al.*, Jour. Biol. Chem., 1915 (21), 309; Luden, Jour. Lab. Clin. Med., vols. 3 and 4. Roffo, Prensa Med. Argentina, 1924 (11), 28.

[71] Proc. Royal Soc. London (B), 1914 (87), 236.

[72] Amer. Jour. Physiol., 1905 (14), 173.

[73] Biochem. Zeit., 1922 (133), 535.

Ca in old tumors, are not altogether correct, and since they are based on ashing of stroma, parenchyma, and dead tissue together, they cannot be expected to portray the actual distribution of the inorganic elements in the tumor. The process described by Waterman recalls the features of metastatic calcification (*q. v.*) and suggests the possibility that secretion of acid by the cancer cells may render their own cytoplasm reciprocally less acid, with solution of calcium from the stroma and its deposition in the cancer cells, just as in metastatic calcification calcium is deposited in tissues from which acid is being excreted.

Beebe[74] analyzed a number of human tumors with the following results: Phosphorus was found in proportion to the amount of nuclear material, varying from 0.139 per cent. (uterine fibroid) to 1.06 per cent. (sarcoma). Iron varied from 0.013 per cent. to 0.064 per cent., probably depending on the amount of blood and nucleoproteins. Calcium is most abundant in old degenerated tumors, and potassium in rapidly growing tumors. These results, supported by Clowes and Frisbie's findings and by the microchemical studies of Ladreyt[75] and Wolf,[76] indicate the importance of potassium for cell growth. Wolf found that mouse carcinoma tissue has a much greater permeability for both Ca and K than normal tissue, and suggests that the protoplasm liquifying effect of K and the condensing effect of Ca antagonize one another and control the rhythm of proliferating activity.[77] Injection of potassium salts into mice increases their susceptibility to inoculation (Clowes),[78] while calcium decreases cancer growth (Goldzieher).[79] Exposure of isolated cancer cells to calcium salts reduces their growth capacity when inoculated, apparently through reducing their water content; both effects are counteracted by sodium (Cramer).[80] However, Herley[81] found that cancer cells and normal cells were equally damaged by various salt solutions, and Woglom[82] found that rat tumor extracts have about the same *p*H as rat tissue extracts.

A greater proportion of potassium was found in primary than in secondary growths by Mottram;[83] sodium was the same in each; there is more potassium in squamous cell carcinoma than in round cell sarcoma. Robin[84] states that in cancerous livers the cancer tissue contains more inorganic matter than the normal liver tissue about it. Cattley[85] found the microchemic distribution of potassium the same in cancer as in normal cells, and the same seems to be true of manganese.[86]

[74] Amer. Jour. Physiol., 1904 (12), 167.
[75] Compt. Rend. Soc. Biol., 1923 (88), 1026.
[76] Compt. Rend. Acad. Sci., 1923 (176), 1932.
[77] See also Roussy and Wolf, Arch. Néerl. Physiol., 1922 (7), 562.
[78] British Med. Jour., Dec. 1, 1906.
[79] Verhandl. Deut. Path. Gesellsch., 1912 (15), 283.
[80] Biochem. Jour., 1918 (12), 210.
[81] Jour. Cancer Res., 1924 (8), 389.
[82] Jour. Cancer Res., 1924 (8), 34.
[83] Arch. Middlesex Hospital, 1910 (19), 40.
[84] Compt. Rend. Acad. Sci., 1913 (156), 334.
[85] Lancet, 1907 (172), 13.
[86] Medigreceanu, Proc. Royal Soc., B, 1912 (86), 174.

Schwalbe[87] found that cancer-cells contain iron in a condition demonstrable by the Berlin-blue reaction, and occurring independent of hemorrhages. Tracy[88] found that tumors reacted microscopically for iron, either free or in the form of an albuminate, only in areas where hemorrhages had occurred. Nuclear or organic iron could be detected in the nuclei, occurring in a network arrangement. In other words, iron occurs in tumors, both quantitatively and qualitatively, exactly as in normal cells of the same type. The same writer[89] found in tumors, by microchemical reactions, that phosphorus in the form of nucleo-proteins likewise shows no essential differences from its distribution in normal tissues. Crystalloids, such as KI, diffuse readily into cancer tissue,[90] because the necrotic tissue has lost its impermeability, but this is probably not a selective action as some have thought.[91] This probably explains the observation of White[92] that degenerating tumors contain more copper than tumors that are not degenerating; also that malignant tumors contain more zinc than benign tumors (Cristol)[93] as well as other inorganic elements (Si, Mg, Ca).[94] Karczag[95] found that the necrotic portions of tumors accumulate certain electrotopic carbinol dyes through electrostatic attraction, although the living cancer cells have no such effect.

(4) **Enzymes.**—The rapid and extensive autolysis that occurs in tumors, as shown both morphologically and by the presence of the products of protein cleavage in them, indicates that tumor cells resemble all other cells in possessing intracellular proteolytic enzymes. Because of autolysis, puncture fluids in cancer of serous surfaces show an increased amount of incoagulable nitrogen (Morris),[96] and they may show free amino-acids (Wiener),[97] while there is a slight increase in the incoagulable nitrogen of the blood (Takemura).[98]

There is considerable but not undisputed evidence that cancer tissue autolyzes somewhat more rapidly than corresponding normal tissues,[99] and, according to Neuberg, Blumenthal and others,[1] that cancer extracts digest other tissues than themselves (heterolysis), a property not exhibited by extracts of normal tissues. Müller and others would ascribe this heterolysis to the abundant leucocytes present in the tumors. The

[87] Cent. f. Path., 1901 (12), 874.
[88] Jour. Med. Research, 1905 (14), 1.
[89] Martha Tracy, Jour. Med. Research, 1906 (14), 447.
[90] Van den Velden, Biochem. Zeit., 1908 (9), 54; Jess, Münch. med. Woch., 1921 (68), 323.
[91] Wells and Hedenburg, Jour. Infect. Dis., 1912 (11), 349.
[92] Lancet, Oct. 1921, p. 701.
[93] Compt. Rend. Acad. Sci., 1922 (174), 887; (not corroborated by Taverne, Nederl. Tijd. Geneesk., 1923 (67), 2810).
[94] Robin, Bull. Acad. Med., 1922 (87), 128.
[95] Zeit. Krebsforsch., 1924 (21), 273.
[96] Arch. Int. Med., 1911 (8), 457.
[97] Biochem. Zeit., 1912 (41), 149.
[98] *Ibid.*, 1910 (25), 505.
[99] See Yoshimoto, Biochem. Zeit., 1909 (22), 299; Daels and Delenzé, Bull. Acad. Med. Belg., 1913 (26), 833.
[1] Bibliography by Hamburger, Jour. Amer. Med. Assoc., 1912 (59), 847.

numerous studies of Falk[2] on the enzymatic action of rat and human tumor extracts on esters and proteins, indicate, however, that at least under the conditions of these experiments, both proteolysis and lipolysis are less active with extracts of tumors than with extracts of most normal tissues,[3] and of somewhat different character. Robin[4] found autolysis more rapid in the non-cancerous portions of cancerous livers than in normal livers.

Nucleases have been found in tumors as in other tissues,[5] and in general the enzymes which deamidize adenine and guanine (adenase and guanase) are usually present if the original tissue possessed these enzymes, but no instance of the presence of xanthine oxidase or uricolytic enzyme has been obtained (Wells and Long, *loc cit.*).[5,6]

Hamburger finds that the enzymes of cancer tissue upon which the glycyl-tryptophan and other enzyme tests for cancer are based, are ereptases, resembling in all their properties the ereptases of normal tissues, and not present in particularly large amount. However, Abderhalden[6] has found evidence that certain peptids may be split in a different way by cancer than by normal tissues, supporting those who hold that cancer enzymes are different from normal tissue enzymes. Autolysis of tumors is said to be augmented by x-ray, and especially by radium (Neuberg), and tumor tissue is readily digested by trypsin. Arginase has been found in a secondary carcinoma of the liver.[7]

The presence of ereptases in carcinomatous gastric juice has been especially studied because of its diagnostic possibilities, and Jacques and Woodyatt[8] show that such an enzyme is rarely present in gastric juice except when derived from a cancer present in the wall of the stomach, provided peptolytic bacteria are excluded by filtration. Deaminizing enzymes may also be found in gastric cancer secretions.[9] In the blood of cancer patients there is usually an increased antitryptic activity, ascribable to the reaction against enzymes absorbed from the cancer; it is less pronounced with sarcoma.[10] The body tissues of patients dying with cancer show a low ereptic activity, but the same occurs in persons dying from other wasting diseases (Colwell).[11] This is also true of other tissue enzymes;—at least purine oxidizing enzymes are deficient in the liver tissue between secondary cancers (Wells and Long)[5,6] and the catalase is also reduced in liver tumors.[12] Brahn[13] reports that the liver tissue between secondary cancer nodules, and also the liver in cases of cancer in the portal area, show diminished catalase, lipase, and lecithinase activity,

[2] "Chemistry of Enzyme Actions," K. G. Falk, Chem. Catalog Co., 1924.
[3] Jour. Biol. Chem., 1924 (59), 183.
[4] Bull. Acad. Med., 1923 (89), 174.
[5] Goodman, Jour. Exp. Med., 1912 (15), 477.
[6] Zeit. Krebsforsch., 1910 (9), 266.
[7] Fuchs, Zeit. physiol. Chem., 1921 (114), 101.
[8] Arch. Int. Med., 1912 (10), 560.
[9] Halpern, Mitt. Grenz. Med. Chir., 1915 (28), 709.
[10] Citronblatt, Med. Klin., 1912 (8), 138.
[11] Arch. Middlesex Hosp., 1909 (15), 96.
[12] Blumenthal and Brahn, Zeit. f. Krebsforsch., 1910 (8), 436; Weidenfeld, Wien. klin. Woch., 1918 (31), 324.
[13] Zeit. Krebsforsch., 1917 (16), 112.

although autolysis is increased, but these changes are not observed in the liver when the cancer is in other parts of the body. However, choline has been found in necrotic sarcomas of rats,[14] which would seem to indicate the presence of enzymes disintegrating lecithin. Glycerophosphatase, which is found in normal epithelial but not in mesoblastic tissues, is also present in both primary and secondary carcinomas.[15]

Other enzymes are also present in tumor cells. Buxton[16] examined a large number of tumors for their enzymes by the plate (*auxanographic*) method, and found considerable variations in different growths. All contained amylase (splitting starch) and lipase (splitting butyrin). Most, but not all, tumors coagulated milk (rennin), and liquefied casein, and gelatin (proteases). Peroxidase was nearly always, and catalase always, present. Digestion of fibrin, coagulated serum, and coagulated egg albumen could not be observed. Practically all tumors split glycogen. Tyrosinase could not be demonstrated. The fact that early embryonic tissues were found poor in enzymes[17] speaks against the common assumption that tumors represent strictly an embryonic formation, but Long[18] found that xanthine-oxidase, which in normal development does not appear until late in fetal life, was absent from primary carcinomas of sheep livers, although normal adult sheep liver tissue is rich in this enzyme.

As mentioned elsewhere (See Melanin), melanotic tumors may contain enzymes oxidizing tyrosine, epinephrin, pyrocatechin, or other related aromatic substances, with the formation of pigmentary substances. (See also, Autolysis in Tumors, Chap. III.)

Brahn[19] reports that cancer tissue is unable to oxidize salicylic acid. An extensive study of the so-called oxidase reaction of tumors by means of the indole-blue synthesis method has been made by Menten,[20] who found that, in general, degenerative changes lead to stronger reactions in tumor cells, while rapidly proliferating cells may show diminished reactions, but there is nothing characteristic about this reaction in tumors, and it is not evidence of a true enzyme action in any case.

MacFadyen and Harden[21] studied the juices obtained by grinding up tumor cells made brittle by liquid air, and found by direct methods (chiefly in breast cancers) invertase, maltase, amylase, proteases acting in both acid and alkaline solutions, catalase, oxidase, with perhaps traces of lipase and peroxidase, but no lactase.

Tumors arising from the gastric mucosa, according to Waring,[22] contain both pepsin and rennin; those from the pancreas, both primary and secondary growths, contain trypsin, steapsin, amylase, and rennin.

[14] Ellinger, Münch. med. Woch., 1914 (61), 2336.
[15] Forrai, Biochem. Zeit., 1923 (142), 282; Zeit. ges. exp. Med., 1924 (43), 647.
[16] Jour. Med. Research, 1903 (9), 356.
[17] *Ibid.*, 1905 (13), 543.
[18] Jour. Exper. Med., 1913 (18), 512.
[19] Zeit. Krebsforsch., 1920 (17), 417.
[20] Jour. Cancer Res., 1920 (5), 321.
[21] Lancet, 1903 (ii), 224.
[22] Jour. Anat. and Physiol., 1894 (28), 142.

(5) Chemistry of the Blood.—The specific gravity of the blood is sometimes decreased, the coagulation time is normal in early but increased in late stages. No evidence of abnormal hemolysis, such as free hematin, increased free hemoglobin or bilirubin in the blood, or siderosis of the liver, is usually demonstrable.[23] Israel, and also Engelmann, have reported the occurrence of a marked increase in the lowering of the freezing-point of the blood in carcinoma (as low as $-0.60°$ to $-0.63°$, the normal being $-0.56°$), which they attributed to the presence of excessive products of protein decomposition in the blood. Engel,[24] however, found no such increased depression of the freezing-point in his cases, and questions the significance of the results of Israel and Engelmann. There may be a dietary increase in the blood sugar in cancer,[25] which rises more rapidly and remains high longer than normal,[26] but this effect has no constant relation to the site or stage of the disease.[26a] Langston[27] found that the blood sugar curve in the sugar tolerance test usually resembles that seen in heightened activity of the thyroid.

The total protein of the blood is said to be increased in the late stages, with some increase in the proportion of globulin as is usual in cachexia;[28] with improvement the globulin may decrease.[29] In early stages the blood proteins are within normal range, but the amount may be decreased under roentgenization.[30] There often is a compensatory decrease in the serum albumin as the globulin rises.[31] However, these changes are not noticeably different from those seen in other chronic diseases.[32] Fibrinogen is usually increased.[33] Galehr[34] found the viscosity of the serum increased with the rise in globulin and residual-N, all these changes paralleling the development of cachexia. The speed of sedimentation of corpuscles in cancer blood is high,[35] because of the high globulin and fibrinogen content. After operative removal of the cancer the sedimentation time may return to normal.[36] High blood albumose figures were obtained in gastric cancer by Wolff.[37]

According to Moore and Wilson,[38] the acid-neutralizing power of the blood is increased in cancer; this is probably related to if not the cause of

[23] See Roessingh, Deut. Arch. klin. Med., 1922 (139), 310.
[24] Berl. klin. Woch., 1904 (41), 828.
[25] Williams and Humphreys, Arch. Int. Med., 1919 (23), 537.
[26] Rohdenburg, Bernard and Krehbiel, Jour. Amer. Med. Assoc., 1919 (72), 1528.
[26a] Kelly, Amer. Jour. Med. Sci., 1925 (169), 216.
[27] Jour. Lab. Clin. Med., 1922 (7), 293; See also Friedenwald and Grove, Amer. Jour. Med. Sci., 1920 (160), 313; Biermer, Deut. med. Woch., 1924 (50), 593.
[28] Loebner, Deut. Arch. klin. Med., 1918 (127), 397; Kennaway, Quart. Jour. Med., 1924 (17), 302.
[29] Bircher, Jour. Lab. Clin. Med., 1922 (7), 660.
[30] Mahnert, Zeit. physiol. Chem., 1920 (110), 1.
[31] See Kennaway (loc. cit.[28]); Gussio, Tumori, 1923 (10), 1.
[32] See Theis, Jour. Cancer Res., 1921 (6), 127.
[33] Foster, Arch. Int. Med., 1924 (34), 301.
[34] Wien. Arch. inn. Med., 1924 (9), 379.
[35] Roffo, Prensa Med. Argentina, 1924 (11), 121.
[36] Gragert, Arch. Gynäk., 1923 (118), 421.
[37] Acta med. Scand., 1921 (53), 810.
[38] Biochem. Jour., 1906 (1), 297; Watson, Jour. Path. and Bact., 1909 (13), 429; Sturrock, Brit. Med. Jour., 1913 (2), 780.

the decreased HCl content of the gastric juice, which occurs whether the cancer is in the stomach or not. As this alkalinity is not associated with an increase in the inorganic bases of the blood, it may be that the proteins have an increased basicity. Although numerous other observers describe a decreased alkalinity, as in other cachectic conditions,[39] Menten,[40] making direct H-ion measurements, found an increase in alkalinity in the serum of nearly all cases of carcinoma and sarcoma. Chambers[41] also found that the dialyzate from blood in cancer shows a distinct decrease in H-ions, (*p*H 7.45 average in cancer, 7.31 normal) but determination of the CO_2 tension showed much less difference, and he believes that the greater alkalinity of the dialyzate in cancer is to be explained by Donnan's theory, as due to an increase in non-diffusible anions in the plasma. Waterman[42] also found an increase in the titrable alkalinity of the blood in cancer, and suggests that since such a change must of necessity decrease the content of the blood in calcium ions this fact may have an effect on cell growth and on other important biological processes, in view of the marked influence of calcium ions on the physical state of colloids. Indeed, several analysts have found that the blood in cancer contains less calcium than normal, which results in a tendency to osteoporosis[43] and to metastatic deposition of calcium in the kidney epithelium.[44]

According to Mottram[45] there is an increase in the potassium of both the blood and tissues, but Theis and Benedict[46] found both calcium and potassium low and sodium normal in the blood, although Krehbiel[47] had found normal calcium values. Probably the natural mechanism for preserving normal salt balances in the blood is usually able to prevent any considerable change in the inorganic constituents of the blood,[48] except with advanced renal changes.

Blood analyses in 189 cases of cancer, by Theis and Stone,[49] gave usually low figures for non-protein and urea nitrogen, but with amino-N slightly above normal; uric acid and sugar were within normal limits. Killian and Kast,[50] however, found increase in urea and creatinine, and especially often of uric acid, in the majority of cancer cases, particularly when the abdominal organs were involved. There were also other evidences of a reduced excretory capacity on the part of the kidneys, and often acidosis was demonstrable but without ketosis. The hyperglucemia without glucosuria, so commonly found in cancer, these authors attribute to the kidney involvement, which also is associated with an increased diastatic

[39] See Traube, Int. Zeit. Physik-Chem. Biol., 1914 (1), 389.
[40] Jour. Cancer Res., 1917 (2), 179. Corroborated by Chambers, Jour. Biol. Chem., 1923 (55), 229, but not by Corran and Lewis, Biochem. Jour., 1924 (18), 1358.
[41] Jour. Biol. Chem., 1923 (55), 257.
[42] Biochem. Zeit., 1922 (133), 535.
[43] Goldzieher, Verh. Deut. Path. Ges., 1912 (15), 283.
[44] M. B. Schmidt, Verh. Deut. Path. Ges., 1913 (16), 329.
[45] Arch. Middlesex Hosp., 1910 (19), 40.
[46] Jour. Cancer Res., 1924 (8), 499.
[47] Jour. Cancer Res., 1920 (5), 199.
[48] See Rhodenburg and Krehbiel, Jour. Cancer Res., 1924 (8), 423.
[49] Jour. Cancer Res., 1919 (4), 349.
[50] Arch. Int. Med., 1921 (28), 813.

activity in the blood. Schweriner[51] found an increase in polypeptids, with normal amino acid figures.

According to DeNiord,[52] cholesterol, fatty acids, and total fats are generally increased in the blood in malignancy, and no noticeable changes occur during Roentgen treatment. The phosphorus content of the whole blood has been found much increased in cancer, Gröbly[53] stating that a figure of over 1.526 parts per thousand is always indicative of malignancy. He calculated a phosphorus quotient, this being P_2O_5 in mg. per 10 c.c. blood divided by the number in millions of red cells, the normal being 2.5–2.9, in cancer usually over 3.17.[54] These findings, however, could not be confirmed by Buckman,[55] who found little alteration in the phosphorus content of the blood, but Wohlfarth[56] found an increase in the P content of the red cells as in other cachectic conditions. There is a marked increase in the blood sulfur both as total and as inorganic and ethereal sulfur.[57]

Kothmann[58] finds that in cancer, as in exophthalmic goiter, the serum colloids show a much greater degree of dispersion than normal human serum or serum from other diseases, and blood coming from cancer tissue showed more dispersion than blood from other tissues of the same individual. Normal serum exposed to cancer cells also acquires an increased dispersion. Cancer serum diluted and heated to 80° is said to show less turbidity than normal serum, which may indicate a greater stability of the colloids.[59]

The rate of reduction of methylene blue by cancer extracts is said to be accelerated with serum from cancer subjects, or the serum of non-cancerous subjects with a cancer parent.[60] Apparently there is no difference in the isoagglutinin blood grouping of the cancerous and non-cancerous.[61] There is a decrease in catalase, proportionally greater than the fall in red cells, giving a low catalase index, at least in advanced cases according to Zerner,[62] but Rohdenburg[63] found the figures for blood catalase to vary either side of normal.

Cancer serum is said to inhibit saponin hemolysis less than normal serum because of a low cholesterol content.[64] Sodium oleate hemolysis is also less inhibited by cancer serum because of a difference in the pro-

[51] Zeit. exp. Path. Pharm., 1920 (21), 129.

[52] DeNiord *et al.*, Arch. Int. Med., 1920 (25), 32. Currie (Brit. Jour. Exp. Path., 1924 (5), 293) found a slight hypocholesterolemia in cancer, and Roffo and Correa (Zeit. f. Krebsforsch., 1925 (22), 79) found a reduction in cholesterol after irradiation.

[53] Arch. klin. Chir., 1921 (115), 261.

[54] Corroborated by Zerner, Zeit. Krebsforsch., 1924 (21), 157.

[55] Buckman *et al.*, Arch. Int. Med., 1924 (34), 191.

[56] Deut. Zeit. Chir., 1924 (186), 20.

[57] Kahn and Postmontier, Jour. Lab. Clin. Med., Res., 1925 (10), 317.

[58] Schweiz. med. Woch., 1920 (50), 160.

[59] Ehrentheil and Weis-Ostborn, Klin. Woch., 1923 (2), 585.

[60] Thomas and Binetti, Compt. Rend. Soc. Biol., 1922 (86), 29; Neoplasmes, 1922 (1), 54.

[61] See Buchanan and Highley, Brit. Jour. Exp. Path., 1921 (2), 247.

[62] Zeit. Krebsforsch., 1922 (19), 263.

[63] New York Med. Jour., 1913 (97), 824.

[64] Luger *et al.*, Zeit. Immunität., 1923 (36), 17.

teins.[65] Sodium taurocholate hemolysis is also inhibited less by cancer than by normal serum.[66] According to Chodat, cancer serum inhibits the action of tyrosinase less than normal, and he has devised a reaction which demonstrates this property.[67]

(6) **Internal Secretion.**—If tumors are derived from an organ with an important internal secretion, the tumor cells in many cases produce the same internal secretion, which seems to have the same functional properties as the normally produced secretion; thus, a metastatic growth from a thyroid tumor has been said to function in place of the resected gland. Gierke[68] found in 20 grams of material from metastatic thyroid tissue in the vertebral column about 5 mg. of iodine, which was a trifle larger proportion than was present in the thyroid itself. Carlson and Woelfel[69] found much iodine in the metastases of a thyroid carcinoma of a dog, while in another dog whose cancerous thyroid contained no iodine the secondary tumors were also devoid of this element. I have also analyzed metastases from a carcinoma of the thyroid which contained no demonstrable iodine despite the presence of colloid. Marine and Johnson[70] found that in two cases of cancer of the thyroid in man, and one in the dog, the cancer tissue showed no ability to retain iodine given by mouth, in contrast to normal thyroid and simple adenomas. Meyer-Hürlimann and Oswald[71] have described a remarkable case of cystic carcinoma of the thyroid, from which in six weeks 2840 c.c. of secretion was obtained by puncture. This fluid contained 0.077 mg. iodine per 10 c.c. (the patient having previously been given KI) as compared with normal thyroid which contains 0.4 to 4 mg. per 10 gm. It contained both globulin and albumin, the former corresponding to true thyroglobulin, even to increasing vagus irritability experimentally. The "adenomatous" nodules of the thyroid often show evidence of active secretion, Goetsch[72] having found their cells rich in mitochondria, while Graham[73] found that they take up iodine and metabolize it so that the adenomatous tissue produces the typical thyroid effect on the development of tadpoles. Exophthalmic goiter symptoms may result from cancer of the thyroid,[74] although this is exceptional.

Pubertas precox has been observed in children with tumors of the endocrine glands, sometimes disappearing with removal of the tumor.[75] Numerous cases of marked vascular hypertension have been observed associated with adrenal tumors, both of cortical and medullary type, with no other explanation for the high blood pressure, and in one case

[65] Weis-Ostborn and Silberstern, Zeit. Immunität., 1924 (39), 239.
[66] Dietrich, Klin. Woch., 1922 (1), 2382; Lundwall, Arch. Gynaek., 1923 (119), 468.
[67] Chodat and Kotzareff, Compt. Rend. Soc. Biol., 1923 (177), 460.
[68] Hofmeister's Beitr., 1902 (3), 286.
[69] Amer. Jour. Physiol., 1910 (26), 32.
[70] Arch. Int. Med., 1913 (11), 288.
[71] Korr.-Bl. Schweizer Aerzte, 1913 (43), 1468.
[72] Bull. Johns Hopkins Hosp., 1916 (27), 129.
[73] Jour. Exp. Med., 1916 (24), 345.
[74] Holst, Norsk. Mag. Laegvid., 1922 (83), 527.
[75] Review by Leiner, Endocrinology, 1920 (4), 369.

Orth[76] found a large amount of epinephrin in an adrenal paraganglioma with hypertension. Adrenal cancers do not usually cause Addison's disease, perhaps because they functionate in the place of the destroyed gland (Lubarsch).

Cancer Cachexia.—In the characteristic production of cachexia, often apparently out of all proportion to the amount of tumor tissue, there would seem to be evidence that a peculiar and abnormal product of metabolism is formed by cancer-cells, and extracts from cancers have been found toxic for protozoa.[77] As yet, however, it has been impossible to demonstrate any characteristic toxic substance in cancers.[78] Girard-Mangin[79] claims that malignant tumors contain colloidal poisonous substances in proportion to their softness, extracts causing paralysis and fall of blood pressure; but others have failed to substantiate this.[80] Seyderhelm and Lampe[81] in a preliminary report state that extracts obtained at low temperatures are toxic, the active autolysis at higher temperatures destroying the cancer poison, but this work has not yet been confirmed. Because of the constant disintegration of the tumor tissues, products of autolysis are formed, and undoubtedly enter the circulation in small quantities; possibly they are a factor in the systemic manifestations of malignant growths, analogous to the action of cleavage products of foreign proteins which may produce "protein fever"[82] and other toxic effects. It has often been observed that when extensive necrosis is produced in experimental tumors in rats and mice the animals may show profound toxemia, presumably because of absorption of autolytic products.

Since all normal tissue-cells produce substances through their metabolism that enter the circulation, it is quite certain that tumor-cells do likewise, and it is highly probable that the presence of abnormal quantities of such products, even if they are of quite normal composition, may cause disturbances in the body. As yet, however, no such substances, either normal or abnormal, have been isolated, nor has their presence been demonstrated. Numerous isolated observations of ptomaines or similar substances in the urine of cancer patients may be found in the literature,[83] but their importance is extremely questionable. A large proportion of cases of malignant tumors exhibit renal injury (Kast and Killian)[84] but whether from products of the tumor or from bacterial infection has not been determined. The ability of cancer cells to attack adjacent tissues without phagocytic processes, indicates that they produce some tissue solvent substance, and the readiness with which they attack bone

[76] See review by Oppenheimer and Fishberg, Arch. Int. Med., 1924 (34), 631.
[77] Woodruff and Underhill, Jour. Biol. Chem., 1913 (15), 401; Calkins, Jour. Cancer Res., 1916 (1), 205 and 399.
[78] See Blumenthal, Festschr. f. Salkowski, Berlin, 1904; Hansemann, Zeit. Krebsforsch., 1906 (4), 565.
[79] Presse Méd., 1906, p. 1709; Compt. Rend. Soc. Biol., 1909 (67), 117.
[80] See Bruschettini and Barlocco, Cent. f. Bakt., 1907 (43), 664.
[81] Deut. med. Woch., 1924 (50), 1050.
[82] Concerning fever in cancer see Briggs, Amer. Jour. Med. Sci., 1923 (166), 846.
[83] See Kullmann, Zeit. klin. Med., 1904 (53), 293.
[84] Proc. Soc. Exp. Biol. Med., 1919 (16), 141.

is in support of the view that this may be lactic acid, as Bierich[85] and others believe. In diffuse cancer of the serous membranes the severe hemorrhagic exudation, so commonly seen, suggests the presence of a toxic agent, which, as Grawitz[86] points out, resembles tuberculin in its effects.

Hemolytic Substances.—A number of observers have described the finding of hemolytic substances in cancer extracts. Bard[87] observed that in hemorrhagic carcinomatous exudates in serous cavities the blood is rapidly hemolyzed, which is not the case in exudates from other causes, but this was not corroborated by Weil.[88] Kullmann[83] found that extracts of carcinomas contain hemolytic substances acting energetically both in the body and *in vitro;* these are soluble in alcohol and in water, are not complex in composition, are not specific for human corpuscles, but are toxic for all varieties of corpuscles. Micheli and Donati[89] likewise found hemolytic substances in 8 of 15 tumors, of which 5 acted on all varieties of corpuscles, and 3 acted on only certain varieties; they regard the hemolytic substances as the products of autolysis in the tumors. Weil[90] also found the hemolytic property of tumor extracts to vary with the amount of necrosis, and to depend on dialysable hemolytic substances distinct from the hemolysins of normal tissues. It is well known that among the products of autolysis of normal tissues are hemolytic substances. Whether the severe anemia frequently present in carcinoma is due, either largely or in part, to these products of autolysis is unknown, but it is very probable that they have some effect. However, anemia is seen chiefly in cancer with ulceration and loss of blood, although it is possible that toxic injury to the bone marrow may play a part.[91]

Hemolysis in Cancer.—The blood serum of cancer patients has often a hemolytic action on the corpuscles of normal persons (Crile), but this property is quite inconstant, being present in 67 per cent. of a series of 472 cancer cases collected by Krida, while 15 per cent. of cases of other diseases and 2.6 per cent. of normal persons showed hemolytic activity of the serum.[92] Elsberg found that normal corpuscles injected subcutaneously into cancer patients are hemolyzed, but Gorham and Lisser found this reaction positive in but 60 per cent. of their cases, the subcutaneous hemolysis not corresponding at all to the hemolytic activity of the patient's serum in the test tube. The stomach contents in ulcerated cancer of the stomach are hemolytic (Grafe and Röhmer).[93] The red corpuscles of cancer patients are said to have usually a greater resistance to hemolysis by cobra venom than normal corpuscles, but this is not characteristic, there being similar alterations in other diseases.[94] The reputed power of the serum in cancer to protect corpuscles from hemolysis by oleic and lactic acid could not be demonstrated by Sweek and Fleisher,[95] but was found to be marked by Decker[96] with sodium oleate hemolysis.

An extensive review of the literature and methods led Cohnreich[97] to the conclusion that resistance of erythrocytes to hypotonic solutions and to poisons vary independently of one another. He has devised an improved method for testing resistance to hypotonic solutions, which seems to vary directly with the amount of stroma and PO_4 content, and finds that determination of maximum and minimum resistance is of little value, as these concern only a small part of the corpuscles; he therefore determines the "plurimum" resistance, involving most of the corpuscles. The most

[85] Klin. Woch., 1924 (3), 221.
[86] Deut. med. Woch., 1917 (43), 961.
[87] La Semaine Méd., 1901 (21), 201.
[88] Jour. Med. Res., 1910 (23), 86.
[89] Riforma Med., 1903 (19), 1037.
[90] Jour. Med. Res., 1907 (16), 287.
[91] See Roessingh, Deut. Arch. klin. Med., 1922 (139), 310.
[92] Literature by Gorham and Lisser, Amer. Jour. Med. Sci., 1912 (144), 103.
[93] Deut. Arch. klin. Med., 1908 (94), 239.
[94] Kraus, Ranzi and H. Ehrlich, Sitz. Ber. Akad. Wien., 1910 (119), 3; see also Grünbaum, Jour. Path. and Bact., 1912 (17), 82.
[95] Jour. Med. Res., 1913 (27), 383.
[96] Zeit. ges. exp. Med., 1924 (38), 174.
[97] Folia Hematol., 1913 (16), 307, full bibliography.

significant results were obtained in cancer of the alimentary tract, in which an increased resistance was always demonstrable. Farmachidis[98] finds the cobra venom resistance more specific for cancer than do most other investigators.

(7) **Metabolism in Cancer.**—There are numerous observations indicating that cancer cachexia is in no way different from the cachexia of other conditions. The behavior of the nitrogen metabolism seems to be quite the same as in tuberculosis and other wasting diseases. There is the same excessive elimination of aromatic substances (phenol,[99] indican) and oxyacids (Lewin,[1] Blumenthal[2]), which Lewin considers to arise from the abnormal metabolism of proteins, and not from putrefactive decomposition in the tumor or in the intestines. In rats with sarcoma, increased excretion of uric acid and creatine has been observed.[3] There is also said to be excessive elimination of mineral salts, termed "demineralization" by Robin,[4] but no alteration in the excretion of chlorides.[5] There is also said to be an increased excretion of iron with a depletion of the iron of the blood and body tissues.[6] As in other cachexias, the creatine content of the muscles is decreased,[7] but Meyer[8] found no creatinuria in cancer cachexia.

Fraenkel[9] finds evidence that there may be some difficulty in tryptophan metabolism in tumors and in tumor patients, especially marked with melanotic tumors. The excretion of amino acids is not different from that of normal persons except with cancer of the liver, when a marked increase in both amino acid and colloidal N is often found in the urine.[9a]

Recently studies have been made on the respiration of tumor tissue *in vitro*.[10] Drew found that tumor tissue is less active in reducing methylene blue than normal tissues, but Russell and Gye found the rate of oxygen consumption of an emulsion of either normal or tumor tissue to increase directly with the rate of growth of the tissues, and similar results were obtained by Russell and Woglom,[11] whose figures on respiratory quotients suggest that rapidly growing tumors burn chiefly carbohydrate, slowly growing tumors chiefly fat. Also Waterman and Dirken found higher oxygen consumption by tumor than by normal tissue. Ahlgren points out that the discrepancies observed may depend on variations in the H-

[98] Gaz. degli Osped., 1915 (36), 689.

[99] Somewhat higher than average figures for phenol in the blood were found in sarcoma cases by Theis and Benedict (Jour. Biol. Chem., 1918 (36), 99).

[1] Deut. med. Woch., 1905 (31), 218.

[2] Festschr. f. Salkowski, Berlin, 1904.

[3] Ordway, Jour. Med. Res., 1913 (23), 301.

[4] Quoted by Lewin, *loc. cit.*[1] Clowes *et al.* (5th Ann. Rep., N. Y. State Dept. of Health, 1903–4) report observing a slight chloride retention in cancer patients, and review the literature of metabolism in cancer.

[5] Robin, Compt. Rend. Acad. Sci., 1913 (156), 1262.

[6] Robin and Bournigault, Bull. acad. Med., 1921 (86), 198.

[7] Chisholm, Biochem, Jour., 1912 (6), 243.

[8] Deut. Arch. klin. Med., 1920 (134), 219.

[9] Wien. klin. Woch., 1912 (25), 1041.

[9a] Labbé and Mouzaffer, Compt. Rend. Soc. Biol., 1924 (91), 1029.

[10] Reviewed by Ahlgren, Brit. Jour. Exp. Path., 1923 (4), 196.

[11] Brit. Jour. Exp. Path., 1920 (1), 244.

ion concentration in the preparations studied. Warburg[12] and his associates find that cancer tissue may utilize no more oxygen in a given time than normal tissue, but that the cancer tissue destroys much more glucose with the same oxygen consumption because it carries the conversion of much of the glucose only to the stage of lactic acid, and produces three to four times as much lactic acid as normal tissue. Drew[13] found that slowly-growing tumor tissue has a relatively low respiratory quotient, not because of any incapacity to burn glucose but because of a lack of available sugar in the cancer cells.

Neuschlosz[14] used m-dinitrobenzene as his indicator of oxidative activity, and found that it was reduced less by the organs of tumor rats (except the spleen) than by corresponding tissues from normal rats. Furthermore, the reduction activity of normal tissues is more depressed by HNC and more stimulated by iron than the activity of tumor cells, which fact supports the view that the reaction which liberates energy in the cancer cells is fermentative rather than respiratory, thus agreeing with the observations of Warburg and Minami.

Extensive respiratory studies by Wallersteiner[15] showed enormous variations in the amount of heat production in different cases, in about 10 per cent. of which, figures as high as those of severe fevers or exophthalmic goiter were obtained repeatedly; most of the patients showed high normal figures. Nitrogen loss did not ordinarily occur if the calorimetric findings were considered in the calculations; nitrogen equilibrium was maintained if sufficient nourishment was obtained and utilized. In general, metabolism in cancer resembles that of fever, and warrants the assumption of a toxic stimulation of tissue destruction.[16] It is entirely possible that the products of cancer protein destruction are responsible for this toxicogenic metabolic abnormality, since Vaughan has demonstrated that the effects of bacteria and foreign proteins are quite the same in their pyretic and toxic action.

Salkowski demonstrated that the amount of colloidal nitrogenous material, precipitated from the urine by strong alcohol, is increased in cancer. Numerous observers have corroborated this, but find that a similar condition obtains in other cachectic diseases, although in cancer the amount of colloidal nitrogen seldom is as low as normal unless the tumor is removed.[17] Much of this colloidal nitrogen seems to be in the form of "oxy-proteic acid" (Salomen and Saxl),[18] which is a mixture of incompletely oxidized polypeptids, containing much unoxidized sulfur.[19]

[12] Biochem. Zeit., 1923 (142), 317,334; 1924 (152), 309; reviewed in Naturwissenschaften, 1924 (12), 1131. Also see Klin. Woch., 1924 (3), 1062.

[13] Brit. Jour. Exp. Path., 1923 (4), 46.

[14] Klin. Woch., 1924 (3), 57.

[15] Deut. Arch. klin. Med., 1914 (116), 145.

[16] Rats with cancer show a low basal metabolism (Händel and Tadenuma, Zeit. Krebsforsch., 1924 (21), 197).

[17] See Mancini, Deut. Arch. klin. Med., 1911 (103), 288; Semenow, Folia Urol., 1912 (7), 215; de Bloeme, *et al.*, Biochem. Zeit., 1914 (65), 345.

[18] Wien. klin. Woch., 1911 (24), 449.

[19] Killian reports finding in the blood two to three times the normal amount of nonprotein sulfur, while the total sulfates remain normal, ("Cancer: Its Nature, Causes, Diagnosis and Treatment." By R. H. Greene, New York, 1918).

The proportion of neutral sulfur in the total sulfur in the urine seems to be increased in cancer (Weiss), but not so constantly or characteristically as to be of great diagnostic value.[20] Much clinical investigation has been made of these urinary changes, which has generally substantiated the fact that there usually is more increase in colloidal nitrogen and ethereal sulfate in the urine of cancer, especially in cancer of the digestive tract,[21] than in other diseases, but that in no sense are these changes specific for cancer, and the fundamental metabolic disturbances responsible have not been ascertained.[22] They seem more indicative of the excessive catabolism of cachexia than of cancer tissue itself. Saxl[23] has ascribed part of the increased sulfur elimination to abnormal excretion of sulfocyanid, and, as small doses of sulfocyanides lead to increased oxyproteic acid in the urine, he suggests that in cancer there is a specific disturbance in sulfocyanid metabolism, an hypothesis that awaits confirmation. Of similar status is the excessive excretion of glucuronic acid described by Roger.[24] Associated with the increased colloid excretion is a lowering of surface tension.[25] There is often an increased capacity of the urine to decolorize methylene blue.[26]

(8) **Diet and Tumor Growth.**—In general, any condition that decreases the nutrition of the body as a whole, or of the tissue in which a tumor is located, decreases the rate of growth of the tumor, in which respect neoplasms exhibit quite the opposite behavior to infectious processes. Thus, the older the individual the more slowly the tumor usually grows; ligation of the lingual artery retards the growth of cancer of the tongue; repeated pregnancy and lactation delay the progress of cancer in mice,[27] suggesting that tumor cells have a greater avidity for nutritive elements in the blood than have ordinary somatic cells, but less than the cells of the fetus or of the active mammary gland. Numerous attempts have been made to determine the relation of tumor growth to specific dietary deficiencies. Sweet, Corson-White, and Saxon[28] found that rats kept upon a diet deficient in specific amino-acids (lysine), so that body growth did not occur although nutrition was maintained, show a slower growth of implanted tumors than animals on an adequate diet. Rous[29] obtained similar results with some transplanted tumors, but not with all, nor with spontaneous tumors. Van Alstyne and Beebe[30] found that rats living on casein and lard showed much less growth of inoculated tumors than when

[20] Stadtmüller and Rosenbloom, Arch. Int. Med., 1913 (12), 276; Interstate Med. Jour., 1916 (23), No. 2; bibliography. Kahn, Jour. Cancer Res., 1917 (2), 379.

[21] Labbé and Mouzaffer, Compt. Rend. Soc. Biol., 1924 (91), 1029.

[22] See Goodridge and Kahn, Biochem. Bull., 1915 (4), 118; Damask, Wien. klin. Woch., 1915 (28), 499; Sassa, Biochem. Zeit., 1914 (64), 195.

[23] Biochem. Zeit., 1913 (55), 224.

[24] Bull. Soc. Med. Hôp., Paris, 1915 (31), 499.

[25] Bechhold and Reiner, Biochem. Zeit., 1920 (108), 98; Schemensky, Mitt. Grenz. Med. Chir., 1922 (34), 451.

[26] Fuhs and Lintz, Neoplasms, 1923 (2), 62.

[27] See Maud Slye, Jour. Cancer Res., 1919 (4), 25.

[28] Jour. Biol. Chem., 1913 (15), 181; 1915 (21), 311.

[29] Jour. Exp. Med., 1914 (20), 433.

[30] Jour. Med. Res., 1913 (24), 217.

lactose was added to the diet. A tryptophan-free diet, on which marked marasmus results, does not prevent growth (Cramer),[31] nor does a diet containing a great variety of proteins increase the number of successful transplants.[32]

Overfeeding with glucose and injection of insulin are said to stimulate growth of transplanted rat tumors.[33] Borrel[34] says that injections of glycogen have an accelerating effect. Attempts to modify tumor growth by changes in the salt supply have met with little success. Händel[35] thought that feeding potassium increased and calcium decreased slightly the growth of mouse cancer, addition of phosphates or decrease in total salts having no effect. Injections of HCl have no effect, $NaHCO_3$ slightly increase the rate of growth (Woglom).[36]

Robertson and Burnett[37] observed that the addition of cholesterol to the diet increases the rate of growth and the development of metastases in inoculated rat tumors, which has been corroborated by others.[38] This accelerative action depends on the hydroxyl radical, although other hydroxy-benzol derivatives do not have this effect.[39] The growth-promoting principle of the hypophysis, tethelin, is also said to stimulate cancer growth.

Numerous attempts have been made to modify the growth of tumors by altering the vitamin supply, but without success.[40] Funk[41] found greater growth of inoculated sarcoma in fowls given normal diets than in those fed polished rice deficient in vitamin B. Benedict and Rahe[42] supplied vitamins by adding to an otherwise inadequate diet, just enough yeast to keep the rats in fair condition, and found that inoculated tumors grew, although extremely slowly, even when the animal itself could not grow. Evidently tumor cells cannot manufacture substances essential for growth, *i. e.*, vitamins. Corson-White[43] states that, generally, vitamin-rich diets favor tumor growth, especially if there is also an abundance of cholesterol. Fraenkel,[44] however, observed no stimulating effect from rice polishings or yeast extracts, and Wyard[45] observed no clinical effects from a diet deficient in vitamin A. Robertson and Ray[46] found that mice which develop spontaneous tumors are characterized by relatively energetic growth during the period of adolescence, as

[31] Sci. Rep. Imp. Cancer Res. Fund, 1923 (8), 17.
[32] Marsh, Jour. Cancer Res., 1924 (8), 385.
[33] Händel and Tadenuma, Zeit. Krebsforsch., 1924 (21), 288.
[34] Compt. Rend. Soc. Biol., 1922 (86), 1096.
[35] Zeit. Krebsforsch., 1924 (21), 281.
[36] Jour. Cancer Res., 1922 (7), 149.
[37] Jour. Exp. Med., 1913 (17), 344; corroborated by Roffo.[79]
[38] See Borst, Zeit. Krebsforsch., 1924 (21), 337.
[39] Jour. Cancer Res., 1918 (3), 75. See also Robertson, "The Chemical Basis of Growth and Senescence," Lippincott, 1923.
[40] See Rep. Imp. Cancer Research Fund, London, 1923.
[41] Zeit. physiol. Chem., 1913 (88), 352.
[42] Jour. Cancer Res., 1917 (2), 159.
[43] Penn. Med. Jour., 1919 (22), 348.
[44] Wien. klin. Woch., 1916 (29), 483.
[45] Lancet, Apr. 29, 1922, p. 840.
[46] Jour. Cancer Res., 1921 (6), 7.

if their anabolic processes which result in growth of parenchyma were exceptionally rapid.

(9) **Immunity Reactions in Cancer.**—The fact that a certain degree of specific immunity can be developed against normal tissue cells (see Cytotoxins, Chap. IX), has encouraged study of the possibility of securing immune antibodies which might be specific for cancer, and has led to much research on this subject,[47] with results as yet of little value. There is no doubt that the body has distinct powers to inhibit to a greater or less degree the growth of tumors, and to destroy many of the cells which escape from cancers into the lymph and blood,[48] while in experimental animals inoculated tumors are in most instances unable to grow, and they may, after growth has once begun, recede or even disappear. Furthermore, animals may be made immune to transplantable tumors to which they would otherwise be susceptible. On the other hand, there is evidence that animals with cancer are much more susceptible to this same cancer (autotransplantation) than other animals (heterotransplantation). Erdmann[49] states that cancer cells grown in plasma from cancer animals retain their ability to produce cancer on inoculation into animals, whereas plasma from normal animals destroys their cancer-producing character. Gay[50] and Kross[51] found that the blood of rats refractory to a certain tumor, when injected into susceptible rats, does not protect them against the tumor growth, but rather increases susceptibility.

Many schemes of immunization of patients by injection of extracts or autolysates made from their own tumors, or similar tumors of others, have been tried,[52] but in the hands of competent and critical observers the results seem to have been practically negative.[53] It is not always kept in mind that inoculated cancers in rats and mice represent an artificial condition behaving very differently from spontaneous tumors. The transplanted tumors have a strong tendency to spontaneous retrogression and a much greater susceptibility to injurious influences, and hence are not at all a fair test object for immunological or therapeutic procedures.[54] Furthermore, animals immunized against transplantable cancers are quite as likely to develop experimental tar cancers as any others,[55] and they have often been observed to develop spontaneous cancers.

There is no lack of evidence that animals immunized with cancers do produce, in greater or less amounts, various antibodies of some degree

[47] Literature by Coca, Zeit. Immunität., 1912 (13), 525; Kraus *et al.*, Wien. klin. Woch., 1911 (24), 1003.

[48] Reviewed by Wells, Jour. Amer. Med. Assoc., 1909 (52), 1731.

[49] Zeit. Krebsforsch., 1923 (20), 322. Review of lit.

[50] Jour. Med. Res., 1909 (20), 175.

[51] Jour. Cancer Res., 1921 (6), 25.

[52] Review by Fichera, Jour. Cancer Res., 1918 (3), 303.

[53] See Blumenthal, Zeit. Krebsforsch., 1914 (14), 491; Bauer, Latzel and Wessely, Zeit. klin. Med., 1915 (81), 420.

[54] During immunization of rats to homologous proteins those with progressing tumors show much less disturbance of the blood sugar equilibrium than normal rats, and human subjects generally show a weak sugar reaction. (Rohdenburg *et al.*, Amer. Jour. Med. Sci., 1922 (164), 361; Jour. Cancer Res., 1921 (6), 223.)

[55] Fibiger, Acta Chir. Scand., 1922 (55), 343.

of specificity for cancer, which must be interpreted as evidence that cancer proteins are in some respects different from the normal proteins of the host; however, the amount and specificity of these antibodies seem to be low,[56] and in many observations they have failed to be demonstrated. Indeed, Coca[47] in his review states unqualifiedly, "The usual biological tests of complement deviation and specific precipitation fail to show the hypothetical antibodies, though a distinct cytotoxic influence can be demonstrated in the plasma of animals of foreign species that have been actively immunized against a tumor." His own experiments failed to demonstrate specific complement-fixation antibodies in patients injected with extracts of their own tumors. Lewin[57] also failed to find conclusive evidence of the demonstration of specific antibodies in cancer, yet accepts the immunity which is produced by injections of virulent cancer material as an active immunity dependent upon cancer antibodies. It may, however, depend on a stimulation of the local cellular reactions that inhibit cancer growth.[58] Pfeiffer[59] reported the presence of specific anaphylactic antibodies in the blood of cancer patients, but this has not been confirmed by several other observers.[60]

v. Dungern[61] has described positive complement fixation reactions, partially specific for cancer and benign tumors, by using alcoholic extracts of the tumors or acetone extracts of human erythrocytes as antigen, but he interprets these reactions as not due to specific antibodies, but to abnormal products of metabolism.[62] The complement content of the blood is said to be slightly increased in cancer (Engel),[63] but there is nothing characteristic about this. A flocculation reaction, similar to the Sachs-Georgi test in syphilis (*q. v.*) is said to give strikingly specific reactions with serum from cancer patients (Roffo),[64] but this has not yet been confirmed. A flocculation-meiostagmin reaction, with ricinic acid as antigen, has also been recommended by Izar.[65] Since flocculation reactions, as seen in syphilis, do not depend on the presence of specific antibodies, but merely on the physical state of the blood colloids, it is altogether possible that some specific reaction may be developed along these lines, for the studies of the physical chemistry of diseases in general and of tumors in particular, indicate that there are differences in colloidal dispersion in many pathological states. As yet, however, this field has

[56] See Morgenroth and Bieling, Biochem. Zeit., 1915 (68), 85.
[57] Folia Serologica, 1911 (7), 1013; literature.
[58] Tyzzer, Jour. Cancer Res., 1916 (1), 125.
[59] Wien. klin. Woch., 1909 (22), 989; Zeit. Immunität., 1910 (4), 455.
[60] See Weil, Jour. Exp. Med., Oct., 1913.
[61] Münch. med. Woch., 1912 (59), 65, 1093 and 2854; also Rosenberg, Deut. med. Woch., 1912 (38), 1225.
[62] Farmachidis (Riforma med., 1918 (34), 382) states that only with malignant disease occurs the activation by cobra venom of the hemolytic action of the serum in the complement fixation reaction.
[63] Deut. med. Woch., 1910 (36), 986. Not corroborated by Ordway and Kellert, Jour. Med. Research, 1913 (28), 287.
[64] Prensa Med. Argentina, 1924 (10), 809.
[65] Klin. Woch., 1923 (2), 641.

not been sufficiently developed to permit of evaluation of the results so far described.[66]

Ascoli and Izar[67] have applied the meiostagmin test (*q. v.*) and state that this gives very positive results in determining the existence of cancer, their work having been corroborated by many but not by all of those who have repeated it.[68] Burmeister[69] could obtain no reliable results with the epiphanin reaction.

Freund and Kaminer[70] have reported that the serum of cancer patients is unable to dissolve cancer cells, as normal serum does, and even protects them against the lytic power of normal serum. The lysis is ascribed to a non-nitrogenous fatty acid, while the protective agent of cancer serum is said to be a "nucleo-globulin" which is increased in the serum in cancer. They also find that cancer extracts give a specific turbidity or precipitation with cancer serum, which is attributed to the carbohydrate content of the extract. On the other hand, Körbler[71] found that the plasma of patients has a greater toxic effect than normal serum on tissues, especially those of a person with cancer. Schmitz[72] found the Freund-Kaminer reaction too uncertain for diagnostic purposes, but did observe an increased carcinolytic power in the serum of cancer patients after radiation. Numerous observers, however, have failed to corroborate the findings of Freund and Kaminer.[73] In support of Freund and Kaminer's observation is the experiment of Neuberg[74] who found that cancer cells plus normal serum underwent digestion more rapidly than cancer cells plus cancer serum, as measured by the incoagulable nitrogen.

Boyksen found that a commercial antiserum against gastrointestinal adenocarcinoma gives a specific allergic cutaneous reaction in persons with the same type of carcinoma, which is corroborated by Wigand,[75] but not by Harke,[76] at least in respect to specificity. Mertens[77] has described a cutaneous reaction produced in cancer patients by intracutaneous injection of serum from cancer patients recently roentgenized, and Hoff and Schwarz have confirmed this,[78] but it has not yet been extensively enough employed to determine its reliability.

[66] See Kahn, Klin. Woch., 1923 (2), 1364 (sodium oleate flocculation); Fischer, Bull. Acad. Med., 1923 (88), 71 ("prostaxis" reaction), also (90), 334; Wilbouchevitch, Compt. Rend. Soc. Biol., 1922 (87), 1339 (Botelho's reaction, precipitation with iodine); also Sabrazes and Muratet, Arch. Mal. Coeur, 1923 (16), 841.

[67] Münch. med. Woch., 1910 (57), 2129; Biochem. Zeit., 1910 (29), 13.

[68] See Rosenberg, Deut. med. Woch., 1913 (39), 926; Wissung, Berl. klin. Woch., 1915 (52), 998; Roffo, Revista Inst. Bact., Buenos Aires, 1917 (1), 53; Waterman, Neder. Tijd. Geneesk., 1921 (2), 2388.

[69] See Burmeister, Jour. Inf. Dis., 1913 (12), 459; Bruggemann, Mitt. Grenz. Med. u. Chir., 1913 (25), 877.

[70] Wien. klin. Woch., 1923 (36), 863, full review.

[71] Arch. klin. Chir., 1924 (132), 75.

[72] Amer. Jour. Obst. Gyn., 1924 (7), 449; also Knipfer, Neoplasmes, 1922 (1), 177.

[73] See Nather, Schweiz. med. Woch., 1923 (53), 54; Herly, Jour. Cancer Res., 1921 (6), 337; Frankenthal, Zeit. Krebsforsch., 1920 (17), 250.

[74] Biochem. Zeit., 1910 (26), 344. See also Koritschoner, Biochem. Zeit., 1920 (104), 259; 1922 (129), 605.

[75] Zeit. Immunität., 1923 (36), 202.

[76] Münch. med. Woch., 1924 (71), 102.

[77] Deut. Zeit. Chir., 1923 (179), 216.

[78] Münch. med. Woch., 1924 (71), 816.

Many observations have been made on the antitryptic activity of the blood in cancer (see Chapter III) which has usually shown an increase (in all but about 10 per cent. of the cases); but many other conditions, especially cachexia, may cause positive reactions. Cancer serum is said to have a lessened power to activate pancreatic lipase[79] when the disease is progressive, but on improvement or recovery this effect is increased, the reaction not being specific for cancer.

A critical test of many recommended methods of serum diagnosis of cancer by Halpern[80] gave disappointing results. With the von Dungern technic he obtained 80 per cent. of positive results, with the meiostagmin reaction 85 per cent., but with the Abderhalden method only 30 per cent. The other methods he finds of little value, which has been the usual experience of critical investigators. Thus, Coca[81] obtained entirely unsatisfactory results with both the von Dungern complement fixation test and the Freund-Kaminer reaction; and Renaud[82] says that the reactions for cancer generally indicate merely the existence of cachexia. The testimony concerning the specificity of the Abderhalden reaction (*q. v.*) in cancer is so conflicting that it seems unprofitable to discuss it, the results varying from such as those cited by Halpern above, to 100 per cent. correct reactions described by others.[83]

B. CHEMISTRY OF CERTAIN SPECIFIC TUMORS

In the literature are to be found a few studies of chemical features of some forms of tumors, which may be briefly discussed.

1. Benign Tumors

(*a*) **Fibromas and Myomas.**—The few specimens studied show only a small amount of nucleoprotein, as might be expected from the small amount of their nuclear material. Because of the tendency of fibromas to undergo retrogressive changes, the amount of calcium is likely to be large. No studies as to the special features of their collagen, as compared with normal connective-tissue collagen, seem to have been made. Lubarsch[84] found no glycogen (microscopically) in any of 66 fibromas he examined. Wells and Long[85] found that in uterine fibromyomas only one per cent. of the total nitrogen is purine nitrogen, distributed as guanine, 44 per cent.; adenine, 31 per cent.; hypoxanthine, 25 per cent. The relatively large proportion of preformed hypoxanthine corresponds with the abundance of this purine free in normal unstriated

[79] Shaw-Mackenzie, Lancet, Nov. 8, 1919; 1922 (2), 759.

[80] Mitt. Grenz. Med. Chir., 1913 (27), 370. See also Mioni, Tumóri, 1914 (3), 697.

[81] Jour. Cancer Res., 1917 (2), 61.

[82] Bull. soc. méd. hôp., 1923 (39), 192. See also Lavedan, Paris Med., 1925 (55), 185.

[83] See de Crinis and Mahnert, Fermentfrsch., 1918 (2), 103; Zacherl, Arch. f. Gyn., 1923 (119), 440.

[84] Virchow's Arch., 1906 (183), 188.

[85] Zeit. Krebsforsch., 1913 (12), 598.

muscle. Fibromyomas are able to deamidize their guanine and adenine to xanthine and hypoxanthine, and contain guanase but not adenase. Extracts from uterine fibromyomas show practically the same composition as extracts of normal uterus.[86]

A uterine fibroid analyzed by Beebe[87] contained 14.56 per cent. of nitrogen, 0.981 per cent. of sulfur, 0.139 per cent. of phosphorus, 0.013 per cent. of iron, 0.12 per cent. of calcium oxide, 0.44 per cent. of potassium, and 1.115 per cent. of sodium. The proportions of nitrogen and sulfur are high as compared with most tumors; the phosphorus, iron, and potassium are low, corresponding to the small amount of nucleoprotein and the slow rate of growth. If degeneration is marked, the amount of calcium is greatly increased. E. F. Hirsch[88] found 80 per cent. of water, 3.38 per cent. of fat, and 13 per cent. of protein in a leiomyoma. He also found that leiomyoma tissue develops, after removal, a progressive increase in acidity up to *p*H 6.0, associated with loss of carbohydrate and appearance of rigor mortis; but fibroids showing "red degeneration" developed neither acidity nor rigor.

Krawkow[89] found a trace of chondroitin-sulfuric acid in a uterine fibroid. Lubarsch found glycogen occasionally in richly cellular uterine leiomyomas, and in the vicinity of degenerating areas; however, 76 out of 85 showed no glycogen. Pfannenstiel[90] analyzed the alkaline fluid of a cystic fibromyoma, which coagulated spontaneously; it contained sugar, but no mucin or pseudomucin. The cysts were dilated lymph-spaces, and the fluid corresponded to lymph in composition. A similar result was obtained by Oerum,[91] who found in the fluid, serum-albumin, serum-globulin, and 0.358 per cent. of fibrin; the total proteins constituted 6.3056 per cent. Sollmann[92] found in the "colloid" of a cystic degenerated fibromyoma both pseudomucin and paramucin (see "Ovarian Cysts"), which differed somewhat from the same substances found in ovarian tumors.

The common occurrence of marked *cardiac weakness* in patients with uterine fibroids has led to the suggestion that in the fibroids some toxic product is formed which acts on the heart, or that both the fibroid and the heart defect might result from a common cause. The experimental evidence concerning the relationship is not convincing, and there is much ground for the belief that the heart suffers solely from the anemia common in these cases.[93] There is said to be a hemolytic poison, a lipoid according to Murray,[94] formed in the degenerating fibroids which causes local hemolysis and "red degeneration," and there are cases of acute aseptic

[86] Winiwarter, Arch. f. Gynäk., 1913 (100), 530.
[87] Amer. Jour. Physiol., 1904 (12), 167.
[88] Jour. Biol. Chem., 1921 (45), 297.
[89] Arch. exp. Path. u. Pharm., 1898 (40), 195.
[90] Arch. f. Gyn., 1890 (38), 468.
[91] Maly's Jahresber., 1884 (14), 462.
[92] Amer. Gynecol., 1903 (2), 232.
[93] See Jaschke, Mitt. Grenz. Med. u. Chir., 1912 (15), 249; McGlinn, Surg. Gyn. Obst., 1914 (18), 180.
[94] Jour. Obs. and Gyn., 1910 (17), 534.

degeneration of fibromyomas which seem to have caused systemic intoxication.

(*b*) **Myxomas.**—From a myxoma of the back Oswald[95] obtained a mucin with the following elementary composition: C, 50.82; H, 7.27; N, 12.24; S, 1.19; P, 0.25 per cent. This differs from other mammalian mucins in containing phosphorus, but Oswald does not consider this a contamination. It also contained 12 per cent. of carbohydrate, apparently glucosamine.

(*c*) **Chondromas,** like normal cartilage, always contain much glycogen (Lubarsch). Mörner[96] found chondroitin-sulfuric acid in several chondromas that he examined, although Schmiedeberg had failed to do so.

(*d*) **Lipomas**[97] have been studied by Schulz[98] and by Jaeckle.[99] The former found in a retroperitoneal lipoma 75.75 per cent. of fat, 2.25 per cent. of connective tissue, and 22 per cent. of water. Of the fat, 7.31 per cent. was in the form of the free fatty acids and 92.7 per cent. as neutral fats. The fatty acids of the fat consisted of 65.57 per cent. oleic acid; 29.84 per cent. stearic acid; 4.59 per cent. palmitic acid. Cholesterol was only qualitatively demonstrable. In the connective tissue was found chondroitin-sulfuric acid. Lubarsch found glycogen in lipomas only when they were degenerated. Jaeckle observed the formation of calcium soaps in a calcifying lipoma, the calcium being distributed as follows: Calcium soaps, 29.5 per cent.; calcium carbonate, 28.61 per cent.; calcium phosphate, 41.89 per cent. The fats of lipomas he found practically identical with those of the subcutaneous tissues, except sometimes for a deficiency in lecithin, as shown by the following figures:

[95] Zeit. physiol. Chem., 1914 (92), 144.

[96] Zeit. physiol. Chem., 1895 (20), 357.

[97] In *xanthoma tuberosum multiplex*, which shows local deposits composed largely of cholesterol esters and contains also pigment with the properties of a lipochrome, the presence of hyper-cholesterolemia seems to be responsible (see Cholesterol, Chap. XVIII), but xanthomas are not true neoplasms.

Edsall found the composition of the fat in the fatty tumors of *adiposis dolorosa* (*Dercum's disease*) but little different from that of normal fat. (Quoted by Dercum and McCarthy, Amer. Jour. Med. Sci., 1902 (124), 994.) Martelli (Tumori, 1918 (6), 1) on histological grounds, states that in 2–3 per cent. of the cells the fats are mixed with fatty acids, while cholesterol, phospholipins, and chromolipins are very scanty; he attributes the condition to disturbed lipogenesis from endocrin-sympathetic dysfunction.

[98] Pflüger's Arch., 1893 (55), 231.

[99] Zeit. physiol. Chem., 1902 (36), 53.

	Composition of Fats in—			
	Subcutaneous tissue	Lipoma I	Lipoma II	Lipoma III
Refraction, at 40°	50.6	50.1	50.9	50.5
Saponification number	197.3	197.7	197.7	195.9
Reichert-Meisser number	0.25	0.33	0.35	0.35
Iodine number	63.7	59.0	64.0	64.1
Olein	74.1	68.6	74.4	74.5
Oleic acid	70.9	65.7	71.2	71.3
Acid number	0.39	0.31	0.48	0.67
Free acid	0.196	0.155	0.24	0.34
Palmitic acid	18.5	24.9	.....	18.5
Stearic acid	6.2	5.1	.....	5.9
Lecithin	0.084	.....	.....	0.015
Cholesterol	0.32	.....	0.34	

Lipomas are able to hydrolyze fats and esters, their lipase behaving in all respects like the lipase of normal areolar tissue.[1] Lipoma fat is hydrolyzed by lipase as readily as is normal human fat. No reason for the reputed unavailability of lipoma fat for the metabolism of the host could be found. It is doubtful if the fat of benign lipomas is entirely unavailable for metabolism, at least in all cases, but in malignant fatty tumors this seems to be true. Hirsch and Wells[2] have studied such a tumor in which, despite most complete exhaustion of the fat from the normal fat depots, about two pounds of fat and four and one-half pounds of protein were stored up in the growing tumor. This tumor was an edematous *retroperitoneal lipo-sarcoma*, weighing 69 pounds and its chemical composition is given below as compared with the composition of granulation tissue (castration granuloma of swine).

	Fibro-lipo-sarcoma, per cent.	Granuloma, per cent.
Alcohol ether residue	6.53	15.84
Alcohol ether extract	2.94	2.28
Total solids	9.47	18.12
Water	90.53	81.88
	Alcohol ether residue (per cent. of solids)	Alcohol ether residue (per cent. of solids)
Total protein	66.91	91.16
Protein sulfur	0.65	0.33
Protein phosphorus	0.44	0.31
Total purine nitrogen	0.13	0.07
	Lipins contained, per cent.	Lipins contained, per cent.
Total nitrogen	0.17	0.09
Total sulfur	0.13	0.05
Total phosphorus	0.19	0.09

(*e*) **Ovarian cyst contents** have been studied more than almost any other tumor constituents, because in their gelatinous or slimy sub-

[1] Wells, Arch. Int. Med., 1912 (10), 297; full review.
[2] Amer. Jour. Med. Sci., 1920 (159), 356.

stance are contained numerous interesting forms of proteins, many of which are combined with carbohydrates and related to the true mucins. These substances are frequently referred to under the names of *pseudomucin, paralbumin, metalbumin,* and *ovarian "colloid,"* and belong to the class of *"mucoids."*[3] In view of the fact that the fluids in the Graafian follicles of the ovary do not contain these particular forms of protein, their presence in cysts derived from adventitious structures (Pflüger's epithelial tubes) suggests a specific form of metabolism on the part of the epithelium of these structures.

Serous cysts, formed by dilatation of Graafian follicles, usually are small in size, and the contents resemble those of the normal follicles (Oerum),[4] consisting of a serous fluid with a specific gravity usually from 1.005 to 1.014 (occasionally 1.020 or more), and containing 1.0–4.0 per cent. of solids. Occasionally in these cysts the contents become solidified through absorption of the water, and a gelatinous or glue-like "colloid" content results. Mucoids are never present (Pfannenstiel).[5]

Proliferating cystomas contain the peculiar characteristic mucoid proteins mentioned above. Usually the contents are fluid, but of a peculiar slimy, stringy character, due to the mucoid substance, and often opalescent or slightly turbid. The specific gravity is generally high—1.015–1.030. The reaction is usually slightly alkaline to litmus, and neutral or slightly acid to phenolphthalein. If hemorrhage has occurred into them, the fluid is discolored, and may contain blood-pigments in crystalline and amorphous forms. Small cysts often show a condensation of the proteins into a semisolid "colloid" material, but sometimes their contents resemble those of a serous cyst. Often masses of proteins fall out of solution, forming yellowish flocculi or large deposits half filling the cysts. As with all stagnant fluids of this type, cholesterol crystals are frequently found. The characteristic proteins are members of the class of pseudomucins, which are constantly present (Oerum).

Intraligamentary papillary cysts contain a yellow, yellowish-green, or brownish-green liquid, which contains little or no pseudomucin; the specific gravity is usually high (1.032–1.036) and the fluid contains 9 to 10 per cent. of solids. The principal constituents are the simple proteins of blood serum (Hammarsten).

According to the same author, the rare *tubo-ovarian cysts* contain a watery serous fluid with no pseudomucin.

Chemistry of the Mucoids of Ovarian Cysts.—Pseudomucin has the following elementary composition: C, 49.75; H, 6.98; N, 10.28; S, 1.25; O, 31.74 per cent. (Hammarsten). In common with the true mucins it yields a sugar-like reducing body, which has been investigated by numerous chemists (Müller, Panzer, Zangerle, Leathes, Neuberg, and Heymann[6]). Panzer considers that this reducing substance is in the form of a sulfuric-acid compound, similar to, but not identical with, chondroitin-sulfuric acid. Hammarsten, however, did not find this substance constantly present. Leathes determined for the carbohydrate group the composition $C_{12}H_{23}NO_{10}$,

[3] Concerning mucoids see Mann's "Chemistry of the Proteins," 1906, pp. 541–551.
[4] See Maly's Jahresbericht, 1884 (14), 459.
[5] Arch. f. Gynæk., 1890 (38), 407.
[6] Hofmeister's Beitr., 1902 (2), 201 (literature).

named it "*paramucosin*," and considers it a reduced *chondrosin* (which is the carbohydrate group of chondroitin-sulfuric acid). Neuberg and Heymann established, however, that the reducing body must come from *chitosamin* ($C_6H_{13}NO_5$), and do not consider paramucosin a constant constituent of ovarian mucoids. Schmiedeberg[7] maintains that the carbohydrate complex is not chondrosin, but a substance which he calls *hyaloidin* ($C_{26}H_{46}N_2O_{20}$) identical with the carbohydrate of ovomucoid. The amount of reducing substance varies greatly in the mucoids found in different cysts; in some the mucoid yields only about 3 to 5 per cent., in others as much as 30 to 35 per cent., of reducing substance.

Pseudomucin dissolves readily in weak alkalies, and differs from true mucin in that it is not precipitated by acetic acid, and from the simple proteins in that its solutions are not coagulated by boiling. With water a slimy, stringy, semisolution is formed, resembling in appearance the material found in ovarian cysts. Leathes distinguishes two forms of ovarian mucoids: One, *paramucin*, occurs as a firm, jelly-like substance, which is converted by peptic digestion into easily soluble pseudomucin. *Ovarian "colloid"* probably consists of a thickened pseudomucin, often mixed with other proteins,[8] especially globulins.[9] Pfannenstiel[5] considers the "colloid" material as representing a modified pseudomucin, strongly alkaline and relatively insoluble, which he calls "*pseudo-mucin β.*" He also describes a very soluble mucoid found only in certain ovarian cysts, naming it "*pseudo-mucin γ.*"

The reason why these variations in the pseudomucins exist is not understood; they cannot be explained as due to variations in the cell type in the cyst wall, although pseudomucin is probably the result of true secretion. The smallest cavities of ovarian cystadenomas contain nearly pure pseudomucin, which presents a clear, glassy structure; the larger the cysts become, and the more turbid and thinner the fluid is, the more simple are the proteins it contains. True mucin is never present in ovarian cysts. Pseudomucin occurs only in the glandular proliferating cystomas and the papillary proliferating cystadenomas, in the former appearing constantly and abundantly, in the latter not constantly and never abundantly (Pfannenstiel). *Paralbumin* (Scherer) is a mixture of pseudomucin with variable amounts of simple proteins. *Metalbumin* (Scherer) is the same body that is called pseudomucin by Hammarsten. *Paramucin* (Mitjukoff)[10] is a mucoid differing from mucin and pseudomucin in reducing Fehling's solution directly, without having the carbohydrate group first split off by boiling with an acid. Hydrolysis of paramucin by Pregl[11] showed an absence of glycine, but traces of diamino-acids, and the presence of leucine, alanine, proline, aspartic and glutamic acids, tryptophan and tyrosine.

Substances similar to pseudomucin have been occasionally found in cancerous ascitic fluid and in cystic fibromyomas (Sollmann); and they are abundant as constituents of the contents of the peritoneum in the condition known as "*pseudomyxoma peritonei*,"[12] when the material is in reality the product of cells implanted on the peritoneal surface through the bursting of an ovarian cyst (or a cyst of the vermiform appendix (Fränkel)).[13] The physically similar substance found in pathological synovial membranes by Hammarsten differs in yielding no reducing substance. Parovarian cysts arising from the Wolffian body present an entirely different content, which is a clear, watery fluid, with specific gravity usually under 1.010; the solids amount to only 1 or 2 per cent., and consist chiefly of salts (the ash being often over 80 per cent.), mostly sulfates and chlorides.[14] They are usually (or always) free from pseudomucin, mucin, or other sugar-containing substances, and other proteins occur only in small amounts, unless the cyst is inflamed. Apparently mucoids do not form in cysts lined by ciliated epithelium (Pfannenstiel).

[7] Arch. exp. Path. Pharm., 1920 (87), 1.

[8] A certain type of colloid ovarian tumor, "*struma colloides ovarii*," seems to represent teratomas composed principally of thyroid tissue, for the colloid gives the staining reactions of thyroid colloid and in one case iodine was found. (See Kafka, Arch. f. Gynäk., 1921 (114), 587.)

[9] Leary, Hartz and Hawk, N. Y. Med. Jour., 1916, July 1. These authors found ovarian cyst contents non-toxic for guinea pigs.

[10] Arch. f. Gynæk., 1895 (49), 278.

[11] Zeit. physiol. Chem., 1908 (58), 229.

[12] Literature by Peters, Monatschr. f. Geb. u. Gyn., 1899 (10), 749; Weber, St. Petersb. med. Woch., 1901 (26), 331.

[13] Münch. med. Woch., 1901 (48), 965.

[14] 0.03 per cent. of the ash was found to be SiO_2 by Schultz (Pflüger's Arch., 1901 (84), 95).

Santi[15] has studied the physical chemistry of ovarian cysts, and finds the freezing point very near that of blood, having no relation to density, viscosity or nitrogen content; the specific electrical conductivity is higher than that of blood serum. The physicochemical properties are less dependent upon chlorides, and more on other substances (Gruner).[16]

(*f*) **Dermoid cysts** of the ovary contain, as their chief and most characteristic constituent, a yellow fat, which melts at 34°–39° and solidifies at 20°–25°. Ludwig and Zeynek[17] have examined over sixty such tumors, and found that the fatty material constantly contains two chief constituents: one, crystallizing out readily, they believed to be *cetyl alcohol*,

$$(CH_3—(CH_2)_{14}—CH_2OH);$$

the other, remaining as an oily fluid, seems to be closely related to cholesterol, although not consisting of one substance alone. Small quantities of *arachidic acid* ($C_{20}H_{40}P_2$), as well as *stearic*, *palmitic* and *myristic acid* ($C_{14}H_{28}O_2$), existing as glycerides, are also present. Ameseder,[18] however, found evidence that the supposed cetyl alcohol is really eikosyl alcohol ($C_{20}H_{24}O$), although later he also obtained cetyl alcohol.[19] These acids were found by Muck[19] to be bound to an unsaturated alcohol of unknown composition, derived from, related to, but different from cholesterol. These substances are secreted by the glands of the cutaneous structures of the cyst, and resemble in composition sebaceous material, which is characterized by containing a large proportion of cholesterol partly combined with fatty acids. Dermoids sometimes contain masses of fatty concretions which seem not to depend on chemical changes but on the presence of formative nuclei and framework of desquamated epithelium; they consist of a mixture of neutral fats and cholesterol esters, with some free cholesterol.[20] A mediastinal dermoid cyst was found by Patein[21] to contain half its solids as ether-soluble material which was mostly neutral fats. A small ovarian cyst of undetermined character was found by Arnold[22] to consist almost entirely of cholesterol crystals. The fat of an ovarian dermoid examined by Kreis[23] contained much cholesterol, volatile fatty acids, and a non-volatile acid resembling, but not identified as, arachidic acid.

Cholesteatomas, in addition to their abundant cholesterol content, contain keratin.[24]

(*g*) **"Butter" Cysts.**[25]—In the mammary gland, retention cysts form, filled with products of alteration of the milk, including butyric

[15] Folia clin. chimica et microscop., 1910 (2), 73.
[16] Biochem. Jour. 1907 (2), 383.
[17] Zeit. physiol. Chem., 1897 (23), 40.
[18] *Ibid.*, 1907 (52), 121.
[19] Quoted by Muck, Zeit. physiol. Chem., 1922 (122), 125.
[20] Lippert, Frankf. Zeit. Path., 1913 (14), 477.
[21] Jour. pharm. Chim., 1922 (26), 369.
[22] Jour. pharm. chim., 1920 (21), 305.
[23] Schweiz, Apoth. Ztg., 1921 (59), 629.
[24] Risel, Verh. Deut. Path. Gesell., 1909 (13), 322.
[25] An "oil cyst" behind the ear has been fully analyzed by Kreis (Schweiz. Apoth. Ztg., 1918 (56), 81) and found to contain, in addition to much neutral fat and cholesterol, considerable amounts of high unsaturated hydrocarbons.

acid and lactose (Klotz),[26] and these are called "butter cysts" or milk cysts. Analysis of the contents of such a cyst by Smita[27] gave the following results, as compared with human milk:

	Cyst Contents	Human Milk
Fat	72.97	3.90
Casein	4.37	0.63
Albumin	1.91	1.31
Milk-sugar	0.88	6.04
Ash	0.36	0.49
Water	20.81	87.09
Fats consisted of—	Cyst	Cow's Milk
Stearin and palmitin	37.0	50.0
Olein	53.0	42.2
Butyrin	9.0	7.8

Occurring independent of lactation usually, but not always, are the "*soap cysts*," which contain chiefly Ca and Mg soaps, but also neutral fats, free fatty acids, and traces of cholesterol (Freund).[28]

2. Malignant Tumors

The chief general features of the composition of these growths have been considered in the discussion of the chemistry of tumors in general. A malignant tumor differs from a similar benign tumor chiefly in having usually a larger proportion of the primary cell constituents, and a smaller proportion of the secondary constituents and intercellular substances, since these are largely the product of the functional activity of the cells, which, in malignant tumors, do not often develop sufficiently to function extensively. Hence malignant tumors usually show a rather high proportion of the characteristic constituents of nucleoproteins; *i. e.*, phosphorus and iron. The H-ion concentration of extracts of rat tumors does not differ appreciably from that of most normal tissues (Woglom).[29]

On account of the extensive disintegration, the products of autolysis are usually much more abundant than in benign tumors. The composition varies greatly with the origin, although to a less extent than with the benign tumors. In Fraenkel's laboratory[30] it was found that cancers are often defective in tryptophan, and from a squamous cell carcinoma of the skin little or none of this amino-acid could be obtained, although normal squamous epithelium is rich in tryptophan. Fasal,[31] however, found usually a high tryptophan figure in cutaneous epithelioma, but very irregular results in other tumors. As Bang and Beebe have shown, the tumors arising from lymphatic tissues show the chemical characteristics of these structures, and contain histone nucleinate. Tumors from squamous epithelium develop keratin in direct proportion to the amount of

[26] Arch. klin. Chir., 1880 (25), 49.
[27] Wien. klin. Woch., 1890 (3), 551; see also Zdarek, Zeit. physiol. Chem., 1908 (57), 461.
[28] Virchow's Arch., 1899 (156), 151.
[29] Jour. Cancer Res., 1924 (8), 34.
[30] Wien. klin. Woch., 1912 (25), 1041.
[31] Biochem. Zeit., 1913 (55), 88.

maturity the cells reach. Even the most complex and specific products of metabolic activity may be developed by malignant tumors (*e. g.*, thyroiodine, epinephrine, bile), and in a form and condition capable of performing function. As Buxton and others have shown, malignant tumors produce a great variety of intracellular enzymes. The idea that glycogen is present in tumors in proportion to their malignancy has been disproved by Lubarsch, Gierke, and others; among the malignant tumors glycogen is found particularly in chorioepitheliomas, hypernephromas, and squamous cell carcinomas. Beebe reported that the composition of metastatic growths is modified by the organ in which they are growing, so that they tend to resemble the organ serving as their host; which, however, does not hold for certain of their enzymes (Wells and Long). In a case of primary carcinoma of the liver, Wolter[32] found the tumor tissue richer in nuclein phosphorus and poorer in phosphatids than the adjacent liver tissue; cholesterol was 0.25 per cent. of the fresh weight, fatty acids 1.67 per cent. and water 82.33 per cent., the water of the normal tissue being 79.34 per cent.

Studies on the influence of blood plasma and tumor extracts on cell growth, have shown that cancer cells resemble embryonic tissues in containing growth-activating agents, the "*trephones*" of Carrel, whereby cancer cells can grow at once in a suitable medium, whereas adult cells do not begin growth until through degenerative or autolytic processes the activating substances are formed (Drew).[33]

It has been found[34] that the blood coming from the wing of a fowl bearing a transplantable sarcoma contains less sugar than the blood from the opposite wing, has a stronger glucolytic effect and contains less diastase. The glucolytic action of cancer tissue *in vitro* is very marked, and Warburg[3] has also found that rat carcinoma tissue in sugar-containing Ringer solution produces much more lactic acid, even 70 times more, than normal tissues, while Posener[36] found that benign tumors are intermediate between cancer and normal tissues in this respect. Possibly this lactic acid production facilitates invasion of other tissues by cancer cells through the softening by hydrophilic swelling of the tissue colloids,[37] and Fleisch[38] found that rat sarcoma oxidizes lactic and succinic acid *in vitro* less rapidly than does rat muscle. Braunstein[39] reports that development of cancer in diabetics may increase the sugar tolerance through the glucolytic action of cancer cells. Russell[40] finds that mice carcinoma and sarcoma attack, or fail to attack, in the same way as normal tissues a series of different hexoses, but are able to destroy pentoses that normal tissues do not attack.

[32] Biochem. Zeit., 1913 (55), 260.
[33] See 21st Report Imp. Cancer Research Fund, London, 1923.
[34] Tadenuma, Hotta and Homma, Deut. Med. Woch., 1924 (50), 1051.
[35] Klin. Woch., 1923 (2), 776; 1924 (3), 1062.
[36] Klin. Woch., 1924 (3), 1490.
[37] See Bierich, Klin. Woch., 1924 (3), 221.
[38] Biochem. Jour., 1924 (18), 294.
[39] Klin. Woch., 1924 (3), 788.
[40] Sci. Rep. Imp. Cancer Res. Fund, 1923 (8), 11.

Waterman has carried out extensive studies on the physical chemistry of cancer cells,[41] and finds the electrical resistance lowered, and even more reduced is the polarization tension of the cell surface. The ratio $\frac{\text{P(olarization)}}{\text{R(esistance)}} = \text{K}$, therefore, is much reduced, often approaching zero, and averages about one-fourth that of normal tissues examined under the same condition. This indicates that the selective permeability of ions, which determines the polarization, has been reduced on the cell surfaces. It was found that addition of calcium ions increases the polarization of cancer cells, which is explained by Clowes' observations on the effects of calcium ions on the dispersion of surface colloids. Apparently cancer cells are characterized by having the surface colloids highly dispersed, whereby entrance of water-soluble ions is facilitated, with lowering of the polarization tension. Calcium ions decrease this dispersion, and so by measuring the relation of resistance and polarization in a tumor, which can be done either *in vivo* or *in vitro*, and observing the effect of calcium solutions on this relation, it is theoretically possible to measure the degree of malignancy of a tumor, *i. e.*, the deviation from normal of the cells. Waterman says that by studying the surface epithelium of mice during the steps of formation of an experimental tar cancer, he has found that when the cells show histological criteria of malignancy the fall in K is observed, and the effect of calcium in raising the value can be demonstrated. The high capacity of cancer cells to migrate and to infiltrate may perhaps depend on the low surface tension observed in the cancer tissue fluids and in cancer cells.[42]

As to the special varieties of malignant growths, there is little as yet determined concerning their chemistry beyond what has been stated previously. The variations in composition of tumors are largely the direct result either of their resemblance to some normal tissue or of degenerative changes that they have undergone.

"Colloid" carcinoma may be mentioned specially, in view of the confusion caused by the lax use of the term "colloid" (*q. v.*). The fluid contents of colloid cancers of the gastro-intestinal tract are usually chiefly epithelial mucus, containing mucin mixed with a greater or less quantity of proteins from degenerated cells and serous effusion. This mucin is acid in reaction, is precipitated by acetic acid, and has an affinity for basic dyes.[43] The colloid cancers of the mammary gland, in which the "colloid degeneration" involves the stroma, probably contain a connective-tissue mucin, analogous to that of the umbilical cord, as also do the myxosarcomas, if we may judge by their origin and staining reactions, but no exact chemical study of these substances can be found. Colloid cancers of the ovary, arising usually from the same structures as the ovarian cysts, contain pseudomucin or allied bodies (see "Ovarian Cysts"). Colloid tumors of thyroid tissue often contain the typical colloid of normal thyroid tissue, even when metastatic in other organs; in the tumor colloid may be a relatively normal proportion of iodine (Gierke[44]).

[41] Reviewed in Zeit. Krebsforsch., 1922 (19), 101; Biochem. Zeit., 1922 (133), 535.

[42] Bauer, Zeit. Krebsforsch., 1923 (20), 358; Kragen, Zeit. Krebsforsch., 1924 (21), 155 and 453; Solowiew, *ibid.*, (21), 456.

[43] The fluid of a colloid cancer of the peritoneum examined by Hawk contained a protein resembling serosa mucin, containing 11.5 per cent. of N and 0.8 per cent. of S. (McCrae and Coplin, Amer. Jour. Sci., 1916 (151), 475.) The colloid fluid formed in a chondrosarcoma contained chondroitin sulfuric acid (Blanchetiére and Sannie, Compt. Rend. Soc. Biol., 1924 (90), 1398).

[44] Hofmeister's Beitr., 1902 (3), 286.

Hypernephromas possess several interesting chemical features. For example Gatti[45] brought forward the fact that such a tumor analyzed by him contained 3.47 per cent. of lecithin, which agreed very well with the amount of lecithin in normal adrenals. Beebe[46] found in the watery extract of a hypernephroma the following substances: Tryptophan, proteoses, glycogen, leucine, and tyrosine, indicating the occurrence of autolysis. About 29 per cent. of fat was present, which was all extractable without pepsin digestion, and the fat contained about 18 per cent. of its weight as cholesterol. Lecithin was also present, but not quantitatively determined. Benign adrenal cortex adenomas contain much cholesterol (2.58 to 16.4 per cent.) as compared with normal cortex (1.3 to 5.5 per cent.).[47]

A study of the fats and lipoids of hypernephromas and other tumors gave me the results shown in the following table:[48]

	Normal adrenal	Hypernephromas				Carcinoma of gall bladder	Carcinoma of breast	Sarcoma, secondary, in liver
		1	2	3	4			
Ether-soluble material	36.3	28.0	33.0	38.4	85.0	8.6	21.4	14.5
Cholesterol, per cent. total dry weight	7.6	4.6	6.7	8.7	0.5	2.2	0.9	1.6
Cholesterol, per cent. dry, fat-free substance	11.9	6.4	10.0	14.0	3.3	2.4	1.2	1.9
Cholesterol, per cent. ether-soluble substance	20.6	16.9	20.4	22.9	0.7	26.1	4.3	11.0
Lecithin, per cent. total dry weight	11.8	6.0	9.0	8.3	2.0	1.7	0.7	6.2
Lecithin, per cent. dry, fat-free substance	18.4	8.3	13.4	13.4	13.3	1.9	0.9	7.3
Lecithin, per cent. ether-soluble substance	33.0	22.7	27.5	21.4	2.4	20.0	3.0	39.8

Hypernephroma No. 1.—Typical specimen, with the usual amount of hemorrhage and necrosis; cells much vacuolated.

Hypernephroma No. 2.—Similar to No. 1.

Hypernephroma No. 3.—Primary growth resembled more a papilloma than an ordinary hypernephroma in most places; no vacuolization of cells, little necrosis, and no hemorrhage.

Hypernephroma No. 4.—Tumor resembling a lipoma, with a stroma in places resembling a fibrosarcoma in structure. In on y a few areas were cells present resembling adrenal tissue, most of the tissue resembling fatty areolar tissue.

It will be at once observed that the two typical hypernephromas, Nos. 1 and 2, show a marked resemblance to the normal adrenal in the proportion of fat and lipoids. (The lower figure for lecithin in No. 1 probably is due to the fact that this specimen had been preserved longer than the others.) This was what was to be expected from the microscopic resemblance of these tumors to adrenal tissue, and corroborates the results of Gatti's and Beebe's observations on isolated cases. More surprising is the fact that equally comparable results were obtained in the hypernephroma (No. 3), which contained only cells free from vacuolization and not at all resembling adrenal cells. From this it may be concluded that in these tumors of adrenal origin the amount of fats and lipoids present cannot be estimated from the degree of cytoplasmic vacuolization of the cells, or the extent of necrosis; *the fatty materials are an integral part of the cells, present in them as an essential constituent and not as the result of degeneration.*

The results of analysis of two carcinomas and a sarcoma indicate that the hypernephromas are peculiar in their close resemblance to adrenal tissue in respect to fat

[45] Virchow's Arch., 1897 (150), 417.

[46] Amer. Jour. Physiol., 1904 (11), 139.

[47] Yavanovitch, Compt. Rend. Soc. Biol., 1924 (91), 158.

[48] Wells, Jour. Med. Res., 1908 (12), 461; see also Steinke, Frankfurt. Zeit. Path., 1910 (5), 167.

and to lipoid content. The amount of all these constituents in these three tumors is far below that found in the hypernephromas, although in the carcinoma of the breast the amount of simple fats is relatively large, as might be expected in view of the function of the cells from which it arose. The large proportion of lecithin in the sarcoma of the liver may possibly be due to the influence of the soil upon which the tumor was growing, but we need more information concerning the lipoid content of other malignant tumors arising in different sites.

Renal hypernephromas reproducing the adrenal cortex in structure do not contain epinephrine,[49] but tumors of the adrenal arising in the medulla may do so.[50] Microscopically, hypernephromas contain much glycogen. The special tests for hypernephroma tissue recommended by Croftan seem not to be specific.[51]

Melanotic tumors produce melanin, which seems not to differ at all from the melanin found in normal pigmented structures. Helman[52] found as high as 7.3 per cent. by weight of melanin in melanosarcomas. (See also Melanin, p. 529, and Enzymes in Tumors, p. 88. Concerning Chloromas[53] see p. 535.)

Multiple Myelomas and Myelopathic "Albumosuria"

Multiple myelomas are of particular chemical interest, because of the appearance in the urine in such cases of the peculiar protein first described as an *albumose* by Bence-Jones,[54] and now, because of lack of grounds for its definite classification, generally known as the "*Bence-Jones body*" or "*Bence-Jones protein.*" Because of the extensive bone destruction there is also an excessive excretion of calcium,[55] and sometimes metastatic calcification may occur.[56] This variety of tumor differs from the standard types of malignant tumors in that it involves the marrow of many bones simultaneously, in a very diffuse manner, without usually giving evidence of visceral metastasis. In many respects it resembles the leukemias, pseudoleukemia, and chloroma, and it is extremely uncertain as to where in the classification of tumors and of the diseases of the blood-forming organs this disease should be placed. Histologically, the tumors show evidence of being derived from the specific cells of the marrow, either from the plasma cells (Wright) or from the neutrophile myelocytes[57] or their predecessors (Muir). Cases of myeloma without the proteinuria have been described, and also a few instances of the presence of apparently typical Bence-Jones protein in the urine without myelomas, but with bone carcinomas, leukemia, or chloroma.[58]

[49] Greer and Wells, Arch. Int. Med., 1909 (4), 291; Brooks, Jour. Exp. Med., 1911 (14), 550; Ciaccio, Deut. Zeit. f. Chir., 1910 (104), 277.

[50] Wegelin, Verh. Deut. Path. Ges., 1911 (15), 255; Oppenheimer and Fishberg, Arch. Int. Med., 1924 (34), 631.

[51] Koerber, Virch. Arch., 1908 (192), 356.

[52] Arch. internat. Pharmacodyn., 1903 (12), 271.

[53] Metabolism in chloroma does not differ from leukemia (Sakaguchi, Mitt. Med. Fak., Tokio, 1914 (13), 198).

[54] For literature, see Rosenbloom, "Endocrinology and Metabolism," 1924 (4), 485; Vance, Amer. Jour. Med. Sci., 1916 (152), 693.

[55] Blatherwick, Amer. Jour. Med. Sci., 1916 (151), 432.

[56] Tschistowitsch and Kolessnikoff, Virchow's Archiv., 1909 (197), 112.

[57] Forman and Warren (Jour. Cancer Res., 1917 (2), 79) found the cells to contain granules giving the indol phenol blue reaction and hence belonging to the myeloid group, but no positive oxidase reaction could be obtained in three cases studied by Morse, Jour. Cancer Res., 1920 (5), 345.

[58] Glynn has described a glucoprotein resembling Mörner's body, in the urine during myeloma (Liverpool Med. Chir. Jour., 1914, p. 82). A crystallizable protein, resembling the Bence-Jones body, has been found in the urine of a woman with gastric cancer without any bone involvement (Schumm and Kimmerle, Zeit. physiol. Chem., 1914 (92), 1).

I & in Kidney destruction, 13 cases by Bennick & Greene. Archives Internal Medicine. 44. Oct. 1929.

Properties of the "Bence-Jones Protein."—Not to go into details, which are given in the literature cited, the important facts concerning the "*Bence-Jones protein*" and its appearance in the urine ("*myelopathic albumosuria*," Bradshaw), are as follows: It is a protein, the exact nature of which has not been determined; at first considered an albumose because of its peculiar reactions to heat, its nature has since been contested, but the weight of evidence seems to be in favor of the contention of Simon that it is most closely related to the water-soluble globulin of the blood. In certain cases it partly precipitates spontaneously from the urine;[59] rarely it crystallizes in the urine or after isolation,[60] and it may crystallize in the renal tubules.[61] Its most characteristic properties are the following:

The coagulation temperature is low, varying from 49°–60° in various cases, and being considerably modified by the amount of salts and urea present in the solution.[62] Probably the protein forms a molecular compound with the salts which is more stable at 100° than at lower temperatures (Hopkins and Savory).

In many cases the coagulum is redissolved on heating, and reappears on cooling, but this characteristic feature is not always present, and often disappears in cases where at first it is present.

A precipitate is formed by strong (25 per cent.) nitric acid, which disappears on heating and reappears on cooling. Strong hydrochloric acid causes a dense precipitate, which is quite typical.

No precipitate is produced by acetic acid, even in excess, and the addition of acetic acid to a hot coagulated specimen causes prompt solution of the coagulum.

Unlike albumoses, this substance does not dialyze; the salt-free solution left in the dialyzing bag does not precipitate.

A purplish-violet color is usually given with the biuret reaction, but it may be more reddish in color, especially if little copper is present.

Sulfur is readily split off by alkalies, reacting with lead acetate to produce lead sulfide (Boston).

After standing in alcohol, by which the protein is precipitated, it loses its solubility (differing in this respect from albumose).

As to the exact nature of this protein, little can be said at the present time. Since protoproteoses, deuteroproteoses, and peptone are split off on digestion with pepsin, the molecule is evidently larger than that of any of the albumoses. The well-purified substance is free from phosphorus, and hence contains no nucleins; but it contains considerable sulfur (between 1 and 2 per cent.), which is readily split off. Like casein, it contains no hetero-group (lack of heteroproteoses on digestion), but differs in containing a carbohydrate group (in small amount) and in the absence of phosphorus. On hydrolysis Magnus-Levy[63] obtained glutaminic acid, tyrosine, and leucine, but no glycine. He found the nitrogen distributed as follows: amid-nitrogen, 9.9 per cent.; humin-nitrogen, 9.8 per cent.; diamino-nitrogen, 6.4 per cent.—which last was composed of: histidine, 0.9 per cent.; arginine, 2.4 per cent.; lysine 3.0 per cent. The extensive analytic studies of Hopkins and Savory[64] show that the amino-

[59] Rosenbloom, Arch. Int. Med., 1912 (9), 255.

[60] See Krauss, Deut. Arch. klin. Med., 1921 (137), 257; Wilson, Jour. Biol. Chem., 1923 (56), 203.

[61] Loehlein, Cent. allg. Path., 1913 (24), 953.

[62] See Malengreau, Arch. internat. physiol., 1921 (18), 151.

[63] Zeit. physiol. Chem., 1900 (30), 200.

[64] Jour. of Physiol., 1911 (42), 189.

acid grouping is that of a typical protein, with a high proportion of aromatic radicals, similar proteins not being found in the tumors or muscles of a typical case. In fact, the amino-acid content, as given below, indicates that Bence-Jones protein is as distinct from other proteins in chemical composition as in its physico-chemical properties. The amino-acids, in round numbers, were isolated in the following percentage proportions of the entire protein: Valine-leucine fraction, 14; glutamic acid, 8; aspartic acid, 2; proline, 2.7; phenylalanine, 4.8; tyrosine, 4.2; tryptophan, 0.8; cystine, 0.6; arginine, 6; histidine, 0.8; lysine, 3.7; sulfur, 1.2. An important point in this work is the agreement in composition of the proteins from two different cases, being identical within the limits of the analytic methods. Lüscher[65] has analyzed a sample of the same protein as one analyzed by Hopkins and Savory[83] by the van Slyke method, and finds a close resemblance to serum globulin in respect to mono- and di-amino acid distribution and tryptophan content, but the proline fraction is much higher in Bence-Jones protein. He agrees with others that this protein is different in constitution from any other known protein. Estimates of the molecular weight of Bence-Jones protein, based on available analytical data, indicate that this is about 24,500,[65a] which, compared with the molecular weights for fibrin (42,000), serum albumin (45,000), and serum globulin (81,000), suggests that perhaps this protein is, after all, the result of protein cleavage.

The more recent studies of the immunological reactions of Bence-Jones protein have shown that it is distinguishable from the proteins of normal serum, even in the presence of serum,[66] although there are possibly two or three different proteins included under this term, and Hektoen and Welker[67] found their crystalline preparation to differ from that of Krauss in containing less carbohydrate and sulfur. The crystalline globulin known as Noel Paton's protein, was found to be entirely distinct from the Bence-Jones protein,[68] and Hektoen[69] has observed still another crystallizable urinary protein.

Occurrence of "Myelopathic Albumosuria."—Not all cases of multiple myeloma show the presence of Bence-Jones protein in the urine, however, and it is present occasionally in other conditions. Multiple bone involvement by other tumors does not often cause "albumosuria."[70] There is no evidence that it occurs in the normal body, even in the bone-marrow, or that it is produced as a step in the splitting of any form of proteins. A few cases of supposed osteomalacia have been reported, with the Bence-Jones body in the urine, but on more careful investigation these seem to have been unrecognized myelomas (*e. g.*, the cases of Bence-Jones and of Jochmann and Schumm). Similarly the

[65] Biochem. Jour., 1922 (16), 556.

[65a] Cohn, Hendry and Prentiss, Jour. Biol. Chem., 1925 (63), 721.

[66] See Hektoen, Jour. Amer. Med. Assoc., 1921 (76), 929; Bayne-Jones and Wilson, Johns Hopkins Hosp. Bull., 1922 (33), 37 and 119.

[67] Jour. Infect. Dis., 1924 (34), 440.

[68] Everett, Bayne-Jones and Wilson, Johns Hopkins Hosp. Bull., 1923 (34), 385.

[69] Jour. Amer. Med. Assoc., 1924 (83), 1154.

[70] Boggs and Guthrie, Amer. Jour. Med. Sci., 1912 (144), 803; Wallgren, Hygeia, 1921 (83), 1.

case reported by Askanazy as leukemia with Bence-Jones protein in the urine, on reëxamination was found to be multiple myeloma. However, numerous cases of true chronic leukemia with Bence-Jones proteinuria have been reported.[71] Occasionally this or a somewhat similar protein has been found in persons without demonstrable bone diseases, *e. g.*, Hodgkin's disease,[72] lymphosarcoma,[73] nephritis.[74] Coriat[75] describes a substance found in a pleuritic fluid which gave the reactions of the Bence-Jones body, and he believes that it may have been formed from serum globulin through the digestive action of the leucocytes or bacteria. Zuelzer reports finding the same body in the urine of a dog poisoned with pyridin.[76] It is a striking fact that the kidneys eliminate such great quantities of this protein without being permeable to the very similar normal blood proteins,[77] but they often show evidence of structural changes; also, that when injected into animals it does not escape freely in the urine as it does in man, but is partly utilized, and nephritic dogs excrete it partly hydrolyzed into proteose.[78] It may be found in the blood and exudates of patients with myeloma,[78] as much as 7.8 per cent. having been found in the blood by Jacobson.[79]

Simon[80] has observed that the protein may be accompanied by dialyzable substances giving the ninhydrin reaction, probably amino-acids or peptids. Sometimes an amyloid-like material is deposited in the tissues, which may be a consolidation of the Bence-Jones protein.[81]

Origin of the Protein.—As to the place of formation of this peculiar protein, there is much diversity of opinion. Magnus-Levy advanced against the idea that it is formed by the tumor cells, the following arguments: In the urine of myeloma patients are excreted great quantities of the protein,—as much as 30 to 70 grams per day,—whereas the total amount of protein in all the tumor tissue in the body seldom exceeds, or, indeed, equals this quantity. It seems improbable that so little tumor tissue can form so much urinary protein, and Magnus-Levy suggests that it must come from the food proteins as a result of altered protein metabolism. Against this view, however, are the following facts: (1) The Bence-Jones body has been found (but not constantly) in the myeloma tissue, but not in other organs or tissues; (2) the quantity in the urine is not dependent upon diet;[82] (3) it is associated almost exclusively with this form of tumor or extensive bone marrow disease. Simon

[71] Boggs and Guthrie, Bull. Johns Hopkins Hosp., 1913 (24), 368; Decastello, Wien. Arch. inn. Med., 1920 (1), 335.
[72] Galloway, Brit. Med. Jour., 1922 (2), 1201.
[73] Micheli, Hematologica, 1921 (2), 1.
[74] Miller and Baetjer, Jour. Amer. Med. Assoc., 1918 (70), 137.
[75] Amer. Jour. Med. Sci., 1903 (126), 631.
[76] Wolgemuth (Arb. a. d. Path. Inst. zu Berlin, Festschrift, 1906, p. 627) states that normal human bone marrow may contain true albumoses.
[77] See Guggenheimer, Zeit. f. Urol., 1924 (18), 523.
[78] Taylor, *et al.*, Jour. Biol. Chem., 1917 (29), 425.
[79] Jour. Urol., 1917 (1), 167.
[80] Jour. Amer. Med. Assoc., 1918 (70), 224.
[81] Glaus, Virchow's Arch., 1917 (223), 301.
[82] See Walters, Jour. Amer. Med. Assoc., 1921 (76), 641.

considers it probable that the protein is formed from serum-globulin, perhaps by an enzymatic action of the tumor cells, and once formed, it is rapidly eliminated by the kidneys, as are all foreign proteins. Normal bone marrow does not contain this protein (Nerking).[83] Rosenbloom[84] has found evidence that Bence-Jones protein may possibly be derived from the osseo-albumoid of the bones. Weber and Ledingham[8] have suggested that it comes from the cytoplasmic residue of karyolyzed plasma cells. The observation that under benzol treatment the amount of Bence-Jones protein in the urine of leukemic patients is reduced (Boggs and Guthrie[71]) is also good evidence of its myelogenous nature. On the other hand, roentgenization of the bone tumors does not alter the urinary out-put of the protein.

The fact that Abderhalden and Rostoski[86] found that the serum of rabbits immunized with Bence-Jones protein gives the precipitin reaction with human serum, is evidence that the protein is a human tissue protein and not merely an absorbed and excreted food protein. This has been corroborated by Hopkins and Savory,[87] who also found that the amount of protein in the urine, which contained about one-third the total nitrogen excreted, varied with the general metabolism and was not controlled by the diet. Massini[88] reports securing positive complement fixation tests with immune sera, differentiating the Bence-Jones protein from normal serum proteins; positive sensitization tests were not obtained by cutaneous injections of the protein by Boggs and Guthrie. Injected into the blood it is non-toxic and does not lower coagulability as a proteose would. It is capable of acting as an antigen in anaphylaxis and other immunological reactions, which also indicates that it is a complete protein and not a cleavage product.[89]

[83] Biochem. Zeit., 1908 (10), 167; corroborated by Hopkins and Savory, Jour. Physiol., 1911 (42), 189.

[84] Arch. Int. Med., 1912 (9), 236.

[85] Folia Hematol., 1909 (8), 14.

[86] Zeit. physiol. Chem., 1905 (46), 125.

[87] Corroborated also by Boggs and Guthrie, Amer. Jour. Med. Sci., 1912 (144), 803; Folin and Denis, Jour. Biol. Chem., 1914 (18), 277.

[88] Deut. Arch. klin. Med., 1911 (104), 29.

[89] Taylor and Miller, Jour. Biol. Chem., 1916 (25), 281; 1917 (29), 425; Abderhalden, Zeit. physiol. Chem., 1919 (106), 130; Hektoen *et al.*[66]

CHAPTER XXII

PATHOLOGICAL CONDITIONS DUE TO, OR ASSOCIATED WITH, ABNORMALITIES IN METABOLISM, INCLUDING AUTOINTOXICATION

DURING the course of metabolism innumerable organic compounds are formed, some of which are of a more or less poisonous nature. As long as the body is in a normal condition, these injurious substances are kept from accumulating in sufficient quantities to do harm; this is accomplished in one of the following ways: (1) Elimination from the body in the urine, feces, etc.; (2) combination with other substances into harmless, or relatively harmless, compounds; (3) chemical alteration into compounds that are non-toxic or relatively innocuous. Therefore a harmful accumulation of metabolic products may be the result of any one of the following conditions:

(1) Failure of elimination because of abnormal conditions in the eliminating organs; *e. g.*, uremia.

(2) Failure of neutralization by chemical combination, presumably due to abnormalities in the organs or tissues through whose activities the neutralization is normally accomplished; *e. g.*, diseases of the liver.

(3) Failure in the chemical transformation of the metabolic products; this may result either from abnormalities in the functionating tissues, or through a checking of the normal steps of metabolism by the failure of elimination of the end-products.

(4) Excessive formation of certain normal products of metabolism; *e. g.*, hyperactivity of the thyroid.

(5) Production of abnormal toxic chemical substances; *e. g.*, the intoxication following superficial burns.

Numerous classifications of autointoxication have been proposed by various authors, some excluding from the causes of autointoxication all but the products of metabolism within the blood and tissues of the body, as has been done in the preceding consideration; many including intoxications caused by the products of gastro-intestinal fermentation and putrefaction; and still others (v. Jaksch) including even the intoxications produced by bacterial invasion of the body.[1] It is extremely difficult to draw the line as to just what should be included under the term autointoxication, and particularly difficult to decide the proper placing of the intoxication resulting from fecal retention and from processes of decomposition in the alimentary canal. For example, the poisoning following the eating of partially decomposed food could not be looked

[1] See résumé by Weintraud, Ergeb. der Path., 1897 (4), 1.

upon as an autointoxication, and yet there is no fundamental difference whether the decomposition occurs, as in this case, before the food enters the body, or whether it occurs in the intestinal tract because of abnormal bacteriological or anatomical conditions. On the other hand, since many of the obnoxious products of metabolism are eliminated through the bowels, failure of elimination through this channel may lead to a true autointoxication as much as may deficient renal elimination. On the whole, it seems best to restrict the term autointoxication, as far as possible, to the disturbances produced by products of metabolism that have been formed within the tissues of the body (*intermediary metabolism*) considering as a distinct but related subject gastro-intestinal autointoxication.

In the discussion of autointoxication from the standpoint of chemical pathology, we are interested particularly in the chemical nature of the substances that cause the intoxication, and in the chemical processes by which their action is kept at a minimum, rather than in the clinical features or anatomical results that may be produced. Unfortunately, in but a few instances have the exact chemical substances causing these intoxications been accurately determined, probably because in most cases not one but a number of poisonous substances are present; and, furthermore, we do not always know exactly when a certain disease is to be ascribed to autointoxication, nor can we always determine that the cause of a certain intoxication lies in an abnormality in metabolism and not in an infection of hidden nature. It is, therefore, quite impossible with the uncertain information available at this time, to consider autointoxication in a systematic way, and we must limit ourselves to a consideration of certain pathological conditions in which there appears to be an element of abnormal metabolism with resulting intoxication. In some cases this intoxication is a prominent feature of the disorder, in others it is subordinate to other manifestations of the disease; and, finally, we may see marked alterations in metabolism without evidences of disturbance of health (*e. g.*, cystinuria, alkaptonuria).

Of the autointoxications due to the retention of poisonous products of metabolism that should be excreted from the body, first in order of importance stand uremia and cholemia (the latter has already been considered in connection with the discussion of Icterus, Chap. XX). Of apparently less significance are autointoxications due to failure of elimination of gaseous metabolic products by the lungs, and failure of the excretory function of the skin.

UREMIA[2]

The cause or causes of the severe, often fatal, intoxication that may occur when the outflow of urine is completely checked, or when it is

[2] General résumé with earlier literature by: Honigmann, Ergeb. der Pathol., 1894 (Bd. 1, Abt. 2), 639; 1902 (8), 549; Ascoli, Vorlesungen über Urämie, Jena, 1903. More modern literature reviewed by Leiter, Arch. Int. Med., 1921 (28), 131. See also W. L. Brown, Proc. Roy. Soc. Med. (Urol.), 1923 (16), 19; Foster, Jour. Amer. Med. Assoc., 1921 (76), 281; Mosenthal, "Endocrinology and Metabolism," 1924 (4), 311.

qualitatively and quantitatively altered for long periods of time, have not yet been definitely determined. As the kidney seems to be the chief organ for the removal of the products of nitrogenous metabolism, it is naturally assumed that uremia is the result of a retention of these products, but as yet it has not been ascertained which of the many products is responsible, and, indeed, there are very good reasons for questioning if the substances present in normal urine do or can cause uremia when their elimination by the kidney is defective. There is no question but that the urine contains toxic substances. Among them are the salts of potassium, which, however, cannot alone explain all the urinary toxicity, for the symptoms produced by the injection of urine are different from those produced by potassium salts, and it has been found that the inorganic constituents (ash) of urine are less poisonous than the entire urine. Furthermore, toxic mixtures of organic, ash-free substances have been obtained from normal urine.[3] Of the known normal constituents of the urine there are few, however, that are toxic to any considerable degree, and these occur in but very small quantities. Urea is generally considered as almost absolutely non-toxic, the animal body withstanding injection of large quantities without appreciable injury. Uric acid, the purine bases, hippuric acid, creatinine, and the urinary pigments are all possessed of very slight toxicity, and their effects do not explain uremia. Injections of urine into animals may cause more or less disturbance, but it is different, on the whole, from the manifestations of uremia.[3a]

For these and other reasons, it has been generally considered that the intoxication of uremia is not due solely or chiefly to the substances that are normally eliminated in the urine, but rather to more toxic antecedents of the nitrogenous constituents of the urine. Urea represents but the final product of a long series of reactions by which the huge protein molecule is broken up into its "building-stones," the various amino-acids, and these in turn are decomposed in such a way that their NH_2 groups are combined with carbonic acid[4] and eliminated as the diamido-compound of carbonic acid, namely urea, $O = C\langle^{NH_2}_{NH_2}$. We know that the liver is able to accomplish the conversion of amino-acids to urea, for it has been experimentally shown that if leucine and glycine are passed through the vessels of the isolated liver they disappear in part, while an increased amount of urea escapes from the hepatic veins. It is probable that the liver is the chief site of urea formation, but it is also probable that urea can be formed in other organs. We do not know, however, the intermediate steps by which the amino acids of the protein molecule are converted into urea. In the breakdown of the amino acids they may either be deaminated, setting free the NH_2 group to form urea, or they may be decarboxylated, which latter process yields amines,

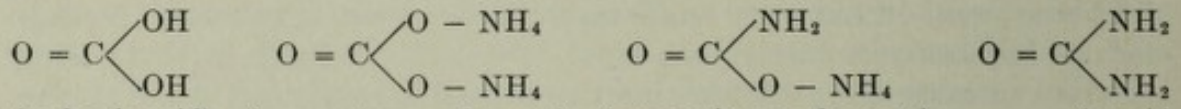
[3] See Dresbach, Jour. Exp. Med., 1900 (5), 315.

[3a] The old experiments of Bouchard and his school present such serious errors of technic and interpretation that they are now largely disregarded.

[4] Arginine alone of all the amino-acids splits off urea directly from its molecule.

many of which are commonly toxic, (See Chapter xxiii). To what extent such toxic amines are found in either normal or abnormal protein metabolism we do not yet know, for ordinarily they are soon or immediately destroyed and so are not readily followed in the living organism. It can only be said that amines have not been found in the blood and tissues during life, although they have occasionally been found in the urine, *e. g.*, putrescine and cadaverine in cystinuria.

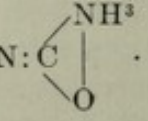
It has been repeatedly shown that urea can be formed from ammonium salts of organic acids (including ammonium carbonate), and ammonia is a constant product of autolysis, being characteristically more abundant as a product of autolytic proteolysis than as a product of tryptic proteolysis; therefore, one of the antecedents of urea is probably ammonia, which is somewhat toxic and especially hemolytic.[5] Another antecedent of urea is ammonium carbamate, which stands in structure intermediate between urea and ammonium carbonate, as shown by the following graphic formulæ:



(carbonic acid) (ammonium carbonate) (ammonium carbamate) (urea)[6]

That ammonium carbamate is possibly an important precursor of urea has been shown particularly through the results of studies of dogs with Eck's fistula,[7] which consists of a fistula between the portal vein and the inferior vena cava, the blood from the portal system then passing directly into the general circulation without first passing through the liver. In such animals the urine becomes poor in urea and relatively rich in ammonium carbamate. At the same time, the dogs show severe symptoms of intoxication from which they die, and which are similar to the symptoms that follow intravenous injection of ammonium carbamate. Ammonium carbamate, being a substance of considerable toxicity[8] when free in the blood, it has, therefore, been quite widely considered that it may be an important factor in the production of uremic symptoms. On the other hand, it seems most probable that the condition of uremia does not depend upon one but upon many various and varying substances, especially as Hawk[9] found that sodium carbamate did not produce uremic symptoms in his Eck fistula dogs, while Liebig's extract did.[10] Clinically, the symp-

[5] Concerning the toxicity of ammonium salts see Rachford and Crane, Medical News, 1902 (81), 778.

[6] Werner believes that the formula of urea is  (Chemistry of Urea, New York, 1923.)

[7] See Hahn, Massen, Nencki, and Pawlow, Arch. f. exp. Path. u. Pharm., 1893 (32), 161.

[8] See Bickel, "Exp. Untersuch. über Cholaemie," Wiesbaden, 1900.

[9] Amer. Jour. Physiol., 1908 (21), 260.

[10] Fischler believes the intoxication which occurs after feeding meat to Eck fistula dogs to be an alkalosis, probably from NH_3 salts (Deut. Arch. klin. Med., 1911 (104), 300).

toms of uremia in different cases are widely different; thus, if uremia is due to complete suppression of urine through mechanical obstruction or loss of all renal substance, the symptoms of this "urinemia" or "asthenic uremia," are quite different from those observed in the uremia following a chronic nephritis; drowsiness, weakness of heart action, and syncope being the chief manifestations of obstructive uremia, the convulsions and other characteristics of nervous irritation characteristic of uremia in chronic nephritis being absent.[11]

Hypertension.[12]—The cause of the heightened blood pressure of nephritis is still undetermined. The fact that relief of urinary obstruction in patients with high blood pressure from prostate trouble regularly reduces the blood pressure (O'Conor),[13] indicates that retained substances normally excreted in the urine are responsible. Urea, uric acid, and most of the known retained metabolites do not have a hypertensive effect. Cash[14] has observed that rise of blood pressure may be obtained in dogs when at least 50 per cent. of the renal tissue has been removed, provided that at the same time a portion of devascularized kidney tissue is left in the body, either procedure alone being ineffective. The relation of this observation to the occurrence of hypertension with and without chronic nephritis is not yet clear. Major and Stephenson[15] observed that guanidine and methyl guanidine salts produce a marked prolonged rise in blood pressure, and since these are substances normally excreted in the urine they may be concerned with hypertension, but their relation to this condition clinically has not yet been determined. Gheorghian[16] found that the blood from patients with hypertension causes a rise in blood pressure in animals.

Marked arterial hypertension often occurs without any evidence of nephritis, and if there is no cardiac insufficiency to impair renal function, the blood chemistry may be normal,[17] although there often is an increase in uric acid[18] and cholesterol. With[19] hypertension there is often hyperglucemia, perhaps associated with the vascular changes in the pancreas,[20] and usually an increase in uric acid. Substances with vasoconstricting effects similar to epinephrine have been described in the blood in nephritis,[21] of unknown nature and source. Hülse[22] suggests that nephritic blood sensitizes the vessels so that they react more strongly to epinephrine.[23]

Chemical Changes in Uremia.—The attempts to isolate from the blood and organs of uremic patients or animals toxic substances that explain the manifestations of uremia, have thus far failed. That there is an actual retention of organic substances in the blood in uremia is shown conclusively, however, by the studies of the physico-chemical properties of the blood. It has been repeatedly found that in uremia the *freezing-point* of the blood is reduced markedly below the normal,[24] instead of the normal depression of 0.55°–0.57° the freezing-point is usu-

[11] Chiari, however, observed true uremia, both clinical and antomical, in a man with ureteral obstruction (Verh. Deut. Path. Gesell., 1912 (15), 207).
[12] See review by Fahr, Deut. med. Woch., 1917 (43), 227.
[13] Jour. Urol., 1923 (10), 135.
[14] Johns Hopkins Hosp. Bull., 1924 (35), 168.
[15] Johns Hopkins Hosp. Bull., 1924 (35), 140 and 186.
[16] Compt. Rend. Soc. Biol., 1924 (90), 1163.
[17] See Williams, Arch. Int. Med., 1921 (27), 748; 1921 (28), 426.
[18] Fishberg, Arch. Int. Med., 1924 (34), 503.
[19] Pribram and Klein, 1924 (20), 572.
[20] See Schwab, Virchow's Arch., 1923 (242), 1.
[21] See Kato and Watanabe, Tohoku, Jour. Exp. Med., 1920 (1), 426.
[22] Zent. inn. Med., 1922 (43), 1.
[23] See also Kylin, Acta. med. Scand., 1923 (58), 342.
[24] See Tieken, Amer. Med., 1905 (10), pp. 393, 567, and 822; Butterfield *et al.*, Amer. Jour. Med. Sci., 1916 (151), 63.

ally reduced to more than –0.60°, and sometimes to as low as –0.75°, which shows that the number of molecules in the blood is increased.[25] At the same time, the *electrical conductivity* may not be at all increased (Bickel),[26] but may even be reduced; and as the electrical conductivity of the blood depends upon the number of dissociable molecules, chiefly inorganic salts, these are evidently not increased.[27] Therefore, the increased number of molecules must represent an excess of organic molecules that dissociate but little if at all, and hence are not conductors of electricity. Some authors, indeed, have ascribed uremia to the increased osmotic pressure of the blood from the retained molecules, but this is improbable, according to Strauss,[28] who found that a marked increase in molecular concentration may occur without uremia, and that a severe uremia may exist without increased osmotic pressure.

Careful metabolic studies have shown that nephritics (chronic interstitial) are not able to convert proteins into urea as rapidly or as completely as do normal persons.[29] Erben[30] has studied the variations in the normal components of the blood during nephritis, and found the albumin generally decreased in proportion to the globulin, especially in cases of parenchymatous nephritis; lecithin and calcium are also decreased. There is often an increase in the blood fats and cholesterol, especially in tubular nephritis or nephrosis, rather than in the forms of nephritis usually associated with uremia,[31] this nephrosis being associated with marked deposition of cholesterol esters in the kidneys.[32]

Rowe[33] found the serum proteins greatly lowered in chronic nephrosis with edema, an increased proportion of globulin being present; with uremia the total protein content is normal or slightly higher, usually with increased globulin, while nephritis without edema or uremia produces a marked increase in the globulin. Quite similar results have been obtained by others; with the nephrotic type of kidney the fall in plasma proteins often occurring without edema and affecting chiefly the albumin,[34] while in the nephrosclerosis type the plasma proteins may be normal if there is no cardiac failure. The decrease in red corpuscles and hemoglobin in nephritis is a well-known feature, but may depend in part on a dilution of the blood.[35]

Numerous attempts have been made by both chemical and immunological methods to determine whether the proteins in the urine in nephritis come from the food, the blood, or from the renal cells themselves. In alimentary albuminuria the urinary proteins seem not to be those of the

[25] See table of freezing points of blood and effusions on page 397.
[26] Deut. med. Woch., 1902 (28), 501.
[27] See Bienenstock and Csaki, Biochem. Zeit., 1917 (84), 210.
[28] Die chronischen Nierenentzündungen, etc., Berlin, 1902.
[29] Levene *et al.*, Jour. Exper. Med., 1909 (11), 825.
[30] Zeit. klin. Med., 1903 (50), 441; 1905 (57), 39.
[31] See Bloor, Jour. Biol. Chem., 1917 (31), 575; Hiller *et al.*, Jour. Exp. Med., 1924 (39), 931.
[32] Dyke, Quart. Jour. Med., 1924 (18), 77.
[33] Arch. Int. Med., 1917 (19), 354.
[34] Linder *et al.*, Jour. Exp. Med., 1924 (39), 887.
[35] See Underhill and Kapsinow, Jour. Urol., 1922 (8), 307.

food, but human proteins.[36] In nephritis differentiation between serum proteins and kidney proteins has not yet been satisfactorily accomplished.[37]

Measurements of the partial pressure of CO_2 in the alveolar air in uremia indicate a certain degree of acidosis.[38] This seems to occur to a sufficient degree to be responsible for definite clinical symptoms of acidosis only in advanced nephritis, but earlier in nephritis an acidosis may be demonstrable by the alkali tolerance test when it is not sufficient to affect the alveolar air.[39] The maximum degrees of acidosis found in uremia are about equal to those of diabetic coma, and may be an important feature of uremia, although usually the convulsive features of uremic coma are quite different from the air hunger of diabetic coma. It seems to depend on a failure of the kidney to excrete acids, and is associated with a phosphatemia and a decreased amount of calcium in the blood. (Further discussion under Acidosis in Nephritis, p. 644.)

The development of this terminal acidity, together with the finding of albumose in the blood of a nephritic by Schumm,[40] suggests the probability of active autolytic processes occurring in uremia. Neuberg and Strauss[41] have also found glycine in considerable quantities (1.5 per mille) in the blood-serum of a uremic patient and in the blood of nephrectomized rabbits. The amount of colloidal material present in the urine is decreased in nephritis, according to Pribram,[42] who suggests that retention of this material, which is rich in aromatic radicals, may be of importance in the toxicity of uremia. Rumpf found that the organs of nephritics contain an excess of potassium, and Blumenfeldt[43] attributes this to a defective elimination of potassium salts which he observed in nephritis. Basal metabolism is somewhat lowered.[44]

Denis and Hobson[45] found the blood sodium, chlorine, potassium and magnesium usually normal, inorganic phosphates and sulfates usually increased, calcium sometimes decreased; but these changes are usually insignificant except the sulfate retention, which sometimes reaches 30 times the normal figure of 0.5 mg. per 100 c.c., so that sulfate resembles uric acid in its selective retention by diseased kidneys. Most observers find more marked changes in the calcium and phosphate, the proportion of the two usually being inverse, a high blood phosphate figure indicating an unfavorable prognosis, while the low calcium of the blood may be responsible for the nervous irritability of uremia.[46] The normal figures are 2 to 4 mg. of P as phosphates and 9 to 11 mg. Ca per 100 c.c. blood, but in advanced uremia figures of 12 to 20 mg. of P and 4 to 6 mg. Ca

[36] Wells, Jour. Amer. Med. Assoc., 1909 (53), 863.
[37] Cameron and Wells, Arch. Int. Med., 1915 (15), 746.
[38] Straub and Schlayer, Münch. med. Woch., 1912 (59), 569; Whitney, Arch. Int. Med., 1917 (20), 931.
[39] Peabody, Arch. Int. Med., 1915 (16), 955.
[40] Hofmeister's Beitr., 1903 (4), 453.
[41] Berl. klin. Woch., 1906 (43), 258.
[42] Fortschr. d. Med., 1911 (29), 951; Biochem. Zeit., 1920 (111), 30.
[43] Zeit. exper. Pathol., 1913 (12), 523.
[44] Aub and DuBois, Arch. Int. Med., 1917 (19), 865.
[45] Jour. Biol. Chem., 1923 (55), 183.
[46] See de Wesselow, Quart. Jour. Med., 1923 (16), 341; Lancet, 1923 (2), 163.

may be found. In non-uremic cases the fall in calcium accompanies a fall in total plasma proteins, while with uremia the fall in calcium is not associated with the total proteins but with the increased phosphate of the blood.[47] In some cases of nephritis, however, instead of being low the blood calcium is increased to the point of saturation and metastatic calcification (*q. v.*) may result. Here the calcium seems to be drawn from the bones to help the deficient alkali reserve.[48] However, in several cases of nephritis Rabinowitch[49] found no changes from the normal ratio of Na, K, Ca and Mg, except in a few of the advanced uremics. Others have found a low concentration of the blood chloride in uremia.[50]

Non-protein Nitrogen of Blood.—The development of improved methods of analysis of small quantities of blood, and other fluids, especially by Folin and Denis, Marshall, and Van Slyke, has enabled us to obtain exact knowledge of many of the chemical changes of nephritis and uremia.[51] It has been found that the normal blood contains from 25 to 40 mg. of nitrogen in noncoagulable form in each 100 c.c., there being usually about 5 mg. increase after meals, and ordinarily about one-half of this nitrogen is in the form of urea.[52] In all conditions that impair renal function, whether renal changes or circulatory deficiency, there is a rise in this noncoagulable nitrogen, and when there is excessive tissue destruction there may also be a rise independent of renal injury. As a general rule, but with some exceptions, the amount increases with increased renal impairment, the highest figures being seen in uremia, in which figures as high as 658 mg. have been obtained. In 130 nephritics, Foster found the average to be 84 mg. of nitrogen in the interstitial variety, with an average of 135 mg. in convulsive uremia. The highest figures as a rule are found in the chronic hypertensive cases, which usually are associated with fibrotic glomerulo-nephritis, whereas the degenerative tubular lesions, or "nephrosis," commonly show less marked retention of nitrogenous catabolites. In anuria the urea forms a much larger proportion of the non-protein N of the blood than in nephritic uremia.

Analysis of the blood in 600 cases of nephritis by Gettler and St. George[53] gave the following figures in mg. per 100 c.c. of blood:

	Normal	Nephritis
Nonprotein nitrogen	25 to 40	40 to 460
Urea nitrogen	10 to 18	20 to 375
Creatinine[54]	0.1 to 0.8	2 to 42
Uric acid	0.5 to 3.0	3 to 17
Sugar	60 to 110	75 to 160
Alkali reserve—per cent	63 to 80	40 to 75

[47] Salvesen and Linder, Jour. Biol. Chem., 1923 (58), 617.
[48] See Hubbard and Wentworth, Proc. Soc. Exp. Biol. Med., 1921 (18), 307.
[49] Jour. Biol. Chem., 1925 (62), 667.
[50] See Atchley *et al.*, Arch. Int. Med., 1923 (31), 606.
[51] Reviews and bibliographies are given by Feigl, Biochem. Zeit., 1919 (94), 84; Folin, Physiol. Reviews, 1922 (2), 460; Myers, Physiol. Reviews, 1924 (4), 274.
[52] See Kast and Wardell, Arch. Int. Med., 1918 (22), 581.
[53] Jour. Amer. Med. Assoc., 1918 (71), 2033.
[54] Later investigators have established the normal value for creatinine at 1.0–1.3 mg.

From their observations these authors conclude: All the waste nitrogen products, non-protein nitrogen, urea, creatinine and uric acid, are present in increased amounts in cases of true nephritis, and generally, but not invariably, present in greater concentration in the blood of those cases which are primarily considered as chronic interstitial nephritis (retention nephritis). The degree of retention (when taking into account the functional efficiency of the cardiac muscle) is a direct criterion of the severity of the lesion. According to Floyd,[55] the increase in non-protein N is more directly related to an increase of kidney stroma, and corresponding loss of active glomerular and tubular structure, than to any other renal lesion.

There is no constant relationship between the blood pressure and the nitrogen figure, but functional tests usually show a correspondence between the excretory power of the kidney and the retention of metabolites in the blood. The symptoms of asthenic uremia are rarely well defined when the concentration of urea in the blood is less than 100 mg. per 100 c.c., and they are rarely absent when the concentration exceeds 200 mg.[56] With these high blood nitrogen figures there is also an increase in nonprotein nitrogen in the tissues (Foster)[57] which may contain more urea than the blood,[58] and metabolism studies show nitrogen retention of considerable degree, sometimes over 1 gram retention when the intake is but 10 grams per day. Becher[59] found that although the muscles take up a particularly large proportion of the retained metabolites, all tissues hold more or less for there is no special storage tissue, and the various retained substances are distributed in the tissues in the same proportion as in the blood. In one uremic cadaver the total retained urea amounted to about 200 grams. The residual nitrogen is also found increased in the cerebrospinal fluid, where figures 60 to 80 per cent. as high as those of the blood are ordinarily found (Brun).[60]

Along with the other nitrogenous constituents the uric acid is increased from a normal 2 to 3 mg. up to 7 to 10 mg., and even higher. Creatinine rises from 1 to 2 mg. up to 5 to 20 mg.[61] It is generally agreed that a rise in the blood creatinine is particularly significant of serious renal damage, and when there is over 5 mg. per 100 c.c. the prognosis is extremely grave.[62] On the other hand, the amino-acid nitrogen may be normal in the blood even with extremely high nonprotein nitrogen figures,[63] although sometimes it is much increased, as high as 30 mg. amino-acid N having been found by Bock[64] in uremia (the normal figure being about 7 mg.). Feigl[51] reports

[55] Jour. Metabol. Res., 1923 (3), 759.
[56] Hewlett, Gilbert and Wickett, Arch. Int. Med., 1916 (18), 636.
[57] Arch. Int. Med., 1919 (24), 242; see also Barát and Hetényi, Deut. Arch. klin. Med., 1922 (138), 154.
[58] See Weiss and Garner, Jour. Lab. Clin. Med., 1922 (7), 229.
[59] Deut. Arch. klin. Med., 1921 (135), 1.
[60] Acta Med. Scand., 1919 (52), 367, full review.
[61] See Myers and Fine, Arch. Int. Med., 1915 (16), 536; 1916 (17), 570.
[62] Review by Feinblatt, Amer. Jour. Med. Sci., 1923 (166), 249; see also Major, Arch. Int. Med., 1924 (33), 89.
[63] Foster, Arch. Int. Med., 1915 (15), 356.
[64] Jour. Biol. Chem., 1917 (29), 191.

finding as high as 125 mg. amino-N, with frequently 60 to 85 mg. Most modern investigators, however, find that the amino-N is practically constant in uremia as in nearly all other conditions.[65]

Myers[66] says that the healthy kidney can concentrate creatinine 100 times, urea 80 times, and uric acid but 20 times, so that with renal disease there is first retention of uric acid, then urea, and last of creatinine.[61] No significant figures on creatine have been obtained.[67] The reduced capacity of the kidney to concentrate uric acid is an early and characteristic manifestation of nephritis,[68] but in uremic coma the previously high uric acid often becomes greatly reduced (Feinblatt).[69]

Folin[51] says that the true values for *ammonia* in the blood still remain uncertain, and Myers even says that there is some question at the present time whether ammonia actually exists in the blood. Folin and Denis found but from 0.03 to 0.08 mg. ammonia-N per 100 c.c. of blood. Nash and Benedict[70] obtained similarly low figures which were not increased after nephrectomy, even with very high non-protein-N. In view of these results and the many sources of errors in analysis, the figures quoted in the literature on blood ammonia in nephritis cannot be accepted.

Indicanemia may also be present but it is not a toxic factor (Dorner).[71] The blood normally contains about 0.05 mg. indican per 100 c.c.; it often increases before there are other evidences of retention; in uremia it may rise to 0.2 mg., and as much as 2.2 mg. has been found in one case.[72] Baar[73] holds that indican retention is the most accurate of evidences of renal impairment, for no matter how much indican is absorbed from the intestine the blood content remains constant if the kidneys are functioning normally, whereas without intestinal putrefaction there is a marked indicanemia if the kidneys are injured, sometimes even when there is little change in the non-protein N of the blood.[74]

Bath[75] says that the blood bilirubin and urinary urobilinogen are decreased in chronic nephritis, but not with arteriosclerotic kidneys. There may also be an accumulation of hippuric acid with renal insufficiency.[76] Imidazole excretion is much reduced in nephritis with nitrogen retention, the amount of excretion varying inversely with the severity of the disease.[77]

[65] Greene *et al.*, Jour. Biol. Chem., 1924 (58), 845.
[66] Jour. Lab. Clin. Med., 1920 (5), 418, 566.
[67] Feigl, Biochem. Zeit., 1920 (105), 225. See review on Creatine and Creatinine, by Hunter, Physiol. Reviews, 1922 (2), 586.
[68] See Upham and Higley, Arch. Int. Med., 1919 (24), 557.
[69] Arch. Int. Med., 1923 (31), 758.
[70] Jour. Biol. Chem., 1922 (48), 463; review on ammonia in blood. See also R. F. Loeb *et al.*, Jour. Biol. Chem., 1924 (60), 491.
[71] Deut. Arch. klin. Med., 1914 (113), 342; Rosenberg, Arch. exp. Path., 1920 (86), 15.
[72] Hass, Deut. Arch. klin. Med., 1916 (119), 177.
[73] "Die Indicanämie," Berlin, 1922.
[74] See also Becher, Deut. med. Woch., 1921 (47), 142.
[75] Wien. Arch. inn. Med., 1922 (3), 309.
[76] Snapper and Grunbaum, Nedr. Tijd. Geneesk., 1922 (2), 2910.
[77] Koessler and Hanke, Jour. Biol. Chem., 1924 (59), 803.

The total N of the urine may be practically all accounted for by the known nitrogenous constituents, but in the blood not all of the non-protein nitrogen is accounted for by known substances, there being in normal blood about 20 per cent. of the non-protein N in unidentified form (residual-N) and in uremia the amount of this unidentified N may be much increased. This suggests that the usual steps of normal nitrogenous metabolism are not carried out when the accumulation of the end-products blocks the reactions, but against this is the fact that usually a high urea content does not result in an increase in the amino-N of the blood. Some of this undetermined fraction may be hippuric acid, some of it histones,[78] and Jackson[79] has found significant amounts of a nucleotid, besides which indican and imidazoles are known constituents of the blood.

The Pathogenesis of Uremia.—The fact that the highest figures for non-protein nitrogen are usually found in uremia might be accepted as proving that uremia is caused by poisoning with these metabolites, were it not for certain contradictory observations.

(1) Occasionally quite typical attacks of uremia are observed without high non-protein N figures for the blood,[80] even as low as 28 mg. having been recorded in a fatal case.[81]

(2) Extremely high nonprotein nitrogen content may be observed without uremia. Thus, Tileston and Comfort found 169 and 150 mg. in two cases of acute intestinal obstruction without uremic symptoms, and still higher figures have been obtained in bichloride of mercury poisoning,[82] and mechanical anuria without convulsive uremia. The occurrence of albuminuric retinitis also seems to bear no relation to the nitrogen retention (Woods).[84]

(3) None of the known nitrogenous constituents of the urine can be held responsible for all the manifestations of typical uremic poisoning. The highest purine, uric acid and creatinine concentration in a given case may occur entirely independent of uremic conditions,[83] the amino-nitrogen is not increased in uremia, urea is not supposed to be toxic in this degree and uremia may occur without high urea concentration or be absent when there is much urea in the blood. To be sure, an unknown toxic substance may be responsible, but in some cases of uremia the total non-protein nitrogen can be accounted for by the known nitrogenous components found in the blood (Foster).

We may consider one of the following alternatives:

(1) The nerve cells may be made hypersensitive to some one of the known constituents by the excessive amounts of the other metabolites or the deficiency in calcium. This is a purely speculative hypothesis, without any actual evidence in its support.

(2) The portion of unidentified nitrogen usually present in the blood may contain a specific, highly efficient poison.

In support of this hypothesis is the finding in a series of cases that the proportion of noncoagulable blood nitrogen that could not be accounted for by the known nitrogenous metabolites seemed to vary directly with the severity of the symptoms (Woods).[84]

[78] Folin and Berglund, Jour. Biol. Chem., 1922 (51), 395.

[79] Jour. Biol. Chem., 1924 (59), 529.

[80] See Wordley, Quart. Jour. Med., 1920 (14), 88.

[81] There are few who would go to the extreme of Strauss (Berl. klin. Woch., 1915 (52), 368) and limit the term uremia to cases showing a high non-protein nitrogen in the blood, no matter what the symptomatology and pathology may be. A totally different viewpoint is expressed by Reiss, Zeit. klin. Med., 1914 (80), 97, 424, 452.

[82] See Foster, Arch. Int. Med., 1915 (15), 754.

[83] Myers and Fine, Jour. Biol. Chem., 1915 (20), 391.

[84] Arch. Int. Med., 1915 (16), 577.

Hartman[85] has suggested that the substance which causes the characteristic odor of the urine may be responsible for at least some of the intoxication of uremia. This substance, which he has isolated and described under the name "urinod," he believes to be a cyclic ketone with the empirical formula C_6H_8O; it is highly toxic, and causes mental symptoms. This important observation awaits confirmation.

Foster[86] has described the finding of a toxic base in the blood of uremics, absent from the blood in other conditions, which causes death of guinea pigs with symptoms suggestive of the eclamptic type of uremia. Further development of this work is also lacking.

(3) Uremia may not depend on intoxication of the nerve cells, but upon the mechanical effects of edema involving these cells.

One of the striking features of autopsies of uremics is often the "wet brain" and the excessive amount of cerebrospinal fluid which, during life, may be found to be under a heightened pressure. We know that not only general but localized edemas occur in nephritis, and that localized edema in the brain may be associated with and apparently responsible for paralyses, convulsions, hyperirritability and mania. The wet brain of nephritis is similar to the wet brain of acute alcoholism and delirium tremens. Oftentimes the nervous symptoms of uremia are distinctly focal, and a complete hemiplegia from hemorrhage may be exactly simulated; convulsive seizures identical with those of brain tumor may be seen. It is extremely difficult to explain these localizations by the action of a soluble poison, and simple if we assume a local edema. It is, of course, as difficult to explain the localization of the edema, but we know that in nephritis localized edemas do occur, so we have a basis for the assumption of localized cerebral edemas. A general acidosis (*q. v.*) is usual in nephritis and marked in uremia[87] but we have no means of knowing whether local acidosis occurs in the nervous system that may be responsible for local edemas according to Fischer's hypothesis. Or, osmotic effects may be responsible, in view of the demonstrated high osmotic pressure of the blood in uremia, and the fact that the life of nephrectomized rabbits is prolonged by giving them water.[88] In any event, the existing evidence on the pathogenesis of uremia does not explain it on a toxicologic basis, and hence the alternative explanation by cerebral edema must be taken into consideration. Ervin[89] proposes the hypothesis that convulsions occur when the blood pressure becomes lower than the intracranial pressure so that the blood supply of the brain is reduced; the convulsion raises the blood pressure. He comments on the difficulty in explaining the transient character of uremic convulsions if caused by concentration of chemical poisons.[90]

On the other hand the pathologist recognizes evidence of systemic intoxication in uremia. The uremic pericarditis and endocarditis, which have often failed by ordinary methods to yield bacteria, are apparently toxic processes. The diphtheritic colitis indicates vicarious excretion of poisonous substances. Structural changes are found in cells that suggest poisoning; chromatolysis of the cortical ganglion cells has been repeatedly observed in uremia, and in nephrectomized rabbits Lewis[91] found acute parenchymatous and fatty degeneration of the myocardium and endothelial cells of the liver. Electrocardiograms in uremia also indicate that the heart is under toxic influences.[92] The localized edemas

[85] *Ibid.*, 1915 (16), 98.

[86] Trans. Assoc. Amer. Phys., 1915 (30), 305.

[87] Henderson, Bull. Johns Hopkins Hosp., 1914 (25), 141; Peabody, Arch. Int. Med., 1915 (16), 955; Sellards, "Principles of Acidosis," Harvard Press, 1917.

[88] Couvee, Zeit. klin. Med., 1904 (54), 311.

[89] Jour. Amer. Med. Assoc., 1918 (70), 1208.

[90] Monakow (Schweiz. Arch. Neurol. Psych., 1920 (6), 183) suggests that the sudden onset of uremic attacks depends on yielding of the choroid plexus to passage of the toxic constituents from the blood into the cerebral fluids.

[91] Jour. Med. Res., 1907 (17), 291.

[92] Wood and White, Amer. Jour. Med Sci., 1925 (169), 76.

of nephritis often show a fluid of the character of an exudate rather than a transudate.[93]

It would seem, despite the prevailing opinion to the contrary, that it is entirely possible that the manifestations of uremia may be caused by the known nitrogenous substances that the kidneys have failed to excrete, and that the only difficult thing to explain is the failure of investigators to consider the *time element* in experimental intoxications. The presence of 200 mg. and upwards of nonprotein nitrogen per 100 c.c. of blood, which is often found in uremia, indicates that the blood plasma that is bathing the tissue cells contains somewhere between 0.5 and 1.0 per cent. of soluble organic substances, a strength of solution that certainly does not require any very high degree of toxicity to produce marked effects when *continuously maintained* at this concentration, as it is in nephritis. The reported experimentally determined toxicities with these substances have only represented transitory conditions which are entirely dissimilar to the actual conditions in uremia. They correspond to the cases of high nonprotein nitrogen in the blood in intestinal obstruction, bichloride poisoning, etc., in which absence of the uremic symptom complex has been noted and remarked upon. To study the relation of uremia to retained metabolites we need observations on their effects when maintained in the organism for long periods at the concentrations occurring in uremics, and this perhaps can be done by such methods as have been devised by Woodyatt.[94] A start in this direction is furnished by Hewlett, Gilbert, and Wickett,[56] who found that when large doses (100 to 125 gm.) of urea were given to normal men there occurred symptoms comparable to those of asthenic uremia, which appeared only when the the urea concentration of the blood had reached levels of 160 to 245 mg. of urea per 100 c.c., *i. e.*, just the concentrations that are usually seen in well developed uremia. If in these experiments of brief duration such marked symptoms were produced by urea, striking effects must be expected when these same urea concentrations are continued in the blood for days and weeks at a time.[95] We must find out what results not only from urea, but from creatinine and uric acid kept in the blood at the concentration found in uremia for long periods, as well as any other substance that may be increased in the blood in uremia. An experiment of a few minutes' or hours' duration cannot be expected to duplicate or elucidate a condition of weeks duration. In chronic diseases our experimental investigations must be of some reasonably comparable duration, and this principle of investigation is now made possible by Woodyatt's methods. Leiter[2] has partly followed

[93] The recognized susceptibility of uremics to infection may depend on the leukopenia and interference with complement function produced by urea (Sherwood, Jour. Infect. Dis., 1922 (31), 252). The complement titer of the blood in uremia was not found reduced, however, in 9 cases examined by Rockwood and Beeler, Jour. Infect. Dis., 1924 (34), 625.

[94] Jour. Amer. Med. Assoc., 1915 (65), 2067; Jour. Biol. Chem., 1917 (29), 355.

[95] However, in *Selachians* the normal urea content in the blood is over two per cent. and ammonium salts exceed $n/10$ NH_3, (see A. B. Macallum, Amer. Jour. Med. Sci., 1918 (156), 1), but it may well be that in such species the tissues are adapted to their environment.

out these suggestions in my laboratory, and has found that slow injection of pure urea into dogs *does* produce a train of symptoms strikingly similar to convulsive uremia in man, and his results indicate that we cannot exclude high urea retention as one factor in the manifestations of uremia, especially if the time factor is taken into consideration.[96]

And finally, in view of the extremely varied symptomatology of renal incompetence, we must recognize that it is highly probable that in different cases these symptoms vary because of different conditions. In one case, urea may be the chief factor, in another the action of urea may be complicated by the effects of acidosis or of high blood pressure *per se*, while in others cerebral edema may be the chief influence. Some continental writers hold that there is a true uremic picture due solely to cerebral edema from salt and water retention, occurring especially in the young, to be distinguished from the uremia of nitrogenous retention, and from a pseudo-uremia resulting from the circulatory disturbances of arteriosclerosis.[97] Howland and Marriott[98] call attention to the reduced calcium in the blood in uremic acidosis, and as nervous irritability is increased by reduction of calcium this may also be a factor in the nervous manifestations of uremia. All possible shades of coöperating influences may be expected to occur when the kidneys fail, and explain the confused, variable, changing picture of the uremic state.[99]

TOXEMIAS OF PREGNANCY[1]

Under this heading are included eclampsia, as characterized by convulsions and certain anatomical changes, together with those instances of intoxication with similar anatomical changes and no convulsions, and the related pernicious vomiting of pregnancy. Acute yellow atrophy of the liver belongs in the same category, although often occurring independent of pregnancy.

ECLAMPSIA

In many respects eclampsia resembles uremia; so much so, indeed, that Frerichs and others have referred to eclampsia as "puerperal uremia." Considering it as a simple uremia occurring in pregnancy, uremia and eclampsia have in common the occurrence of renal disturbance with albuminuria and decreased elimination of urea, and also violent convulsions and profound coma terminating in death. On the other hand,

[96] See also Becher, Zent. inn. Med., 1924 (45), 229.

[97] See Haim and Tchertkoff, Rev. Méd. Suisse Romande, 1918 (38), 15.

[98] Arch. Int. Med., 1916 (18), 708.

[99] The influence of a hypothetical internal secretion of the kidney (Brown-Sequard), or of the products of nephrolysis (Ascoli), as a cause of uremia, may now be considered as of historical interest only. (See Pearce, Arch. Int. Med., 1908 (2), 77; 1910 (5), 133.) The same is true of the attempt to explain the high blood pressure as the result of adrenal hypertrophy. (Pearce, Jour. Exp. Med., 1908 (10), 735; 1910 (12), 128.)

[1] Review and bibliography by Ewing, Amer. Jour. Med. Sci., 1910 (139), 829; Kosmak, "The Toxemias of Pregnancy," Appleton, 1922; Metabolism of pregnancy reviewed by Bailey in "Endocrinology and Metabolism," 1924 (4), 835.

eclampsia differs greatly from uremia in the anatomical changes observed in the organs of the body other than the kidneys;[2] these are of such a nature that in some cases it becomes difficult to distinguish eclampsia from acute yellow atrophy of the liver,[3] while in other cases the picture resembles that of a profound bacterial intoxication, so that numerous authors have urged that eclampsia is the result of a bacterial infection.

Puerperal Nephropathies.—While eclampsia is usually associated with marked signs of renal injury, this is by no means constant or severe, and on the other hand renal changes without eclampsia are common. These puerperal nephropathies have been divided into two classes:[4] One form is a nephrosis with edema, with retention of NaCl and H_2O but without rise of blood pressure, or increase in the residual N in the blood or changes in the fundus of the eye. The other form is a nephritis, with N retention, rise of blood pressure, increase of residual N in the blood and albuminuric retinitis. The first form reacts well to a restriction of H_2O and NaCl, but this is of no avail in the second form and the rapid progress of the eye changes generally calls for artificial interruption of the pregnancy. Eclampsia more commonly occurs with the first form. Wallis points out that in contrast with acute nephritis, in toxemias of pregnancy the permeability of the glomerules is increased, wherefore in the urine in acute nephritis the ratio, albumin:globulin = 6.1, in eclampsia it is more nearly 2:1; the output of diastase in the urine is low in nephritis and high in eclampsia; the high blood urea and sugar figures are not obtained in the latter.

At the present time the cause of puerperal eclampsia is quite unknown, but there is a decided tendency to assume that poisonous substances are developed in the placenta or fetus, or are formed in the body as a reaction of the maternal organism to the foreign fetal elements. These theories will be discussed after considering the known facts concerning the chemical changes of the disease that have been reported by various observers.

Chemical Changes in Eclampsia.—*Urinary changes* are practically invariably present, and usually they are profound, although there are no known characteristic qualitative or quantitative differences from the urinary changes of puerperal albuminuria without eclampsia. Proteins are abundant, including a large proportion of globulin, decreasing rapidly after delivery as a rule. The urea is usually very low, but generally increases with great rapidity after delivery, until two or three times the normal amount is passed per day; as urea and ammonia do not seem to be greatly increased in the blood, this has been interpreted as indicating that during eclampsia there is an accumulation of the precursors of urea in the system. However, the involution of the uterus itself results in an increased nitrogen excretion which probably accounts for much if not all of these findings (Slemons).[5] There is an excessive elimination of nitrogen in the form of ammonia, which is said to be due to the formation of abnormal quantities of sarcolactic and other organic acids in the body, which are combined with ammonia in the blood and eliminated in the urine,[6] often associated with acetone bodies in considerable quantities.

[2] See Harbitz, Surg. Gyn. Obst., 1923 (36), 767.

[3] Concerning the liver changes see Konstantinowitsch, Ziegler's Beitr., 1907 (40), 483.

[4] v. Fekete, Fuchs, Molnór, Wien. Arch. Inn. Med., 1922 (3), 397.

[5] Bull. Johns Hopkins Hosp., 1914 (25), 195.

[6] See Zweifel and Lockmann, Münch. med. Woch., 1906 (53), 297; Cent. f. Gyn., 1909 (33), 847.

This fact once led many to look with favor upon the idea that eclampsia is due to an acid intoxication, and there often is a high alkali tolerance[7] and a low alkali reserve.[8]

Other nitrogenous urinary constituents may also be increased, so that the relative proportion of nitrogen eliminated as urea is often greatly reduced. It is said that the toxicity of the urine, which is high in normal pregnancy, is increased if the kidneys are not impaired, but decreased if their permeability is impaired by nephritis, the character of the toxicity being such as to indicate that it is from substances derived by disintegration of proteins (Franz). The proportion of sulfur eliminated in an unoxidized form, as compared with that eliminated as SO_4 is much greater than normal.

These findings all indicate that oxidation within the body is impaired. There is more or less retention of chlorides, but there is nothing characteristic in this.[9] In spite of the hepatic lesions of eclampsia the tolerance for levulose was not found reduced by Alsberg.[10]

Blood Chemistry.—The nonprotein nitrogen of the blood is not greatly increased in eclampsia and it bears no definite relation to the severity of the symptoms (Farr and Williams).[11] They found from 25 to 72 mg. per 100 c.c. in seven cases. These figures can be reasonably explained as the result of tissue disintegration rather than renal retention, and indicate that the renal changes are the result rather than the cause of the intoxication.[12] In normal pregnancy the nonprotein- and urea-N are commonly somewhat low, with the uric acid the same as in normal women; but in all forms of pregnancy toxemia, whether nephritic or not, the uric acid tends to increase.[13] A relatively small proportion of the nonprotein-N is urea, the undetermined fraction being correspondingly increased as well as the uric acid.[14] Part of this undetermined-N may be indican, which may increase from a normal of below 1.67 mg. to 60 mg. or over in eclampsia so that a rise of blood indican indicates oncoming eclampsia or uremia (Rübsamen).[15] In some cases the renal damage becomes sufficient to cause the blood picture of acute nephritis with marked creatinemia, and there may be a decreased capacity to excrete urea even when there is no urea retention.[16] Beker[17] reported an increase

[7] See White, Lancet, 1920 (2), 1248.
[8] Bokelmann and Rother, Zeit. ges. exp. Med., 1924 (40), 13.
[9] Zinsser, Zeit. f. Geb., 1912 (70), 200.
[10] Cent. f. Gyn., 1910 (34), 6.
[11] Amer. Jour. Med. Sci., 1914 (147), 556.
[12] With the observed low blood urea of eclampsia it is difficult to account for Hammett's (Jour. Biol. Chem., 1918 (34), 515) finding of a high urea content in eclamptic placentas.
[13] Stander, Johns Hopkins Hosp. Bull., 1924 (35), 133; Williams, Jour. Amer. Med. Assoc., 1921 (76), 1297; Plass, Jour. Amer. Med. Assoc., 1924 (82), 266; King and Denis, Amer. Jour. Obst. Gyn., 1924 (7), 409.
[14] See Killian and Sherwin, Amer. Jour. Obst. Gyn., 1921 (2), 6; Caldwell and Lyle, *ibid.*, p. 17; Harding *et al.*, Jour. Obst. Gyn. Brit. Emp., 1924 (31), 595.
[15] Arch. Gynäk., 1922 (117), 397.
[16] de Wesselow, Jour. Obst. Gyn. Brit. Emp., 1922 (29), 21.
[17] Neder, Tijd. Geneesk., 1919 (1), 431.

in the creatine in the blood, presumably derived from the uterus. There may be a disturbance in the exchange of non-protein nitrogenous materials between the blood plasma and the corpuscles (Plass).[18] After delivery the non-protein nitrogen rises and the plasma protein value decreases to give figures more abnormal than in the period of most acute physical signs.[19] Losee and Van Slyke could find no increase of amino-acids or other intermediates of protein destruction in either blood or urine in pregnancy toxemias,[20] their total non-protein blood nitrogen figures ranging from 25 to 46 mg. Similar results are reported by Slemons,[21] who also found normal amounts of fat, with increased cholesterol and decreased lecithin, and after the convulsions some increase in blood sugar. Others have found a greater cholesterolemia in eclampsia than in normal pregnancy,[22] and the sugar content varies slightly around the normal average, with marked lability.[23]

Macallum describes a high proportion of potassium in the blood in eclampsia.[24] The blood calcium is usually somewhat lowered as it is in normal pregnancy but the extremely low calcium of nephritic uremia is not seen in eclampsia[25] nor the correlated rise in inorganic phosphate. The surface tension of the serum is said to be lowered.[26] The decrease in the alkalinity of the blood observed by Zangmeister and others[27] has been ascribed to the formation of sarcolactic acid by Zweifel,[28] who failed to detect oxybutyric acid or oxalic acid in the blood. Bokelmann,[29] however, found that pregnant women have 20 to 30 per cent. more acetone bodies in the blood than normal women, and these are a factor in the progressive decrease in blood alkali observed even in normal pregnancy.[29a]

Ballerini[30] found that the physico-chemical changes in the blood are quite the same as in corresponding conditions of nephritis. Blood lipase is much increased because of the hepatic injury (Whipple).[31] As to the blood proteins, fibrinogen is increased;[32] fibrin ferment has been found increased and antithrombin decreased (Dienst),[33] while Schmidt found a relative increase in the globulin. The antitryptic titer of the blood may

[18] Jour. Biol. Chem., 1923 (56), 17.
[19] Plass, Johns Hopkins Hosp. Bull., 1924 (35), 345.
[20] Amer. Jour. Med. Sci., 1917 (153), 94; corroborated by Morse, Bull. Johns Hopkins Hosp., 1917 (28), 199.
[21] Amer. Jour. Obst., 1918 (77), 717.
[22] Wallis and Williams, Lancet, 1922 (1), 784.
[23] See Obata and Hayashi, Arch. Gynäk., 1923 (119), 80.
[24] Amer. Jour. Med. Sci., 1918 (156), 1.
[25] Plass and Bogert, Amer. Jour. Obst. Gyn., 1923 (6), 427.
[26] Fuhrmann and Kisch, Zeit. ges. exp. Med., 1921 (24), 84.
[27] See Mahnert, Arch. Gynäk., 1923 (119), 407.
[28] Arch. f. Gyn., 1905 (76), 537.
[29] Zeit. Geb. u. Gynäk., 1924 (88), 408; also Bock, Klin. Woch., 1924 (3), 2294.
[29a] Rowe, Amer. Jour. Physiol., 1925 (71), 660.
[30] Annali Ostet. e. Gin., 1910 (32), 273.
[31] Jour. Med. Res., 1913 (24), 357.
[32] de Wesselow and Wyatt, Proc. Roy. Soc. Med. Obst., 1922 (15), 57; Foster, Arch. Int. Med., 1924 (34), 301.
[33] Arch. f. Gyn., 1912 (96), 43; 1922 (117), 56; Zeit. Geb. u. Gyn., 1919 (82), 102.

be much increased.[34] In late toxemia of pregnancy the degree of plasma dilution varies with the amount of clinical edema, and is apt to be less marked in those who develop general convulsions than in those who do not.[35]

The statements to be found in the literature concerning the toxicity of the blood in eclampsia leave nothing proved concerning this point, but Graf and Landsteiner[36] and a few others describe an increase of toxicity of the blood, not due to any special poison but to an increase in the amount of the toxic substances ordinarily present; however, any studies of toxicity of the blood are of doubtful value because of the reactions produced by injections of even normal blood. More attention may be given to the observation of Hüssey,[37] that while the serum of pregnant women has a slight vasodilator effect,[38] in eclampsia and other pregnancy toxicoses there is a marked vasoconstrictor effect, ascribed by him to amines which have not yet been demonstrated by chemical means.

Despite the absence of marked changes in either blood sugar or carbohydrate metabolism, an increased glycogen store seems to protect the liver from injury, as with other hepatotoxic conditions.[39] Although the liver changes often are so severe as to dominate the anatomical picture, functional tests show that the hepatic injury may follow rather than precede the eclampsia,[40] and reduction of phthalein excretion is observed only in severe cases.[41] Cornell[42] could find no characteristic changes in the basal metabolism.

Theories as to Etiology.—The anatomical changes of eclampsia are such as to leave little or no room for doubt that there is a severe intoxication with poisons that have a markedly toxic effect upon all the organs of the body, thus differing from the toxic materials at work in uremia, which seem to affect chiefly the central nervous system and to produce no marked tissue changes. Repeated bacteriological and histological studies have failed to demonstrate that infection with either vegetable or animal parasites is the cause, and clinical observations do not support such an hypothesis. The association of the condition with pregnancy, and particularly the rapid improvement that often follows the removal of the contents of the uterus, almost compels us to admit that the causative agent is produced by the fetus or the placenta. Some investigators (Politi, Liepmann) believe that they have found a greater degree of toxicity in extracts from the placentas from eclamptic than from normal women. We have no exact ideas as to the nature of the supposed toxic substances, but they may come from proteolysis of tissue

[34] Franz. Arch. f. Gyn., 1914 (102), 579; Ecolle, Arch. Mens. Obst. Gyn., 1917 (6), 97.
[35] Plass and Bogert, Johns Hopkins Hosp. Bull., 1924 (35), 361.
[36] Cent. f. Gyn., 1900 (33), 142.
[37] Corrbl. Schweiz. Aerzte, 1918 (48), 691; Schweiz. med. Woch., 1920 (50), 857.
[38] Not confirmed by Theodor, Arch. Gynäk., 1924 (122), 675.
[39] See Titus *et al.*, Jour. Amer. Med. Assoc., 1920 (74), 777.
[40] Linzenmeier, Arch. Gynäk., 1923 (120), 80.
[41] J. S. Smith, Amer. Jour. Obst. Gyn., 1924 (8), 298.
[42] Surg. Gyn. Obst., 1923 (36), 53.

proteins, presumably from the placenta. The hypothesis of Zweifel that lactic acid is responsible seems untenable, and the degree of acidosis present is not sufficient to account for the intoxication (Losee and Van Slyke).

The Placenta as a Source of Intoxication.—Histologists having frequently observed placental cells in the blood and vessels of eclamptic patients, it was once suggested that multiple *capillary emboli of placental cells*, detached from chorionic villi and forced into the placental circulation, cause the manifestations of the disease; this theory is entirely inadequate, however, to explain all the features of eclampsia. Related to this hypothesis is the idea that the placental tissues, being foreign to the maternal organism in so far as they are derived from the ovum, give rise to the production of antibodies (*syncytiolysins*) by the mother, which are toxic for pregnant animals (Ascoli), and which may have to do with eclampsia in some unknown way. Rosenau and Anderson found that guinea pigs could be sensitized to guinea-pig placenta, showing conclusively that the placenta contains proteins foreign to the mother. Attempts to establish the anaphylactic nature of eclampsia[43] have, like so many other theories, foundered on the fact of the characteristic anatomy of this disease, which is never seen in anaphylaxis.[44] The studies of Abderhalden have shown that the blood of every pregnant female animal contains enzymes which have a specific proteolytic action, and so the possibility exists that abnormal or excessive products of such proteolysis, or a lack of adequate defensive digestive action, may be responsible for the toxemias of pregnancy. Esch[45] and Franz[46] have, indeed, found evidence of the presence in the serum and urine of eclamptics, of substances resembling anaphylactic poisons in their action, and presumably derived from proteolysis somewhere in the body. Franz found that if the poison injures the kidneys seriously it is retained in the body, the urine ceasing to be toxic, which has, presumably, a relation to the toxicosis of eclampsia.[47]

Liepmann[48] and others have reported the finding of a considerable degree of toxicity in eclamptic placentas, but this is probably related to the increased autolysis observed in eclamptic placentas by Dryfuss.[49] It is also reported that eclamptic placentas digest perfused protein more

[43] Review by Zweifel, Zeit. Immunität., 1921 (31), 22.

[44] See Felländer, Zeit. Geb. u. Gyn., 1911 (68), 26; Mosbacher, Deut. med. Woch., 1911 (37), 1021. Vertes (Monat. Geb. u. Gyn., 1914 (40), 361, 466) states that animals dying from anaphylaxis may show typical eclamptic tissue changes, which is not in accordance with the observations of many others.

[45] Münch. med. Woch., 1912 (59), 461.

[46] *Ibid.*, page 1702.

[47] Hull and Rhodenburg (Amer. Jour. Obst., 1914 (70), 919) ascribe importance to leucine derived from proteolysis of the placental elements, while Kiutsi (Zeit. Geb. u. Gyn., 1912 (72), 576) considers the nucleins of the placenta the toxic agents, both statements being unconfirmed and improbable.

[48] Münch. med. Woch., 1905 (52), 687 and 2484; Boos, Boston Med. and Surg. Jour., 1908 (158), 612; Oden. Mich. State Med. Soc. Jour.,1925 (24), 110.

[49] Biochem. Zeit., 1908 (7), 493.

rapidly than normal placentas (Liepmann).[50] Obata[51] found no great difference in the toxicity of eclamptic and normal placentas, but believes that in eclampsia there is a reduced capacity of the maternal blood to neutralize this placenta poison.[52] According to Mohr and Heimann,[53] the eclamptic placenta shows a great decrease in lecithin, which they ascribe to the increased autolysis, and to the hydrolyzed lecithin they attribute the hemotoxic effects. On the other hand, Murray and Bienenfeld[54] report the finding of an increased amount of lipoids in eclamptic placenta,[55] and Schönfeld[56] describes toxic lipoids derived from normal placentas.

Another hypothesis is that eclampsia may result when there is infarction or partial detachment of the placenta of some duration, the toxic products of placental necrosis producing the renal and hepatic lesions, which in turn are in large part responsible for the symptoms.[57] The high blood pressure in eclampsia has naturally suggested that pressor bases derived from the placenta might be responsible, but this hypothesis remains unproved. Hüssy[58] believes that he has proved the presence of amines by physiological tests, and reports the demonstration of substances in eclamptic blood with a pressor effect similar to that of histamine. The fact that the capillary circulation has been found to be extremely sluggish with intermittent spasm,[59] may be related to the known capillary toxicity of histamine.[60]

The Fetus as a Source of Intoxication.—A reasonable view of the cause of eclampsia is that it is initiated by the excessive or abnormal products of metabolism thrown into the blood of the mother, both from the fetus and from her own overactive tissues; these cause injury to the kidneys, leading to a further retention, or injure the liver so that the normal metabolic processes of that organ (particularly oxidation) cannot be carried on; or, perhaps more often, both liver and kidney as well as other organs are injured. In this way a vicious circle might be established and rapidly lead to an overwhelming of the maternal system with toxic products derived from both her own and the fetal tissues. It must be admitted, however, that the rapid improvement that so often follows removal of the products of conception indicates strongly that the poisonous substances

[50] Arch. Gynäk., 1923 (120), 71.

[51] Jour. Immunol., 1919 (4), 111; bibliography on etiology of eclampsia; also Arch. Gynäk., 1923 (118), 586; (119), 69.

[52] See also Hayashi, Arch. Gynäk., 1923 (119), 29, 505.

[53] Biochem. Zeit., 1912 (46), 367.

[54] Jour. Obst. and Gyn. Brit. Empire, 1910 (18), 225; Biochem. Zeit., 1912 (43), 245.

[55] The hypothesis of Mohr and Freund that oleic acid from the eclamptic placenta is a hemolytic factor, is not corroborated by Polano (Zeit. Geb. u. Gyn., 1910 (65), 581).

[56] Arch. Gynäk., 1921 (115), 80.

[57] Young and Miller, Brit. Med. Jour., 1921 (1), 486.

[58] Schweiz. med. Woch., 1920 (50), 857.

[59] See Hinselmann, Arch. Gynäk., 1923 (116), 443.

[60] Another theory is that eclampsia results from injury, by unknown poisons, of the choroid plexus, so that its function of protecting the central nervous system from toxic substances is lost. (Franke *et al.*, Compt. Rend. Soc. Biol., 1924 (90), 305.)

arise chiefly, if not exclusively, in the fetus or the placenta. But, as Liepmann points out, the child shows relatively little evidence of intoxication, while, on the other hand, eclampsia may develop *after* delivery of the fetus, which facts speak in favor of the place of the origin of the poison being the placenta and not the fetus, and death of the fetus seems to have no effect on the eclampsia.[61] Especially important in this connection is the observation of cases of eclampsia in patients with a hydatid mole and no fetus.[62]

The modern studies of the compatibilities of human bloods have been applied to mother and fetus with interesting results. McQuarrie[63] found that over 70 per cent. of his cases of toxemia occurred in women whose blood interagglutinated with that of the fetus. Gruhzit[64] also says that in eclampsia the mother's blood agglutinates the red corpuscles of the fetus, which is not the case in normal pregnancy, but these authors do not establish that any agglutination occurs in the mother's blood *in vivo*. Ottenberg[65] calls attention to the early work of Dienst,[65a] who found that eclampsia occurred in women whose blood agglutinated or laked the fetal blood, *and who also had an abnormal connection between the placental and maternal circulation*, and finds much to support this hypothesis, since injections of incompatible blood may produce conditions similar to those of eclampsia, especially the agglutination thrombi with necrosis in the liver. On the other hand, the occasional occurrence of typical eclampsia in women with a hydatid mole and no fetus,[62] is difficult to explain on this basis.

The Ductless Glands in Eclampsia.—In view of the mystery surrounding the cause and effect of the enlargement of the thyroid during pregnancy, it is not strange that the suggestion has been made that the enlargement is for the purpose of neutralizing the excessive amounts of toxic materials in the maternal blood, and that failure of this enlargement is responsible for eclampsia. In support of this idea, Lange[66] states that absence of the normal thyroid enlargement is usual in eclampsia, and Fruhinsholz and Jeandelize[67] note the frequency of eclampsia in myxedematous women. The notable influence of calcium upon convulsions, and the possible deficiency in calcium during pregnancy, has led to the suggestion that this may be responsible for eclampsia,[68] and, since the parathyroids are related to calcium metabolism, that they are concerned;[69] but such theories fail to explain the many changes other than the convulsions, and have not been accorded much importance. Kastle and Healy[70] consider that parturient paresis of cattle, which bears some resemblance to human eclampsia, is caused by absorption of toxic substances produced in the formation of the colostrum; it is cured by dilating the lacteal ducts by oxygen or other means. This observation

[61] See Lichtenstein, Zeit. f. Gyn., 1912 (36), 1419.

[62] Hitschmann, Cent. f. Gyn., 1904 (28), 1089. See also Frey (Schweiz. med. Woch., 1924 (54), 121) who found records of seven cases of eclampsia with hydatid mole without a fetus.

[63] Johns Hopkins Hosp. Bull., 1923 (34), 51.

[64] Amer. Jour. Obst. Gyn., 1923 (5), 400.

[65] Jour. Amer. Med. Assoc., 1923 (81), 295.

[65a] Zent. f. Gynäk., 1905 (29), 353.

[66] Zeit. f. Geb. u. Gyn., 1899 (40), 34.

[67] Presse Méd., 1902 (10), 1023.

[68] See Silvestri, Gaz. degli Osped., 1910 (31), 689; Mitchell, Med. Record, 1910 (78), 906.

[69] Massaglia, Arch. ital. Biol., 1907 (48), 109; Endocrinology, 1921 (5), 309.

[70] Jour. Infec. Dis., 1912 (10), 226.

lends support to the theory advanced by Sellheim[71] that human eclampsia is of mammary gland origin. Wallis and Williams[72] believe that an overproduction of a toxic substance of unknown nature, which they have found in the corpus luteum, is responsible.

Pernicious Vomiting of Pregnancy.—This condition is inseparably associated with eclampsia and non-convulsive toxemias of pregnancy, there being transitional and border-line cases of all sorts. In fatal cases of pernicious vomiting, anatomical changes resembling those of eclampsia have been found, and albuminuria and icterus are often observed.[73] The blood usually shows normal figures for non-protein N except in some severe cases,[74] but the proportion of urea is often reduced. There is less renal injury than in typical eclampsia, and less diastase in the urine.[75] The chief chemical interest in these cases lies in the urinary findings, there being commonly observed a relatively high proportion of ammonia and undetermined nitrogen with decreased urea, which findings have been considered indicative of defective oxidation or deaminization (Ewing and Wolf) and of prognostic and diagnostic significance (Williams). There is also excretion of acetone bodies and other evidences of more or less acidosis,[76] but without much if any reduction in the alkali reserve. The excretion of acetone bodies generally parallels the clinical condition (Harding).[74] Underhill and Rand[77] hold that the observed urinary changes are entirely compatible with those which can be produced by the starvation which is present in pernicious vomiting; but Ewing[78] contends that there are other underlying factors besides those of starvation. Possibly in pregnancy the demand for carbohydrate is increased, and hence the effects of carbohydrate deficiency are correspondingly more intense;[79] in any event, much benefit is often obtained by administration of glucose.

Summary.—Most of the known facts at hand speak against the idea that one definite chemical substance is responsible for the anatomical changes and symptomatic manifestations of eclampsia. More probably there are present not only the poisonous substances that initiate the tissue changes and which probably originate in the placenta itself or from digestion of placenta proteins in the maternal blood or organs, but also toxic substances that accumulate because of the disorganization of the liver and kidney cells, and which are possibly similar to the toxic substances most prominent in uremia and in acute yellow atrophy, for eclampsia seems to stand intermediate between these two diseases, encroaching upon the characteristics of each. Acid intoxication, which undoubtedly exists

[71] Zent. f. Gyn., 1909 (34), 1609.
[72] Lancet, 1922 (1), 784.
[73] See Ewing and Wolf, Amer. Jour. Obstr., 1907 (55), 289.
[74] Harding and Drew, Jour. Obst. Gyn. Brit. Emp., 1923 (30), 507.
[75] Wallis, Brit. Med. Jour., 1920 (2), 73.
[76] See Gilliatt and Kennaway, Quart. Jour. Med., 1919 (12), 61; Losee and Van Slyke, Amer. Jour. Med. Sci., 1917 (153), 94; Duncan and Harding, Canad. Med. Assoc. Jour. 1918 (8), 1057.
[77] Arch. Int. Med., 1910 (5), 61.
[78] Amer. Jour. Med. Sci., 1910 (139), 828.
[79] Harding and Potter, Brit. Jour. Exp. Path., 1923 (4), 105.

to a greater or less degree in some cases of eclampsia, is not an important cause of the clinical manifestations of the disease. The finding of minute quantities of lactic acid in the blood, urine, and in the cerebrospinal fluid is not of great significance, for similar amounts may be found in other conditions associated with convulsions and partial asphyxia, or in partial starvation, such as results from the vomiting of pregnancy. The excretion of these organic acids, as well as the large proportion of unoxidized sulfur in the urine, indicates that incomplete oxidation is an important feature of eclampsia, and under such conditions a large number of imperfectly known toxic substances may accumulate in the blood and tissues. The defective oxidation indicated by the urinary findings are probably the result of the injury to the liver-cells, which have such a prominent oxidizing function. The hypotheses which ascribe the intoxication to products of specific proteolysis of the foreign proteins of the placenta which have entered the maternal organism, are suggestive, but as yet are not sufficiently developed to permit of any definite conclusions as to the extent to which they apply. The similarity of the anatomical changes seen in the liver to those produced by injection of hemagglutinating agents, gives significance to the suggestion that agglutination resulting from incompatability of maternal and fetal blood may be the cause of eclampsia. If this hypothesis can be established it will be adequate to explain the features of the disease.

DIFFUSE TOXIC NECROSIS ("ACUTE YELLOW ATROPHY") OF THE LIVER[80]

In this condition there is presented a striking picture of autolysis, in that a large parenchymatous organ undergoes a rapid reduction of size because of a solution of its structural elements, while at the same time products of protein digestion (leucine, tyrosine, etc.) appear free in the liver, the blood, and the urine. Because of these prominent features and their relation to the questions of metabolism in general, and the function of the liver in particular, diffuse toxic necrosis, or as it is more commonly but less properly called, acute yellow atrophy of the liver, has been the object of much greater interest and investigation than its clinical importance would warrant, for it is not a common disease. However, of late years more cases have been observed, a fact ascribed to increasing use of various cyclic organic compounds, which are held to be responsible for many cases.[81]

The etiology of the condition is quite unknown, but it is very probably not a specific one for we find that numerous forms of intoxication may lead to a condition closely resembling acute yellow atrophy,[82] particularly puerperal eclampsia, and some cases of septicemia (especially with the strepto-

[80] Full review of anatomy of acute yellow atrophy and related conditions given by Herxheimer, Ziegler's Beitr. Path. Anat., 1923 (72), 56 and 349.

[81] See Discussion in Verh. Deut. path. Gesell., Apr. 1921.

[82] It is to be borne in mind that the color is yellow only during the earlier stages, "red atrophy" occurring later, but the name acute "yellow atrophy" has come through usage to apply to the disease as a whole.

coccus),[83] and poisoning with phosphorus, arsenic, nitrophenols, and mushrooms.[84] It seems probable that *any poison which does not directly cause death, but which causes a severe injury to the liver-cells without at the same time destroying the autolytic enzymes, so that the cells die and undergo rapid autolysis, may produce a condition identical with or similar to acute yellow atrophy* (Wells and Bassoe).[85] In the typical cases of the disease, of "idiopathic" origin, the poisonous agent possibly comes from the alimentary canal, as indicated by a preliminary period of gastro-intestinal disturbance that usually precedes the onset of the disease, and secondly by the fact that the liver seems to receive the chief effect of the poison. Whether these hypothetical poisons are produced by abnormal fermentation and putrefaction in the alimentary tract, or by a specific organism elaborating its poison in this location, is quite unknown. Bacteriological studies of the disease have so far given inconstant and non-instructive results. In the countries where phosphorus poisoning is common (especially Austria) there has been found much difficulty in distinguishing in many cases the results of phosphorus poisoning from acute yellow atrophy of the liver, and many have contended that there is no real difference; *i. e.*, that phosphorus, as well as unknown poisons, may cause acute yellow atrophy.[86] The present trend of opinion, however, seems to favor the view that there is a primary liver necrosis which is different from that caused by phosphorus in several essential respects.[87]

Phosphorus Poisoning.—*Between phosphorus poisoning and "primary" hepatic necrosis the following chief differences may be discerned:* Phosphorus produces a general injurious effect upon all the organs of the body, the liver merely showing the most marked anatomical changes, which at first consist of a fatty metamorphosis of the liver, due to migration of the body fat from the fat deposits into the injured cells (Rosenfeld, Taylor); subsequently the liver cells disintegrate, the cytoplasm being affected before the nucleus, and the liver may become smaller than normal, although it is usually enlarged because of the fat deposition. Typical acute yellow atrophy is characterized by an early necrosis of a large proportion of the liver cells, the nucleus becoming unstainable while the cytoplasm is still little altered in appearance, and fatty changes play a subordinate rôle or are absent. As Anschütz says, the poison seems to strike at the life of the cell, its nucleus, while phosphorus attacks the cytoplasm. Furthermore, the poison of yellow atrophy seems to be very specific, for it attacks the other organs of the body almost not at all, and within the liver it affects only the hepatic cells proper, while the bile-duct epithelium and the stroma cells are so little injured that they are able to proliferate greatly, this proliferation being a prominent feature. There are also clinical and chemical differences that will be discussed later, but yet, on the whole, the resemblances of yellow atrophy and phosphorus poisoning are so great that we have obtained much information concerning the former by means of experimental studies of phosphorus poisoning.

Delayed Chloroform Poisoning.—After *chloroform narcosis*, and rarely after ether, there occasionally develops a severe intoxication, with clinical and anatomical

[83] Babes, Ann. Inst. Past. Bucarest, vol. 6.

[84] Frey, Zeit. klin. Med., 1912 (75), 455; Prym, Virchow's Arch., 1919 (226), 229.

[85] Jour. Amer. Med. Assoc., 1904 (44), 685.

[86] The difficulty seems largely a question of definition, since the cases resembling phosphorus poisoning anatomically in the material which is called by some acute yellow atrophy, would not be so regarded by many careful pathologists, but merely as examples of severe toxic injury with fatty changes.

[87] See Anschütz, Arb. a. d. Path. Inst. Tübingen, 1902 (3), 230; Paltauf, Verh. Deut. Path. Gesell., 1903 (5), 91; Riess, Berl. klin. Woch., 1905 (42), No. 44a, p. 54: Herxheimer, Klin. Woch., 1922 (1), 1441.

findings very similar to acute yellow atrophy and phosphorus poisoning;[88] in point of the fatty changes the cases usually stand intermediate between acute yellow atrophy and phosphorus poisoning. This action of chloroform would seem, from the studies of Evarts Graham,[89] to be produced by the hydrochloric acid formed from it within the liver cells, and he found that other fat-soluble substances liberating halogens within the cells produce a similar effect.[90] Such poisons as chloroform are especially likely to attack livers that are already fatty, presumably because the fat-soluble poisons accumulate in greater amounts in such fatty livers.[91] Judged by the reduction in the capacity of the liver to conjugate phenols, chloroform produces more hepatic injury than phosphorus.[92] Some cases of puerperal eclampsia also present such profound liver changes that they are distinguished as eclampsia chiefly on the basis of the convulsive manifestations, rather than on the ground of anatomical changes. So, too, the hepatic changes in certain septicemias and acute syphilis may resemble those of acute yellow atrophy to a greater or less degree. The distinction between acute yellow atrophy and the liver changes of poisoning with chloroform and phosphorus as outlined above, although not recognized by some pathologists, is supported by the extensive study of Herxheimer,[87] and is in harmony with my own observations on these conditions.

Summary of Views on Etiology.—From a review of the literature and the study of a few cases, I have reached the following understanding of the condition described as acute yellow atrophy of the liver: The "atrophy" is due entirely to autolysis of necrotic liver cells by their own enzymes. In the most typical cases of "primary" or "idiopathic" yellow atrophy we have to do with a poison having a very specific effect on the liver cells, which destroys their "life" (*i. e.*, stops synthetic activities) without injuring their intracellular proteolytic enzymes, and consequently autolysis occurs. As the poison affects other organs but little, the necrosis and autolysis continue until there is so much loss of liver function that systemic poisoning results from the hepatic insufficiency and from the resulting accumulation of poisonous products of incomplete metabolism. That the intoxication comes in large measure from the changes in the liver, even in phosphorus poisoning, is shown by the greater resistance to phosphorus of dogs with Eck's fistulas.[93] The patient dies from this poisoning, and the liver is found at autopsy to have decreased by from one-third to one-half or more in its volume. This great change would not be possible if the poisons affected the heart, kidneys, or brain as much as they do the liver structure, which is probably the reason that phosphorus, bacterial poisons, snake poisons, and other poisons that affect many sorts

[88] Complete review and literature by Bevan and Favill, Jour. Amer. Med. Assoc., 1905 (45), 691; Muskens, Mitt. Grenz. Med. u. Chir., 1911 (22), 568. Full discussion of chemistry of chloroform necrosis by Wells, Jour. Biol. Chem., 1908 (5), 129. Experimental necrosis—see Whipple and Sperry, Johns Hopkins Hosp. Bull., 1909 (20), 278; Graham, Jour. Exper. Med., 1912 (15), 307; Simonds, Arch. Int. Med., 1919 (23), 362; Davis and Whipple, *ibid.*, p. 612.

[89] Jour. Exp. Med., 1915 (22), 48. This hypothesis receives support from the conclusion reached by many investigators that dichlorethylsulphide ("mustard gas") also injures tissues through intracellular dissociation liberating HCl. (See Lillie *et al.*, Jour. Pharmacol., 1919 (14), 75.)

[90] Carbon tetrachloride poisoning, see Davis, Jour. Med. Res., 1924 (64), 601; Schultz and Marx, Amer. Jour. Trop. Med., 1924 (4), 469. See also Underhill and Kapsinow, Jour. Metabol. Research, 1922 (2), 57.

[91] The same is true of fatty kidneys (McNider, Jour. Pharmacol., 1921 (17), 289).

[92] Pelkan and Whipple, Jour. Biol. Chem., 1922 (50), 513.

[93] Fischler and Bardach, Zeit. physiol. Chem., 1912 (78), 435.

of cells do not ordinarily produce the typical picture of liver atrophy. When these poisons affect the liver more and the other tissues less, we approach the condition of acute yellow atrophy; *e. g.*, if the dose of phosphorus is not so great as to kill the patient through injury of other more vital organs, after a few days the necrosed liver cells undergo autolysis, and if enough liver cells have been destroyed, hepatic insufficiency may cause death, with the finding of an anatomical condition in the liver that can be properly designated as acute atrophy. Hence it is possible for many poisons to cause this condition under certain circumstances, and there seem to be certain unknown poisons (possibly of intestinal origin) that are of such a nature that they cause specifically acute hepatic atrophy.

The above hypothesis seems to explain all the known facts concerning this disease. That phosphorus, chloroform, and some other poisons lead particularly to fatty changes may, perhaps, be due to their acting especially upon the oxidizing enzymes,[94] leaving the autolytic enzymes and the lipase free to digest the cell and to form fat.[95] That it is particularly the oxidizing enzymes that are attacked is well shown by the chemical findings, and also by Loewy's[96] observation that in poisoning with CNH, which acts by impairing oxidation, the alterations in protein metabolism are very similar to those of phosphorus poisoning.[97] To be sure, Lusk[98] found no deficiency in general oxidation in phosphorus poisoning, but this does not signify that the local changes do not depend upon *local* defective oxidative processes. Furthermore, the marked power of sugar to protect the liver from such poisons as phosphorus and chloroform seems to depend on its furnishing easily oxidizable material to cells with reduced oxidative capacity (Simonds).[99]

Not only phosphorus but many metals, especially mercury, seem able to cause the anatomical changes of acute yellow atrophy, for the condition has been observed very frequently in persons receiving mercurial and arsenical treatment for syphilis.[1] Here the syphilis has been held responsible by some,[2] but the fact that in many of the cases the syphilis was quiescent or chronic at the time, and that mercury and arsenic are known to kill cells

[94] See Verworn, Deut. med. Woch., 1909 (35), 1593; Joannovics and Pick, Arch. ges. Physiol., 1911 (140), 327.

[95] Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

[96] Cent. f. Physiol., 1906 (19), 23.

[97] The hypothesis suggested by Quincke (Nothnagel's Handbook, 1899, vol. 18, p. 307) that possibly regurgitation of pancreatic juice up the bile ducts might be responsible for the degenerative changes in the liver, is contradicted by the fact that the bile pressure is greater than the pancreatic juice pressure, and that the bile-ducts and peripheral portions of the lobules are least affected. Nor could Best (Thesis, Univ. of Chicago, 1903), prove that trypsin injected into the liver by way of the bile-ducts is able to cause such changes. (See Wells and Bassoe.[85])

[98] Science of Nutrition, Philadelphia, 1909.

[99] Arch. Int. Med., 1919 (23), 362. Ervin, however, ascribes the protective action of carbohydrate feeding to the glycogen within the liver cells, where it protects the protein-fat emulsion of the protoplasm from the action of acids (Jour. Lab. Clin. Med., 1919 (5), 146).

[1] Severin, Zeit. klin. Med., 1912 (76), 138. Bendig, Münch. med. Woch., 1915 (62), 1144.

[2] Herxheimer[87] says that about 20 per cent. of the 700 cases he could collect from the literature, occurred with syphilis, mostly in the secondary stage.

and stimulate autolysis, seems to incriminate the metals,[3] at least in some cases. On the other hand, Stewart,[4] calls attention to the fact that acute yellow atrophy occurs especially often after poisoning with picric acid, trinitro-toluene, dinitro-benzene, and aromatic arsenicals; as in all these substances the only common component is the benzene radical, its responsibility is strongly suggested.

Chemical Changes of Acute Toxic Necrosis of the Liver

The Urine.—Most striking, and long regarded as pathognomonic, is the presence of *leucine* and *tyrosine* in the urine, first described by Frerichs. While we now know that these and other amino-acids may occur in the urine in any conditions in which there is a great breaking down of tissue within the body, yet it is true that in no other condition are they found so abundantly as in acute hepatic atrophy (as high as 1.5 gm. of tyrosine per diem has been found).[5] They were formerly said to be constantly present (in thirteen out of fourteen cases studied by Riess),[5a] tyrosine being usually the more abundant. However, it is probable that not infrequently crystals of bile pigments or other substances have been mistaken for tyrosine in the urine[6] and Schumm[7] was able to find tyrosine by the most delicate chemical methods in the urine of but 2 out of 7 cases. Therefore, it is probable that the frequency and amount of tyrosine excretion may not be so great as is generally believed.

Deutero-proteose is also frequently (but not constantly) found, as further evidence of abnormal protein splitting.[8] Uric acid and other purines are often somewhat, but not characteristically, increased, probably resulting from the nuclear destruction in the liver. Carbamates do not seem to be present in recognizable amounts, and sugar is also absent. There is often an increase in ethereal sulfates (Salkowski),[9] and in phosphorus poisoning various bases have been found in the urine,[10] which presumably might also be found in acute yellow atrophy if sought for. The total elimination of nitrogen is increased[11] (particularly if the scanty

[3] Tileston (Boston Med. and Surg. Jour., 1908 (158), 510) has described a case of acute yellow atrophy from mercurialism without syphilis.

[4] Stewart, Vining and Bibby, Jour. Path. Bact., 1919 (23), Proc. Path. Soc., p. 120.

[5] An interesting exception has been reported by W. G. Smith (Practitioner, 1903 (70), 155) who found great quantities of leucine in the urine of a young woman who was apparently not at all ill. Rosenbloom has found tyrosine crystals in the urine of a healthy pregnant woman, and cites other cases of tyrosinuria without hepatic atrophy (N. Y. Med. Jour. Sept. 19, 1914). He also gives a review on aminoaciduria in "Endocrinology and Metabolism," 1924 (4), 475.

[5a] Berl. klin. Woch., 1905 (42), No. 44a., p. 54.

[6] Goegel, Klin. Woch., 1922 (1), 1158; Dorner, Deut. med. Woch., 1922 (48), 453.

[7] Zeit. physiol. Chem., 1922 (121), 1.

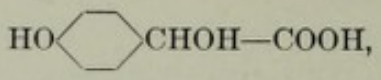
[8] Salkowski (Berl. klin. Woch., 1905 (42), 1581) found in the urine of a case of acute yellow atrophy a large quantity of nitrogen in a colloidal but non-protein form, apparently of carbohydrate nature. Mancini (Arch. di farm. sperim., 1906 Bd. v) also observed an increase in the colloidal nitrogen of the urine in liver diseases.

[9] Virchow's Arch., 1909 (198), 188.

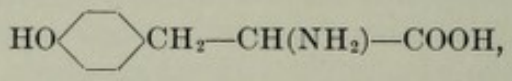
[10] Takeda, Pflüger's Arch., 1910 (133), 365.

[11] See Welsch, Arch. int. pharm. et thér., 1905 (14), 211.

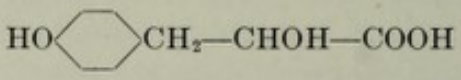
intake is considered), and the proportion that appears as urea is decreased, largely because of the presence of much ammonia,[12] part of which, at least, is eliminated combined with organic acids. Chief of these acids is *sarcolactic* acid, but of particular interest is the supposed appearance of *oxymandelic* acid,



which might be derived from tyrosine (Schultzen and Riess),



by the splitting out of the NH_2 group, the benzene nucleus failing to be completely oxidized as it normally is. The researches of Ellinger and Kotake,[13] however, make it probable that the supposed oxymandelic acid is something else, most likely *p-oxyphenyl-lactic acid*,



which can be demonstrated in the urine of dogs poisoned with phosphorus, and which represents a simple deaminization of tyrosine without further oxidation. It is evident from the urinary findings, therefore, that oxidation is decreased, which is presumably because of the destruction of liver tissue with its important oxidizing functions. The reduction of oxidation can also be shown experimentally by studying the respiratory exchange, Welsch having found the oxidation decreased by from ⅑ to ⅕ in phosphorus poisoning.

In phosphorus poisoning the urinary findings are similar, but with marked quantitative differences. Tyrosine cannot usually be detected, at least by ordinary methods, being found by Riess in but 7 of 36 cases of (human) phosphorus poisoning, and in but 4 of these was it abundant. Leucine is even less frequently found. With experimental animals glycine and other amino-acids have been found[14] in the urine, and they could probably be found in acute hepatic atrophy if the same delicate methods were employed. Wohlgemuth[15] has indeed found glycine, alanine, and arginine in human urine after phosphorus poisoning. The small quantity of amino-acids in phosphorus poisoning is probably due to the relative slowness of the autolytic changes. On the other hand, the deficiency of oxidation in phosphorus poisoning is shown by the abundant elimination of organic acids. Riess having obtained as high as 4 to 6 grams of zinc salt of *paralactic* acid from the urine (per liter) in human cases, and its presence seems to be constant.

[12] See Voegtlin, Johns Hopkins Hosp. Bull., 1908 (19), 50; White, Boston Med. and Surg. Jour., 1908 (158), 729.

[13] Zeit. physiol. Chem., 1910 (65), 397 and 402; also Fromherz, *ibid.*, 1911 (70), 351.

[14] Abderhalden and Barker, Zeit. physiol. Chem., 1904 (42), 524; Abderhalden and Bergell, *ibid.*, 1903 (39), 464.

[15] Zeit. physiol. Chem., 1905 (44), 74.

The Liver.[16]—In the liver may be found an abundance of the free amino-acids that have not yet escaped by diffusion, their presence having been first detected by Frerichs microscopically. Taylor[17] was able to isolate from a liver weighing 900 grams, 0.35 gm. of leucine and 0.612 gm. aspartic acid, which probably represent much less than the total amount present. Deuteroalbumose was also found, but no peptone, arginine, histidine, or lysine, and glycogen was also absent. In another case that appeared to be the result of chloroform intoxication, Taylor[18] obtained 4 grams of leucine, 2.2 grams of tyrosine, and 2.3 grams of arginine nitrate. Stadie and Van Slyke[19] estimated that the liver in their early case contained 13 to 14 grams of free amino-acids. Strauss and Becher[20] found 514 mg. non-protein N per 100 grams of liver tissue in their case, although the figures for other tissues were not high. Wells found several amino-acids free in sufficient quantity to identify in the liver in cases of acute yellow atrophy and chloroform necrosis, an increase in gelatin-forming substance in the former, and of organic non-lipoidal phosphorus in both, sulfur being unchanged. The increase in tissue phosphorus is striking, and agrees with Slowtzoff's and Wohlgemuth's[21] finding that the tissue phosphorus persists in experimental phosphorus poisoning. Wakeman[22] found that in phosphorus poisoning of dogs the liver shows a diminution of the hexone bases as a whole, the arginine being especially reduced; but no such change was found by him in acute yellow atrophy, nor by Wells in chloroform necrosis. Jackson and Pearce[23] found an increase in the diamino nitrogen with extensive necrosis of the liver in dogs and horses. Wohlgemuth[24] found arginine in the urine in phosphorus poisoning. The lecithin of the liver is also decreased (Heffter,[25] Kahn and Barsky,[26] and Wells), and the increase in P_2O_5 observed in the urine presumably comes partly from this source; cholesterol is unchanged, and sulfatids are decreased.[26] Beebe[27] found the pentose of the liver not greatly altered from the normal relations. The typical idiopathic atrophied liver shows *little or no increase in fat*, either chemically or microscopically, whereas there is considerable replacement of the lost liver substance by water, as shown in the following table:

[16] Full analyses and discussion of the chemistry of the liver in actue yellow atrophy and chloroform necrosis given by Wells, Jour. Exper. Med., 1907 (9), 627; Arch. Int. Med., 1908 (1), 589; Jour. Biol. Chem., 1908 (5), 129. Studies on the composition of the liver in various pathological conditions are reported by Hoppe-Seyler, Zeit. physiol. Chem., 1923 (130), 217.

[17] Zeit. physiol. Chem., 1902 (34), 580; Jour. Med. Research, 1902 (8), 424.

[18] Univ. of Calif. Publications (Pathol.), 1904 (1), 43.

[19] Arch. Int. Med., 1920 (25), 693.

[20] Zent. inn. Med., 1921 (42), 345.

[21] Biochem. Zeit., 1911 (32), 172.

[22] Jour. Exper. Med., 1905 (7), 292; Jour. Biol. Chem., 1908 (4), 119.

[23] Jour. Exper. Med., 1907 (9), 520.

[24] Zeit. physiol. Chem., 1905 (44), 74.

[25] Arch. exp. Path. u. Pharm., 1891 (28), 97.

[26] Arch. Int. Med., 1921 (28), 142.

[27] Amer. Jour. Physiol., 1905 (14), 237.

	Water	Fat	Fat-free Dried Substance
Normal liver (Hoesslin)[28]	75.8	2.5	21.7
Normal liver (Quincke)	76.1	3.0	20.9
Normal liver (Wells)	77.6	5.0	17.4
Acute atrophy (Perls)	81.6	8.7	9.7
Acute atrophy (Perls)	76.9	7.6	15.5
Acute atrophy (Hoesslin)	78.9	3.6	17.5
Acute atrophy (v. Starck)	80.5	4.2	15.5
Acute atrophy (Taylor)	85.8	2.0	12.2
Acute atrophy (Kahn and Barsky)	82.7	2.2	15.1
Acute atrophy (Kahn and Barsky)	75.4	8.3	16.3
Acute atrophy (Stadie and Van Slyke)	71.7	13.5	14.8
Acute atrophy (Wells)	83.8	2.5	13.7
Acute atrophy (Voegtlin)	78.0	6.6	15.4
Phosphorus poisoning (v. Starck)	60.0	29.8	10.0
Phosphorus poisoning (Hoesslin)	66.7	17.2	16.1
Phosphorus poisoning (Hoesslin)	56.5	32.2	11.3
Fatty degeneration (v. Starck)	64.0	25.0	11.0
Fatty degeneration (Hoesslin)	48.6	40.9	10.5
Chloroform necrosis (Wells)	72.4	8.8	18.8

Similar results have been obtained frequently by other observers, Taylor estimating that in his case about three-fourths of the liver parenchyma had disappeared. Feigl[29] has reported analyses of 24 livers which show in general a high water and low fat content, but there are several exceptions and wide variations. Unfortunately, there is no adequate description of his cases to permit a pathologist to determine just what sort of livers were being analyzed, and since there are no positive criteria to distinguish all forms of liver degenerative changes from one another, it is possible that some of the specimens examined in this series might exhibit changes not acceptable as acute yellow atrophy by all pathologists, in view of the high fat content recorded in two of the cases.

The yellow color of the liver tissue characteristic of this condition seems to be due to bilirubin rather than to fat, because as soon as the tissues are put into oxidizing agents (*e. g.*, dichromate hardening fluids) they turn grass-green from the oxidation of the bilirubin into biliverdin. There seems to be a marked increase in free fatty acids, probably the unsaturated higher fatty acids, which are strongly hemolytic.[30] Glycogen is greatly reduced in phosphorus and chloroform poisoning, and in acute yellow atrophy the liver has been found to be free from glycogen.[31]

Jacoby[32] found that the livers from phosphorus-poisoned dogs underwent autolysis with greater rapidity than normal livers, which was attrib-

[28] Deut. Arch. klin. Med., 1883 (33), 600.
[29] Biochem. Zeit., 1921 (115), 22.
[30] Joannovics and Pick, Berl. klin. Woch., 1910 (47), 928.
[31] Holm, Virchow's Arch., 1925 (254), 236.
[32] Zeit. physiol. Chem., 1900 (30), 174; see also Porges and Pribram, Arch. exp. Path. u. Pharm., 1908 (59), 20.

uted to increased activity or amount of the autolytic enzymes, although addition of phosphorus to a solution containing liver ferments was not found to increase their activity. The aldehydase was not found decreased, and tyrosinase could not be demonstrated, but Slowtzoff[33] found both peroxidase and protease decreased, and attributed the increased autolysis to a greater acidity of the liver. Burge[34] describes decrease in the catalase of liver and blood in experimental phosphorus poisoning, and Simonds[35] found hepatic ereptase also decreased, but not in chloroform poisoning; esterase was not altered. (See also Autolysis of Liver, p. 85.)

The Blood.—In the blood marked changes are found, one of the most prominent, besides the icterus, being the *decreased coagulability* of the blood. This seems due to a loss of fibrinogen,[36] which, with the globulin, is greatly decreased,[37] the albumin remaining less altered.[38] The fibrin-ferment also seems to be decreased. These changes may be due to direct autolysis of the blood constituents (Jacoby having found that thrombi become rapidly dissolved in phosphorus-poisoning) or to the changes in the liver. The icterus depends apparently upon lesions of the finest bile capillaries,[39] although there is also some increase in hemolysis, and a decrease in the total blood and all its elements (Welsch);[40] and both bile salts and pigments appear in the urine. In all these diseases with marked liver changes there is an increase in the lipase of the blood.[41] Neuberg and Richter[42] have analyzed the blood drawn during life from a patient with acute hepatic atrophy, and isolated from 355 c.c. of blood 0.787 gm. tyrosine, 1.102 gm. leucine, and 0.240 gm. of lysine; they estimated the amount of free amino-acids in the entire blood to be about 30 grams.[43] This amount is so large that they question the possibility of it all arising from the degenerated liver tissue. In dogs suffering from chloroform necrosis of the liver or phosphorus poisoning the amount of free amino acids in the blood and urine is usually very small.[44] In a case

[33] Biochem. Zeit., 1911 (31), 227.

[34] Amer. Jour. Physiol., 1917 (43), 545.

[35] Jour. Exp. Med., 1918 (28), 673.

[36] Whipple and Hurwitz (Jour. Exper. Med., 1911 (13), 136) find a great decrease in fibrinogen during experimental chloroform necrosis of the liver.

[37] See Foster, Arch. Int. Med., 1924 (34), 301; Isaac-Krieger and Hiege, Klin. Woch., 1923 (2), 1067.

[38] Jacoby, *loc. cit.*;[53] see also Doyon, Compt. Rend. Soc. Biol., 1905 (58), 493; and 1909, Vol. 66.

[39] Lang (Zeit. exp. Path., 1906 (3), 473) found fibrinogen in the bile of a dog poisoned with phosphorus, which may account for the occlusion of the bile vessels and the resulting jaundice.

[40] Arch. int. Pharm. et Thér., 1905 (14), 197.

[41] Whipple *et al.*, Bull. Johns Hopkins Hosp., 1913 (24), 207 and 357. Quinan found the lipase content of liver tissue much reduced in chloroform necrosis (Jour. Med. Res., 1915 (32), 73). A review of work published on blood changes and liver function in phosphorus and chloroform poisoning is given by Marshall and Rowntree, Jour. Exp. Med., 1915 (22), 333.

[42] Deut. med. Woch., 1904 (30), 499.

[43] v. Bergmann (Hofmeister's Beit., 1904 (6), 40) was able to isolate 2.3 grams of amino-acids combined with the chloride of naphthalene sulfonic acid, from 270 c.c. of blood in a case of acute yellow atrophy.

[44] See Van Slyke, Arch. Int. Med., 1917 (19), 77.

of human chloroform necrosis. Stander[45] found an increase in non-protein N and a hypoglucemia.

By the use of Van Slyke's method it has been found that acute yellow atrophy is sometimes accompanied by the highest amino-N figures in the blood recorded in any disease, Feigl and Luce[46] having reported 200 mg. per 100 c.c., or over 1 per cent. of amino acids. Other observers, however, have not obtained such high figures. Thus, Stadie and Van Slyke[19] found from 14 to 26 mg. amino-N per 100 c.c. of blood in their case, as compared with the normal 5 to 8 mg.[47] In two cases Falkenhausen[48] found 11.25 and 13.8 mg. The urea content is usually below 50 mg. The lipin content of the blood is also greatly increased;[49] with cholesterolemia[50] but a decrease in phospholipins, the total ether extract being as high as 1.9 per cent. Sugar is at first increased and later decreased; acetone bodies are but slightly increased. Because of the amount of liver destruction, sugar tolerance is reduced.[51] The urobilin quotient, (*i. e.*, the relation of the urobilin of the urine to that of the feces), is extremely high, indicating the degree of liver injury.[52]

Origin of the Amino-acids.—The earliest conception of the source of the leucine and tyrosine found in the urine was that they came from the products of tryptic digestion absorbed from the intestinal tract, which the liver could not convert into urea because of its damaged condition. On the demonstration by Jacoby[53] that these same bodies were present in the livers of phosphorus-poisoned animals because of autolysis, it became probable that the leucine and tyrosine found in the urine were formed from the degenerated liver-cells rather than in the intestine, which view has become generally accepted. It seems most probable, however, that the urinary amino-acids are derived partly (and perhaps chiefly) from the autolysis of the liver, and partly from amino-acids produced both in the intestine and within the body during tissue metabolism, and which the liver cannot transform into urea as it normally does, for several observers have reported that even relatively slight disturbances in hepatic function are accompanied by a considerable rise in the amino-acids in the urine,[54] and there may be an abnormally high amino-N content of the blood after feeding amino acids when there is liver disease (Falkenhauser[48]).

[45] Johns Hopkins Hosp. Bull., 1924 (35), 46.
[46] Biochem. Zeit., 1917 (79), 162.
[47] See also Feinblatt and Shapiro, Arch. Int. Med., 1924 (34), 690.
[48] Arch. exp. Path. Pharm., 1924 (103), 322.
[49] Biochem. Zeit., 1918 (86), 1.
[50] Weigeldt found droplets of cholesterol esters in the leucocytes (Deut. med. Woch., 1921 (47), 1221).
[51] See Bodasky, Jour. Biol. Chem., 1923 (58), 515.
[52] Linzenmeier, Arch. Gynäk., 1923 (120), 80.
[53] Zeit. physiol. Chem., 1900 (30), 174.
[54] See Masuda, Zeit. exp. Path., 1911 (8), 629; Labbé and Bith, Compt. Rend. Soc. Biol., 1912 (73), 210.

ACID INTOXICATION AND ACETONURIA[55]

If a rabbit is given in repeated small doses by mouth considerable quantities of inorganic acids, such as hydrochloric or phosphoric acids, which it cannot destroy by oxidation, it soon becomes extremely ill. The manifestations are characteristic—unsteadiness of motion and stupor being followed by coma, in which the striking feature is the excessively active respiration, as if the animal were being asphyxiated (the so-called "air hunger"),[56] although at the same time there is no cyanosis and the blood is bright red, containing much less CO_2 than normal, while the amount of oxygen remains quite normal. The current explanation of this interesting condition is as follows: Normally the blood carries the CO_2 away from the tissues to the lungs in combination with the inorganic alkalies of the blood, of which sodium is by far the most abundant. This combination is the bicarbonate of sodium (or other base), which in the lungs is decomposed into the carbonate, the CO_2 escaping into the alveolar air, according to this equation:

$$2NaHCO_3 \rightleftarrows Na_2CO_3 + H_2O + CO_2$$

The carbonate thus formed goes back to the tissues to combine again with more CO_2 and form bicarbonate. If inorganic acids are introduced into the blood they combine with the alkalies there, forming neutral salts which are eliminated in the urine, and in this way the amount of alkali in the blood is reduced, with a consequent reduction in the capacity of the blood to carry CO_2 away from the tissues, the amount of CO_2 in the blood sinking to as low as 2.5 and 3 per cent. (Walter). Consequently, in acid poisoning the CO_2 produced in metabolism accumulates in the tissues where it is formed, and blocks the processes of oxidation, so that the animal suffers from asphyxia exactly as if it were deprived of air. In other words, the lack of alkalies in the blood in acid intoxication checks the "internal respiration," as intracellular gas exchange is called, by preventing the removal of CO_2 from the cells. The acids stimulate the respiratory center, which is extremely sensitive to them, and the increased respiration tends to reduce the acidity by getting rid of the CO_2, but under the conditions of the experiment this is not sufficient to prevent asphyxia.

If the urine of such an animal is analyzed, it is found to contain increased quantities of the four chief inorganic bases, Na, K, Ca, and Mg (the last two apparently being derived from the bones);[57] but in addition to these it is found that the amount of ammonia in the urine is

[55] General literature to 1908, given by Ewing, Arch. Int. Med., 1908 (2), 330; also see Magnus-Levy, Ergebnisse inn. Med., 1908 (1), 374. More recent literature given by Hurtley, Quart. Jour. Med., 1916 (9), 301; Myers, Jour. Lab. Clin. Med., 1920 (5), 700; Sellards, "Principles of Acidosis," Harvard Univ. Press, 1917; Wilson, Physiol. Reviews, 1923 (3), 295; Van Slyke, "Endocrinology and Metabolism," 1924 (4), 51; Henderson, Physiol. Reviews, 1925 (5), 131.

[56] Dyspnoea is fully reviewed by Means, Medicine, 1924 (3), 309.

[57] See Goto, Jour. Biol. Chem., 1918 (36), 355.

decidedly increased.[58] If instead of a rabbit a carnivorous animal, such as a dog, is given acids, it will be found relatively insusceptible, so that much larger quantities can be given without causing acid intoxication. Examination of the urine of such a dog will show that the elimination of ammonia is increased much more than it is in the herbivora, while the inorganic alkalies are increased but little. From this it is deduced that in acid intoxication part of the nitrogen that normally goes to form urea becomes, while in the antecedent form of ammonia, combined with part of the acid that has entered the blood.[59] In this way much of the neutralization of the acids is accomplished by ammonia, and the inorganic alkalies of the blood are spared. As the amount of protein metabolism is much greater and more rapid in carnivora than in herbivora, the ammonia available for neutralization of acids is much greater in the former, and hence the relative lack of susceptibility of carnivora to acid poisoning.[60] The proteins of the blood also combine some of the acid, perhaps one-fifth of the neutralizing capacity of the blood being attributable to them. Another factor is the possible accumulation of acids within the cells, which must modify greatly any conclusions based upon studies of the blood and urine. It is within the cells that the effects of acids must be manifested, and we may have degrees of acidity and alkalinity in the cells which are quite different from those in the blood.[61]

Normal life processes cannot be carried out unless the hydrogen ion concentration of the body fluids is kept practically constant, the blood varying little from the slightly alkaline condition of $p\mathrm{H} = 7.4$, (neutrality

[58] Marked histological alterations are found in the liver of such an acid-poisoned rabbit (See Hess and Goldstein, Wien. klin. Woch., 1924 (37), 689), and the tissues show an increased permeability for vital stains (lithium carmine) according to Asada (Amer. Jour. Physiol. 1919 (50), 20).

[59] Nash and Benedict have advanced the hypothesis that the ammonia excreted in the urine is formed exclusively in the kidney during the elimination of acids (Jour. Biol. Chem., 1921 (48), 463. See also Bigwood, Bruxelles-Méd., Nov. 1, 1923). But Keeton (Jour. Biol. Chem., 1921 (49), 411) found evidence that the neutralization of inorganic acids takes place especially in the portal area.

[60] This has been clearly shown by Eppinger (Wien. klin. Woch., 1906 (19), 111), who found that administration of considerable quantities of amino-acids (glycine, alanine, aspartic acid) to rabbits greatly increased their resistance to acid intoxication, presumably by yielding ammonia through normal steps of protein metabolism.

[61] Van Slyke (See Physiol. Reviews, 1921 (1), 141) has pointed out that in addition to the buffer action of the bicarbonates, phosphates, and proteins in the plasma, the hemoglobin is a very important factor, because of the fact that when hemoglobin changes from reduced to oxidized hemoglobin, at the same time it changes from a weak to a stronger acid. He says that the chief carbon dioxide carrier of the blood is the hemoglobin. It is almost as completely responsible for the transport of carbon dioxide in the blood as it is for the transport of oxygen. Of the alkali furnished to neutralize the CO_2 that enters the venous blood, from 80 to 95 per cent., sometimes possibly all, comes from the hemoglobin. Of such alkali, the greater part is set free when the relatively strong acid, oxyhemoglobin, is changed by loss of oxygen to the weaker one, reduced hemoglobin. A smaller part of the alkali is furnished by the unchanged oxyhemoglobin, which is itself an efficient buffer at blood reaction. The relatively slight remainder is furnished by the other buffers, viz., the phosphate in the cells, the proteins in the plasma, and the bicarbonate. By means chiefly of the exchange of carbonic and hydrochloric acids with the plasma, the cells are enabled to use their reserves of buffer alkali to maintain constancy of reaction in the plasma, although the buffers themselves (hemoglobin and phosphates) do not leave the cells.

being at pH = 7.0) while the tissue fluids are generally slightly less alkaline. As pointed out especially by Henderson,[62] the normal reaction of the body is kept practically constant chiefly by: (1) The salts of CO_2 and H_3PO_4, existing in such proportions of carbonate, bicarbonate, and carbonic acid, or disodium- and monosodium-hydrogen-phosphate, as to produce an almost neutral solution. These being salts of weak acids with strong bases it follows that when a stronger acid, such as lactic or butyric, combines with the bases there is only the weak acid liberated and hence the influence of the strong acid on the blood reaction is greatly reduced. (2) The acid most abundantly formed in metabolism, CO_2, is volatile and hence is rapidly excreted by the lungs without withdrawing bases from the blood. (3) The kidneys can eliminate the other buffer acid, PO_4, with but a minimum of base attached in the form of NaH_2PO_4; and they also remove the basic product of metabolism, ammonia. However, the kidneys play a subordinate rôle in removing acid, since, according to Wilson,[55] the equivalent of 20 to 40 liters of normal acid are excreted by the lungs and but 50 to 150 c.c. by the kidneys during 24 hours. Much of the excretion of base is by the bowels, particularly if there is an excess of bases.[63]

By the combined influence of these factors the acids formed in metabolism are passed out with a maximum rapidity and with a minimum alteration in the reaction of the fluids by which they are carried through the body. In addition to these we have, as mentioned before, the capacity of the proteins to combine with both acids and alkalies, the reserve neutralizing capacity of ammonia formed in metabolism, and also the enormous reserve supply of bases in the bone salts.[64] So effective is this mechanism that accurate determination of the H-ion concentration of the blood shows that very rarely is there more than the slightest deviation from the normal proportion of free H and OH ions, which is slightly on the alkaline side of exact neutrality. This neutrality is one of the most fixed of all the constants of the body, and, as stated by Hawk, a change equivalent to the difference between tap water and distilled water might be fatal.

Wilson[55] says that although the limits of variation in reaction within which life is possible are still in doubt, blood as acid as pH 6.95 and as alkaline as pH 9.00 has been reported. The limits of variation in the normal individual which result from temporarily taxing the available mechanisms for maintaining neutrality, are nearly as great as the extremes just mentioned. A pH of 7.05 has been found after short periods of strenuous muscular exercise. On the alkaline side a pH of about 7.85 has been observed after forced breathing. The difference in

[62] See Harvey Society Lectures, 1914–5; see also Jour. Biol. Chem., 1924 (59), 379.

[63] Shohl and Sato, Jour. Biol. Chem., 1923 (58), 235.

[64] The significance of Ca and Mg in binding acids is shown by the observations of Rabl and of Bernhardt (Zeit. klin. Med., 1924 (100), 735), who found that by administering NH_4Cl, which causes acidosis, so much of these salts may be removed from the bones that the change is visible in roentgenograms, and the bones may even become pliable so that corrective procedures for deformity may be undertaken after 6–14 days NH_4Cl treatment.

*p*H between arterial and venous blood has been studied by several investigators, and Parsons calculated the change in *p*H as but 0.02.

Acidosis, therefore, is a condition in which the essential feature is not an actual acidity of the blood, but the impoverishment of the body in available bases, whereby there results a decreased capacity of the tissues to get rid of CO_2 and other acids formed in their metabolism. This reduction in bases may be, and most usually is, the result of excessive production of acids, in excreting which the bases are eliminated in excess, but it may also result from deficient capacity of the kidneys to excrete acids, since the kidneys play an important rôle in regulating acidity. Macleod[65] summarizes the conditions that might give rise to changes in the hydrogen ion concentration (*c*H) in the blood as follows:

	Increase of *c*H
Addition or accumulation of acid	Accumulation of CO_2 (asphyxial conditions). Incomplete oxidation of carbohydrate (lactic acid in muscular exercise).
	Defective oxidation of fat (ketosis).
	Renal insufficiency (nephritis).
	Decomposition of protein (as in acidosis of fever).
	Intestinal fermentation.
	Administration of acid (experimental).
Decrease of base	Diarrhea and hemorrhage, respectively (may explain acidosis in cholera and in certain forms of shock).[66]
	Decrease in *c*H.
Addition or accumulation of base	Ammonia (faulty metabolism of urea).
	Intestinal putrefaction (infantile conditions).
	Administration of alkalies (experimental and therapeutic).
Removal of acids	Excretion of CO_2 (excessive pulmonary ventilation, as in faulty ether administration).
	Excretion of acid urine.

Practically, acidosis results either from defective oxidation of organic acids formed in metabolism or from defective elimination of mineral acids (acid phosphate) because of impaired renal function. The chief example of the former is the acidosis of diabetes, of the latter the acidosis of nephritis, and mixed forms may occur. The degree of acidosis may be estimated in several ways, as follows:[65]

1. By determining the CO_2 content of the blood, which must decrease as other acids increase, or as the bases decrease.
2. Direct estimation of the H-ion concentration of the blood.
3. By determining the amount of acid or alkali necessary to change the reaction of the blood to different indicators.
4. Determination of the CO_2 tension of the alveolar air, this varying directly with the CO_2 tension of the arterial blood.
5. The "alkali tolerance test" of Sellards, which consists in ascertaining the amount of sodium bicarbonate that must be taken by mouth in order to produce an alkaline urine.[67]
6. Estimation of the amount of organic acids, H-ion concentration, and ammonia content of the urine; a method which is fundamentally defective since it indicates

[65] See reviews by Frothingham, Arch. Int. Med., 1916 (18) 717; Macleod, Jour. Lab. Clin. Med., 1919 (4), 315.

[66] To this may be added loss of base from biliary or pancreatic fistulæ.

[67] See critique by Palmer *et al.*, Jour. Biol. Chem., 1920 (45), 101.

merely the acids and bases that have been removed from the body and not those that remain to modify its reactivity.

7. Determination of the capacity of the blood serum to bind CO_2. Normal serum binds about 55 to 75 per cent. of its volume of CO_2, whereas in acidosis it may bind but 20 per cent. (Van Slyke).

Diabetic Coma[55]

In man, poisoning with inorganic acids, as in the experiments cited above, is a rare occurrence, but not infrequently acid intoxication results from the presence of undue quantities of organic acids produced in metabolism. The most striking example of this is the coma of diabetes, in which the asphyxia without cyanosis, dependent upon failure of the blood to carry CO_2, is sometimes strikingly similar to that observed in experimental animals poisoned with acids. In diabetic coma the acid intoxication is due chiefly to the accumulation in the blood or tissues of large quantities of *beta-hydroxybutyric acid* (*β-oxybutyric acid*). Associated with it, in smaller quantities, are usually found *diacetic* (*acetoacetic*) *acid* and *acetone*, which are chemically so closely related that it was once generally considered that they are derived from the hydroxybutyric acid, as follows:

β-hydroxybutyric acid is—

$$CH_3—CHOH—CH_2—COOH,$$

and by oxidation this readily forms—

$$CH_3—CO—CH_2—COOH,$$

which is diacetic acid (being two molecules of acetic acid united to each other, as follows):

$$CH_3—CO—\overline{|OH—H|}—H_2C—COOH.$$

Diacetic acid is, in turn, readily deprived of its carbon dioxide, forming acetone,

$$CH_3—CO—CH_3.$$

All these reactions are easily accomplished in the laboratory, and there seemed to be reason for believing that they may normally occur in the same way in the animal body.[68] Wakeman and Dakin,[69] and others, however, found evidence that the liver cells may also *reduce* diacetic to β-hydroxybutyric acid, and it seems probable that this is the usual direction of the reaction, which is believed to be produced by a specific enzyme. In what tissue these ketone bodies are chiefly formed is not known, but the liver is probably not the sole, and perhaps not the chief site of their formation.[70]

Hurtley[55] concludes that the reduction of aceto-acetic acid to hydroxybutyric acid is accomplished by the body under ordinary conditions far

[68] Small quantities of acetaldehyde and perhaps other aldehydes have also been found in diabetic urine (See Fricke, Zeit. physiol. Chem., 1920 (116), 129) and there is evidence that other acids than the acetone-bodies may at times assume importance in diabetic acidosis (See Bock *et al.*, Jour. Metabol. Res., 1923 (4), 27); Starr and Fitz, Arch. Int. Med., 1924 (33), 95).

[69] Jour. Biol. Chem., 1909 (6), 373; 1910 (8), 105.

[70] See Allen and Ebeling, Jour. Metabol. Res., 1923 (4), 423.

more readily than the oxidation of hydroxybutyric to aceto-acetic acid. Marriot[71] gives the following scheme as indicating the normal path of fatty acid catabolism:

Fatty acid ⟶ Butyric acid(?) ⟶ Aceto-acetic acid ⟶ d-hydroxybutyric acid (readily burned) / l-hydroxybutyric acid (difficultly burned)

Hence the present understanding is that aceto-acetic acid is first formed, and if not destroyed as it normally should be, is partly decomposed to form acetone and CO_2, or reduced to hydroxybutyric acid. Since these substances depend on the formation of a keto acid, their accumulation is called "ketosis." Folin says that perfectly fresh diabetic urine does not contain any acetone, that which is commonly found being derived from diacetic acid which rapidly decomposes into acetone. Acetone may be demonstrated in the breath, however.[72] By usage, we speak of acetonuria or acetone-bodies, with the understanding that the term covers all three substances and that acetone may or may not be present.

The study of the utilization of the acidosis substances when injected intravenously at accurately measured rates for considerable periods by Wilder,[73] furnishes conclusive evidence of the origin of β-hydroxybutyric acid from acetoacetic acid. He found that normal dogs excrete hydroxybutyric acid when it is injected at the rate of 0.4 gm. (0.0032 gm. molecule) of the sodium salt per kilo of body weight per hour, but not with lower rates. Sodium acetoacetate was excreted when injected in rates of 0.2 gm. (0.0016 gm. molecule) per kilo per hour, and when injected at 0.4 gm. rate it was excreted accompanied by hydroxybutyric acid. Evidently, then, the acetoacetic acid must be converted almost quantitatively into hydroxybutyric acid. On the other hand, acetoacetic acid did not appear in the urine when larger quantities of hydroxybutryic acid were injected, and hence it is apparent that not much if any urinary acetoacetic acid is derived from this source.

As long as a normal individual is burning at least one molecule of carbohydrate to three of higher fatty acids the urine is free from more than traces of these three "acetone bodies,"[74] and hence carbohydrates and other substances which prevent ketosis are spoken of as "antiketogenic."[75] But when for any reason the daily oxidation of carbohydrates falls below this minimum the acids appear, combined largely with ammonia, but partly with mineral bases. "Fats burn in the fire of the carbohydrates," and, as Woodyatt[76] puts it, when the proportion of fat is

[71] Jour. Biol. Chem., 1914 (18), 241.

[72] See Higgins, Johns Hopkins Hosp. Bull., 1920 (31), 447; Briggs and Shaffer, Jour. Biol. Chem., 1921 (48), 413.

[73] Jour. Biol. Chem., 1917 (31), 59.

[74] Veeder and Johnstone (Amer. Jour. Dis. Chil., 1916 (11), 291) give as the normal daily average excretion 32 mg. of ketones (diacetic acid and acetone) and 38 mg. hydroxybutyric acid. The old statement that acetone appears in advance of the two acids is incorrect, the error being due to faulty methods (See Howland and Marriott, Amer. Jour. Dis. Chil., 1916 (12), 459).

[75] See review on Antiketogenesis by Shaffer, Harvey Soc. Lectures, 1922–3, p. 105.

[76] Jour. Amer. Med. Assoc., 1916 (66), 1910.

too great for the fire it "smokes" with unburnt fats and acetone bodies. Since glycerol and the amino acids which are capable of preventing ketosis are also capable of forming glucose, it may be that only carbohydrates are antiketogenic. Apparently some product formed in the oxidation of glucose interacts with the ketone substances, and further oxidation destroys the compound; in the absence of glucose oxidation the ketone bodies cannot themselves be oxidized (Woodyatt).[77]

The presence of large quantities of these keto-acids in the urine presages coma, during which the amount of hydroxybutyric acid often reaches 15–20 grams per day, and has been known to reach 150 grams (Külz reported finding 226 grams). Diacetic acid appears in relatively small amounts, rarely exceeding 10 per cent. of the total organic acids of the urine; as a rule, when any one of the three acetone bodies is present in large amounts, there is an abundance of each of the others.[78] The greater part of the acetone is eliminated in the breath, and, when given by mouth, large doses are required to cause an increase in the urine. Normally but 2 to 5 per cent. of the nitrogen of the urine is in the form of ammonia, but in diabetic *acidosis* the proportion may reach from10 to 25 per cent., the proportion of urea being correspondingly reduced.[79]

In the internal organs the acetone bodies may also be detected, especially in the liver.[80] In normal blood Marriott found less than 4 mg. of hydroxybutyric acid, and 1.5 mg. of acetone and aceto-acetic acid together, per 100 c.c.; but in diabetic coma the figures rose as high as 45 mg. and 28 mg. respectively for each fraction, the amount in the blood not corresponding to the urinary excretion,[81] or to bicarbonate content of the blood.[82] Associated with acidosis is usually an increase in the blood lipins.[83] (See Lipemia, chap. xviii.)

Relation of Acidosis to Diabetic Coma.—There seems to be little room for doubt that the typical diabetic coma with "air hunger" depends upon an excess of these substances in the blood—*i. e.*, is an acid intoxication—for the following reasons: (1) The coma usually appears when the amount of organic acids in the urine is highest, and is absent when there is little or none of them in the urine. (2) Because of the resemblance of the symptoms to those of experimental acid intoxication. (3) Because of the repeated demonstration of a reduced amount of alkali in the blood, as determined by titration, and a great reduction of the amount of CO_2 carried in the venous blood. The capacity of the serum to absorb CO_2 *in vitro* is also greatly reduced, from a normal 55 to 75 per

[77] Jour. Amer. Med. Assoc., 1910 (55), 2109. See also Shaffer, Jour. Biol. Chem., 1921 (47), 433.

[78] Kenneway (Biochem. Jour., 1913 (8), 355) confirms Neubauer's statement that hydroxybutyric acid is rather constantly from 60 to 80 per cent. of the total acetone bodies excreted in the urine.

[79] According to Edie and Whitley (Biochemical Jour., 1906 (1), 11), administration of excessive amounts of alkali causes, conversely, elimination of increased amounts of organic acids.

[80] Sassa, Biochem. Zeit., 1914 (59), 362.

[81] Marriott, Jour. Biol. Chem., 1914 (18), 507.

[82] Fitz, Trans. Assoc. Amer. Phys., 1917 (32), 155.

[83] Gray, Bost. Med. Surg. Jour., 1917 (178), 16–156.

cent. to as low as 20 per cent. (Van Slyke). By means of gas-chain measurements Rolly[84] found that the lowest OH values observed in the blood are in diabetic coma;[85] and Sellards[86] showed that in diabetes the tolerance for alkalies may be increased. He states[55] that a deficit of 20 to 30 grams of sodium bicarbonate produces a degree of acidosis demonstrable by blood examination only, 40 to 50 grams deficit usually causes dyspnoea only on exertion, 75 to 100 grams deficit may produce persistent dyspnoea, 150 grams deficit may accompany coma, while the maximum deficits reported have been about 200 grams.[87] (4) The marked improvement that sometimes results from the administration of alkalies (usually sodium bicarbonate). Associated with this improvement is an elimination of greatly increased amounts of organic acids, indicating their previous retention in the body because of lack of alkali with which they could combine.

But there are cases of diabetic coma without typical air hunger, and it is the exception rather than the rule for alkali therapy to produce a marked improvement in the fully developed coma of diabetes. Furthermore, coma may occur in diabetics who are producing no such quantity of organic acids as would seem theoretically to be necessary to cause enough acid intoxication to result in acidosis, and coma develops in diabetics who are being supplied with sufficient bases for all requirements. Hence it must be concluded that only a part of the symptomatology of diabetic coma depends on acids as such, but as yet we do not know what other agents are acting.[88]

β-hydroxybutyric and diacetic acid, according to many authorities, seem to have no specific poisonous effects, but act simply as acids in the blood. Acetone does not have this effect, not being an acid, and seems not to be toxic to any considerable degree; doses of 4 grams per kilo cause effects similar to ethyl alcohol in dogs, 8 grams per kilo being fatal, which corresponds to a dose of 500 grams for an adult man. Widmark[89] observed no toxic symptoms in rabbits given acetone until the blood contained 0.8 per cent., and Briggs and Shaffer[90] observed no toxic effects in dogs given 1.0 to 1.45 gms. per kilo intravenously, although very large doses will kill.[91] According to Rhamy,[92] acetone is more toxic (for guinea-pigs) than methyl alcohol, while for rabbits Desgrez and Saggio[93] found acetone the least toxic of the acetone bodies, diacetic acid next, and β-hydroxybutyric acid most. Ehrmann[94] also says that he has produced

[84] Münch. med. Woch., 1912 (59), 1201.

[85] Menten, however, reports that in diabetes before acidosis the OH concentration is usually somewhat above normal (Jour. Cancer Res., 1917 (2), 179).

[86] Johns Hopkins Hosp. Bull., 1912 (23), 289.

[87] Death occurs in diabetic acidosis when the *p*H falls as low as 7.00–6.94, according to Endres (Deut. Arch. klin. Med., 1925 (146), 51).

[88] See Allen and Wishart, Jour. Metabol. Res., 1923 (4), 223.

[89] Acta med. Scand., 1919 (52), 87.

[90] Jour. Biol. Chem., 1921 (48), 413.

[91] See Schlomowitz and Seybold, Amer. Jour. Physiol., 1924 (70), 130; Salant and Kleitman, Jour. Pharm., 1922 (19), 293.

[92] Jour. Amer. Med. Assoc., 1912 (58), 628.

[93] Compt. Rend. Soc. Biol., 1907 (63), 288.

[94] Berl. klin. Woch., 1913 (50), 11.

typical coma with the sodium salts of butyric and of β-hydroxybutyric acid, but as high as 40 grams of β-hydroxybutyric acid have been found in the day's urine of a non-diabetic without any evidence of intoxication. Ewing suggests that the acetone bodies may cause renal injury, which is usually evident in acidosis. It has been found that β-hydroxybutyric acid has an inhibitory effect on many different sorts of enzymatic actions, but trypsin and autolytic enzymes are not depressed,[95] which may explain in part the heightened tissue destruction observed in diabetic acidosis. The withdrawal of the inorganic bases, especially Ca and Mg, may also be responsible for symptoms, as it is well established that a proper balancing of these ions is necessary for normal cell activity, especially in the nervous system.

Hurtley[55] sums up the evidence on the toxicity of the acetone bodies by saying that aceto-acetic acid seems to be highly toxic only in depancreatized animals, while hydroxybutyric acid is practically non-toxic. He favors the view that aceto-acetic acid poisoning is responsible for diabetic coma, for it increases in the urine on the approach of coma, and the ratio of aceto-acetic to hydroxybutyric acid in the urine increases with the severity of the intoxication. The increased proportion of aceto-acetic acid presumably means that it is being produced in such quantities throughout the body that the liver cannot reduce as large a proportion to hydroxybutyric acid as it normally does. In considering the possibility that the acetone bodies may be responsible for at least part of the intoxication of diabetic coma we must bear in mind that the evidence of their low toxicity is based on short time experimental intoxications, and that they may be found to be much more toxic than is generally assumed when they are allowed to act for many days and weeks on the nervous tissues, as they do in diabetes. That is, the experimental evidence concerning the toxicity of the acetone bodies has not been obtained under conditions comparable to those of diabetic acidosis.

Origin of the Acetone Bodies.—The chemical nature of the acetone bodies is such that they might readily be produced from any or all of the three classes of foodstuffs.

They might be derived from *carbohydrates*, as is the closely related lactic acid, but we know that this is not the usual source. On the contrary, administration of a proper amount of carbohydrates under certain conditions may cause the acids to disappear from the urine, and acetone bodies may be eliminated in large quantities while the patient is on a diet almost free from carbohydrates. Carbohydrates are, indeed, the most active agents in preventing the formation of these ketone bodies, *i. e.*, they are *antiketogenic*.[96]

They might readily be formed from *proteins* through splitting out of the NH_2 group from the amino-acids; indeed the amino-acids are generally considered as a source of the acetone bodies,[97] particularly because, whenever there is considerable

[95] Harpuder and Erbsen, Klin. Woch., 1924 (3), 2010.

[96] Concerning antiketogenesis see Woodyatt, Jour. Amer. Med. Assoc., 1910 (55), 2109; Shaffer, Harvey Soc. Lectures, 1922–3, p. 105.

[97] Embden and his associates have (Hofmeister's Beitr., 1906 (8), 121; 1908 (11), H. 7–9) demonstrated that the liver can form acetone from many substances perfused through it in the blood, including not only amino-acids of the fatty acid series, but also the aromatic radicals of the protein molecule.

pathological breaking-down of proteins, these bodies, especially acetone, may appear in the urine; *e. g.*, during absorption of exudates, in carcinoma, and in starvation or other conditions with great wasting of the tissues. Dakin[98] has shown that only leucine, histidine, phenylalanine and tyrosine yield diacetic acid when perfused through the liver, while most of the other amino-acids except lysine and tryptophan are able to yield sugar in diabetic animals, and hence are antiketogenic.

On the other hand, the amount of acids sometimes found in the urine seems to be greater than can be explained by the protein destruction that occurs (Magnus-Levy),[99] and in diabetes it is often observed that feeding of fats and fatty acids increases the output of acetone bodies, and hence it is evident that acetone bodies may be derived from the *fats*. β-hydroxybutyric acid can be produced readily from fatty acids, especially, of course, from butyric acid, and we usually observe an increase in the acetone excretion in a diabetic given large quantities of butter. Other higher fatty acids are also found to cause increased acetone excretion.

The studies of Knoop,[1] and his associates have indicated that in the catabolism of fatty acids, the chains are broken down by oxidation of the carbon atom third from the end, that is, the β-position, and the two end carbon atoms are then split off. Therefore, two carbon atoms are always split off at a time, and hence there can be oxidized into hydroxybutyric acid only those fatty acids which contain an *even* number of carbon atoms, which includes the ordinary fatty acids (oleic, palmitic, and stearic) of fat tissue, which have each an even number of carbon atoms (16 or 18), and also butyric, caproic, and similar acids. Normal fatty acids which contain an odd number of carbon atoms cannot yield hydroxybutyric acid.[2] However, according to A. Loeb,[3] aceto-acetic acid may be built up from acetic acid in the liver, and the urine in diabetes may contain acetic acid. "The formation of oxybutyric acid and of diacetic acid in all these cases may be said to be due to the fact that the diabetic organism is not able quite to finish the attack on the beta-carbon atom of butyric acid" (Folin).

From the results of these studies it seems that the acetone bodies *can*, theoretically, be formed from any of the three classes of food-stuffs, but that ordinarily they come chiefly from the fats, and in severe diabetes also to a considerable extent from fatty acids formed by deaminization of amino-acids. Although it is probable that the acetone bodies are formed in many if not all tissues, yet there is abundant evidence that the liver plays an important part in ketogenesis, as shown by the decrease in acetone bodies in Eck fistula dogs, and their great increase when the blood supply of the liver is augmented.[4]

Acidosis and Acetonuria in Conditions Other than Diabetes[5]

When our chief method of recognition of acidosis consisted of determining the presence of acetone bodies in the urine, the term acetonuria was used as synonymous with acidosis, but we now know that we may have varying degrees of acetonuria without significant acidosis as determined by examination of the blood, and severe acidosis may occur with little or no acetonuria.

Furthermore, we may have high urinary ammonia not only because of excretion of diacetic and hydroxybutyric acids as in diabetic acidosis, but also from excretion of excessive quantities of lactic acid, as in puer-

[98] Jour. Biol. Chem., 1913 (14), 328.

[99] Arch. exp. Path. u. Pharm., 1899 (42), 149; Ergeb. inn. Med., 1908 (1), 374.

[1] Full bibliography and discussion by Porges, Ergebnisse Physiol., 1910 (10), 6. See also Ringer, Jour. Biol. Chem., 1913, Vol. 14; Dakin, Physiol. Reviews, 1921 (1), 394.

[2] If diabetics receive a fat with an odd number of carbon atoms (intarvin) they may excrete acids with an odd number of carbon atoms instead of the usual acetone bodies (Lundin, Jour. Metabol. Res., 1923 (4), 151; Modern, *ibid.*, 177).

[3] Biochem. Zeit., 1912 (47), 118.

[4] Fischer and Kossow, Deut. Arch. klin. Med., 1913 (101), 479.

[5] See Sellards "Principles of Acidosis," Harvard Press, 1917; also Frothingham Arch. Int. Med., 1916 (18), 717.

peral eclampsia, pernicious vomiting of pregnancy, acute yellow atrophy, and other conditions associated with severe injury to the liver. As high ammonia excretion is characteristic of diabetic acidosis, the presence either of acetone bodies or high urinary ammonia was formerly considered to indicate the existence of acidosis, but it is now recognized that as a general rule there is not a severe acidosis in these hepatic diseases, the intoxication being dependent on neither lactic acid nor acidosis, but upon poisons of unknown character. This group of diseases has been considered in previous pages. Because of its significance as one of the chief organic acids formed in metabolism, rather than as a cause of serious acidosis, we may in this chapter briefly consider lactic acid and its relation to disease.

Sarcolactic Acid often is found in the urine, but in origin and significance it is entirely different from the acetone bodies, and it probably is never present in sufficient amounts to cause an acid intoxication by abstraction of alkalies from the blood. During muscular activity lactic acid is formed in the muscle, and Hill[6] says that in violent exertion as much as 3 grams may be liberated, in which case there may be as much as 0.2 per cent. in the blood and 0.35 per cent. in the muscles. This is compensated by hypernoea with removal of CO_2 by the lungs, and a little lactic acid escapes in the urine. Embden[7] says that lactic acid increases progressively in the active muscle and is responsible for maintaining contraction.

In vitro, we obtain sarcolactic acid whenever sugar is placed in an alkaline solution, *provided the supply of oxygen to the solution is deficient;* but if the oxygen supply is adequate, sugar will not yield lactic acid with alkalies (Nef). Similarly, an isolated surviving muscle, when asphyxiated by any means, shows a rapid accumulation of lactic acid, which fails to occur when sufficient oxygen is supplied. This lactic acid comes chiefly from sugar, but about 25 to 30 per cent. of it can have its origin in protein (or fat?) (Woodyatt). If an organism as a whole is insufficiently supplied with oxygen, lactic acid accumulates in the tissues and appears in the urine, disappearing when the oxygen supply is restored. Lactic acid often appears after poisoning with a large number of drugs, which Loewy has classified as drugs whose action in the body resembles that of lack of oxygen (arsenic, phosphorus, hydrazine, chloroform, etc.). These poisons are all characterized by causing impoverishment of glycogen, fatty liver, and acute degenerative changes, especially in the liver cells and the endothelium. Therefore the assumption seems justified that the poisons and conditions which lead to lactic acid excretion depend ultimately upon impairment of the interchange of oxygen in the cells. Woodyatt states that, as far as known, lactic acid has never been demonstrated in any tissue in which deficient oxygenation can be excluded, and regards lactic acid as the metabolite of asphyxia or its equivalent. Over against this view is that of Embden and his associates, which is shared by others, that lactic acid is a normal intermediary in the breakdown of the sugars in the body, its direct antecedent being a triose, but perusal of their work only emphasizes that in all the conditions in which their data were obtained asphyxial conditions were present; furthermore, this conception of lactic acid as a chief intermediate in normal sugar catabolism is not in harmony with the best ideas of carbohydrate chemistry (Woodyatt). This author has furthermore found, by direct observation of the utilization of lactic acid when injected intravenously, that it cannot well be an important intermediate in carbohydrate catabolism.[8] (See also discussion of lactic acid under Diabetes, chapter XXVI.)

It is possible that the presence of lactic acid in the urine may also result from defective transformation of ammonia into urea by a diseased liver, the acid neutralizing, and being excreted with, the ammonia; in this case no defective oxidation need be assumed. However, administration of phlorhizin to phosphorus poisoned dogs causes both ammonia and lactic acid to disappear from the urine, indicating that the ammonia is the protective substance which neutralizes the lactic acid, and not the

[6] Lancet, 1924 (2), 361.
[7] Klin. Woch., 1924 (3), 1393.
[8] Harvey Society Lectures, 1916.

reverse. Likewise, lactic acid acts as a defensive mechanism when excess of alkali is administered, appearing in the urine in slightly increased amounts.[9]

Sarcolactic acid, which is dextrorotary, must be distinguished from its optical isomer, the inactive lactic acid that is produced by fermentation. When this fermentation lactic acid is formed in the stomach and enters the blood, it ordinarily, like other ingested organic acids, is combined by the blood alkalies and oxidized to carbonates. It is doubtful if it ever enters the urine.[10]

As a general rule sarcolactic acid is not found abundant in the urine together with the acetone bodies, but is, indeed, antiketogenic. Its appearance in the urine indicates that glycogen is not completely burned, and this condition is usually accompanied with fatty changes in the liver, which also may depend on lack of oxidation. Throughout the clinical forms of acidosis, lactic acid and fatty degeneration are always associated (Ewing). To assume, as has been generally done, that the lactic acid appears in the urine when hepatic alterations are marked, because of the loss of the liver tissue which should destroy it, is probably not warranted. Rather, the liver conditions and the formation of lactic acid depend upon the same cause, which is a defective oxygen supply or interchange, either general or local.[11]

Acidosis in Nephritis.—This presents a very different urinary chemistry from diabetic acidosis, in that there is no excessive excretion of organic acids or ammonia, and indeed no other striking urinary change to account for the acidosis which may be severe, undoubtedly often terminating life.[12] There is a definite increase in the inorganic phosphate content of the blood in nephritis with acidosis (Marriott and Howland[13]), and often the urinary acidity is decreased, so the acidosis is attributed to reduced capacity of the kidney to excrete acid phosphates which is one of the most important normal mechanisms for maintaining the neutrality of the blood. Chace and Myers[14] found that all fatal cases of chronic nephritis with marked nitrogen retention show severe acidosis, often fatal. In fatal cases the plasma phosphate may reach ten times the normal figure, whereas in non-fatal cases the phosphate increase is relatively slight.[15] The nocturnal hyperpnœa of nephritis probably is the result of acidosis (Whitney).

The most marked acidosis is observed in those types of nephritis that are associated with uremia, *i. e.*, advanced chronic glomerulo-nephritis and acute nephritis, but not in the chronic parenchymatous types. Measuring the acid-binding power by determining the amount of acid that can be added to the blood before agglutination of the corpuscles occurs, Rieger and Freund[16] found evidence that this is reduced in glomerulo-nephritis before there is demonstrable nitrogen retention or other evidence of renal lesions, whereas tubular and circulatory changes do not produce this effect.

[9] Macleod and Knapp, Amer. Jour. Physiol., 1918 (47), 189.

[10] The theory of Boix that cirrhosis of the liver may be produced by butyric acid formed in gastric fermentation could not be corroborated by Joannovics, Arch. int. Pharmacodyn., 1905 (15), 241.

[11] See Macleod and Wedd (Jour. Biol. Chem., 1914 (18), 446) who found that reducing the oxygen supply to the liver caused a marked rise in the lactic acid content of the hepatic blood. Also Mendel *et al.* (Klin. Woch., 1925 (4), 306) who found an increased lactic acid content in the venous blood obtained from veins distal to a constricting band.

[12] See Whitney, Arch. Int. Med., 1917 (20), 931.

[13] Arch. Int. Med., 1916 (18), 708.

[14] Jour. Amer. Med. Assoc., 1920 (74), 641.

[15] Denis and Minot, Arch. Int. Med., 1920 (26), 99.

[16] Arch. Int. Med., 1922 (30), 517.

Acidosis generally parallels in degree the impairment in excretory capacity of the kidney, and, in contrast to diabetes, it does not reach a high grade so long as the excretion of acid by the kidney is comparatively efficient (Sellards).[55] While administration of sodium bicarbonate corrects the acidosis it has little effect on the course of the disease, or on the amount of phosphate in the blood. With this high phosphatemia there is a reduction in the blood calcium, which may have an unfavorable influence on the irritability of the nervous tissues. Whitney[12] believes that in nephritis with acidosis there is probably some excessive production of acid, since a kidney with greatly impaired excretory capacity may be able to maintain a normal acid threshold. Begun and Münzer[17] attribute part of the acidosis to a decreased formation of NH_3 in metabolism, while Nash and Benedict,[18] believing that the urinary ammonia is formed only in the kidney in eliminating acids, suggest that acidosis may result from either decreased ability of the kidney to eliminate acids, so that acid retention occurs, or because of lessened ability to form ammonia so that other bases are excreted with the urinary acids and alkali depletion results.

Other Diseases with Acidosis.—While a slight degree of acidosis undoubtedly may occur in many conditions, there are few conditions except diabetes and nephritis in which it is of importance. Chief of these are the following:

Asiatic Cholera exhibits often a severe acidosis, presumably because of the frequency of severe renal lesions (Sellards[55]). Loss of bases in the evacuations may also be a factor. The acidosis differs from that of nephritis in the excretion of large amounts of ammonia, but usually without acetone bodies. Other infectious diseases do not commonly exhibit any significant degree of acidosis, rheumatic fever alone possibly excepted.[19]

Acidosis in Infancy.—Both true acidosis and acetonuria, with or without acidosis, occur frequently and easily in the young. Normally the urine of infants and children contains about 3 mg. of acetone per kilo of body weight, which may be increased to ten times that amount by fasting,[20] or removed by giving carbohydrates.[21] Also the amount of acetone bodies in the blood is greater and more readily increased by fasting than in adults.[22] Presumably the infantile organism has a lower oxidative capacity, since it excretes unoxidized organic acids from relatively slight causes, in corroboration of which is the observation of Pfaundler[23] that the proportion of nitrogen in the urine of infants in forms other than urea is higher than in adults. Acidosis is especially important in severe choleriform diarrhea, possibly because of excretion of bases in the discharges, and in burns and severe nephritis, acidosis is of significance. In ileocolitis true acetonemic acidosis has been observed. Acetonuria of mild degree is often seen in children with acute infections.[24]

However, according to Howland and Marriott[25] serious acidosis in infancy and childhood, although frequent, is usually not due to the acetone bodies, and McNider[26]

[17] Zeit. exp. Path., 1919 (20), 78.
[18] Jour. Biol. Chem., 1921 (48), 463.
[19] Frothingham, Arch. Int. Med., 1916 (18), 717.
[20] Veeder and Johnstone, Amer. Jour. Dis. Child., 1917 (13), 89.
[21] Schick and Wagner, Zeit. Kinderheilk., 1924 (37), 336.
[22] See Moore, Amer. Jour. Dis. Chil., 1916 (12), 244. The statement that there is usually a relative acidosis in newborn infants could not be corroborated by Seham, *ibid.*, 1919 (18), 42.
[23] Jahrb. f. Kinderheilk., 1901 (54), 247.
[24] Veeder and Johnstone, Amer. Jour. Dis. Chil., 1920 (19), 141.
[25] Amer. Jour. Dis. Child., 1916 (12), 459.
[26] Arch. Int. Med., 1920 (26), 1.

found that young animals have a greater capacity to maintain a normal alkali reserve despite injured kidneys than old animals.

Terminal Acidosis.—In many dying persons the final symptomatology is strikingly like that of death from acidosis, and Whitney[12] has found that the final figures for alkali reserve in the blood of animals killed by acid intoxication are of the same order as those that may sometimes be obtained from dying patients with many different diseases. Of forty cases studied by him, in all but three there was marked acidosis, and in many there was a degree of acidosis sufficient of itself to cause death. Sellards notes typical acidosis in advanced *atrophic cirrhosis*, but this has not yet been sufficiently studied to permit of proper interpretation. Whenever the blood pressure becomes greatly lowered, as in *shock*, there may occur an apparent acidosis, with lowered alkali reserve but actually with decreased *c*H.[27] The blood alkali reserve of animals injected with bacteria shows a temporary fall coincident with the initial leucopenia, returning to normal as the leucocyte count rises.[28] Fasting and starvation cause acidosis which often becomes severe.[29]

Severe anemia, both primary and secondary, may exhibit a moderated degree of acidosis, but in only about one fifth of all cases examined by Sellards.

Pregnancy Toxicoses.—The blood plasma of women with toxemias of pregnancy shows ordinarily a loss in alkali reserve, amounting to a distinct acidosis which is not in direct proportion to the degree of intoxication or the amount of renal injury. With convulsive eclampsia the acidosis is increased by the severe muscular activity, but severe acidosis sometimes occurs in eclamptic conditions without convulsions.[30] Even normal pregnant women have 20 to 30 per cent. more acetone bodies in the blood than non-pregnant women,[31] and show a mild acidosis.[31a]

ALKALOSIS[32]

The occurrence of an increase in OH ions of the blood, or of an abnormally high alkali reserve and increased capacity to carry CO_2, seems to occur infrequently, presumably since the trend of metabolic processes is to produce acid substances. Clinically, alkalosis may depend on either an alkali excess or a deficit in CO_2, the *p*H usually being from 7.5 to 7.8. With excessive breathing the CO_2 may be driven off until the *p*H is above normal and an alkalosis exists although there may be a diminution in the blood alkali.[33] Alkalosis may result from administration of alkalies therapeutically, or from loss of acid through vomiting or from a gastric fistula, and hence is sometimes present in pyloric stenosis.[34] If the alkalosis is from excess of alkali the alveolar CO_2 is increased, whereas it is decreased if the alkalosis is the result of loss of CO_2.

The characteristic result of alkalosis from whatever cause is tetany, characterized by muscular irritability and even convulsions, but it is thought that not all tetany depends on alkalosis, *e. g.*, parathyroid tetany (*q. v.*, Chap. XXIV). Alkalosis may cause an upset of carbohydrate metabolism resembling diabetes, with acetonuria and decreased glucose toler-

[27] Moore, Lancet, 1919 (2), 473.

[28] Hirsch, Jour. Amer. Med. Assoc., 1920 (75), 1204; Dragstedt, Jour. Infect. Dis., 1920 (27), 452.

[29] See Asada, Amer. Jour. Physiol., 1919 (50), 1.

[30] See Bokelmann and Rother, Zeit. ges. exp. Med., 1924 (40), 13.

[31] Bock *et al.*, Klin. Woch., 1924 (3), 2294.

[31a] Rowe, Amer. Jour. Physiol., 1925 (71), 660.

[32] See review by Kast, Myers and Schmidt, Jour. Amer. Med. Assoc., 1924 (82), 1858; also Koehler, Arch. Int. Med., 1923 (31), 590; Grant, *ibid.*, 1922 (30), 355.

[33] See Moore, Lancet, 1919 (2), 473.

[34] See Wilson *et al.*, Jour. Biol. Chem., 1915 (21), 169; 1915 (23), 89; McCann, *ibid.*, 1918 (35), 553.

ance (Haldane).[35] There is also a fall in the calcium content of the blood, which is believed to be an important factor in producing the symptoms of tetany. Tetany is improved by giving acids, and presumably the convulsions of the disease have a similar effect through increased acid production. Also, as Greenwald[36] points out, the excess alkalinity of the blood diminishes the dissociation of oxyhemoglobin and causes an oxygen deficiency in the tissues, leading to formation of lactic acid which serves to reduce the alkalinity. Therapeutically, ammonium chloride has been used with success,[37] since it liberates acid by the following steps:

$$2NH_4Cl + CO_2 \rightarrow (NH_2)_2CO + 2HCl + H_2O$$

Menten[38] has found an increased OH-ion concentration in the blood to be characteristic of cancer, whether involving the stomach or not, although sometimes observed in other conditions, especially diabetes before the stage of acidosis. A slight degree of alkalosis may be produced by feeding large amounts of alkali, in which case some of the alkali is removed in the urine combined with lactic acid.[39] In fever, alkalosis may occur of sufficient extent to cause symptoms (Koehler), probably resulting from increased lung ventilation.

ACETONURIA WITHOUT MARKED ACIDOSIS

Not infrequently acetone bodies are found in the urine of patients suffering from the most diverse diseases. It was formerly customary to refer to this condition as "*acetonemia*" or "*acetonuria*," and to ascribe many of the observed symptoms to "acid intoxication." The presence of these substances in the urine, however, is by no means evidence of acidosis, for excretion of considerable amounts of acetone bodies may occur without reduced CO_2-carrying capacity of the blood and they may be absent with marked acidosis; indeed, acetonuria may occur in alkalosis.[40] In addition, it must be kept in mind that acidosis may result from other causes than over-production of organic acids; *e. g.*, acid phosphate retention in nephritis, or loss of bases from biliary or pancreatic fistula. In no other conditions do the amounts of organic acids in the urine approximate the amounts found in diabetic coma. Therefore, the intoxication in these cases is probably not due to the acids, but, on the contrary, the presence of the acetone bodies is due more often to the effects of toxic substances of diverse origins and natures.

Anesthesia.—As shown first by Greven (1895), and especially by Brewer and by Helen Baldwin[41] acetone is nearly always present in the urine during the first twenty-four hours after administration of chloroform or ether, and occasionally diacetic acid appears on the second or third day after; but ordinarily there is no increase in organic acids in the urine. There is usually little or no clinically demonstrable

[35] Lancet, 1924 (1), 536.
[36] Jour. Biol. Chem., 1922 (54), 283.
[37] See Haldane, Jour. Physiol., 1921 (55), 265.
[38] Jour. Cancer Res., 1917 (2) 179.
[39] Macleod and Knapp, Amer. Jour. Physiol., 1918 (47), 189.
[40] See Cammidge, Brit. Med. Jour., Aug. 25, 1923, p. 318.
[41] Jour. of Biol. Chem., 1906 (1), 239.

acidosis,[42] but there is a definite reduction in the alkali reserve with an increase in the *c*H of the blood even without acetonuria, which may depend on accumulation of lactic acid derived from the muscle.[43] When there is a ketosis it does not usually manifest itself for some hours after the anesthesia. Stehle and Bourne[44] found evidence that discharge of phosphoric acid from the muscles may be an important factor in the acidosis of anesthesia, but CO_2 increase is also a factor, especially with rebreathing methods.[45] The starvation preceding and following operation is also a factor of considerable importance. Ethylene anesthesia produces much less change in the blood than either ether or chloroform, which are about alike in their effects.[46]

It does not seem probable that the symptoms observed in typical cases of delayed chloroform-poisoning (*q. v.*) are due chiefly, if at all, to acid intoxication *per se*, but rather are the result of extensive injury to the parenchymatous organs, particularly the liver, by the chloroform, which causes a condition resembling acute yellow atrophy or phosphorus-poisoning.

Cachectic Acetonuria.—Acetone and diacetic acid, but less abundantly hydroxy butyric acid, are found in the urine in many *conditions associated with wasting*, among which may be especially mentioned:

Starvation.—Acetone, which is normally excreted through the lungs for the most part (80–90 per cent. of that produced), appears in excess in the urine very soon after fasting begins, more being produced than can be exhaled. It is associated with diacetic acid and hydroxybutyric acid, which may reach 10 to 20 grams per day in starvation, and even higher figures are recorded. The urinary ammonia nitrogen runs parallel to the acid excretion. The use of a carbohydrate-free diet is also accompanied by a marked acetonuria,[47] no matter how much fat is supplied, and may reach a point where several grams of hydroxybutyric acid are being excreted per day without symptoms of serious intoxication. A relatively small amount of carbohydrate (80 grams) is sufficient to prevent this acidosis. If the meat-fat diet is continued for some time, however, there seems to be some sort of adaptation so that the acetonuria diminishes until practically normal figures may be reached.

Pregnancy.—During pregnancy the urine usually contains acetone bodies in slight excess, and occasionally in large excess in women who are suffering from the toxemias of pregnancy. Here there is a rise in ammonia far beyond the proportion of acetone bodies, partly because of the large amounts of lactic acid which are excreted, and partly from abnormal protein metabolism and tissue destruction, but the proportion of the urinary nitrogen which is constituted by ammonia is too inconstant to serve as a prognostic and operative guide. Ewing has observed a case of pernicious vomiting with 75 per cent. of the total nitrogen as ammonia, and no urea,—while there may occur fatal cases without large excess of ammonia. Higher ammonia figures are usually reached in pernicious vomiting of pregnancy than in eclampsia; in neither is the acidosis present sufficient to account for the intoxication. (See discussion of "Eclampsia.") Even normal pregnant women seem to show a reduced ability to tolerate a deficiency in the carbohydrates of the diet,[48] and often exhibit an increase in the acetone body content of the blood.[48a]

Retention of placenta or fetus, acetonuria being considered of diagnostic value in determining the death of the fetus *in utero*,[49] but not in extrauterine pregnancy (Wechsberg).[50]

Cyclic Vomiting.—Here the urine usually shows acetone bodies, lactic acid, indican in excess, and a rise in the proportion of neutral to oxidized sulfur (Howland and Richards). As these findings may persist in spite of absorption of carbohydrates, they are not entirely due to starvation, and there are severe fatty changes in the liver

[42] See Caldwell and Cleveland, Surg. Gyn. Obst., 1917 (25), 22; Morriss, Jour. Amer. Med. Assoc., 1917 (68), 1391; Short, Jour. Biol. Chem., 1920 (41), 503.
[43] Ronzoni, Koechig and Eaton, Jour. Biol. Chem., 1924 (61), 465.
[44] Jour. Biol. Chem., 1924 (60), 17.
[45] Koehler, Jour. Biol. Chem., 1924 (62), 435.
[46] Leake, Brit. Jour. Anæsthesia, 1924 (2), No. 2.
[47] See Higgins, Peabody and Fitz, Jour. Med. Res., 1916 (34), 263.
[48] Porges and Novak, Berl. klin. Woch., 1911 (48), 1757.
[48a] Bokelmann, Zeit. Geb. Gynäk., 1924 (88), 408.
[49] See Frommer, Berl. klin. Woch., 1905 (42), 1008.
[50] Wien. klin. Woch., 1906 (19), 953.

and kidneys,[51] indicating a toxemic origin associated with defective oxidation. Mellanby[52] found a considerable creatine elimination in a typical case. There is, however, usually no acidosis, although it may develop.

Inanition and Cachexia.—Under this heading may be grouped the acetonuria observed in intestinal disturbances in children,[53] hysterical vomiting, psychoses, and cancer. In each of these conditions coma of the type of diabetic coma has sometimes been observed, and in all of them acetonuria is common, the reasons being obvious after the above discussion. A relative acidosis may also result from deficiency of bases in the diet of growing infants. In many cases of acidosis of infants there is not sufficient increase in the acetone bodies of the blood to account for the acidosis;[54] on the other hand, most of the children excreting acetone bodies in the urine do not have acidosis.

In uremia, as previously mentioned, organic acids may appear in the urine, but apparently as a result, and not as the cause, of the uremia (Orlowski). There is usually some acidosis in advanced nephritis, but marked only in uremia as discussed above.

Other Conditions.—Acetonuria is observed inconstantly in fever, especially in children;[55] also after poisoning by many drugs, including, besides the heavy metals, morphine, atropine, antipyrine, and phlorhizin. Pneumonia is said to be accompanied by acidosis,[56] often of serious degree, subsiding, rapidly after the crisis, but later studies have shown that the blood *p*H and the alkali reserve are usually normal in pneumonia.[57] Acidosis seems to be an important feature in gas gangrene.[58] At high altitudes there is always an acidosis, which stimulates the respiratory center to increased activity. In asphyxial conditions of all sorts more or less acidosis is present, *e. g.*, uncompensated cardiac defects, severe anemia, gas poisoning, surgical or traumatic shock. With intestinal putrefaction indicanuria may be associated with slight acetonuria.[59]

FATIGUE[60]

The symptoms of fatigue, whether general or local, seem to be due to an intoxication with the products of the excessive metabolic activity, and part of the symptoms, at least, seem to be due to acid intoxication. Among the metabolic products of muscular activity are known to be creatine, creatinine, sarcolactic acid, and carbon dioxide. A large amount of acid is developed in an active muscle, and when the activity is violent or prolonged the sarcolactic acid accumulates, being formed faster than it can be removed. Part of the acidity of the muscle is due, however, not to the sarcolactic acid itself, but to monopotassium phosphate (KH_2PO_4), which is formed by the action of the sarcolactic acid upon the dipotassium phosphate present in the blood and muscle. The effect of these various substances upon muscular fatigue has been studied experimentally, and while the creatine seems not to be a "fatigue substance," sarcolactic acid, monopotassium phosphate, hydroxybutyric acid, and carbon dioxide all cause muscle tissue to react to stimuli in the same way that a fatigued

[51] See Moser, Frankf. Zeit. Path., 1920 (23), 56.
[52] Lancet, July 1, 1911.
[53] See Howland and Marriott, Amer. Jour. Dis. Child., 1916 (11), 309; (12), 459.
[54] Moore, Amer. Jour. Dis. Child., 1916 (12), 244.
[55] See Garland, Arch. Pediat., 1919 (36), 468; Veeder and Johnston, Amer. Jour. Dis. Chil., 1920 (19), 141.
[56] Lewis and Barcroft, Quart. Jour. Med., 1915 (8), 108.
[57] Hastings *et al.*, Jour. Clin. Invest., 1924 (1), 25.
[58] Wright and Fleming, Lancet, Feb. 9, 1918.
[59] Wilson, Jour. Lab. Clin. Med., 1920 (5), 515.
[60] Full bibliography by Spaeth, Jour. Indust. Hyg., 1919 (1), 22.

muscle does (Lee[61]). Presumably these substances act chiefly by virtue of their carrying hydrogen ions, although there is some evidence that the negative ions of lactic and hydroxybutyric acids and some positive ions, especially potassium, are capable of producing certain fatigue phenomena (Scott). Indole, skatole and phenol may also produce fatigue conditions.

It is quite probable that the muscular weakness of diabetics, and the exhaustion associated with many conditions in which organic acids appear in the urine in abnormal quantities, depend, at least in part, upon the effect of these acids upon the muscle tissue, for Lee found that β-hydroxybutyric acid causes the same fatigue reaction in muscles as does sarcolactic acid. Furthermore, sarcolactic acid itself often appears in the urine in these conditions. Fatigue in man causes an increased urinary acidity.[62]

The blood shows but slight chemical changes during fatigue, there being some concentration in solids, increase in viscosity, in total and non-protein N, and in uric acid, the sugar being increased with short periods of exertion and decreased when the exercise is protracted (Rakestraw).[63] Violent exercise in dogs may induce hemolysis, although the resistance of the corpuscles to laking by hypotonic solutions is increased (Hastings).[64] In experiments with rats and guinea pigs, fatigue did not lower resistance to pneumococcus infection and to tetanus toxin, but if anything resistance was increased.[65]

The "Toxins" of Fatigue.—In extreme exhaustion the evidences of a general intoxication often become severe, so that the condition may resemble an acute febrile disease and last for several days. It seems very probable that substances more toxic than the above-mentioned acids are involved. Weichardt[66] claimed that he had demonstrated a toxic substance, produced by muscular fatigue, which in structure resembles the bacterial toxins, called by him *kenotoxin*,[67] and against which an antitoxin may be obtained. This toxic material is, he believes, formed from the protein molecule in the first stages of its decomposition, as a side product which is normally protected against by a formation of an antitoxin, rather than by being split up further, as is the case with the rest of the protein molecule. The study of anaphylaxis has led to so many evidences of the remarkable toxicity of the products of protein cleavage, that the possibility that some of these may be responsible for fatigue cannot be entirely disregarded at present,[68] although Lee and Aronovitch[69] could not demonstrate toxic substances in fatigued muscles, and no one has been able to confirm Weichardt's specific findings. The following observation of Mosso indicates that the blood of fatigued animals contains toxic substances: If blood is transfused from an exhausted dog to a normal dog, from which an equivalent amount of blood has been withdrawn, this second dog will show the usual manifestations of fatigue. Mendenhall[70] has

[61] Jour. Amer. Med. Assoc., 1906 (46), 1491, where is given a complete review of the subject of fatigue, with the literature. Also see Scott, Public Health Reports, 1918 (33), 605.

[62] Hastings, Publ. Health. Rep., 1919 (34), 1682; Barach, Amer. Jour. Med. Sci., 1920 (159), 398.

[63] Jour. Biol. Chem., 1921 (47), 565; 1923 (56), 121.

[64] U. S. Public Health Bull., 1921, No. 117.

[65] Oppenheimer and Spaeth, Amer. Jour. Hyg., 1922 (2), 51; Nicholls and Spaeth, *ibid.*, 527.

[66] "Ueber Ermüdungsstoffe," Enke, Stuttgart, 1912; Kolle and Wassermann's Handbuch, 1913 (2), 1499.

[67] See Weichardt and Schwenk, Zeit. physiol. Chem., 1913 (83), 381.

[68] The failure of various investigators to corroborate Weichardt is discussed by Konrich, Zeit. f. Hyg., 1914 (78), 1; Korff-Petersen, *ibid.*, p. 37.

[69] Amer. Jour. Physiol., 1924 (69), 92.

[70] Amer. Jour. Physiol., 1919 (48), 13.

found that the heart is also affected by the products of muscular fatigue. Recent studies in shock (Bayliss, Cannon) attribute the manifestations of shock, at least in part, to toxic cleavage products of injured tissues, and many of these manifestations are allied to fatigue.

Mental Fatigue.—The chemical changes of mental fatigue are not known, but the ganglion-cells show marked structural alterations as a result of fatigue, chromatolysis often being very striking. Since lecithin forms so important a part of the nervous system, it is tempting to imagine that in fatigue excessive quantities of its toxic decomposition-product, *choline*, and the still more toxic derivative of choline, *neurine*, are formed in considerable amounts and cause part, at least, of the intoxication. That choline or neurine actually are the cause of any of the symptoms of fatigue, however, has not been established; but Donath[71] considers choline an important factor in the production of *epileptic convulsions*. Animals kept for a long time from sleeping are said to show the presence in their blood, cerebro-spinal fluid, and brain tissues, of a poisonous property causing somnolence in other animals (Legendre and Piéron).[72] This cannot well be choline or any similar substance, for it does not filter, is insoluble in alcohol, and is destroyed by heating at 65°.

THE POISONS PRODUCED IN SUPERFICIAL BURNS[73]

In a certain proportion of cases of extensive but superficial burns, death follows after an interval of from six hours to a few days, apparently because of a profound intoxication. As evidence of intoxication we have not only clinical manifestations, such as delirium, hemoglobinuria, and albuminuria, vomiting, bloody diarrhea, etc., but, more convincingly, the anatomical findings at autopsy, which are strikingly similar to those resulting from acute intoxication with bacterial products. Bardeen found quite constantly cloudy swelling and focal and parenchymatous degeneration in the liver and kidneys; softening and enlargement of the spleen with focal degeneration in the Malpighian bodies; and particularly degenerative changes in the lymph-nodes and intestinal follicles resembling those observed in diphtheria, which McCrae[74] considers due to proliferation and phagocytosis by the endothelial cells of the lymphatic structures. The severe degenerative changes seen in the adrenals and myocardium especially recall diphtheria intoxication (Weiskotten).[75] Marked changes are usually present in the blood, consisting of fragmentation and distortion of the red corpuscles, hemoglobinemia, loss of water with a relative increase in the number of corpuscles by from one to four millions per cubic millimeter, an increase in the blood platelets, and a rise in the number of leucocytes as high as 30,000 to 50,000.[76] Hemoglobinuria is also frequently present,[77] and almost constantly gastrointestinal irritation occurs, with anatomical evidences of acute enteritis, acute gastritis,

[71] Zeit. physiol. Chem., 1903 (39), 526.

[72] Zeit. allg. Physiol., 1912 (14), 235.

[73] Literature given by Bardeen, Johns Hopkins Hosp. Reports, 1898 (7), 137; Eyff, Cent. Grenzgeb. Med. u. Chir., 1901 (4), 428; Pfeiffer, Virchow's Arch., 1905 (180), 367. Full discussion of theories by Vogt, Zeit. exp. Path. u. Pharm., 1912 (11), 191.

[74] Amer. Med., 1901 (2), 735.

[75] Jour. Amer. Med. Assoc., 1917 (69), 776; 1919 (72), 259; also Nakata, Corr. Bl. Schw. Aerzte, 1918 (48), 1283; Olbrycht, Rev. de Méd., 1924 (41), 81.

[76] Locke, Boston Med. and Surg. Jour., 1902 (147), 480.

[77] According to Becky and Schmitz[83] the urine often exhibits indicanuria independent of intestinal putrefaction, and perhaps derived through dry distillation of the burned proteins.

and occasionally gastric or duodenal ulcers. According to Korolenko,[78] the sympathetic nervous system is seriously involved.

It therefore seems probable that poisons are formed as a result of superficial burns, which have the effect of causing hemolysis, and which are also cytotoxic for parenchymatous cells. These hypothetical poisons seem to be eliminated by the intestines and kidneys, which are injured by the poisons in their passage through these organs. The attempts to explain all the observed effects of burns as due to thrombosis or to embolism by altered corpuscles seem to have failed, for the peculiar location of the lesions (*e. g.*, duodenal ulcers, necrosis in the Malpighian bodies of the spleen, etc.) does not agree with this hypothesis, and there are too many evidences of the presence of some decidedly toxic substance in the blood. There can be no question that the poisonous substance or substances are formed in the burned area, and not in the internal organs as a result of hyperpyrexia, as shown by numerous observations. Thus, if the burned area is removed immediately (in narcotized experimental animals), death will be prevented, whereas if the burned tissue is permitted to remain for a few hours, death will occur. If the burned skin is transplanted to a normal animal, this animal will develop symptoms of intoxication, while the burned animal may be saved by the transplantation (Vogt,[73] Robertson and Boyd[89]). When one of a pair of animals united to another by operative procedure (parabiosis) is burned, the other animal may become intoxicated, while the intoxication of the burned animal is less than it would be if it were alone (Vogt).

The poison appears to be absorbed from the burned area into the blood, for if the circulation is shut off from the burned area, no intoxication results; this probably explains in part why deep destructive burns of small areas, which are associated with local thrombosis, are much less serious than a superficial slight scalding over a large area. However, the older reports[79] that burning of muscle and subcutaneous tissues does not produce toxic effects were not corroborated by Robertson and Boyd.[89]

Numerous investigators have reported finding poisonous substances in the blood, tissues, or urine of burned men and animals, but the reports disagree widely in details.[80] Thus, Dietrichs states that the blood of burned animals contains hemolysins and hemagglutinins, which could not be corroborated by Burkhardt[81] or by Pfeiffer.[82] The latter, however, finds that the urine, serum, and organs of burned animals contain substances poisonous for the same and for different species, which is in accord with the results of numerous earlier investigators. Becky and Schmitz[83] also found the urine highly toxic for mice, and report the pres-

[78] Cent. f. Path., 1903 (10), 663.

[79] Eijkman and Hoogenhuyze, Virchow's Arch., 1906 (183), 377.

[80] Ravenna and Minassian (Ref. in Biochem. Centr., 1903 (1), 348) state that blood heated outside the body to 55°–60° is toxic, and causes the same anatomical changes as does death from burning, which finding is corroborated by Helsted, Arch. klin. Chir., 1906 (79), 414.

[81] Arch. klin. Chir., 1905 (75), 845.

[82] Virchow's Arch., 1905 (180), 367; Zeit. f. Hyg., 1906 (54), 419.

[83] Mitt. Grenz. Med. Chir., 1919 (31), 416.

ence of an unidentified protein in the urine, giving a double ring with nitric acid. The poisons, according to Pfeiffer,[82] are neurotoxic and necrogenic in their properties, and act without a period of incubation; they are rapidly weakened on standing in solution and by the action of sunlight, are absorbed from the gastro-intestinal tract, are soluble in water, alcohol, and glycerol, but not in chloroform or ether, are precipitated by $HgCl_2$ in acid solution, and by phosphotungstic acid, and they are not volatile. Apparently, according to Pfeiffer, they are not ptomaines, nor yet pyridine derivatives, as many investigators have contended, but resemble more closely the labile poisons of snake venom, and have effects similar to the unknown poisons that are concerned in uremia. The neurotoxic substance is more thermostable than the necrogenic substance, which is very easily destroyed by heat. Pfeiffer believes it probable that the poisons are derived from the cleavage of proteins altered in composition by burning, and he finds an enzyme splitting glycyltryptophan in the blood and urine of burned animals.[84] The hemolysis he attributes to direct injury of the blood in its passage through the heated area, and not to the action of poisons; this is very possible, since red corpuscles fragment after being heated to 52°, and may be seriously impaired functionally at 45°. There are many authors, indeed, who consider the blood changes the chief cause of death, but the weight of evidence is in favor of the theory of the development of toxic substances in the burned skin.

Kutscher and Heyde[85] believe methyl guanidine to be the toxic substance eliminated in the urine, stating that it produces effects similar to that caused by injections of the toxic urine from burn cases. These symptoms are quite similar to those characteristic of anaphylaxis, and Heyde states that small burned areas sensitize an animal to later injections of extracts of burned tissue. He, as well as Vogt, are therefore inclined to believe that some cases, especially those dying unexpectedly 12 or 13 days after the burning, may be the result of anaphylactic reaction to proteins made of foreign character by the heat.[86] The newer observations concerning the presence of toxic substances in the urine during anaphylactic intoxication are in harmony with the findings in burn cases,[87] although the identity of methyl guanidine with the toxic agent is questionable. Kok[88] found that the blood of burned dogs has an increased power to cause constriction of blood vessels although it did not cause contraction of either uterine or intestinal muscle.

Robertson and Boyd[89] state that death within the first 24 hours is from shock, but later death is due to a poison formed in the burned area, which circulates either in or adsorbed by the red corpuscles, and which they found to consist of two portions, one of which is thermostabile, diffusible, and neurotoxic, the other is thermolabile, colloidal, and necro-

[84] Zeit. Immunität., 1915 (23), 473.
[85] Cent. f. Physiol., 1911 (25), 441.
[86] Heyde, Med. Klinik, 1912 (8), 263.
[87] See Pfeiffer, Zeit. Immunität., 1913 (18), 75.
[88] Zeit. exp. Med., 1921 (14), 220.
[89] Jour. Lab. Clin. Med., 1923 (9), 1.

toxic. They believe both parts of the poison to consist of proteoses, against which an antitoxin could not be obtained. They found no toxic material in skin burned after death, which fact is in favor of the view that the poison is formed in the blood circulating through the heated area. Extensive superficial burns produce a rapid and marked concentration of the blood, which Underhill[90] believes may be a serious factor in producing the shock effects, but which can scarcely explain all the toxic manifestations.

Burn Blisters.—The contents of burn blisters resemble the fluid of inflammatory edemas generally. K. Mörner[91] found 5.031 per cent. of proteins, which included 1.359 per cent. of globulin and 0.011 per cent. of fibrin; there was also present a substance reducing copper oxide, but no pyrocatechin.

[90] Underhill *et al.*, Arch. Int. Med., 1923 (32), 31.
[91] Skand. Arch. Physiol., 1895 (5), 272.

CHAPTER XXIII

GASTRO-INTESTINAL "AUTOINTOXICATION" AND RELATED METABOLIC DISTURBANCES

Under this heading are commonly included all intoxications that can be ascribed to the absorption from the gastro-intestinal tract of toxic substances that have been formed within its contents, either by the action of the digestive ferments or of putrefactive bacteria. The propriety of considering such conditions as examples of autointoxication is properly questioned, since it is often difficult to determine whether the putrefaction occurred within the body, or had already taken place in the food before it was eaten. But even those who would limit the use of the term autointoxication to intoxication with the products of cellular metabolism, must admit the possibility of products of metabolism reëntering the blood from the contents of the bowels through the intestinal wall, since the bile, and perhaps also the intestinal juice, contain excrementitious substances which may, in case of defective fecal elimination, be reabsorbed into the blood. Therefore, in gastro-intestinal disturbances we have the possibility of both true autointoxication and intoxication by putrefactive products occurring together in an inseparable way, and the usual inclusion of gastro-intestinal intoxication in the discussion of autointoxication would seem to be justifiable as well as expedient.

The possible sources of poisonous substances arising in the gastro-intestinal tract are numerous. They may be formed either from the food-stuffs, or from the secretions and excretions of the body that enter the alimentary canal; and they may be formed either by the digestive ferments or by the bacteria of the intestinal contents. Hence the number of these products is enormous, and we are by no means sure that those that have been identified include the most important or most toxic; furthermore, *at the present time we are far from sure that any of these materials, known or unknown, are important factors in human pathology.* To classify the poisonous substances that are known to be formed in the alimentary canal, and which might, under certain conditions, cause an intoxication, is extremely difficult, because of the uncertainty of our information; but, using as a basis the sources of the substances, they may be classified as follows:[1]

I. The constituents of the digestive secretions, including the bile salts and pigments, pepsin, and trypsin.

II. Products of normal digestion:

(*a*) From proteins—proteoses, peptones, amino-acids.

(*b*) From fats—fatty acids and glycerol.

[1] Modified from Weintraud, Ergeb. allg. Pathol., 1897 (4), 1, who gives exhaustive discussion and bibliography to that date.

III. Products of putrefaction and fermentation:
(*a*) From proteins:
(1) From the aromatic radicals (tyrosine, phenylalanine, tryptophan)—indole, skatole, skatole-carbonic (or indole-acetic) acid, phenol, cresol, dioxyphenols, and the pressor bases.
(2) From the fatty acid radicals—fatty acids (especially butyric and acetic), acetone, ammonia, amino-acids, carbon dioxide, hydrogen, marsh-gas. Also ptomaines; cadaverine, putrescine, ethylidendiamine, isoamylamine.
(3) From the sulfur-containing radicals—H_2S, methyl mercaptan, ethyl mercaptan, ethyl sulfid.
(4) From the heterocyclic amino acid, histidine—histamine.
(*b*) From carbohydrates:
Fatty acids, the following having been detected—formic, acetic, propionic, butyric, valerianic, lactic, oxybutyric, and succinic; also acetone, CO_2, CH_4, H_2.
(*c*) From fats:
Higher fatty acids, as well as butyric acid; also glycerol. From lecithin—choline, neurine, and muscarine-like bodies.
IV. Synthetic products of bacterial activity (*e. g.*, botulismus) which cannot properly be considered as causing "autointoxication."

I. THE CONSTITUENTS OF THE DIGESTIVE FLUIDS

These call for but brief consideration, for, although many of them are known to be toxic, yet there is no evidence that they cause autointoxication, either in health or disease. Both pepsin and trypsin, especially the latter, are decidedly toxic when injected experimentally into the blood in the impure tissue extracts or secretions in which they are found (see Enzymes), but they do not appear ever to pass through the intestinal wall in sufficient quantity to cause harm, although minute traces may appear in the urine; this harmlessness probably depends largely on the known inhibiting action of the blood upon enzymes.

The bile salts are also toxic, especially hemolytic, but such as are reabsorbed from the intestines are taken back into the liver and reexcre ed. This protective arrangement seems to be sufficient for all emergencies. The bile-pigments become converted into *urobilinogen* through reduction, and this is largely absorbed and eliminated as *urobilin*. Icterus and cholemia do not seem ever to be produced by absorption of bile-pigments and bile salts from the intestines.

II. PRODUCTS OF NORMAL DIGESTION

Proteoses and Peptones.—Under normal conditions, these are broken up in the intestinal wall into the amino-acids, through the agency of erepsin, and do not appear in the blood in appreciable quantities in health.[1a] To be sure, certain authors report finding *albumose* in normal blood, but if present the amounts are extremely minute, and the evidence that these traces of nitrogenous material are truly proteoses is not conclusive. In conditions in which ulceration or other lesions are present in the gastro-

[1a] The power exhibited by the intestinal wall to change proteoses into something else is not exhibited by the liver, for proteoses absorbed in large amounts from an isolated loop of intestine do not cause albumosuria, whereas small amounts injected into the portal vein produce albumosuria (Delaunay and Desqueyroux, Compt. Rend. Soc. Biol., 1923 (88), 710).

intestinal tract it is possible to find small amounts of proteoses in the urine, probably absorbed through the abnormal areas, but not in quantities sufficient to account for any appreciable intoxication, although proteoses are distinctly toxic. This last statement has been much contested, because the difficulty of purifying proteoses obtained from ordinary sources has left open the possibility that such toxic effects as have been observed are due to contaminating substances, such as histamine, and not to the proteoses themselves. More recent work, however, by Underhill,[2] Gibson,[3] Zunz,[4] and Hanke and Koessler,[5] seems to have established affirmatively the toxicity of proteoses, whether from animal or vegetable proteins. Commercial Witte's peptone contains measurable amounts of histamine, but Hanke and Koessler[5] have shown that "fibrin-peptone," apparently free from histamine, produces a typical peptone shock and causes contraction of the virgin guinea pig uterus. Abel and Geiling,[6] however, find the albumoses isolated from Witte peptone to be of such low toxicity that they again raise the question as to their actually being toxic of themselves. Clark,[7] nevertheless, finds most of the toxicity of "peptones" to depend on the higher proteoses, but there is also an alcohol-soluble, dialyzable toxic element believed to be distinct from histamine.

Besides the classical effect of inhibiting the coagulation of the blood, the proteoses have a lymphagogue effect (Heidenhain),[8] cause a marked febrile reaction,[9] and in doses of some size are fatal to experimental animals (rabbits being much less susceptible than dogs and many other animals). Locally they cause a mild inflammatory reaction, which is followed by the appearance of much connective-tissue formation.[10] Long-continued injection of proteoses does not produce visceral lesions.[11] The careful studies of Zunz show that intravenous injection of hetero-albumose, thio-albumose, deutero-albumose, and proto-albumose cause a rise in blood pressure, but large doses may cause a fall in pressure; the

[2] Amer. Jour. Physiol., 1903 (9), 345; Jour. Biol. Chem., 1915 (22), 443 (literature).
[3] Philippine Jour. Sci., 1914 (9), 499.
[4] Arch. internat. physiol., 1911 (73), 110.
[5] Jour. Biol. Chem., 1920 (43), 567.
[6] Jour. Pharm., 1924 (23), 1.
[7] Jour. Pharm., 1924 (23), 45.
[8] See also Nolf, Arch. internat. de Physiol., 1906 (3), 343.
[9] Gibson finds that carefully purified proteoses have but a slight pyrogenic effect. (Philippine Jour. Sci., 1913 (8), 475.)
[10] In a paper appearing in the Transactions of the Chicago Pathological Society, 1903 (5), 240, I published the observation that repeated injections of Witte's "peptone" (which consists chiefly of proteoses) into rabbits led to the production of marked cirrhosis of the liver, and suggested the possibility that proteoses escaping through a diseased gastric or intestinal wall into the blood might be a factor in the production of cirrhosis in man. Subsequent observations, however, have shown that repeated injection of almost any foreign protein material (*e. g.*, emulsions of organs, foreign blood, etc., used in immunization experiments) will cause a similar cirrhosis in rabbits, which animals, indeed, often spontaneously show this condition when apparently otherwise normal. "Peptone" injections in dogs and guinea-pigs have failed to cause a similar cirrhosis, and hence the value of these and all other rabbit experiments on cirrhosis of the liver is very questionable; however, the possibility of the correctness of the original conclusions still remains open.
[11] Woolley *et al.*, Jour. Exp. Med., 1915 (22), 114. Boughton describes acute degenerative changes from Witte's peptone. (Jour. Immunol., 1919 (4), 381.)

abiuret products of tryptic digestion are much more actively depressor than the albumoses. As a general rule, however, it has been observed that the first products of protein hydrolysis are the most toxic, and with further cleavage the toxicity lessens and finally disappears, as shown especially in the studies on anaphylaxis and anaphylatoxin formation.[12] Thus, Wolf[13] found that the amino-acids do not cause a fall of blood pressure, nor do polypeptids.[14] Proteoses have little if any power to stimulate antibody formation (see Antigens, Chap. VII). Whipple[15] has described the isolation of highly toxic proteoses from the contents of closed intestinal loops, and injection of these proteoses causes a marked increase in nitrogen elimination, presumably from toxicogenic destruction of tissue proteins. With this is a great increase in the non-protein nitrogen of the blood, partly due to renal injury. He also observed an increased resistance of the animals to these proteoses after repeated injections.

Hahn[16] has determined the difference between the nitrogen precipitated from blood by phosphotungstic acid, which precipitates protein and the higher cleavage products, and the trichloracetic acid precipitate, which contains only the proteins. This difference should theoretically consist of proteose, peptone and some polypeptid nitrogen, and is normally below 10 mg. per 100 c.c. of blood, sometimes none at all being found. Figures higher than this were usually indicative of tissue disintegration, as in suppuration, tuberculosis, uremia, cancer. In normal blood this fraction is found chiefly in the corpuscles, in pathological conditions in the serum.[17] Wolff[18] considers the difference between the precipitates with metaphosphoric and phosphomolybdic acid to be albumose, and finds normally below 8 mg. of this "albumose-N" per 100 c.c. of normal blood. Abnormally high quantities were usually found in typhoid, pneumonia, suppuration, gastric cancer but not gastric ulcer, leukemia, but not in nephritis. Of 48 patients with fever, 61 per cent. had albumosemia, 69 per cent. albumosuria, and in 48 per cent. both occurred.

It is possible that some of the symptoms of these and other conditions are due to intoxication with proteoses, for 0.07 to 0.1 gram deutero-albumose will cause a febrile reaction in a healthy man,[19] but probably the amount is usually too small to cause appreciable effects.[20] It is well known, however, that the characteristic rise of temperature following the injection of tuberculin into tuberculous individuals is also produced if

[12] The statement of v. Knaffl-Lenz (Arch. exp. Path. u. Pharm., 1913 (73), 292) that the toxicity of the cleavage products varies directly with their tryptophan content could not be corroborated by Underhill and Hendrix, Jour. Biol. Chem., 1915 (22), 443.

[13] Jour. of Physiol., 1905 (32), 171.

[14] Haliburton, *ibid.*, 1905 (32), 174.

[15] Jour. Exp. Med., 1917 (25), 461; 1918 (28), 213; 1918 (29), 397.

[16] Biochem. Zeit., 1921 (121), 264.

[17] See also Achard and Feuillié, Compt. Rend. Soc. Biol., 1920 (83), 1535, 1584.

[18] Acta Med. Scand., 1921 (53), 810; Ann. Med., 1921 (10), 185.

[19] See Matthes, Arch. exper. Path. u. Pharm., 1895 (36), 437.

[20] In a series of unpublished experiments I was unable to cause amyloid degeneration in rabbits by protracted intoxication with proteose solutions.

minute quantities of proteose solutions are injected in place of tuberculin; therefore, proteoses arising from autolysis in tuberculosis may be of importance in causing fever and other symptoms.[21] Tuberculous animals are said to succumb to a much smaller dose of deutero-albumose than normal animals.[22]

"Albumosuria."[23]—If proteoses enter the blood stream they appear in large part in the urine, indicating that the tissues do not readily utilize them in this form.[24] Consequently, when proteoses are produced in considerable amounts by autolysis of pathological tissues they appear in the urine, and their presence is considered to be of diagnostic value.[25]

"Albumose," therefore, may be found in the urine whenever any considerable amount of tissue or exudate is being autolyzed and absorbed, and it has been found in the following conditions: Suppuration of all kinds; resolution of pneumonia; involution of the puerperal uterus; carcinoma (two-thirds of all cases—Ury and Lilienthal), and other malignant growths; febrile conditions with tissue destruction (37.5 per cent. of all cases, Morawitz and Dietschy);[26] acute yellow atrophy, phosphorus poisoning, and eclampsia; leukemia, especially under *x*-ray treatment; absorption of simple and inflammatory exudates; ulcerating pulmonary tuberculosis,[27] and after tuberculin reactions (Deist).[28] Albumosuria is present in smallpox and may serve in differential diagnosis.[29] In ulcerative conditions of the alimentary canal albumoses may be absorbed unchanged and cause alimentary albumosuria. By his improved methods, Wolff[18] found albumosuria in 68 per cent. of febrile cases, especially in pneumonia and empyema, but only in two of six cases of gastric cancer, and not at all in uterine cancer or nephritis. The normal kidney seems to be impermeable to the small amounts of proteose that may be present normally in the blood, or even after large oral ingestion of proteoses, but in parenchymatous nephritis it may escape in the urine (Henderson,[30] Pollak[23]).

True peptone seems rarely, and according to many observers never, to appear in the urine; but in view of the observation that polypeptids often appear in the urine,[31] it is probable that true peptones do also, and Richaud[32] reports finding peptone much more frequently than albumoses.

The so-called "Bence-Jones albumose" that appears in the urine of patients with multiple bone-marrow tumors is not a true albumose, but is more closely related to the simple proteins, and is discussed under the head of "Chemistry of Tumors."

III. PRODUCTS OF PUTREFACTION AND FERMENTATION[33]

We may perhaps gain some appreciation of the enormous amount of bacterial action that goes on in the normal intestinal digestive processes by considering the fact that as much as one-third of the total weight of the solids of normal feces may consist of bacteria (Strasburger), their

[21] Simon, Arch. exp. Med., 1903 (49), 449. Concerning relation of tuberculin to proteoses see review by Jolles in Ott's "Chemische Pathol. der Tuberculose."

[22] Kirchheim and Tuczek, Arch. exp. Path. u. Pharm., 1914 (77), 387.

[23] Critical review given by Pollak, Zeit. exp. Med., 1914 (2), 314.

[24] They may be partly hydrolyzed into smaller complexes, however, primary proteoses being partly changed to deutero-proteoses, and the latter partly to peptone (Chittenden, Mendel, and Henderson, Amer. Jour. Physiol., 1899 (2), 142).

[25] See Yarrow, Amer. Med., 1903 (5), 452; Ury and Lilienthal, Arch. f. Verdauungskr., 1905 (11), 72; Senator, International Clinics, 1905 (4, series 14), 85.

[26] Arch. f. exp. Path. u. Pharm., 1905 (54), 88.

[27] See Parkinson, Practitioner, 1906 (76), 219.

[28] Beitr. z. klin. Tuberk., 1912 (23), 547.

[29] Primavera, Gaz. Int. Med. e Chir., 1913 (116), 217.

[30] Lancet, Mar. 6, 1909.

[31] Chodat and Kummer, Biochem. Zeit., 1914 (65), 392.

[32] Jour. pharm. chim., 1921 (23), 376.

[33] Complete bibliography given in the résumé on "Intestinal Putrefaction" by Gerhardt, Ergebnisse der Physiol., 1904 (III, Abt. 1), 107. Chemistry of Putrefaction is reviewed by Ellinger, *ibid.*, 1907 (6), 29.

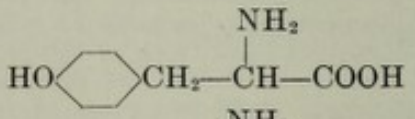
proportion being increased in diarrheal disorders and decreased in constipation. They attack all food-stuffs, and among the decomposition-products formed through their activity are undoubtedly many of considerable toxicity. Most of the products of intestinal putrefaction that have as yet been isolated are, however, not extremely poisonous; but many of them are toxic to some degree, and their long-continued absorption might possibly lead to serious disturbances. Considering them first according to their origin and chemical nature, we take up first the products of:

A. Protein Putrefaction

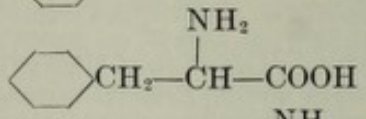
(1) Substances Derived from the Aromatic Radicals of the Protein Molecule

In the protein molecule are contained the following amino-acids with an aromatic nucleus:

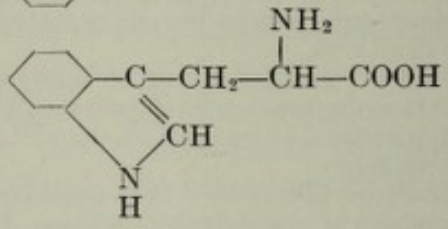
Tyrosine,



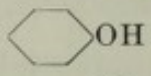
Phenylalanine,



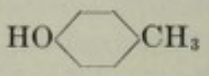
Tryptophan,



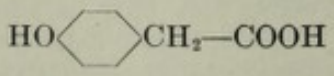
In the intestinal contents have been found a number of substances that are undoubtedly derived from these aromatic radicals. They are (1) *phenol*,



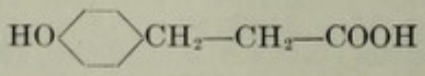
which is formed in small quantities, presumably from tyrosine, as also is the closely related (2) *paracresol*,



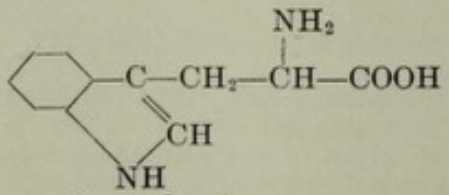
and also (3) *para-oxyphenyl acetic acid*,



and (4) *para-oxyphenyl-propionic acid*,

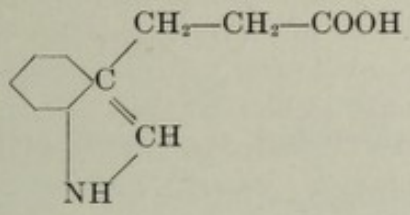


From the tryptophan are formed numerous important substances, as follows:

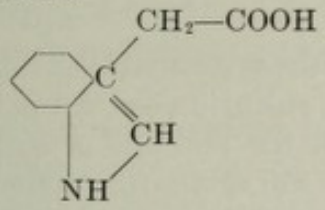


(trytophan)

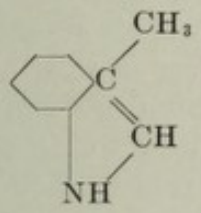
readily yields, through splitting off the NH_2 group and addition of H, *indole propionic acid* (formerly incorrectly called *skatole acetic acid*), which is



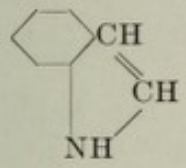
and from which in turn may readily be formed *indole acetic acid* (erroneously called *skatole carboxylic acid*), which is



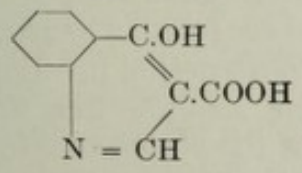
Both of these substances have been found in the intestinal contents. From them are formed the more familiar *skatole*,



and *indole*,

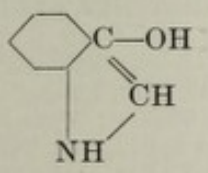


In dogs, but not in man, *kynurenic acid*.



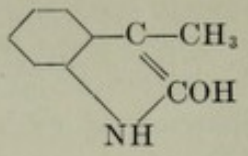
is also formed from tryptophan.[34]

The greatest interest concerning these bodies arises from the fact that after they are absorbed from the intestine they become combined with sulfuric or glucuronic acid and are excreted in the urine as salts of these acids; consequently the amount of sulfuric acid appearing in the urine in such organic combination ("ethereal sulfuric acid") is considered as an index of the amount of intestinal putrefaction. In the case of indole and skatole, which have no hydroxyl group, a preliminary oxidation occurs, whereby indole is converted in *indoxyl*,

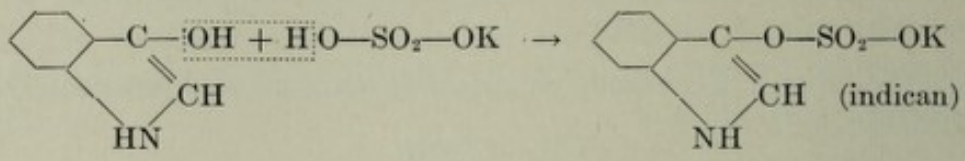


[34] See Ellinger, Zeit. physiol. Chem., 1904 (43), 325.

and skatole into *skatoxyl*,



and they are then combined with sulfuric or glucuronic acid, as follows:



By far the greater part of these aromatic substances, when excreted in the urine, is combined with sulfuric acid,[35] and but a small part with glucurconic acid,[36] but in case the amount of sulfuric acid available is too small to combine with all the aromatic radicals entering the blood, a large amount of the glucuronic acid compound appears in the urine (*e. g.*, after therapeutic administration of phenol, cresol, thymol, camphor, etc.). Both the preliminary oxidation and the combining with acids seem to occur chiefly in the liver, this process constituting one of the most important of the many protective offices of that organ, since the resulting compounds are much less toxic than are the original substances.[37] Herter and Wakeman[38] have shown that living cells have the power of acting upon indole and phenol (and presumably upon the rest of this group) in such a way that they cannot be recovered by distillation. Most active in this respect is the liver, then in order come kidney, muscle, blood, and brain.[39]

Indole.[40]—This is probably the most important member of this group of substances, the striking color of its derivatives making its detection in the urine easy, so that it is generally used as the most available index of the amount of putrefaction that is occurring in the intestines.[41] The greatest quantities are found when intestinal putrefaction is marked, especially in intestinal obstruction involving the small intestine; obstruction of the large intestine, as Jaffe first demonstrated, does not cause marked indicanuria unless the stagnation involves the ileum, as it may in the later stages of obstruction. With marked impairment of renal function indican may accumulate in the blood (see Uremia). There can be no question that the indican of the urine is derived, at least in large part, from the indole formed in the intestine, for administration of indole by mouth to either animals or man causes a considerable increase

[35] See Hoppe-Seyler, Zeit. physiol. Chem., 1916 (97), 171.

[36] Sherwin (Jour. Biol. Chem., 1917 (31), 307) states that phenylacetic acid is excreted by monkeys combined with glycine, but by man it is excreted combined with glutamine.

[37] Metchnikoff insisted that these sulfo-compounds still retain considerable toxicity. (Ann. Inst. Pasteur, 1914 (27), 893).

[38] Jour. Exper. Med., 1899 (4), 307.

[39] For further discussion of this topic, see "Chemical Defences against Poisons of Known Composition," Chapter XII.

[40] See "Die Indicanämie," Gustav Baar, Berlin, 1922.

[41] See Houghton, Amer. Jour. Med. Sci., 1908 (135), 567.

in the indican present in the urine; however, but 40 to 60 per cent. can be recovered in this way, the rest apparently being oxidized to other compounds, part of which may also appear in the urine.[42]

Probably the chief agent in the formation of indole in the intestines and in putrid tissues is the colon bacillus, which, as is well known, produces indole in ordinary culture-media.[43] Bacteria acting on urine containing much indican may liberate indoxyl and form indigo blue.[44] As shown by Baar,[45] an increased absorption of indole from the bowel and a resulting indicanuria are not accompanied by any appreciable rise of the indican content of the blood as long as the kidneys are normal, and hence intoxication from indican or indole is scarcely possible except in nephritis.

Whether part of the urinary indican is derived from tryptophan liberated during intracellular protein metabolism, and not from intestinal putrefaction, has long been a disputed point among physiological chemists.[46] The demonstration by Ellinger and Gentzen[47] that tryptophan, when fed or injected subcutaneously, causes no increase in urinary indican, whereas its injection into the cecum causes much indicanuria, would indicate that indole is formed from tryptophan only through putrefaction, and not in cellular metabolism. Other experiments support the same view.[48] However, it is possible that part of the indican present in the urine during conditions associated with gangrene, putrid cancers, putrid placentas, or putrid purulent exudates, may be derived from these decomposing materials. It is said that large amounts of indican may be excreted in cases of bulky secondary cancers of the liver without putrefaction. The excretion of indole in the breath and sputum may be observed in pulmonary suppuration and gangrene.[49] The statement that indicanuria is of significance in insanity could not be substantiated by Borden,[50] who used quantitative methods and careful controls. A large proportion of the data and conclusions in the early literature concerning indicanuria are valueless because of improper or inadequate methods.

Toxicity of Indole.—Although the toxicity of indole seems to be relatively slight, and this toxicity is further reduced by the conversion of indole into indoxyl and indican, yet Herter[51] found that administration to healthy men of indole in quantities of 0.025 to 2 grams per day caused

[42] If gelatin is substituted for proteins in the dietary, indican is not excreted, because gelatin does not contain tryptophan (Underhill, Amer. Jour. Physiol., 1904 (12), 176).

[43] See Distaso and Sugden, Biochem. Jour., 1919 (13), 153. Logie, Jour. Path. Bact., 1920 (23), 224.

[44] Hoppe-Seyler, Zeit. physiol. Chem., 1916 (97), 250.

[45] Die Indicanämie, Berlin, 1922.

[46] Literature by Gerhardt, Ergeb. der Physiol., 1904 (III, Abt. I), 131.

[47] Hofmeister's Beitr., 1903 (4), 171.

[48] See Scholz, Zeit. physiol. Chem., 1903 (38), 513; Underhill, *loc. cit.*[42] Sherwin and Hawk found an absence of indican in the urine in the latter part of a long fast (Biochem. Bull., 1914 (3), 416).

[49] Cantelli, Riforma Med., 1922 (38), 481.

[50] Jour. Biol. Chem., 1907 (2), 575.

[51] New York Med. Jour. 1898 (68), 89.

frontal headache, irritability, insomnia, and confusion; the continued absorption of enough indole to cause a constant strong reaction for indican in the urine is sufficient to cause neurasthenic symptoms. Long-continued injection of indole is said to lead to hypertrophy of the adrenal medulla and slight interstitial changes in the kidneys,[52] but the reputed responsibility of indole for arteriosclerosis is most doubtful.[53] Lee[54] has also demonstrated that indole, skatole, and methyl mercaptan cause muscles to react to stimuli like fatigued muscles. Indole and paracresol have a vasoconstricting action.[55]

Normal urine contains but about 12 milligrams of indican per day, which amount is so insignificant in proportion to the above-mentioned doses that were found necessary to produce symptoms, that we may well doubt the occurrence of noticeable intoxication from this substance under ordinary conditions. Nesbitt[56] states that twenty times as much indole or skatole as are excreted daily by an adult man may be injected into the jugular vein of a dog of four kilos without causing appreciable effects.[57] Richards and Howland, however, have demonstrated the possibility, that defective oxidation of substances of this group may permit of intoxication.[58] When subcutaneously injected, dissolved in oil, indole and skatole have the property of greatly stimulating epithelial proliferation, similar to the action of scarlet R and other fat stains (Stoeber),[59] but we have no direct evidence that these substances cause similar effects in the human body.

Other Aromatic Compounds.—Skatole seems to accompany indole in small amounts, but apparently in no constant quantitative relation. Herter[60] states that skatole is formed under entirely different conditions from indole, and that *B. coli* does not produce skatole. It is not always present in the contents of the large intestines of healthy persons, and seems to be formed later than indole. Skatol has little effect when given by mouth, Brieger having observed no effect from giving 5 grams to a dog, but intravenously it causes a marked fall in blood pressure (Salant and Kleitman).[61]

Indole-acetic acid appears in the normal urine in extremely minute quantities, and is increased in the same conditions as skatole. It is the mother substance of *urorosein*, and can be found in the intestines of patients who show this substance in the urine (Herter).[62] Ross[63] found indole-acetic acid in the urine in 21 per cent. of normal persons, and in 48 per cent. of dementia precox cases, and obtained evidence in favor of an endogenous origin in two cases studied especially to determine this point.

Phenylacetic acid[64] is formed from phenylalanine on putrefaction, as also are phenylpropionic and benzoic acids. Benzoic acid combines with glycine and is

[52] Woolley and Newburgh, Jour. Amer. Med. Assoc., 1911 (56), 1796.
[53] See Steenhuis, Folia Mikrobiol., 1915 (3), 76.
[54] Jour. Amer. Med. Assoc., 1906 (46), 1499.
[55] Vasoin, Gaz. degli Ospedali, 1919 (40), 673.
[56] Jour. Exper. Med., 1899 (4), 5.
[57] Effects of indole and skatole on the nitrogen metabolism of rabbits is discussed by Hijikata, Acta Schol. Med. Kioto, 1921 (4), 215.
[58] See note in Science, 1906 (24), 979.
[59] Münch. med. Woch., 1910 (57), 947.
[60] Jour. Biol. Chem., 1908 (4), 101; general discussion.
[61] Jour. Pharm., 1922 (19), 307.
[62] Jour. Biol. Chem., 1908 (4), 253.
[63] Arch. Int. Med., 1913 (12), 112 and 231.
[64] See Sherwin and Kennard, Jour. Biol. Chem., 1919 (40), 259.

excreted as hippuric acid, and the phenylpropionic acid is excreted as *p*-hydroxy hippuric acid. Phenylacetic acid combines with glutamine and is excreted as phenylacetylglutamine in man, but in other species different radicals are used for detoxication.[65] It is but slightly toxic, 5 gm. doses in man causing slight symptoms resembling alcoholic intoxication, 16 gms. not producing serious results.

Phenols[66] appear in the urine normally. Folin and Denis[67] found the total excretion of phenols to be from 0.2 to 0.4 gm. per day, the amount varying with the protein intake.[68] They seem to come chiefly, if not entirely, from tyrosine.[69] Rhein[70] ascribes the phenol of intestinal origin to the action of a specific sort of colon bacilli *B. coli phenologenes*, which does not produce indole but produces phenol from tyrosine, and also from *p*-hydroxybenzoic acid which therefore may be an intermediary in tyrosine cleavage. Hanke and Koessler[71] have found that only certain strains of colon bacilli can catabolize tyrosine, forming tyramine as a buffer when acidity develops but forming phenol when the medium is alkaline or neutral. Much more phenol is undoubtedly formed in the intestines than is recovered in the urine, for but a small fraction of phenol given by mouth (2 to 3 per cent., according to Munk) appears in the urine as a sulfuric-acid compound; part of the rest is oxidized to hydrochinon and pyrocatechin, $C_6H_4(OH)_2$, and eliminated as ethereal sulfates. These sulfates, although distinctly toxic are much less so than the phenol itself (Metchnikoff).[72] Pelkan and Whipple[73] say that more than half of the volatile phenols (chiefly phenol and para-cresol) are oxidized, the rest conjugated in the liver with sulfuric and glucuronic acids, and probably no free phenols are present in the blood except in phenol poisoning. Extensive injury to the liver, as by chloroform, greatly reduces the amount of phenol conjugation.

Contrary to prevailing ideas, Folin and Denis found the greater part of the phenols to be excreted normally uncombined. The largest quantities are found in the same conditions as indican except, of course, in "carbolic-acid" poisoning, when the amounts may be so great that practically all the sulfuric acid in the urine is in this organic combination, much of the phenol under these conditions being also combined with glucuronic acid.[74] A small amount of urinary phenol may be of endogenous origin.[68] Underhill and Simpson[75] found that the amount of phenols in the urine varies with the amount of protein in the food and the amount of intestinal putrefaction, constipation having more effect than diet alone on phenol excretion.

Blood contains small amounts of free phenols, about one-third being polyphenols. In a series of pathological cases Theis and Benedict[76] found from 1.87 to 7.96 mg. per 100 c.c. blood, somewhat higher figures being found in sarcoma and hernia cases than in other diseases. Under some ill defined conditions, so much oxyphenol is formed through protein catabolism as to lead to melanuria independent of melanomatosis.[77]

Cresol (chiefly paracresol), *para-oxyphenyl acetic acid*, and *para-oxyphenyl propionic acid* appear under similar conditions as the phenols except that the two oxyacids are possibly also formed within the body through cellular metabolism, as they have been found present in the urine independent of intestinal putrefaction. Presumably these substances are derived largely from tyrosine, and, being nontoxic are not oxidized or conjugated, but excreted in the urine in the free state (Pelkan and Whipple).[73] Paracresol is quantitatively the most important of the urinary phenols, and long continued feeding of paracresol produces no noticeable effects in rabbits.[78]

[65] Shiple and Sherwin, Jour. Biol. Chem., 1922 (53), 463.
[66] Literature given by Dubin, Jour. Biol. Chem., 1916 (26), 69.
[67] Jour. Biol. Chem., 1915 (22), 309.
[68] Moore, Amer. Jour. Dis. Child., 1917 (13), 15.
[69] Tsudji, Jour. Biol. Chem., 1919 (38), 13.
[70] Biochem. Zeit., 1917 (84), 246.
[71] Jour. Biol. Chem., 1924 (59), 867.
[72] Ann. Inst. Pasteur, 1910 (24), 755.
[73] Jour. Biol. Chem., 1922 (50), 499 and 513.
[74] See the observations of Wohlgemuth and of Blumenthal (Biochem. Zeitschrift, 1906 (1), 134), on the detoxication of lysol and similar poisons.
[75] Jour. Biol. Chem., 1920 (44), 69.
[76] Jour. Biol. Chem., 1918 (36), 99.
[77] Haden and Orr, Johns Hopkins Hosp. Bull., 1924 (35), 58.
[78] Denny and Frothingham, Jour. Med. Res., 1914 (31), 277.

Probably part of the benzoic acid that appears in the urine combined with glycine, as hippuric acid, is derived from intestinal putrefaction.[79]

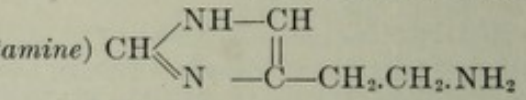
The Pressor Bases[80]

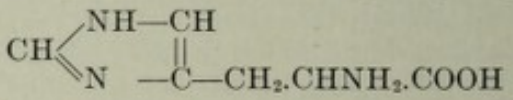
Among the products of protein putrefaction are several amines that have marked power to stimulate the sympathetic nervous system and thus to raise the blood pressure, hence resembling epinephrine physiologically as well as chemically. There are also bases derived from amino-acids which stimulate non-striated muscle without raising general blood pressure. The most important of these bases are:

Phenol-ethylamine $C_6H_5.CH_2.CH_2.NH_2$,
derived from *phenylalanine* $C_6H_5.CH_2.CHNH_2.COOH$.

Para-hydroxy-phenyl-ethylamine (*tyramine*) $OH.C_6H_4.CH_2.CH_2.NH_2$
derived from *tyrosine* $OH.C_6H_4.CH_2.CHNH_2.COOH$.

Its relation to *epinephrine* is seen on comparing with the structural formula of the latter $(OH)_2C_6H_3.CHOH.CH_2NH.CH_3$

Beta-iminazolyl-ethylamine (*histamine*) 

derived from *histidine* 

It will be observed that these are all amines derived from the cyclic amino-acids of proteins by the process of *decarboxylization* (loss of CO_2). The straight chain amines are much less active physiologically. The lowest amine having any considerable pressor action is *isobutylamine*, but *phenylethylamine* is at least five times as active as any aliphatic amine (Barger). Tyramine injected subcutaneously or intravenously increases blood pressure and slows the pulse rate,[81] resembling epinephrine, but it is only one-twentieth as active as the latter. Histamine ordinarily causes a fall of blood pressure, although it does constrict many peripheral vessels. Capillaries are dilated by histamine and Dale and Laidlaw compare it to a capillary poison,[82] which causes increased permeability to plasma and a retardation of blood flow through the capillaries. It kills guinea pigs by bronchiolar spasm and rabbits by obstruction to the pulmonary circulation in which respects its action resembles the syndrome of anaphylactic shock in these animals. When injected in very small doses into man, who seems to be more sensitive than laboratory animals, it causes dizziness, flushing of the skin, tachycardia, and a marked fall in blood

[79] See Prager, Med. News, 1905 (86), 1025; Magnus-Levy, Münch med. Woch., 1905 (52), 2168.

[80] Full bibliography given by Barger, "The Simpler Natural Bases," Monographs on Biochemistry, London, 1914; and Guggenheim, "Die Biogenen Amine," Springer, Berlin, 1924.

[81] See Hewlett, Proc. Soc. Exp. Biol. Med., 1917 (15), 12.

[82] Jour. Physiol 1918 (52), 110, 355; see also Rich, Jour. Exp. Med., 1921 (33), 287.

pressure.[83] Histamine acts as a strong stimulant to the gastric glands (secretin effect).[84] The relative pharmacological effects of epinephrine, tyramine, and histamine are given by Barbour, as follows:

	Blood pressure	Peripheral vessels	Coronary vessels (Ox)	Non-pregnant uterus
Epinephrine	+	+	−	−
Tyramine (*p*-hydroxyphenylethylamine)	+	+	+	−
Histamine (β-iminazolylethylamine)	−	+	+	+

+ means rise of blood pressure or constriction, − the opposite; the last-named amine may have a pressor effect in some animals.

Another difference is the production of severe urticarial reactions by histamine introduced into the skin, while tyramine and epinephrine both cause local blanching (Sollman and Pilcher).[85] Histamine causes inflammatory reactions, but tyramine does not.[86]

These substances have all been found in putrid protein materials, and they are formed in the intestine since colon bacilli are able to form histamine from histidine. Koessler and Hanke[87] found from 6 to 20 mg. of histamine in human stools weighing from 500 to 600 gms. These authors have shown that the production of amines by bacteria is a protective mechanism which serves to protect the microorganisms from excessive acidity. When the culture medium is kept alkaline or neutral no amines are produced from amino-acids. They also have found that some strains of colon bacilli will specifically decarboxylate histidine to form histamine, while others decarboyxlate tyrosine to tyramine, but the same strain will not do both.

Tyramine is also one of the active constituents of ergot, and has been found in the salivary gland of cephalopods, where it functions as a venom in paralyzing the prey. Histamine is said to be an important constituent of ergot. It has been found regularly present in the intestinal mucosa, presumably formed by intestinal bacteria. According to Abel[88] histamine, or at least some substance that has similar physiological effects, is widely distributed in all animal tissues, organ extracts, and Witte's peptone. Abel at one time suggested that histamine is the active agent of the posterior lobe of the hypophysis, but Hanke and Koessler[89] have shown, by using purely chemical methods, that the perfectly fresh

[83] Popielski, Arch. ges. Physiol., 1920 (178), 214; Carnot et al., Compt. Rend. Soc. Biol., 1922 (86), 670.

[84] See Popielski, Arch. ges. Physiol., 1920 (178), 214; Rothlin and Gundlach, Arch. internat. physiol., 1921 (17), 58.

[85] Jour. Pharm., 1917 (9), 391.

[86] E. P. Wolf, Jour. Exp. Med., 1923 (37), 511.

[87] Jour. Biol. Chem., 1919 (39), 539; 1924 (59), 835, 855, 879.

[88] Jour. Pharmacol., 1919 (13), 243.

[89] Jour. Biol. Chem., 1920 (43), 551, 567.

hypophysis contains no histamine. They could further demonstrate that peptone prepared from fibrin under aseptic conditions is free from histamine yet capable of producing typical peptone shock.

All these amines have little effect when given by mouth,[90] perhaps because partly detoxicated in the liver, but probably the intestinal mucosa is of more importance in the detoxification of amines, as has been shown to be true for histamine by Koessler and Hanke.[91] It is probable that no inconsiderable amounts are taken in our food and formed in the intestines every day. Their detoxication is accomplished by deaminization and oxidation, the resulting carboxylic acids being excreted or burned. Therefore it is not certain whether pressor bases formed in the intestines ever have any pathological effect, but it is quite possible that outside the portal territory various infections may give rise to pressor bases which enter the general circulation directly and escape the defensive mechanism of the liver and of the intestine. They may also cause local effects in the tissues where they are formed, *e. g.*, the bronchi.[92]

In some respects their effects resemble those of anaphylactic intoxication, and as the latter apparently results from toxic products of protein cleavage, the possibility at once presents itself that here, too, pressor bases are concerned, but as yet the relation is undetermined (see Anaphylaxis, Chap. X). The resemblance is especially seen in the profound effect on the bronchial musculature, which can be thrown into strong contraction, especially by histamine which in 0.5 mg. doses kills guinea pigs from asphyxia, with distended lungs as in fatal anaphylaxis; also it causes a similar fall in temperature. Another point of similarity is the severe local urticaria when weak solutions (1–1000) of histamine are placed on a scarified area of skin,[93] recalling vividly the fact that urticarial eruptions are conspicuous in some types of anaphylactic reactions. Furthermore, in guinea pigs histamine kills by bronchial spasm, in rabbits by obstruction to the pulmonary circulation (Dale and Laidlaw),[94] which difference is also characteristic of anaphylaxis in these animals. On the other hand, histamine does not produce the profound alteration in the coagulability of the blood that is characteristic of anaphylaxis and of peptone shock, but it may be that in each of these cases some other poison is responsible for the effect on the blood, in addition to histamine or a similar substance. In doses of 1 mg. and upward in cats, histamine causes a condition resembling traumatic shock, there being oligæmia from passage of plasma out of the vessels and retardation of blood in the periphery because of loss of tone by the capillaries. Possibly in traumatic shock histamine is liberated in the injured tissues.

[90] See Guggenheim and Loeffler, Biochem. Zeit., 1916 (72), 325.

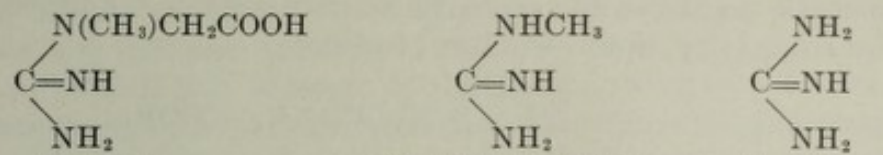
[91] Jour. Biol. Chem., 1924 (59), 889.

[92] Compare Huber and Koessler, "The pathogenesis of bronchial asthma," Arch, Int. Med., 1922 (30), 689. See also Ramirez and St. George, (Med. Jour. and Rec., 1924 (119) 71), who report that histamine causes a greater skin reaction in asthmatics than in normal subjects.

[93] Eppinger and Guttmann, Zeit. klin. Med., 1913 (78), 399.

[94] Jour. Physiol., 1919 (52), 355.

Methyl guanidine is said to be normally present in the urine in minute amounts, coming chiefly from the muscles where it presumably is formed from creatine, the relation being shown by their formulæ



Creatine — Methyl guanidine — Guanidine

It has been said to be found increased in amount in parathyroid tetany (Koch),[95] after extensive burns (Heyde)[96] and in a few other conditions. As methyl guanidine is distinctly toxic the symptoms and effects of these and other disease conditions, including anaphylactic shock, have been attributed to this amine.[97] However, the validity of these claims is doubtful. Parathyroid tetany is alleviated by calcium, which does not combat guanidine poisoning,[98] and the effects of guanidine are quite dissimilar to those of anaphylactic shock[99] and not the same as parathyroid tetany.[1] Furthermore, Greenwald[2] has been unable to confirm, with improved analytical methods the presence of increased guanidine excretion in parathyroidectomized dogs, and he has pointed out that earlier reports on the supposed presence of methyl guanidine in urines and other fluids are probably erroneous because of faulty analytical methods whereby creatine is changed directly into methyl guanidine. Major and Stephenson[3] observed that the guanidine compounds cause a rise of blood pressure, and suggest that this may be of some significance in the production of hypertension, but there is no evidence that there is any increase in these substances in the blood in persons with hypertension.

Alkaptonuria[4]

Alkaptonuria may be appropriately considered in this connection, since it depends on an abnormal metabolism of the aromatic groups, tyrosine and phenylalanine, which are, partly at least, split out of the protein molecule in the intestine. This condition is characterized by the tendency of the urine to turn dark on exposure to air, due to the presence in it of homogentisic acid.[5] Homogentisic acid has been found in the blood but not in the feces of alkaptonurics, and the urine shows no other deviations from the normal except a slight increase in ammonia, with which the acid is combined. It is of rare occurrence, only about eighty cases having been reported, persists throughout life with only little apparent effect upon the health[4] of the individual, and is often hereditary,[6]

[95] W. F. Koch, Jour. Biol. Chem., 1913 (15), 43; also Paton and Findlay, Quart. Jour. Exp. Phys., 1916 (10).

[96] Zent. f. Physiol., 1912 (26), 401.

[97] See Kollwitzer-Meier, Zeit. ges. exp. Med., 1924 (40), 59.

[98] Klinger, Arch. exp. Path. Pharm., 1921 (90), 129.

[99] Loewit, Arch. exp. Path. Pharm., 1913 (73), 1.

[1] Swingle and Nicholas, Amer. Jour. Physiol., 1924 (69), 455; Bayer and Form, Zeit. ges. exp. Med., 1924 (40), 445.

[2] Jour. Biol. Chem., 1924 (59), 329.

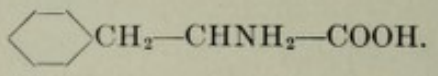
[3] Johns Hopkins Hosp. Bull., 1924 (35), 140, 186.

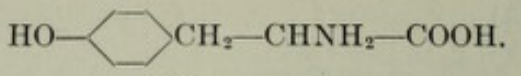
[4] Résumé and literature by Garrod, "Inborn Errors of Metabolism," Oxford Med. Publications, 1909; also Lancet, July, 1908; Gibson and Howard, Arch. Int. Med., 1921 (28), 632; Rosenbloom "Endocrinology and Metabolism," 1924 (4), 501.

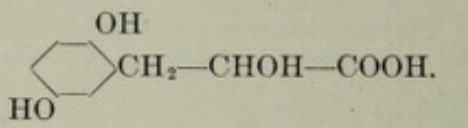
[5] It should be mentioned that *hydrochinon*, when present in the urine (usually after ingestion of large quantities of phenol), may also turn dark on exposure to air; and *melanin* may be excreted as a chromogen which turns dark on exposure, by patients with melanotic tumors or ochronosis (*q. v.*).

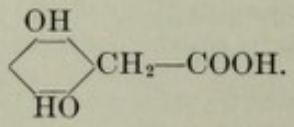
[6] Apparently it behaves as a Mendelian recessive, (see Cuthbert, Lancet, 1923 (1) 593).

being grouped by Garrod along with cystinuria, pentosuria and albinism as a "chemical malformation" of hereditary origin.[7] The relation of these aromatic bodies to the aromatic constituents of the proteins is best shown by comparing their structural formulæ:

Phenylalanine, 

Tyrosine, 

Uroleucic acid,[8] 

Homogentisic acid, 

Apparently the condition depends upon an abnormality in the intermediary metabolism, and not upon an abnormal formation of homogentisic acid through intestinal putrefaction, as was at first believed. Alkaptonuria is never observed in slight degrees; if there is any homogentisic acid in the urine at all it is there in large amounts (4-5 grams per day), depending on the diet, for when the error in metabolism is present at all it is complete. On a mixed diet the ratio of homogentisic acid to nitrogen in the urine is 40 or 50 to 100. The prevailing idea has been that the abnormality consists not in the excessive formation of homogentisic acid, but in a lack of ability on the part of the alkaptonuric individual to split open the benzene ring. It is generally stated that tyrosine and phenylalanine first suffer a splitting out of the nitrogen radical from the alanine side-chain, and then are oxidized into homogentisic acid, following which changes comes a disintegration of the benzene ring, with subsequent complete oxidation. On this basis the alkaptonuric accomplishes the conversion into the oxy-acid but the process stops there. Wakeman and Dakin,[9] however, have obtained evidence that in the normal oxidation of tyrosine and phenylalanine, homogentisic acid is not an intermediary product, and Dakin states that the alkaptonuric can destroy simple derivatives of phenylalanine and tyrosine, provided their structure is such that the formation of substances of the type of homogentisic acid is precluded. He believes that in alkaptonuria there is abnormal formation of homogentisic acid as well as a failure to destroy it when formed. On the other hand, Abderhalden[10] has been able to cause the appearance in the urine of homogentisic acid in a normal individual by feeding large

[7] Alkaptonurics may give a positive Wassermann reaction without other evidence of syphilis, and in one case this reaction disappeared when the patient was given large amounts of tyrosine (Söderbergh, Nord. Med. Arkiv., 1915 (48), 1).

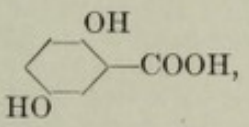
[8] The older writers stated that uroleucic acid commonly accompanied homogentisic acid in the urine of alkaptonuria, but later observations do not confirm this. (Oswald, Zeit. physiol. Chem., 1914 (93), 307).

[9] Jour. Biol. Chem., 1911 (9), 139 and 151.

[10] Zeit. physiol. Chem., 1912 (77), 454.

amounts of tyrosine, which is in favor of the view that it is a normal intermediary in tyrosine catabolism.[11] In any case the administration of tyrosine or phenylalanine, or of tyrosine-rich foods—*i. e.*, proteins—causes a marked increase in the amount of homogentisic acid eliminated in the urine; indeed, this increase is almost quantitative. Normal individuals when given these substances in moderate amounts, or homogentisic acid itself, destroy them completely, so that the latter does not appear at all in the urine.[12] If alkaptonurics are kept without protein food for some time, the elimination of alkaptonuric acid goes on, although in diminished amounts, indicating that the aromatic amino-acids formed in tissue catabolism also fail to be destroyed and, therefore, appear in the urine as these derivatives.

Since *gentisic acid*,



when given by mouth, is also eliminated unchanged by alkaptonurics, although completely destroyed by normal individuals, it seems evident that the difficulty in metabolism concerns the benzene ring itself and does not depend upon the character of the side-chain. Normal organisms seem to be capable of destroying such aromatic compounds as pass through a stage of homogentisic acid in being oxidized, which indicates that the benzene ring can be broken up only when oxidized in this particular manner (the 2, 5 position); the alkaptonuric differs in being unable to break up even this form (Falta). According to Garrod[13] the conversion of tyrosine and phenylalanine into homogentisic acid is so complete that the ratio of homogentisic acid to nitrogen is constant and the same in all cases.[14] Frommherz and Hermanns[15] advance the suggestion that normal oxidation of the aromatic radicals may take place by two routes, one by way of homogentisic acid, the other by way of the 3–4 dioxy-derivatives (*i. e.*, pyrocatechin), since such derivatives can be readily oxidized in the metabolism of alkaptonurics who cannot destroy homogentisic acid. That is, their deficiency involves only one of two possible methods of oxidizing aromatic compounds, leaving them considerable capacity for this important metabolic function. The tissues of the alkaptonuric are probably not chemically affected in this condition, for Abderhalden[16] found that the hair and nails of an alkaptonuric contained normal proportions of tyrosine. There is often an arthritis from deposition of pigment in the joints, designated by Gross[17] as "arthritis alkaptonurica."

[11] Katsch (Deut. Arch. klin. Med., 1918 (127), 210) believes that homogentisic acid is converted into acetone.

[12] Gross states that normal serum destroys homogentisic acid, which property is lacking in the serum of alkaptonurics (Biochem. Zeit., 1914 (61), 165).

[13] Garrod and Clarke, Biochem. Zeit., 1907 (2), 217.

[14] However, Katsch (Deut. Arch. klin. Med., 1920 (134), 159) has observed in an alkaptonuric a disappearance of the alkaptonuria during acidosis produced by fasting.

[15] Zeit. physiol. Chem., 1914 (91), 194.

[16] Zeit. physiol. Chem., 1907 (52), 435.

[17] Deut. Arch. klin. Med., 1919 (128), 249.

In some cases of alkaptonuria a pigmentation of the cartilages also occurs, *ochronosis*, but the association is not constant; ochronosis may occur without alkaptonuria, and conversely. (See "Ochronosis.")

(2) Substances Arising from the Fatty Acid Radicals (Amino Acids) of Proteins

The protein molecule consists of a combination of a great number of organic acids, of various sorts, all of which have as a common characteristic the presence of a NH_2 group attached to the carbon atom nearest the acid radical, the α position; thus, $R—CHNH_2—COOH$. A few of the amino acids contain an aromatic group, and the relation of these to intestinal decomposition has been considered above. The greater number have a simple fatty acid radical (the simplest amino-acid being glycine, $CH_2NH_2—COOH$), and from them are derived by intestinal putrefaction substances that are, for the most part, chemically simple and, as far as known, pathologically unimportant. From leucine alone is derived a substance of known considerable toxicity, the pressor base *isoamylamine*. $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH—CH_2—CH_2—NH_2$ which is less powerful than the cyclic pressor bases described previously. Bain[18] found it the most abundant pressor base of the urine, but this finding is probably incorrect.[19]

Fatty acids may readily be formed by splitting out the NH_2 group; thus acetic acid may be formed from glycine, etc. Apparently butyric and acetic acid are the acids most commonly formed in this way. Gaseous derivatives, such as hydrogen, ammonia, carbon dioxide, and marsh-gas, are also produced. *Acetone* is perhaps formed from these fatty acids; it is often present in the intestinal contents, but may come from other sources.

Certain conditions of cyanosis have been designated as *enterogenous cyanosis*, (see Methemoglobin, Chap. XX) because of the belief that the methemoglobin responsible for the cyanosis is caused by nitrites derived from intestinal putrefaction and demonstrable in the blood.[20] Under the same term are included the cases of *sulfhemoglobinemia*. This condition is ascribed by Wallis[21] to bacteria which produce from the proteins a hydrox lamine derivative, capable of reducing oxyhemoglobin, and which he finds present in the blood of patients with sulfhemoglobinemia (*q. v.*).

Diamines.—Two of these, *putrescine*, $NH_2(CH_2)_4NH_2$, and *cadavarine*, $NH_2(CH_2)_5NH_2$ are of particular interest,[22] because they have been sometimes observed in the feces and urine of persons with *cystinuria* (*q. v.*). The stools in cholera also seem to contain these ptomaines frequently. A few reports describe the findings of traces of these diamines in the urine and feces in other conditions, but the value of these observations is doubtful. Their etiological relation to the cystinuria is no longer accepted, and their toxicity is slight. They are probably derived from the diamino-acids of the protein molecule, putrescine being closely related to ornithine,[23] and is probably formed from it as follows:

$$\underset{\text{(ornithine)}}{CH_2(NH_2)—CH_2—CH_2—CH(NH_2)—COOH} = \underset{\text{(putrescine)}}{CH_2(NH_2)—CH_2—CH_2—CH_2(NH_2)} + CO_2$$

while cadaverine is probably formed from, *lysine*,

$$\underset{\text{(lysine)}}{CH_2(NH_2)—(CH_2)_3—CH(NH_2)—COOH} = \underset{\text{(cadaverine)}}{CH_2(NH_2)—(CH_2)_3—CH_2(NH_2)} + CO_2$$

[18] Quart. Jour. Exp. Physiol., 1914 (8), 229.

[19] Guggenheim and Loeffler, Biochem. Zeit., 1916 (72), 325.

[20] See Gibson, Quart. Jour. Med., 1907 (1), 29; West and Clarke Lancet, Feb. 2, 1907; Davis, Lancet, Oct. 26, 1912.

[21] Quart. Jour. Med., 1913 (7), 73.

[22] For discussion of formation and properties of these two ptomaines, see Vaughan and Novy's "Cellular Toxins."

[23] Ornithine forms part of the arginine molecule, which is universally present in proteins, ornithine being formed when urea is split from arginine.

Ethylidendiamine, $CH_3—CH\begin{matrix}NH_2\\NH_2\end{matrix}$ which is somewhat toxic, has also been detected in the contents of the gastro-intestinal tract.

Apparently these substances are absent from normal feces, but this does not exclude the possibility of their normal formation, absorption, and destruction. There is no evidence that they often, if ever cause symptoms or pathological alterations, although Rominger[23a] attributed the intoxication observed in an infant with megacolon to amines, which were present in the urine. In other conditions of intestinal disturbance, however, no excretion of amines could be observed.

β-iminazolylethylamine (histamine), formed by decarboxylation of histidine, has been discussed under the pressor bases. The other known amines of importance are few, except the normal amine, epinephrine.

(3) Substances Arising from the Sulfur-containing Radical of Proteins

Most if not all of the sulfur in the protein molecule seems to be contained in the amino-acid, *cystine*, which has the following composition:

$$\begin{matrix}S—CH_2—CHNH_2—COOH.\\|\\S—CH_2—CHNH_2—COOH.\end{matrix}$$

From this is formed the *hydrogen sulfide* of the intestinal gases, of which about 0.058–0.066 gram is present in each one hundred grams of normal colon contents. Although Senator has described a case in which an intoxication with H_2S of intestinal origin occurred, this gas seems not to be a frequent cause of intoxication, and Senator's case stands almost alone. Under normal condition H_2S does not appear in the urine, any that may be absorbed probably being oxidized to SO_4. If enough H_2S should enter the blood so that it was not completely destroyed, it might well cause harm, for it is decidedly toxic, particularly affecting the nervous system; but we have no evidence that this often happens. Van den Bergh[24] has observed cases of intestinal obstruction in which the presence of *sulfhemoglobin* in the patient's blood was demonstrated. (See sulfhemoglobin, p. 537.)

Methyl mercaptan, CH_3SH, has also been found in the feces, although it seems not to be abundantly or constantly present, accordin to Herter,[25] who found also that mixed bacteria from normal feces rarely produce mercaptan in cultures. However, bacteria from the feces of persons suffering with various diseases often produce mercaptan. *Ethyl mercaptan*, C_2H_5SH, and *ethyl sulfide*, $C_2H_5—S—C_2H_5$, have also been described as fecal constituents. It is not known that the mercaptans are a cause of intoxication.

Cystine and Cystinuria[26]

Cystine has been observed in the urine in a number of cases, and when present at all it is usually found in considerable quantities. Because of its slight solubility it appears as a deposit of hexagonal crystals, and frequently forms *cystine concretions* (*q. v.*) in the urinary bladder.[27] According to Garrod it is more common than alkaptonuria, and, like the rest of the "Inborn Errors of Metabolism," occurs much more often

[23a] Klin. Woch. 1925 (4) 261.

[24] Deut. Arch. klin. Med., 1905 (83), 86.

[25] Jour. Biol. Chem., 1906 (1), 421.

[26] Literature concerning cystine given by Friedmann, Ergebnisse der Physiol., 1902 (I. Abt. 1), 15. Cystinuria reviewed by Bödtker, Zeit. physiol. Chem., 1905 (45), 393; Garrod, "Inborn Errors of Metabolism." Mörner, Upsala Läkaref. Förh., 1920 (25), 265; Kahn and Rosenbloom, "Endocrinology and Metabolism," 1924 (4), 515.

[27] Abderhalden (Zeit. physiol. Chem., 1903 (38), 557) has described a case in a child in which the organs were infiltrated with masses of the cystine crystals, and two similar cases are described by Lignac, Neder. Tijd. Geneesk., 1924 (1), 2987.

in males than in females. Hofmann[28] was able to collect from the literature to 1907 a total of 175 cases, of which 85 were males and 45 females. Baumann and others observed that in cystinuria the urine often contains, besides the cystine, the diamines *cadaverine* and *putrescine*, which are formed from lysine and ornithine respectively in the intestines through putrefaction, and they naturally suspected that cystine arose in the same way. Another view was that the diamines interfered with the oxidation of sulfur in the body, so that it was eliminated in the unoxidized form of cystine. But it has been demonstrated that neither of these hypotheses is correct, for (1) cystine could not be found in the feces; (2) if given by mouth, it is completely oxidized, and causes only the appearance of excessive amounts of sulfates in the urine; (3) cystinuria has been observed to occur independent of the presence of the diamines, and not to be modified or caused by their administration or pathological formation. The view now prevalent is that the cystine that escapes in the urine in cystinuria is not derived from intestinal putrefaction, but is formed in the tissues from the protein molecule, and fails to be further decomposed because of some anomaly of metabolism. This view is supported by the fact that cystinuria often appears to be an hereditary disease, occurring in families for several generations; it is independent of the diet, small amounts of cystine appearing even if proteins are withheld, and also independent of intestinal putrefaction.[29] In some cases, however, a large part of the urinary cystine is derived from the protein of the food, while in other cases most of the cystine seems to be of endogenous origin.[30] It having been found that *leucine* and *tyrosine* may also occur in the urine in cystinuria,[31] it seems probable that this condition depends upon a general abnormality of protein metabolism, but in a series of cases studied by Looney *et al.*,[32] there was no abnormality in the metabolism of other amino acids. The relation of the diamines to the condition is therefore very uncertain. Cystine does not seem to exert any toxic effect, and patients with cystinuria do not usually appear to suffer greatly from the abnormal metabolism, the chief trouble observed being due to the formation of the concretions in the kidneys or bladder.[33] Sometimes in children, however, emaciation and early death without other apparent cause, have been observed, and Abderhalden[34] suggests that this is because of the role played by amino-sulfur compounds in biological oxidations.

The metabolic error in cystinuria is not complete in cases so far studied, for only a portion of the total cystine of the catabolized proteins is excreted

[28] Cent. Grenz. Med. u. Chir., 1907 (10), 721.

[29] An isolated case of transient cystinuria in a patient with Raynaud's disease is described by Githens (Penn. Med. Jour. 1910 (1), 507).

[30] See Rosenfeld, Berl. klin. Woch., 1917 (54), 957.

[31] See Abderhalden, Zeit. physiol. Chem., 1919 (104), 129.

[32] Looney, Berglund and Graves, Jour. Biol. Chem., 1923 (57), 515.

[33] This may be avoided by decreasing the cystine by means of a low protein diet, and increasing its solubility by keeping the reaction of the urine alkaline (Smillie, Arch. Int. Med., 1915 (16), 503). Administration of sodium bicarbonate greatly decreases the proportion of cystine sulfur in the urine (Looney et al).[32]

[34] Arch. Néerl. physiol., 1922 (7), 234.

as such (Garrod). This would amount to some five grams per day, whereas the average excretion is only about 0.3–0.5 gram, and sulfates and other neutral sulfur compounds are always present in the urine. In no condition other than cystinuria have putrescine and cadaverine been found in quantities which could be detected by ordinary methods in 24-hour specimens; they may also be found in the feces of cystinurics, where cystine is never found. In the urine their presence is inconstant, and the amounts are at best very small. Leucine and tyrosine are found much less often than the diamines; lysine has been found in one case,[35] which supports the view that cadaverine and putrescine come from the diamino-acids of the protein molecule by metabolism rather than by putrefaction. If this is true the formation of amines in the tissues by decarboxylation constitutes a further anomaly of metabolism associated with the "chemical malformation" of cystinuria.

B. Products of Fermentation of Carbohydrates

These include practically all the members of the fatty acid series, from *formic acid* to *valerianic acid;* and the oxy-acids, *lactic*, *succinic*, and *oxybutyric;* also, *oxalic acid*, *acetone*, *ethyl alcohol*, and the following gases: CO_2, CH_4, H_2. For the most part, the various organic acids are absorbed through the intestinal walls, and are oxidized completely in the tissues without causing any harm whatever. The possibility that acid intoxication may be produced in this way has been suggested, but it is generally believed that this does not occur, except possibly in infants. *Lactic* and *butyric*[36] acids are formed particularly in gastric fermentations in persons with deficient hydrochloric acid, motor insufficiency, or organic obstruction. Most of the disturbances observed in these conditions seem to be due to distention of the stomach with gas, chiefly CO_2, which is formed during the fermentation. It is possible, however, that the organic acids exercise some irritant effects on the mucous membrane; and they may also cause diarrhea, lactic and acetic acids often being present in diarrheal discharges due to excessive feeding with carbohydrates (Herter).

These acids or their salts do not appear in the urine, unless possibly as minute traces, except the *oxalic acid.* Minute quantities (0.02 gm. per day) of this substance are present in normal urine, but larger quantities (*oxaluria*) seem to depend either upon the taking of food containing much oxalic acid (rhubarb, spinach, etc.) or upon excessive gastric fermentation of carbohydrates (Baldwin),[37] and perhaps upon excessive destruction of purines, from which oxalic acid may be derived. Of the amino-acids it is presumably the diatomic acids, glutamic and aspartic, which yield oxalic acid (Jastrowitz).[38] Others, however, do not admit that any appreciable amount of oxalic acid is derived from proteins.[39] Probably the small quantities of oxalic acid thus formed do not cause toxic effects, and are important chiefly as causing urinary concretions of calcium oxalate, although there is evidence that long-continued excretion of oxalic acid may cause renal lesions. (See also consideration of oxalate calculi; Chap. XIX.)

C. Products of the Decomposition of Fats and Lipoids

These differ but little from the products of carbohydrate fermentation, the large fatty acid molecules being broken down to smaller ones.

[35] Ackermann and Kutscher, Zeit. f. Biol., 1911 (57), 355.

[36] Coleman (Ann. Inst. Pasteur, 1915 (29), 139) attempts to incriminate butyric acid in the production of arteriosclerosis, while Oswald Loeb believed lactic acid to be important, a view which could not be altogether supported by Denny and Frothingham, Jour. Med. Res,, 1914 (31), 277.

[37] Jour. Exp. Med., 1900 (5), 27.

[38] Biochem. Zeit., 1910 (28), 34.

[39] Wegrzynowski, Zeit. physiol. Chem., 1913 (83), 112.

In infants these fatty acids have been believed to be a cause of acid intoxication and acetonuria,[40] but probably they are seldom, if ever, of pathological importance. It is possible, however, that a serious reduction in the bases of the blood may result from the formation of excessive amounts of fatty acids in the intestines, the bases being combined to unite with the fatty acids, and then excreted in the feces.

The Choline Group.—It is quite otherwise with the products of decomposition of *lecithin.*[41] From its molecule is split off the ptomaine *choline*,

$$(CH_3)_3 \equiv N—CH_2—CH_2OH$$
$$\quad\quad\quad

serum, and about the same proportion in the urine, there being no characteristic variations in disease. Subcutaneous injection of choline does not appreciably increase the amount excreted in the urine, and Shanks[51] found none after feeding one to two grams, so evidently it is largely destroyed.

Normally the largest amounts by far are obtained from the adrenals, which also seem to contain choline derivatives of much greater physiological activity. Choline itself is somewhat toxic, but the closely related body, neurine, into which it may be transformed, is highly poisonous, which makes choline an important indirect source of intoxication. It has been suggested that the intoxication of fatigue may be due, at least in part, to choline and neurine produced from lecithin decomposed during the period of cellular activity. The close structural relation to choline and neurine, of the mushroom poison, muscarine, which produces physiological effects very similar to those of neurine, indicates the close relationship of the putrefactive ptomaines and the vegetable alkaloids. Indeed, a muscarine apparently identical with that of the mushroom has been found in decomposing flesh, and neurine, presumably derived from lecithin, may be found in human urine.[43] Betaine, the fourth member of the group, which has but slight toxicity, is particularly well known as a constituent of plant tissues.

Both neurine and muscarine are extremely poisonous and quite similar in their effects. Subcutaneous injection of but 1 to 3 mg. of muscarine in man produces salivation, rapid pulse, reddening of the face, weakness, depression, profuse sweating, vomiting, and diarrhœa. Neurine, likewise, causes salivation, lachrymation, vomiting, and diarrhœa. In fatal poisoning, respiration ceases before the heart stops. Both poisons resemble physostigmine in their stimulation of secretion and are equally well counteracted by atropine. The toxicity of these substances is so great that not a large amount would need to be formed by oxidation of choline to produce severe symptoms, although it is not known that this actually occurs in the body. When introduced by mouth, the lethal dose of neurine is ten times as great as when injected subcutaneously, indicating that chemical changes in the gastro-intestinal tract or liver offer some protection against intoxication by these substances when taken in tainted food. Choline, although by no means so poisonous as neurine, has a similar action when administered in sufficiently large doses. According to Brieger, it is about one-tenth to one-twentieth as toxic as neurine.[52] Magnus[53] and his pupils maintain that choline is the physiological stimulant of Auerbach's plexus and have used it in the treatment of ileus, finding that an adult can tolerate 0.6 gm. of choline-HCl intravenously, the chief observed effects being redness of the face, salivation, slow pulse and lowered blood pressure.

Donath[54] found that choline injected directly into the cerebral cortex or under the dura is extremely toxic, causing severe tonic and clonic convulsions, and believes that choline may be responsible for epileptic convulsions. This view has been opposed, and properly so, by Handelsmann[48] and others. The attempt to ascribe importance to choline as a cause of either toxic or therapeutic effect of *x*-rays seems also to be entitled to but slight consideration.[55] It is probably a factor in the lowering of blood pressure which results from injection of extracts of various tissues, in which it is commonly present in minute amounts,[56] for very minute amounts of choline will produce a decided fall in blood pressure.[57] Shanks[58] reports finding an increased amount of choline in the blood in parathyroid tetany.

[51] Jour. Physiol., 1923 (58), 230.

[52] Halliburton, "Chem. of Muscle and Nerve," 1904, p. 119, states that choline produces a fall in blood pressure by dilating the peripheral vessels, whereas neurine constricts the peripheral vessels; he uses this difference in physiological effect as a means of distinguishing the two substances. Injected into animals, choline causes a considerable but transient decrease in the number of leucocytes in the blood, followed later by an increase (Werner and Lichtenberg, Deut. med. Woch., 1906 (32), 22).

[53] See Münch. med. Woch., 1923 (72), 249, 251.

[54] Zeit. f. physiol. Chem., 1903 (39), 526; also see Med. News, 1905 (86), 107, for literature and methods of analysis.

[55] See Schenk, Deut. med. Woch., 1910 (36), 1130.

[56] Schwarz and Lederer, Pflüger's Arch., 1908 (124), 353; Kinoshita, *ibid.*, 1910 (132), 607.

[57] Mendel *et al.*, Jour. Pharm. and Exp. Ther., 1912 (3), 648; Hunt and Taveau, Bulletin 73, Hyg. Lab. U. S. P. H. Service.

[58] Jour. Physiol., 1924 (58), 466.

Results of Gastro-intestinal Intoxication[59]

As we have seen from the above, but few of the known products of gastro-intestinal putrefaction are toxic to any considerable degree, and these are probably produced in too small quantities to cause any appreciable effect, especially in view of the detoxicating and eliminatory powers of the intestines, liver, kidney, and other organs. And yet we have abundant clinical evidence that excessive intestinal putrefaction or retention of the intestinal contents causes marked disturbance in health. The slight malaise, headache, and lassitude observed as the result of simple constipation may possibly be adequately accounted for by intoxication with indole and similar substances, although we have no conclusive proof that such is the case, and not a little evidence that such intoxication is unlikely to occur (see Indole, p. 662). Furthermore, people may pass large amounts of indican in the urine for a long time and yet show no evidence of ill health.

Two possible sources of intestinal intoxication may be suggested: One is that the intestinal flora becomes altered because of the changed conditions, and bacteria thrive that produce specific soluble toxic substances, analogous to those formed by B. *botulinus*.

Another suggestion is that many unidentified poisonous substances are produced in the alimentary canal which ordinarily are destroyed, but under certain conditions may be reabsorbed, especially when the detoxicating function or the selective permeability of the intestinal wall is reduced or altered, as might well happen through any extensive toxic injury to the epithelium. That unrecognized toxic substances are formed in the intestines is almost certain, for it has been repeatedly shown that extracts of the contents of the alimentary canal are very poisonous. Although the technic of many of these experiments has been questionable, the results have been obtained so often as to render it probable that the main contention is correct. Thus Magnus-Alsleben[60] has found in the upper part of the small intestine of dogs (except when on milk diet) a very poisonous substance which killed rabbits by respiratory paralysis, but which is inert when injected into the portal vein. Extracts of the wall of the large intestine are also toxic, and lose their toxicity at 60°, by passing through procelain filters and by treatment with alcohol; extracts of fetal intestines are not toxic (Distaso).[61] There is reason to believe that histamine is responsible for much of the toxic effects obtained with intestinal materials injected into the blood and tissues. But the body is protected against this as against many other poisons by the intestinal epithelium, which holds back some poisons and destroys others. For example, Whipple[62] found that the highly toxic substance which is formed in obstructed loops of small intestine is harmless when put into the intestine, and Koessler and Hanke[63] found that 500 mg. of histamine produced

[59] See review by Alvarez, Physiol. Reviews, 1924 (4), 352.
[60] Hofmeister's Beitr., 1905 (6), 503.
[61] Zeit. Immunität., 1913 (16), 466.
[62] Jour. Exp. Med., 1918 (28), 213.
[63] Jour. Biol. Chem., 1924 (59), 889.

no effects whatever when given to a dog by mouth, and Ivy observed no effects from equally large amounts introduced into the stomachs of dogs with Eck fistulas, although as little as 0.0027 mg. per kilo per minute injected into a vein causes a distinct fall in blood pressure. Evidently the intestinal wall is the detoxicating agent in this case, since it has been shown that the histamine disappears from the lumen of the bowel.

In any case, correctly or incorrectly, a great number of disease conditions have been attributed to poisons of gastro-intestinal origin, including not only such minor conditions as headache, malaise, lassitude, etc., but also sciatica, tetany, epilepsy, eclampsia, many forms of dermatitis, various forms of nervous diseases, myxedema and cretinism, chlorosis and pernicious anemia, cirrhosis, nephritis, and arteriosclerosis.[64] While in many cases the severity of these various conditions is apparently augmented by intestinal disturbances, the etiologic relation is not so clear. That long-continued intoxication of intestinal origin may cause serious injury to the tissues is, of course, possible, although not demonstrable as yet. There is, perhaps, reason for believing that some cases of non-alcoholic cirrhosis are due to this cause; possibly chronic nephritis, myocarditis, and arteriosclerosis may occasionally be the result of long-continued intoxication from the same source. Arteriosclerosis especially has been attributed to indole and related substances by Metchnikoff and his associates, who have reported the production of arteriosclerosis in rabbits by injecting indole, but not with skatole. Such results are, however, far from conclusive evidence that these substances have anything to do with human arteriosclerosis. As is well known, Metchnikoff believed that most of the manifestations of senility come from putrefaction in the large bowel,[65] and a number of observers have described as products of intestinal putrefaction certain pressor substances of high potency which, presumably, might cause serious arterial and cardiac injury,[66] but nothing has ever been proved. An elaborate study of a certain type of cases of defective development led Herter[67] to the conclusion that intestinal intoxication is responsible, and hence he designated this condition "intestinal infantilism." The severe intoxications observed in infants and attributed to intestinal putrefaction, may be either the result of absorption of amines from the intestine,[68] or, as many believe, the result of dehydration or of invasion of the blood by intestinal bacteria.

Tetany associated with gastric dilatation was formerly supposed to present the strongest case, numerous observers having reported finding a marked toxicity of the stomach contents.[69] But recent studies indicate

[64] The supposed relation of gastro-intestinal intoxication to these various diseases is reviewed by Weintraud, Ergeb. allg. Pathol., 1897 (4), 17.

[65] See Ann. Inst. Pasteur, 1910 (24), 755.

[66] See Granger, Arch. Int. Med., 1912 (10), 202.

[67] See McCrudden, Jour. Exper. Med., 1912 (15), 107.

[68] See Brown and Boyd, Canad. Med. Assoc. Jour., 1923 (13), 800.

[69] Bibliography by Halliburton and McKendrick, Brit. Med. Jour., 1901 (i), 1607.

that alkalosis is the important factor in tetany, and repeated vomiting with gastric dilatation causes alkalosis by removal of hydrochloric acid,[70] hence gastric tetany is probably not an autointoxication.[71] There is also considerable evidence that the tetany, when present, is associated with a deficiency in calcium in the blood and nervous tissue, and that this is further related to the functional activity of the parathyroids (*q. v.*). Although there are usually evidences of intoxication in *acute dilatation of the stomach*, yet there is no good evidence as to its nature. It is suggested by Woodyatt and Graham[72] that the dilatation is produced by CO_2 secreted into the stomach from its walls.

The relation of intestinal intoxication to the various anemias, particularly chlorosis and pernicious anemia, has been repeatedly suggested and discussed. Clinical evidence strongly indicates that such a relation exists, and there is no doubt that hemolytic substances may be formed in the alimentary tract,[73] but that chlorosis and pernicious anemia do depend upon intestinal putrefaction or infection is far from established (see "Anemia," Chap. XV).

As yet, *we cannot say positively that any human disease is caused by the products of intestinal putrefaction*, and with growing knowledge the importance ascribed to this source of disease is becoming steadily less.[74] The fact must not be overlooked that many persons habitually eat putrefied proteins, from the "high" game of the epicure to the carrion masses that delight many primitive people, without the slightest evidence of intoxication therefrom. Alverez,[59] in his excellent critical review on the subject of intestinal autointoxication, shows how inconclusive is all the evidence so far submitted to prove that substances absorbed from the alimentary canal are common or important sources of intoxication. Even the familiar manifestations of constipation are, he believes, explainable as merely the results of mechanical distention and irritation of the rectum by fecal masses, quite the same effects being produced by filling the rectum with masses of cotton. He sympathizes with the view of Adami that much of what is called autointoxication is subinfection by bacteria coming from the intestine. Adami feels that it is much more rational to regard the evil effects of intestinal stasis in man as due, not to autointoxication, but to conditions favoring subinfection. He feels so strongly on this point that he says: "The term gastrointestinal autointoxication is pernicious and not to be employed by any self-respecting member of our profession save for so limited a set of conditions that for ordinary purposes it may safely be wholly explunged from our medical vocabulary."

[70] See Haden and Orr, Jour. Exp. Med., 1923 (37), 377.

[71] Gollwitzer-Meier (Zeit. ges. Exp. Med., 1924 (40), 83), however, does not believe amine intoxication can be excluded in the etiology of gastric tetany.

[72] Trans. Chicago Path. Soc., 1912 (8), 354.

[73] See Külbs, Arch. exper. Path., 1906 (55), 73; also Herter, Jour. Biol. Chem., 1906 (2), 1.

[74] See Alvarez, loc. cit.,[59] also Jour. Amer. Med. Assoc., 1919 (72), 8; E. O. Jordan "Food Poisoning," Univ. Chicago Press, 1917.

The Detoxifying Function of the Liver.—It seems highly probable that gastrointestinal "autointoxication" would be a much more serious matter were it not for the mechanisms of defence possessed by the body, especially the intestinal epithelium, as mentioned above, and also the liver.[75] For example, Richards and Howland have indicated the increased toxicity of indole when the oxidizing power of the liver is reduced, and Herter and Wakeman have shown the power of the liver to combine indole and thus remove it from circulation. This topic has been discussed more fully elsewhere (Chap. XII).

Although there is no doubt that the liver does have more or less capacity to destroy, bind, or detoxify various poisonous substances reaching it in the blood, as illustrated by numerous examples cited on previous pages, nevertheless I wish to call attention to a common error in reasoning often employed by experimenters studying the supposed detoxicating effect of the liver. Their custom has been to inject a substance into the portal vein and compare its effect with the same doses injected into a systemic vein. When the intraportal injection shows less effect than the injection into the systemic vein, as it almost always does, the difference is attributed to a detoxicating effect of the liver. This conclusion is not at all justified, for it disregards certain elementary anatomical facts. The material injected into the systemic veins goes directly to the heart and lungs, having an opportunity to act upon these vital organs without first passing through any capillary network whatever, and it reaches the brain and all other parts of the body after having passed through only the lung capillaries. We know that capillary walls and reticulo-endothelium are active agents in defense reactions, and it is most probable that every capillary network is a potential area for detoxification. If the material to be tested is injected into the portal blood it passes through the capillary system of the liver before reaching the heart and lungs, and through a second set, in the lungs, before reaching the brain, cord, nerves and other tissues. Hence to determine a specific detoxifying function of the liver the systemic injection used for comparison with the portal injection should be made into a systemic *artery*, whereby the material injected will have to pass through a set of capillaries before it reaches the heart and lungs, just as it does when injected into the portal vein. At my suggestion Koessler and Hanke compared the effect of histamine injection into the portal vein and into the femoral artery, and found that the well known "detoxifying" effect of the liver was shown quite as well by the hind leg of the dog, and may well be in part, as Meakins and Harrington have suggested, merely a buffering effect of the capillaries which delays the spread of the poison through the body, although the endothelial cells may also play an active part in holding or destroying it. In view of these facts it is evident that much of the existing evidence concerning the detoxifying capacity of the liver is in need of revision.

Acute Intestinal Obstruction[76]

The violent effects that follow complete occlusion of the intestine, especially in the upper portion, apparently are due to some highly toxic substance or substances. The toxic effects of obstruction decrease progressively from the duodenum downward, and as the contents of loops formed in each portion are poisonous, the differences probably depend on absorption differences at the several levels. The clinical features of obstructive ileus, namely, vomiting, collapse, complete muscular relaxation, and subnormal temperature, are associated with the excretion of large quantities of indican and other substances combined with sulfuric acid, proving that intestinal putrefaction is active. Presumably in ileus there is a profound and rapidly fatal intoxication with substances formed in the obstructed intestines.

Whipple[77] has demonstrated that closed duodenal loops in dogs come to contain a highly toxic substance of unknown nature, apparently formed

[75] For discussion and literature see Lust, Hofmeister's Beitr., 1905 (6), 132.

[76] Literature reviewed by Gerard, Jour. Biol. Chem., 1922 (52), 111; Jour. Amer. Med. Assoc., 1922 (79), 1581.

[77] Whipple, Stone and Bernheim, Jour. Exp. Med., 1913 (17), 286.

in the epithelium of the gut rather than in its contents (since it is not absorbed when introduced into the lumen of the intestine), which causes severe splanchnic congestion, vomiting, and diarrhœa when injected into normal dogs. The toxic agent is not destroyed by autolysis, filtration, or heating at 60°, yet dogs can be made somewhat refractory or immune. From the contents of such loops, and from the bowel above obstructions, he has isolated a very toxic proteose,[78] which he believes may be responsible for the intoxication.[79] Whether this proteose, or whatever the active poison may be, comes from bacterial infection, autolysis, duodenal secretion, or what, is not yet agreed by the numerous investigators in this field.[80] There is much evidence in favor of the essential importance of bacteria[81] and it is held by some that toxic amines may be produced by bacterial action in the closed loops[82] and that injury to the mucosa facilitates absorption of the poisons. Certainly the fluid formed in closed duodenal loops has toxicological effects very similar to those of histamine, which Gerard[76] has found in closed intestinal loops (2 to 3 mg. per 100 c.c.).[83]

Apparently the liver does not have much detoxicating effect, for dogs with Eck fistula behave much the same when the intestine is obstructed as dogs with normal circulation. A similar material cannot be obtained by hydrolysis or autolysis of normal duodenal mucosa, the obstruction being an essential feature. The normal intestinal secretions do not have any considerable toxicity.[84] Obstruction of lower portions of the intestine has much less effect[85] and it has been suggested that the poison formed in the duodenum is neutralized or destroyed farther down in the intestine.[86] A striking feature of intestinal obstruction is the high non-protein nitrogen content of the blood, figures similar to those of fatal uremic coma being common,[87] which may be the result of absorption of cleavage products from the intestine, or toxicogenic destruction of tissue proteins, or both.[88] There is also probably an element of renal injury and reduced excretion,[89] but although urea is much increased, it constitutes less than half of the non-protein nitrogen.[90] Alkalosis and a fall in blood NaCl has also been

[78] Jour. Amer. Med. Assoc., 1915 (65), 476; 1916 (67), 15; Jour. Exp. Med., 1916 (23), 123; 1917 (25), 231 and 461.

[79] Schönbauer (Arch. klin. Chir., 1924 (130), 427) says that the toxic material may be found in the peritoneal fluid, but this is not corroborated by Foster and Hausler (Arch. Int. Med., 1924 (34), 697).

[80] Review by South and Hardt, Arch. Int. Med., 1918 (21), 292; also Gerard.[76]

[81] Cannon and Dragstedt, Jour. Inf. Dis., 1920 (27), 139.

[82] Dragstedt *et al.*, Jour. Exp. Med., 1919 (30), 109.

[83] This is not corroborated by Ingvaldsen (Ingvaldsen *et al.*, Jour. Exp. Med., 1924 (39), 117), who believes the toxic agent to be a nucleoprotein.

[84] Davis and Stone, Jour. Exp. Med., 1917 (26), 686.

[85] See Bunting, Jour. Exp. Med., 1913 (17), 192.

[86] Maury, Amer. Jour. Med. Sci., 1909 (137), 725.

[87] Cooke, Rodenbaugh and Whipple, Jour. Exp. Med., 1916 (23), 123.

[88] In acute intestinal obstruction, as well as in severe burns or other tissue injury, the urine often shows with the nitric acid test a double ring, supposedly caused by either albumose or mucoid. (See Flesch-Thebesius, Beit. klin. Chir., 1921 (121), 321.)

[89] McQuarrie and Whipple, Jour. Exp. Med., 1919 (29), 397.

[90] Louria, Arch. Int. Med., 1921 (27), 620; Conners *et al.*, Proc. Soc. Exp. Biol. Med., 1923 (20), 357; Rabinowitch, Canad. Med. Assoc. Jour., 1924 (14), 1057.

observed.[91] Hartwell and others have believed that loss of water through vomiting and deficient absorption is an important element in the intoxication of intestinal obstruction, and the rise in non-protein N may be interpreted as pseudouremia from dehydration, with secondary toxic disintegration of tissue proteins, similar to that seen in heat stroke.[92] However, other authors have been unable to demonstrate that there is enough dehydration to account for the observed results,[93] and Foster and Hausler[79] believe that shock combined with the products of bacterial growth within the strangulated loop accounts for the fatal results.

[91] Haden and Orr, Surg. Gyn. Obst., 1923 (37), 465; Jour. Exp. Med., 1923 (37), 365; also McVicar, Amer. Jour. Med. Sci., 1925 (169), 224.

[92] Bacon *et al.*, Arch. of Surg., 1921 (3), 445.

[93] See Ingvaldsen *et al.*[83]

CHAPTER XXIV

CHEMICAL PATHOLOGY OF THE DUCTLESS GLANDS[1]

DISEASES OF THE THYROID[2]

As we have much evidence that the thyroid has a marked influence upon metabolism, and also that it may be of importance in preventing and in producing autointoxication, the chemistry of diseases of the thyroid may be appropriately considered in connection with the autointoxications. In fact, hyperthyroidism is one of the most definitely established instances of true autointoxication that can be cited.

THE FUNCTIONS OF THE THYROID[3]

Metabolic Function.—That the thyroid has an important relation to metabolism, especially of proteins, is shown by the following facts:

(1) Administration of the gland substance, or active preparations made from it, to healthy men or animals, causes a greatly increased elimination of nitrogen in the form of urea.[3a] This nitrogen comes not only from the food, but also from increased tissue-destruction, as is shown by the loss of weight and strength, and by the increased excretion of sulfur and phosphorus. An increased destruction of the body fat also occurs, so that thyroid therapy has been found efficient in the treatment of obesity, but often dangerous because of the relatively great amount of tissue destruction. Basal metabolism is most markedly raised in hyperthyroidism, and is lower in cretinism and myxedema than in any other disease.[4]

(2) Loss of thyroid tissue, either through operation or disease, greatly reduces both nitrogeneous metabolism and oxidative processes. Administration of thyroid preparations under these conditions will bring the nitrogen elimination and the gas exchange back to normal.

(3) Deficient thyroid secretion in young animals prevents their developing normally, the amount of deficiency varing from nearly total lack of development in extreme cretinism to slight grades of defective development (infantilism) or delayed maturity. In adult animals, besides decreased metabolism there occur also various trophic changes in the skin and its appendages, an increased amount of mucin-like material in the tissues, and greatly decreased nervous and mental activity. All these conditions are relieved to greater or less degree by administration of thyroid tissue or its preparations.[5] Evidently, therefore, the thyroid exerts an influence upon growth

[1] Thorough reviews of the entire subject of the ductless glands are given by Biedl, "Innere Skeretion," Urban and Schwarzenberg, Berlin, 1922; Vincent, Ergebnisse Physiol., 1910 (9), 451; 1911 (10), 218; and in "Endocrinology and Metabolism," Barker, Hoskins and Mosenthal, Appleton, 1924.

[2] Concerning the thyroid see besides the above,[1] the review by Bircher, Ergebnisse Pathol., 1911, XV (1), 82; Crotti, "Thyroid and Thymus," Phila., 1922.

[3] See review by Marine, Physiol. Reviews, 1922 (2), 521.

[3a] See Rohde and Stockholm, Jour. Biol. Chem., 1919 (37), 305.

[4] Du Bois, Arch. Int. Med., 1916 (17), 915.

[5] Concerning the influence of thyroid on skeletal growth see Holmgren, Nordiskt Med. Arkiv., 1910 (43), No. 2. Literature given by Basinger, Arch. Int. Med., 1916 (17), 260.

and tissue changes; whether this depends upon its influence upon metabolism, or is an independent and specific function, cannot be determined.[6]

How the thyroid or its secretion modifies metabolism is not yet understood. One is reminded of the effects of kinases upon enzymes and their antecedents, and it may be imagined that the thyroid secretion activates both proteolytic and oxidative enzymes within the cells. Shryver,[7] indeed, did find that administration of thyroid to dogs for some time before killing them causes their liver tissue to undergo autolysis more rapidly than normal, although Wells[8] had been unable to observe any increased amount of autolysis when thyroid extracts acted upon liver tissue *in vitro*. Experimental observations show that carbohydrate metabolism is much influenced by the thyroid, so that thyroidectomized animals may fail to show glucosuria from various procedures that usually produce it, and they are incapable of utilizing sugar injected parenterally as well as normal animals; they also exhibit an excessive creatine output. Starlinger[9] says that the blood leaving the thyroid shows a greater degree of colloid dispersion than that coming to it, suggesting that the thyroid function may be concerned with preliminary steps in transforming large molecular aggregates of proteins into smaller ones. Protracted feeding of thyroid to growing animals reduces the weight attained and causes marked enlargement of suprarenals, heart, liver, spleen, testes, ovaries, and pancreas; the pituitary and uterus are reduced in size.[10]

Detoxicatory Function.—At one time it was thought that the thyroid had for its function the *destruction or neutralization of poisonous substances* formed in metabolism or through intestinal putrefaction. In support of this were advanced the following observations:

(1) After total removal of the thyroid from many species of animals, acute symptoms develop that suggest strongly an intoxication.

(2) After removal of the thyroid, marked changes occur in the blood, there being a severe anemia (as low as 2,000,000 red corpuscles), with some leucocytosis, and there occur structural changes in the blood vessel walls (Kishi).[11] Cytoplasmic degeneration of the liver, kidneys, and myocardium may also result (Bensen).[12] These effects suggest strongly the presence of poisonous substances in the blood of persons or animals lacking sufficient thyroid tissue.

(3) All the effects of thyroidectomy are more marked in carnivorous animals than in herbivora; indeed, the latter may be able to live in fair condition for several years without a thyroid. It is now believed that these results may be due to the fact that in some herbivora the parathyroids are so far separated from the thyroid that they are not ordinarily removed in thyroidectomy, whereas in many carnivora complete removal of parathyroids with the thyroids is more likely to be accomplished.

The effects described above, which were once ascribed to loss of thyroid, are now attributed to the loss of parathyroid tissue (*q. v.*). Suggesting an actual detoxicatory effect on the part of the thyroid itself is the observation of Reid Hunt,[13] who found that mice fed thyroid preparations have a greatly increased resistance to poisoning by aceto-nitrile; however, this is not necessarily nor even probably a direct intoxica-

[6] See Gudernatsch (Arch. Entwickl., 1912 (35), 457; Amer. Jour. Anat., 1914 (15), 431; Anat. Record, 1917 (11), 357), who found that feeding thyroid to tadpoles hastens their differentiation but checks growth; also removal of the thyroid from tadpoles increases growth but delays or entirely prevents metamorphosis (Hoskins, Jour. Exp. Zool., 1919 (29) 1). See review by Uhlenhuth, "Endocrinology and Metabolism" Vol. 1, p. 181.

[7] Jour. of Physiol., 1905 (32), 159.

[8] Amer. Jour. Physiol., 1904 (11), 351; corroborated by Morse, Jour. Biol. Chem., 1915 (22), 125.

[9] Mitt. Grenz. Med. Chir., 1923 (36), 151.

[10] Herring, Quart. Jour. Exp. Physiol., 1917 (11), 231; Kojima, *ibid.*, p. 255; Cameron and Carmichael, Jour. Biol. Chem., 1920 (45), 69; 1921 (46), 35.

[11] Virchow's Arch., 1904 (176), 260.

[12] Virchow's Arch., 1902 (170), 229.

[13] Amer. Jour. Physiol., 1923 (65), 257; full review.

tion, but more likely it results from alterations in metabolism.[14] Rats and guinea pigs behave just the opposite, showing a decreased resistance to aceto-nitrile after being fed thyroid, and according to some authors morphine is more toxic for such animals.[15] Also, it is said that the thyroid has the capacity of methylating and thus detoxifying guanidine acetic acid, which function is lost when the thyroid is removed but restored by feeding thyroid. (Stuber *et al.*)[16]

Whether the thyroid exercises its detoxicating effect, assuming that it has one, by a direct neutralizing action of its secretion upon the toxic substances in the blood or tissues, or indirectly by stimulation of the function of other tissues which perform the detoxication, or in part locally within the gland itself, is an unsettled problem. In relation to the last-named hypothesis is the extreme vascularity of the thyroid, which, according to Burton-Opitz[17] has passed through it much more blood in proportion to its weight than any other gland. Against the idea of a *local* detoxication is the fact that after extirpation of the thyroid all abnormal conditions may be prevented by proper administration of thyroid substance.

Biedl summarizes his views as to the function of the thyroid, in the following statement: "The thyroid is a secretory organ which discharges its secretion eventually into the blood, in the form of an iodine-containing protein. This secretion acts as a hormone, in that it modifies the activities of remote tissues. As far as we now know the thyroid secretion plays the rôle of a 'disassimilatory' hormone, in that it causes an increased disassimilation and increase of normal activity in many tissues. This effect is exemplified by the augmented metabolism, the activity of the heart and many parts of the sympathetic nervous system, and of a series of internal secretory organs (adrenals, hypophysis). In other tissues are found evidences of the action of an inhibiting and assimilatory hormone, as shown in the increase in growth of bone, development of the sex glands, and decreased internal secretion of the pancreas."

Chemistry of the Thyroid[18]

There can now be little question that the thyroid owes its action to constituents of its specific secretion.[19] Furthermore, the chief, if not the sole, active ingredient of the colloid is the iodine containing substance first discovered by Baumann in 1896, and called by him *thyroiodine* (or *iodothyrein*).[20] What seems to be the active iodine compound has been isolated by Kendall, and called *thyroxin*.

[14] Koopman (Endocrinology, 1919 (3), 318) states that administration of thyroid increases antibody formation in immunized animals. Remedi (Lo Sperimentale, 1902; abst. in Cent. f. Path., 1903 (14), 695) claims that tetanus toxin and other bacterial poisons, when injected into the thyroid gland, are harmless, which he attributes to a neutralization by the colloid. This observation is discredited by the work of Basinger, Jour. Infect. Dis., 1917 (20), 131.

[15] See Olds, Amer. Jour. Physiol., 1910 (26), 354.

[16] Biochem. Zeit., 1923 (143), 221.

[17] Quart. Jour. Physiol., 1910 (3), 297.

[18] Reviews are given by Rahel Hirsch, Handb. d. Biochem., 1909 III (1), 271; and A. Kocher, Virchow's Arch., 1912 (208), 86.

[19] Beyond the characteristic colloid secretion product, the thyroid presents no chemical features of interest; it differs from the other endocrine glands in being poor in lipoids (Fenger, Jour. Biol. Chem., 1916 (27), 303), the lipins being chiefly neutral fats (Haeberli, Virchow's Arch., 1916 (221), 333). The thyroid is very rich in lipase, catalase, and peroxidase; extirpation is followed by a decrease in these enzymes in the blood, while thyroid feeding increases them as well as the antitrypsin (Juschtschenko, Biochem. Zeit., 1910 (25), 49; Zeit. physiol. Chem., 1911 (75), 141).

[20] Iodine is present in the thyroid of all species, most in marine forms (Cameron, Jour. Biol. Chem., 1914 (16), 465; Biochem. Jour., 1914 (7), 466).

Proteins of the Thyroid.—The chemical nature of thyroid *colloid* has been studied particularly by A. Oswald.[21] He found that all the iodine of the thyroid is dissolved out in physiological salt solution, and that none of it is present in an inorganic form. In the salt solution extract are two protein bodies; one, precipitated by half saturation with ammonium sulfate, contains all the iodine, and seems to be a globulin; it resembles myosin in being precipitated by weak acids, and it contains an easily separated carbohydrate group. The other, precipitated by saturation with ammonium sulfate (exact limits of precipitation are between 6.4 and 8.2 tenths saturation), is a nucleoprotein, containing 0.16 per cent. phosphorus, but no iodine; it is without marked physiological activity as also is the protein-free watery extract of the thyroid.[22]

According to Tatum,[23] iodine is present both in the colloid and in the cells of the thyroid, about 20 to 40 per cent. being in the cells. It is assumed that the colloid secretion of the thyroid is reabsorbed into the blood, and Hektoen, Carlson, and Schulhof[24] have been able to demonstrate, by means of specific precipitin tests, that thyroglobulin is present in lymph coming from the thyroid.[25] Bensley and Cowdry[26] have found histological evidence that the thyroid cells may discharge their secretion either into the lumen of the acini or in the opposite direction, presumably into either the blood or lymph.

The iodine-containing protein, called *thyreoglobulin*, constitutes one-fourth to one-half the dry weight of the gland, and seems to contain the sole active constituent of the colloid; at least, its administration to animals has the same physiological effects as does the entire colloid (great increase in the urea elimination and decrease in blood pressure in animals, curative effect on myxedematous patients, increased tonus of both sympathetic and peripheral nervous systems).[27] Analysis of the thyreoglobulin from various animals has shown it to be of quite constant quantitative composition except for the iodine, which may vary greatly in amount. Normal human thyreoglobulin (from persons living in non-goitrous districts) has the following percentage composition:

$$C = 51.85,\ H = 6.88,\ N = 15.49,\ I = 0.34,\ S = 1.86.$$

Thyreoglobulin from goitrous districts contains much less iodine (0.18−0.19 per cent.), and from calves born with goiters a thyreoglobulin was obtained that agreed in all respects with normal thyreoglobulin, except that it contained no iodine at all. On the other hand, administration of iodides to patients causes the thyreoglobulin to become rich in organically bound iodine.[28] From these facts Oswald believes that the thyreoglobulin, as first secreted by the glandular epithelium, is free from iodine, and that it combines later with iodine from the circulating blood. Thyreoglobulin is not, however, simply an *iodized protein*, for the iodized proteins that can be artificially prepared do not possess the physiological activity of the thyreoglobulin, nor do other naturally occuring iodine-containing proteins (gorgonin, spongin). F. C. Koch[29] finds that the full activity of the gland is contained in the thyreoglobulin, and also in the metaprotein fraction of this globulin, while simpler cleavage products show less and

[21] His work is reviewed in his dissertation, "Die chem. Beschaffenheit und die Function der Schilddruse," Strassburg, 1900; also see Virchow's Arch., 1902 (169), 444.

[22] A. Oswald, Arch. ges. Physiol., 1916 (166), 169. Kocher and his collaborators, however, ascribe to the thyreonucleoprotein some slight metabolic effects antagonistic to the thyreoglobulin (See Mitt. Grenz. Med. Chir., 1916, vol. 29). Gröbly (*Ibid.*, 1918 (30), 403) suggests that the nucleoprotein decreases the solubility and resorption of the thyroglobulin.

[23] Jour. Biol. Chem., 1920 (42), 47; see also Van Dyke, *ibid.*, 1922 (54), 11.

[24] Jour. Amer. Med. Assoc., 1923 (81), 86.

[25] Hektoen and Schulhof (Jour. Amer. Med. Assoc., 1923 (80), 386) found that thyroglobulins are, at least in the main, species specific.

[26] Amer. Jour. Anat., 1916 (19), 37; 1922 (30), 25.

[27] A. Oswald, Arch. ges. Physiol., 1916 (164), 506.

[28] Nagel and Roos (Arch. f. Anat. u. Physiol., 1902, p. 297) found that administration of bromides had no effect upon the amount of iodine in the thyroid, and no storage of bromine takes place. Administration of pilocarpine does not increase the amount of iodine in the thyroid.

[29] Jour. Biol. Chem., 1913 (14), 101.

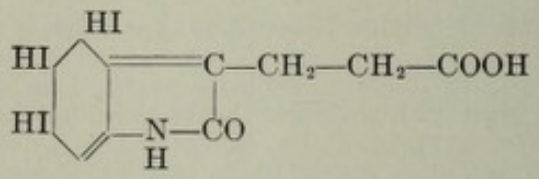
less activity per unit of iodine content. He could find no thyroid activity in iodine compounds of histidine, and di-iodotyrosine was found inactive by Strouse and Voegtlin.[30]

The remarkable influence of the thyroid on the development of tadpoles (Gudernatsch[6]) is exhibited by the thyreoglobulin, by iodized blood proteins[31] and also by diiodotyrosine and diiodotyramine,[32] and seems to be a property of many compounds of iodine but not of other halogens.[33] Swingle[34] states that inorganic iodine itself, even in thyroidless tadpoles, will slowly bring about metamorphosis that would not take place in the absence of iodine,[35] but this does not seem to be true of other species.

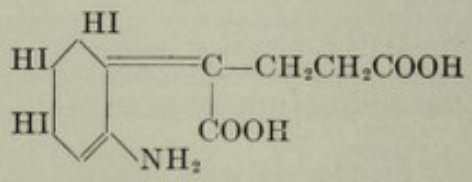
Bromine will not substitute for iodine in causing metamorphosis. The division rate of unicellular organisms (*Paramecium*) is not increased by thyroxin.[36] Lenhart[37] considers the effect of thyroid on tadpoles to be merely an expression of the general stimulation of metabolism, rather than a specific effect on differentiation.[38]

By decomposing thyreoglobulin by boiling with 10 per cent. sulfuric acid, a body is obtained containing as high as 14.5 per cent. of iodine; this is the *thyroiodine* of Baumann, which gives no biuret reaction, yet is physiologically active. The stability of this active constituent of the thyreoglobulin explains the successful administration of thyroid preparations by mouth. By the use of immunological methods it has been possible to demonstrate the absorption of unchanged thyroglobulin from the intestine, by finding it in the portal blood.[39] It appears to be absorbed unchanged and, unless enormous doses are given, none appears in the urine (Oswald).[40] Long-continued digestion with trypsin, or autolysis of thyroid glands, causes a complete splitting out of the iodine. One part of the iodine seems to be more firmly bound than the rest. A small amount of the iodine may exist as inorganic and lipoid compounds.[41]

Thyroxin.—Kendall[42] has isolated from the thyroid, after alkaline hydrolysis, a crystalline compound containing 65 per cent. of iodine which seems to be an indole derivative, designated as "thyroxin" (thyro-oxy-indole) with the following structural formula:



In the body the indole ring seems to exist in the open form shown below, corresponding to the open and closed structure of creatine and creatinine.



[30] Jour. Pharm. and Exp. Ther., 1910 (1), 123.

[31] Rogoff and Marine, Jour. Pharm., 1917 (10), 321.

[32] Abderhalden and Schiffmann, Arch. ges. Physiol., 1922 (195), 167.

[33] See review by Swingle, Biol. Bull., 1923 (45), 229.

[34] Jour. Gen. Physiol., 1919 (1), 593; Jour. Exp. Zool., 1919 (27), 397.

[35] Neither iodine nor thyroid produce any effect on the development of an animal naturally devoid of thyroid tissue (crab). (McLean, Publ. Puget Sound Biol. Stat., 1921 (3), 93).

[36] Woodruff and Swingle, Amer. Jour. Physiol., 1924 (69), 21.

[37] Jour. Exp. Med., 1915 (22), 739.

[38] See also Kahn, Arch. ges. physiol., 1916 (163), 384.

[39] Hektoen, Kanai and Dragstedt, Jour. Amer. Med. Assoc., 1925 (84), 114.

[40] Arch. exp. Path. u. Pharm., 1910 (63), 263.

[41] Blum and Grützner, Zeit. physiol. Chem., 1914 (91), 400.

[42] Jour. Amer. Med. Assoc., 1918 (71), 871; Trans. Assoc. Amer. Phys., 1918 (33), 324; Endocrinology, 1919 (3), 156; Jour. Biol. Chem., 1919 (39), 125; 1919 (40), 265.

This substance is highly active, causing rapid pulse, nervousirritability and increased metabolism, but these effects are not observed until several hours after injection, the maximum effect occurring after about 10 days and it requires about 24 days for all effects to cease. The function of thyroxin is believed by Kendall to be to furnish a compound that can be acted on by mild oxidizing agents, among which is molecular oxygen, and which can then by an intramolecular rearrangement produce an intensely oxidizing substance. Kendall has obtained evidence that the effect of thyroxin on the metabolic rate depends on the CO-NH grouping, for when the H is replaced by acetyl this function is lost; but as the acetylated thyroxin exhibits the usual effect on metamorphosis of tadpoles this latter effect may depend on the iodine. He suggests, however, that the iodine of the thyroxin molecule may render the reactive NH-CO grouping more effective in stimulating metabolism. Whether other halogens could replace iodine in the thyroxin molecule is not known.

Through its action on the metabolism of all the cells of the body thyroxin relieves most of the manifestations of myxedema and cretinism. It is estimated that one-third milligram increases the basal metabolism of an adult by 1 per cent., on which basis it may be calculated that from 23 to 50 mg. are functioning in the entire body. It acts like a true catalyst in carrying on action for two or three weeks after being administered. It contains only one-fourth of the iodine of the thyroid but the nature of the other iodine compounds is unknown, beyond the observation that they have no appreciable effects on normal persons but greatly improve the condition of cretins; presumably they represent intermediate stages in the formation of thyroxin. When fed to tadpoles, Kendall's active principle produces the characteristic thyroid effect.[43]

Persons with complete atrophy of the thyroid have a basal metabolism 60 per cent. of normal, which can be brought to normal by thyroxin, but what carries the 60 per cent. metabolism when the thyroid is functionless is unknown. Presumably thyroxin merely increases the rate of metabolism that goes on at a lower level without it, in which case the function of the thyroid seems to be merely to permit a greater range of flexibility in energy output. A single large injection has little effect while repeated small doses cause death, indicating that thyroxin of itself is not toxic, but acts only through its effect on metabolism. The excess is rapidly excreted, and but little is stored in the thyroid. The limit of tolerance for man is 2 mg. daily. If all the iodine found in normal blood (0.013 mg. per 100 c.c.) is thyroxin, there is about one part in five million of this compound in circulation.

Iodine Content of Thyroid.—The physiological activity of thyroid preparations, according to nearly all investigators, is in direct proportion to the iodine content,[44] which is the best of evidence that the formation of this compound is one of the chief functions of the gland, and that the iodine in the thyroid is not merely stored there as an undesirable foreign substance like copper in the liver. The selective deposition

[43] Rogoff and Marine, Jour. Pharmacol., 1916 (9), 57.

[44] Fonio, Mitt. Grenz. Med. u. Chir., 1911 (24), 123; Frey, *ibid.*, 1914 (28), 349; Hunt, Jour. Amer. Med. Assoc., 1907 (49), 1323; and Jour. Pharm. and Exp. Therap., 1910 (2), 15.

of iodine in the thyroid is remarkable, and when iodine is fed to animals it is stored very rapidly but it seems to require several hours before the active growth-modifying hormone is formed.[45] The perfused extirpated thyroid exhibits the same capacity to take up iodine.

Marine and Lenhart[46] find that the normal human gland contains an average of 0.4 mg. of iodine per gram of fresh weight (2.17 mg. per gram of dry weight), being less than that of domestic animals in the same part of the country. These figures agree closely with those obtained in thyroids from various parts of America by Wells[47] (2.10 mg. per gram dry weight). Zunz[48] has published extensive analyses of thyroids of healthy soldiers killed in the war, obtaining an average of 2.29 mg. of iodine per gram dry weight, the average total content being 15.53 mg. but varying from 3.15 to 44.5. Wells, Marine, and Oswald found that the amount of iodine varies directly with the amount of colloid, being decreased when cellular hyperplasia is present, in direct proportion to the amount of hyperplasia. Administration of iodine causes a reduction in the hyperplasia and a return to the colloid type of gland, while the iodine is deposited in the gland. Kocher, however, disputes the regularity of the variation of iodine and colloid content, stating that it is especially the *concentrated* follicle contents which hold the iodine. Seidell and Fenger[49] have found a marked seasonal variation in the thyroid iodine of animals, there being about three times as much between June and November as between December and May.[50] In man it has been found that at or before birth the thyroid of the fetus contains little or no iodine,[51] but in domestic animals the fetal glands contain not a little iodine (Fenger).[52] Extracts of the thyroid have little effect on the blood pressure, except for an alcohol-soluble fraction, poor in iodine, which is a depressor.[53] On the other hand, thyroid secretion increases the sensitiveness of the sympathetic nervous system to epinephrine.

Wasting diseases are associated with a considerable decrease in the size of the thyroid and the amount of colloid, and with this a decrease in the iodine; especially is this true of tuberculosis.[54] Patients or animals to whom iodine compounds are administered deposit it in the thyroid in large amounts, especially if the gland is previously deficient in iodine and at times there results even an acute thyroiditis from the iodine administration.[55] The variation in iodine content under various condi-

[45] Marine and Rogoff, Jour. Pharm., 1916 (9), 1.
[46] Arch. Int. Med., 1909 (4), 440.
[47] Jour. Amer. Med. Assoc., 1897 (29), 897.
[48] Arch. intern. physiol., 1921 (16), 288. Gives figures for various components.
[49] Jour. Biol. Chem., 1913 (13), 517.
[50] Valuable figures on the iodine content of foods are given by Forbes *et al.*, Bullet. Ohio Agric. Expt. Station, June, 1916, No. 299.
[51] See Thomas and Delhounge, Virchow's Arch., 1924 (248), 201.
[52] Jour. Biol. Chem., 1912 (11), 489; 1912 (12), 55; 1913 (14), 397.
[53] Fawcett *et al.*, Amer. Jour. Physiol., 1915 (36), 113.
[54] See Vitrey and Giraud, Compt. Rend. Soc. Biol., 1908 (65), 405. Aeschbacher, Mitt. Grenz. Med. Chir., 1905 (15), 269; Pellagrini, Arch. sci. Med., 1915 (39), 276.
[55] See Tobler, Mitt. Grenz. Med. Chir., 1924 (37), 622.

tions is given in the following table from Jolin,[56] his figures for normal glands being somewhat lower than found in America.[57]

Number and condition of glands	Dry wt., gms.	Mg. iodine per gm.	Total iodine
152 glands from persons over 10 yrs. old (44 not normal)	7.04	1.63	11.20
28 glands from children (1 mon. to 10 yrs.)	0.54	0.28	0.145
108 normal glands from adults only (both sexes)	5.38	1.56	8.05
67 normal glands from adults (males)	5.07	1.56	7.83
41 normal glands from adults (females)	5.90	1.55	8.40
38 glands from chronic diseases	4.29	1.90	7.81
29 glands from acute diseases	5.54	1.47	8.11
21 glands from sudden death	6.88	1.29	8.45
10 glands showing marked goiter	23.09	1.09	26.49
25 colloid-rich glands	8.25	2.24	18.20
34 glands from persons receiving iodine	5.79	2.56	15.06

Chemistry of Goiter

In connection with his earliest studies of thyroiodine Baumann observed a great difference in the amount of iodine in the thyroid glands of normal individuals living in goitrous districts, as compared with those living in non-goitrous districts. Thus in Freiburg, a goitrous district, the average weight of the dried thyroid was 8.2 grams, each gram containing 0.33 mg. of iodine a total of 2.5 mg. of iodine to each gland. Glands from Hamburg averaged 4.6 gm. in weight, containing 0.83 mg. of iodine per gram, a total of 3.83 mg. per gland. Berlin glands weighed 7.4 grams, and contained 0.9 mg. of iodine per gram, or a total of 6.6 mg. of iodine per gland. Both of the last-named cities are in districts where goiter is not endemic. The thyroids of young children show the same relative paucity of iodine in goitrous districts, as compared with non-goitrous districts. Wells[58] found that the thyroids throughout the United States contain even larger amounts of iodine than the Berlin glands, averaging 10 to 12 mg. per gland, agreeing with the fact that goiter is comparatively rare in this country.[59] Monery[60] has found for France, as Baumann did for Germany, that the amount of iodine contained in the glands of normal individuals is in inverse proportion to the frequency of goiter in districts in which they live.[61]

[56] Festschr. f. O. Hammersten, Upsala Lakarefören. Förh., 1906, XI, Suppl.

[57] In Japan the thyroids are reported as but one-third the weight of European thyroids, but with relatively more iodine because of the fish diet. (Fukushima, Japan Med. World., 1922 (2), 45.)

[58] Jour. Amer. Med. Assoc., 1897 (29), 897.

[59] It is probable, in view of the higher results obtained by later analyses, that the results of Baumann and of Monery are somewhat too low.

[60] Jour. Pharm. et Chim., 1904 (95), 288.

[61] Oswald, and also Aeschbacher (Mitt. a. d. Grenzgeb. Med. u. Chir., 1905 (15), 269), however, state that normal thyroids in goitrous districts contain more iodine than thyroids from goiter-free districts.

Chemical analyses of goiters have given extremely variable results, which are found to depend upon the histological type of the goiter. Baumann found that in a series of twelve cases of goiter, in which the average dry weight was 32 grams, the amount of iodine in each gram was but 0.09 mg., but the total amount, 2.6 mg., was about the same as he found in normal glands of the same goitrous district. However, in two goiters large amounts of iodine were found, namely, 17.5 mg. and 31.5 mg. Wells found that the amount of iodine depends upon the structure, for two hyperplastic goiters contained respectively 8.23 and 8.3 mg. of iodine or about the amount normal for thyroids in this country, whereas two colloid goiters contained 53.16 and 24.59 mg. of iodine. This is corroborated by the more extensive studies of Marine and his co-workers, who have found the proportion of iodine low in all glands showing epithelial hyperplasia, but high in colloid goiters.[62] Administration of iodine causes a reversion of the hyperplastic to the colloid type of gland, while deprivation of iodine causes hyperplasia. Presumably, therefore, during the active growth of a goiter the iodine is low, but in the quiescent colloid state it is high. The physiological activity of colloid or other preparations from goiters is found to be quite the same as that from normal glands, varying in direct proportion to the iodine content.[63] In an adenomatous goiter, in the new growth, Wells found 1.98 mg. of iodine per gram, while the rest of the gland contained but 0.8 mg.; the total amount of iodine was 9.26 mg., or the same quantity as found in normal glands. In nine fetal adenomas Marine and Lenhart found iodine in eight in amounts averaging 0.174 mg. per gram of dry weight. However, when iodine is given to persons with thyroid adenomas the tumor tissue does not take up the iodine to the same extent that the normal gland tissue does. The presence of great numbers of mitochondria in the cells of adenomas indicates their high functional activity.[64]

Oswald found that colloid goiters contain a thyreoglobulin that is relatively very poor in iodine; in goitrous calves the thyreoglobulin contained no iodine; in human goiters it contained but 0.07 to 0.19 per cent. of iodine as against a normal proportion of 0.34 per cent. Administration of iodides to a goitrous patient caused a rise in the proportion of iodine in the colloid to 0.51 per cent., showing that in colloid goiters in goitrous districts the thyreoglobulin is probably poor in iodine because of a lack of iodine for it to unite with, and not because it is of an abnormal nature that prevents its chemical combination with iodine.[65] In general, Oswald[66] found the amount of iodine to vary with the amount of colloid in the goiters, although occasionally goiters with exceptionally large amounts of

[62] Arch. Int. Med., 1908 (1), 349; 1909 (3), 66; 1909 (4), 440; see also Homma, Mitt. Grenz. Med. Chir., 1923 (36), 389.

[63] See Fonio, Mitt. Grenz. Med. u. Chir., 1911 (24), 123. However, Abderhalden and Schiffmann[32] report that goiter tissue does not have the usual effect of thyroid on the metamorphosis of tadpoles.

[64] Goetsch, N. Y. State Jour. Med., July, 1918.

[65] See Kocher, Mitt. a. d. Grenzgeb. Med. u. Chir., 1905, vol. 14.

[66] Virchow's Arch., 1902 (169), 444.

iodine were found, and the proportion of iodine is not usually so great when the amount of colloid is very large. Simple hyperplastic goiters he found poor in iodine, or free from it if they contained no colloid; however, they were found to contain a thyreoglobulin typical in all respects except an absence of iodine. Presumably in such goiters the little thyroiodine present is contained in the parenchymatous cells. The physiological activity of thyreoglobulin obtained from goiters was found to be the same as that from normal glands, except that it was weaker in direct proportion to the amount of iodine it contained, and, therefore, when iodine-free it was without effect.[67] In colloid goiters the greater part of the weight of the gland, three-fourths or more, is made up of this colloid-poor thyreoglobulin. The fluid contents of cystic goiters may be free from iodine, but if they contain much colloid, iodine will be found, and Rositzky[68] found 0.193 mg. of iodine in 20 c.c. of the jelly-like contents of a thyroid cyst.

There is constantly growing evidence that the cause of endemic goiter is a deficiency in the iodine in the food, the drinking-water, or the air of the goitrous district. This is supported by the relative infrequency of endemic goiter in districts on the sea-coasts, where the iodine-containing sea-water is sprayed through the air, and where the inhabitants eat largely of sea-foods. Inland districts have the iodine leached out rapidly because of the great solubility of iodides,[69] unless it is supplied by a subsoil which has more recently emerged from the sea.[70] Also, administration of minute amounts of iodine, even in the air, seems to reduce existing goiter and to prevent its occurrence in inhabitants of goitrous districts.[71] However, there are many exceptions, and it cannot be said that this hypothesis of the etiology of all goiter rests on satisfactory evidence, particularly in view of the abundant iodine content of colloid goiters. Epidemics of goiter presumably are the results of an infection with some unknown organism, and possibly the endemic form has a similar cause,[72] but it is also possible that dietary abnormality, whether a deficiency in iodine or an excessive demand on the function of the thyroid, may play at least a subsidiary part.

There is much evidence, in any event, that whatever the cause of goiter may be, it often is related to the drinking water.[73] Studies in America of the incidence of goiter have shown a striking relationship to iodine deficiency in the water and food supply,[74] and the results of prophylactic

[67] See Oswald, Arch. ges. Physiol., 1916 (164), 506.
[68] Wien. klin. Woch., 1897 (10), 823.
[69] Hayhurst, Jour. Amer. Med. Assoc., 1922 (78), 18.
[70] See McClendon and Williams, Jour. Amer. Med. Assoc., 1923 (80), 600.
[71] See Hunziker, Corr. Bl. Schw. Aerzte, 1918 (48), 220.
[72] See McCarrison, "The Thyroid Gland," New York, 1917.
[73] See de Quervian, Mitt. a. d. Grenzgeb. Med. u. Chir., 1905 (15), 297; Bircher, Zeit. exp. Path. u. Ther., 1911 (9), 1. However, various direct experiments fail to support this hypothesis. (See Münch. med. Woch., 1913 (60), 393 and 1813; Sitzber, Wien. Akad., 1914 (123), 35; Knappenburg, Geneesk. Bladen, 1919 (21), 300.)
[74] See Olin, Jour. Amer. Med. Assoc., 1924 (82), 1328; McClendon and Hathaway, *ibid.*, p. 1668; Keith, Canad. Med. Assoc. Jour., 1924 (14), 284.

iodine administration have been convincing as a demonstration that at least a large proportion of goiter is solely dependent on iodine deficiency. Very probably the causes of colloid and parenchymatous goiters will be found to be different from the causes of cystic and adenomatous goiters, but in the face of the success of iodine prophylaxis there seems to be little doubt that at least the great majority of cases of goiter, represent work hypertrophy due to functional demand with an iodine shortage.[75]

Myxedema and Cretinism

These conditions depend upon a deficiency of thyroid secretion, whether from operative procedure or from pathological alterations in the organ. Consequently we find evidences of a decreased protein metabolism, the urine containing a diminished quantity of nitrogen, especially in the form of urea, while ammonia and other forms of nitrogen are relatively excessive. A retention of nitrogen and phosphorus[76] has been found, but not of calcium and chlorine.[77] The albumin content, viscosity, and refraction coefficient of the serum are high.[78] The temperature is usually subnormal, and the energy metabolism is low.[79] Basal metabolism is lower than in any other known condition (Du Bois),[80] and may be raised to normal by thyroid treatment.[81] Fat and carbohydrate metabolism seem not to be proportionately affected,[82] and hence the elimination of CO_2 is relatively high as compared to the nitrogen elimination. Gastrointestinal disturbances are common, with resulting increase in the amount of indican and ethereal sulfates in the urine. Whether from this cause or from deep-seated metabolic anomalies, there is a decided anemia, and the ability of the corpuscles to combine with oxygen seems to be decreased, so that the arterial blood may contain less oxygen than normal venous blood. There is no constant relation between the anemia and the metabolic rate.[83]

It is impossible to say whether the failure of growth and development of the young (cretinism), and the mental and physical torpidity of the adult, are due to an autointoxication from products of intermediary metabolism which accumulate because of the failure of the thyroid to furnish the "stimulus" necessary for their complete destruction, or to a lack of some essential action of the thyroid secretion upon the nervous tissues and the growing cells themselves. Administration of thyroid extract to cretinoid children causes a marked increase in food requirement,[84] with retention of nitrogen and phosphorus, but more strikingly of calcium,[85] and obese cretins lose weight, chiefly from the non-nitrogenous elements (Scholz). The amount of iodine in human cretin thyroids seems

[75] See review by Marine, Proc. Inst. Med. Chicago, 1924 (5), 31.

[76] Blood phosphate is low (Landsberger, Klin. Woch., 1924 (3), 1360).

[77] Benjamin and Reuss, Jahrb. f. Kinderheilk., 1908 (67), 261. In a cretin Greenwald found little deviation from normal (Arch. Int. Med., 1914 (14), 374). The blood calcium is said to be low in thyroidectomized sheep (Parhon, Endocrinology, 1923 (7), 311).

[78] Deusch. Deut. Arch. klin. Med., 1920 (134), 342; 1922 (138), 175.

[79] Talbot, Amer. Jour. Dis. Chil., 1916 (12), 145.

[80] Arch. Int. Med., 1916 (17), 915; Means and Aub., *ibid.*, 1919 (24), 404.

[81] Flemming, Quart. Jour. Med., 1922 (16), 11.

[82] Rarely myxedema and diabetes have been observed conjointly (see Strasser, Jour. Amer. Med. Assoc., 1906 (44), 765).

[83] Emery, Amer. Jour. Med. Sci., 1923 (165), 577.

[84] Ambrozic, Zeit. Kinderheilk., 1921 (29), 117.

[85] See Hougardy and Langstein, Zeit. f. Kinderheilk., 1905 (61), 633. Full figures are given by Scholz, Zeit. exp. Path. u. Ther., 1906 (2), 270.

not to have been estimated, but in five cretin dogs Marine and Lenhart could find no thyroid iodine at all.[86]

The myxedematous change in the connective tissues is in the nature of a reversion to the fetal type of tissue, and suggests that the thyroid secretion is necessary for proper cell growth. This effect might be either specific, or depend simply on the abnormality of protein metabolism. Horsley[87] describes the appearance of the tissues of animals dying after thyroidectomy as follows: "The subcutaneous connective tissue is swollen, jelly-like, bright and shining, and excessively sticky. The same thing is observed in the loose tissue of the mediastinum, about the heart, and in the omentum. The submaxillary and parotid glands are greatly enlarged, and have a semi-translucent, swollen appearance; from the cut surface a sticky, glairy fluid exudes. Apparently the parotid becomes transformed into a mucous gland; likewise the mucous membrane of the alimentary tract is swollen and transparent." Fetal tissues contain normally more mucin than those of adults (0.76 per cent. as against 0.37 per cent. in the subcutaneous tissues, according to Halliburton), and in the early stages of the formation of excessive subcutaneous tissue in myxedema, such an increase of mucin may be present. But, under ordinary conditions, the term myxedema seems to be entirely a misnomer, for Halliburton's analyses showed that the skin of myxedematous patients contains quite the same amount of mucin as is present in normal skin.[88] When the condition is of long standing, the amount of mucin may even be much reduced, because of the development of a fibroid character in the connective tissue. However, in monkeys upon which thyroidectomy had been performed, Halliburton[89] found a decided increase in the mucin in the tissues throughout the body, especially in the salivary glands, but also in the skin, subcutaneous tissues, and tendons; and mucin was found in the blood.[90]

That the thyroid is connected with general growth is shown not only by the thyroid abnormalities present in cretinism, but also by the frequent observation of thyroid defects in conditions of delayed growth and development of less extreme degree (*infantilism*), and the favorable effects of thyroid feeding in many such cases. Also in certain types of short-limbed dwarfs (*chondrodystrophia foetalis*) some thyroid anomaly may have an etiologic bearing, for in such a case, in which the thyroid was histologically greatly altered and quite free from colloid, I could find

[86] Related to cretinism is the "hairless pig malady," in which hairless pigs are born dead or die soon after birth, the mothers and offspring often having goiter; administration of KI checks the appearance of the disease (see Hart and Steenbock, Jour. Biol. Chem., 1918 (33), 313).

[87] Brit. Med. Jour., 1885 (i), 211.

[88] Jour. of Pathol. and Bact., 1893 (1), 90.

[89] Quoted by Horsley, *loc. cit.*[87] Later experimenters, however, have had difficulty in producing experimental myxedema as described by Horsley, or have failed entirely.

[90] It has been suggested that the thyroid produces an enzyme which destroys mucin, but that such is the case has never been demonstrated (see Parhon, Compt. Rend. Soc. Biol., 1916 (79), 504). Levin (Med. Record, 1900 (57), 184) states that mucin is toxic for thyroidectomized rabbits, but this is not substantiated by Nèfédieff (Vratch, 1901 (22), Oct. 27).

no trace of iodine.[91] On the other hand, the thyroid of a giant which I have analyzed contained 62.9 mg. of iodine, or six times the amount present in normal glands.[92]

Hyperthyroidism and Exophthalmic Goiter

It has by no means been conclusively determined that exophthalmic goiter is due to an intoxication with excessive amounts of thyroid secretion, either normal or abnormal, but there is abundant evidence in favor of this view. Most important is the similarity of exophthalmic goiter to the effects of "hyperthyroidism" or "thyroidismus," produced either experimentally or through overuse of thyroid extract for therapeutic purposes. In thyroidismus there are observed a rapid, weak pulse; greatly increased metabolism, especially of proteins;[93] a striking increase in basal metabolism[94] paralleling the degree of intoxication; a slight reduction in the respiratory quotient;[95] marked mineral loss, especially of Ca and P from the bones;[96] increased secretion, especially of perspiration; marked nervousness and irritability, often with mental confusion and delusions; gastro-intestinal disturbances, especially diarrhea; sweating, flushing, tremors, palpitation of the heart, loss of weight, and slightly increased temperature are also often observed, and not rarely typical exophthalmos may appear.[97] These manifestations, which are common to both thyroidism and exophthalmic goiter, are quite the opposite of the characteristic changes of myxedema, with its general lowering of all metabolic and nervous processes. Alike in experimental hyperthyroidism and exophthalmic goiter there is a greatly increased sensitiveness of the sympathetic nervous system to epinephrine.[98] Reid Hunt's acetonitrile test for thyroid secretion has sometimes been found positive in the blood from patients with exophthalmic goiter,[99] which presumably means the presence of an excess of thyroid secretion circulating in the blood in this disease; also, Hara[1] found evidence that the blood in exophthalmic goiter has an effect like thyroxin in increasing metabolism. Furthermore, the histological changes observed in the thyroid may resemble those of

[91] Reported by Hektoen, Amer. Jour. Med. Sci., 1903 (125), 751.

[92] Reported by Bassoe, Trans. Chicago Path. Soc., 1903 (5), 231.

[93] Metabolism in exophthalmic goiter, see Du Bois, Arch. Int. Med., 1916 (17), 915; Halverson, Bergeim and Hawk, *ibid.*, 1916 (18), 800; Means and Aub, Arch. Int. Med., 1919 (24), 645.

[94] Full review by Boothby and Sandiford, Physiol. Reviews, 1924 (4), 69; also E. F. DuBois, "Basal Metabolism in Health and Disease," Phila., 1924.

[95] See King, Bull. Johns Hopkins Hosp., 1923 (34), 304."

[96] Kummer, Rev. Méd. Suisse Rom., 1917 (55), 442.

[97] Sugar utilization is decreased, as shown by study of the utilization of sugar given intravenously (Wilder and Sansum, Arch. Int. Med., 1917 (19), 311); also a dietary hyperglucemia is readily induced (Denis, Aub and Minot, Arch. Int. Med., 1917 (20), 964; McCasky, Jour. Amer. Med. Assoc., 1919 (73), 243).

[98] See Goetsch, N. Y. State Jour. Med., 1918 (18), 259; Endocrinology, 1920 (4), 389. Also Dryerre, Quart. Jour. Exp. Physiol., 1924 (14), 217.

[99] See Ghedini, Wien. klin. Woch., 1911 (24), 736; Hunt Amer. Jour. Physiol., 1923 (65), 257.

[1] Mitt. Grenz. Med. Chir., 1923 (36), 527.

compensatory hypertrophy, suggesting strongly that the goitrous change of this disease is due to a true hypertrophy, with increased production of the specific secretions. There is a marked increase in the mitrochondria of the thyroid epithelium in exophthalmic goiter, which also is evidence of heightened activity.[2] Kocher[3] says that when iodine is given to patients with cancer of the thyroid they may develop symptoms of exophthalmic goiter, as if an excess of thyroidine were formed. Also speaking strongly in favor of the view that exophthalmic goiter is the result of overactivity of the thyroid, is the frequent cure of the disease through removal of a large part of the diseased gland. Although at times the colloid type of gland is found in exophthalmic goiter, Marine contends that it has been preceded by a hyperplastic stage.

The Anatomical Basis of Hyperthyroidism.—The following statement by L. B. Wilson[4] gives a summary of our present understanding of the anatomic basis of human hyperthyroidism:

"The pathologic changes met with in the thyroid in true exophthalmic goiter are now well agreed on. Briefly, the disease as indicated in the changes in the thyroid may be divided into three general stages:

1. Early exophthalmic goiter, with moderate increase in basal metabolism and usually moderate exophthalmos. In this condition there is moderate thyroid enlargement. The parenchymal cells show marked hypertrophy and moderate hyperplasia. There is diffuse hyperemia through the gland.

2. Advanced exophthalmic goiter with high metabolic rate, usually marked exophthalmos and a well-marked nervous syndrome. There is usually marked thyroid enlargement. There is advanced parenchymal cell hypertrophy and hyperplasia. There is little if any stored colloid. There is diffuse hyperemia throughout the gland.

3. Late exophthalmic goiter with high but sometimes declining metabolic rate, exophthalmos and a well-marked nervous syndrome. Pathologically the changes in the gland are similar to those in the earlier stages of exophthalmic goiter but with beginning or well-marked storage of colloid. Many follicles containing colloid are lined with flattened parenchymal cells. In some instances newly developed follicles are numerous. Hyperemia is usually materially less than in glands in the previous groups.

"The parenchymal changes in the thyroid in true exophthalmic goiter are almost always diffuse and, therefore, the gland is rarely nodular in its gross appearance.

"As pointed out by H. S. Plummer, related to, but clinically distinct from exophthalmic goiter, is a large group of patients with symptoms usually of slow development or, if rapidly developing, only after a long period of non-symptomatic thyroid enlargement, with basal metabolism increased as much as in exophthalmic goiter but usually with no ocular symptoms, and without the nervous syndrome peculiar to exophthalmic goiter. This type of hyperthyroidism is so often associated with nodular thyroids that Plummer tentatively designated the disease first 'toxic non-hyperplastic goiter,' then 'toxic adenoma'[5] and now 'hyperfunctioning adenoma.'"

Wilson says that patients with enlarged nodular thyroids, with symptoms of hyperthyroidism but without exophthalmos, without the nervous syndome peculiar to exophthalmic goiter, and with basal metabolic rates of 20 or more points above normal, have glands characterized by increased activity of the epithelium, indicated by cell hypertrophy and hyperplasia and some colloid storage. These glands differ from those of exophthalmic goiter in having somewhat less hyperplasia and more colloid storage. On the other hand patients of approximately the same age with enlarged nodular thyroids, without symptoms of hyperthyroidism and with basal metabolic

[2] Goetsch, Bull. Johns Hopkins Hosp., 1916 (27), 129.
[3] Deut. Zeit. Chir., 1908 (91), 302.
[4] Amer. Jour. Med. Sci., 1923 (165), 738.
[5] See review on toxic adenoma by Boothby, Endocrinology, 1921 (5), 1.

rates within 10 points of normal, have glands lacking these evidences of hyperactivity, corresponding to the lack of increased basal metabolism. He goes on to discuss the possible relation of thyroid function to hyperthyroidism as follows:

"It is a very inviting hypothesis that the symptoms of non-exophthalmic hyperthyroidism are caused by the adsorption of complete thyroxin in previously stored colloid which is being manufactured more rapidly than in the normal gland but much more slowly than in the thyroid of exophthalmic goiter. This hypothesis receives support from the facts that the symptoms produced by the administration of thyroxin in large doses to human beings are essentially those of non-exophthalmic hyperthyrodism, that stored colloid does contain thyroxin and that the histologic picture indicates that colloid is being transferred from the follicles to the circulation. On the other hand we might go a step further and speculate on the probability that true exophthalmic goiter is caused by an incompletely elaborated thyroid secretion, an antecedent of thyroxin but not with its normal quota of its atoms of iodine in the molecule, for example. Such a hypothesis would harmonize many of the clinical and pathological findings. However, an equally alluring hypothesis is that in true exophthalmic goiter the primary onset of the disease is in the nervous system and that the more significant thyroid hyperfunction is nevertheless a secondary development."

Oswald[6] found that the thyroid in exophthalmic goiter contains generally a smaller proportion of iodine than normal glands, but with the total amount approximately normal. However, the findings are very inconstant, corresponding with the fact that in some cases of exophthalmic goiter the amount of colloid is abundant (in which case the amount of iodine may be large), while usually the amount of colloid is small, and its highly vacuolated condition in hardened sections suggests that it is of unusually fluid consistency. A. Kocher[7] found that either the amount of iodine is small, which is usual, or else very high, but it is seldom the same as in normal thyroids; the more dense the colloid in the follicles the higher iodine content he observed; the phosphorus content is both relatively and absolutely increased. Tobler[55] also found a low iodine content, which is greatly raised by iodine treatment. Marine has found that in exophthalmic goiter as well as in other conditions the amount of iodine is in direct proportion to the colloid and inverse to the hyperplasia. E. V. Smith[8] obtained in simple hyperplastic glands an average of 0.54 mg. of iodine per gram dry weight, as compared with 1.52 mg. in hyperplastic glands showing retrogressive changes with more densely staining colloid. Fonio[63] found that, as with normal thyroids, the physiological effect of exophthalmic goiter glands varies directly with the proportion of iodine and such glands take up iodine administered therapeutically just as a normal thyroid does (Kocher,[9] Marine and Lenhart).[10] These results, therefore, indicate nothing either for or against the hypothesis that exophthalmic goiter is due to autointoxication with the secretion of the thyroid, but Wilson and Kendall[11] find that in the toxic type of goiters there is but $\frac{1}{20}$–$\frac{1}{15}$ as much thyroxin as in normal glands, and hence they suggest that in thyroid intoxication this toxic material has been discharged from

[6] Virchow's Arch., 1902 (169), 475.
[7] Virchow's Arch., 1912 (208), 86.
[8] Jour. Amer. Med. Assoc., 1914 (62), 113.
[9] Arch. klin. Chir., 1910 (92), 442; 1911 (96), 403.
[10] Arch. Int. Med., 1911 (8), 265.
[11] Amer. Jour. Med. Sci., 1916 (151), 79.

the thyroid into the circulation.[12] Plummer has found that large doses of iodine cause a marked decrease in the metabolic rate in exophthalmic goiter, which he explains by assuming that in this disease the thyroxin is not of normal character because of lack of iodine.[13] On the other hand, when iodine is given to patients with adenomatous goiter which is not hyperfunctioning, there often develops a marked condition of hyperthyroidism,[14] indicating that these adenomas are capable of forming thyroxin in excess when provided with sufficient iodine, but the manifestations are never those of typical exophthalmic goiter.

It is not possible to produce a symptom-complex completely resembling exophthalmic goiter[15] in animals by excessive feeding of thyroid,[16] either normal or from exophthalmic goiter; and after extensive study of the subject Marine and Lenhart have come to the conclusion that "the essential physiological disturbance of the thyroid in exophthalmic goiter is insufficiency, its reaction compensatory and its significance symptomatic." This view, however, certainly fails to agree with the excellent results which come from partial extirpation of the thyroid in exophthalmic goiter. Oswald[17] believes the primary trouble to be an abnormally irritable nervous system, which stimulates the thyroid and in turn is stimulated by the thyroid secretion, constituting a vicious circle. Other observers are of the opinion that not an excessive, but a perverted, secretion is at fault,[18] a view not confirmed by tests of the effects of thyroid extracts on animals.[19] However, it is stated by Blackford and Sanford,[20] that extracts of the thyroid in this disease, as well as the blood of patients in the acute toxic stages, exhibit a marked depressor effect on blood pressure, which is distinct from that of choline, and which they believe to be specific for exophthalmic goiter.

There can be no doubt that the thyroid secretion is capable of causing serious intoxication, for patients who have overused thyroid preparations in the treatment of obesity, skin diseases, etc., have often suffered severely from the symptoms mentioned previously, and in at least one such case, a diagnosis of exophthalmic goiter was made before the cause of the disturbance was detected. Not infrequently evidences of acute intoxication have followed immediately after operations upon the thyroid,

[12] In exophthalmic goiter the protective colloid effect of the serum, as shown by the Kottmann reaction, is increased, and the same effect is seen in hyperthyroidism. (Petersen *et al.*, Arch. Int. Med., 1922 (30), 386; see critique of this reaction by Morse, Jour. Lab. Clin. Med., 1923 (8), 692.) This indicates that overactivity of the thyroid results in an increase in the degree of dispersion of the blood colloids, which is supported by the observation of Starlinger. The concentration of proteins in the serum and the blood viscosity are low (Deusch, Deut. Arch. klin. Med., 1922 (138), 175).

[13] See Boothby and Sandiford.[94]

[14] See review by Wallach, Deut. Arch. klin. Med., 1925 (145), 240.

[15] The pathogenesis of the exophthalmos is unknown. See Troell, Arch. Int. Med., 1916 (17), 382.

[16] See Carlson *et al.*, Amer. Jour. Physiol., 1912 (30), 129; Marine, Jour. Amer. Med. Assoc., 1912 (59), 325.

[17] Correspondenzblatt Schweizer Aerzte, 1912 (42), 1130.

[18] Klose *et al.*, Beitr. z. klin. Chir., 1912 (77), 601.

[19] See Schönborn, Arch. exp. Path. u. Pharm., 1909 (60), 390.

[20] Jour. Amer. Med. Assoc., 1914 (62), 117.

and these have been considered as due to intoxication with the large quantities of thyroid secretion that have escaped from the gland during the operative manipulation. The fact that *amblyopia*, resembling that produced by tobacco, etc., may follow overuse of thyroid preparations[21] is indicative of their toxicity, as also is the *glucosuria* that may result from thyroid administration.[22] Hyperthyroidism may also result in focal necrosis in the heart muscle.[23]

Even if the hypothesis that exophthalmic goiter is due to intoxication with thyroid secretion is correct, we have no satisfactory explanation of the cause of the hyperactivity of the thyroid. In some cases degenerative changes have been observed in the superior cervical sympathetic ganglia, and cure or improvement of exophthalmic goiter is said to have followed resection of these ganglia; however, this relation has not been observed at all constantly. In other cases there has been evidence suggesting a primary intoxication with the products of intestinal putrefaction,[24] leading to a secondary hyperplasia of the thyroid, but this also seems to be an exceptional observation.[25] All things considered, it seems most probable that the hyperactivity of the thyroid is secondary to some exciting condition, and is not of itself primary, although the resulting hypersecretion of the thyroid may cause the dominant features of the disease. The frequent association of exophthalmic goiter with puberty and pregnancy suggests that some abnormality in the function of the generative organs may be a frequent starting-point of the thyroid derangement.[26] In not a few cases diabetes or pancreatitis have been associated,[27]

[21] Birch-Hirschfeld and Inouye, Graefe's Arch., 1905 (61), 499.

[22] See Geyelin, Arch. Int. Med., 1915 (16), 975.

[23] Goodpasture, Jour. Exp. Med., 1921 (34), 407.

[24] In view of the presence of a tryptophan nucleus in thyroxin, and his observation that the urine contains no indican in exophthalmic goiter, Harries (Brit. Med. Jour., 1923 (1), 553) has suggested that because of a lack of indole-forming bacteria in the intestine to destroy tryptophan, an excess of this amino acid is provided and so an excess of thyroxin is formed.

[25] *Antithyroid Serum.*—Based on the theory that the normal function of the thyroid is the detoxication of metabolic products, is the serum treatment advocated first by Ballet and Enriquez, and later by Lanz, and Burghart and Blumenthal. (Deut. med. Woch., 1899 (25), 627.) Also Möbius, Münch. med. Woch., 1901 (48), 1853; v. Leyden, Med. Klinik, 1904 (1), 1; Eulenberg, Berl. klin. Woch., 1905 (42), 3.) On the principle that after thyroidectomy the blood should contain an accumulation of those substances, which the thyroid normally neutralizes, they injected the serum of thyroidectomized goats into patients with exophthalmic goiter, in the hope that these accumulated substances might in turn neutralize any excessive thyroid secretion. Favorable results were obtained, and it was subsequently found that the milk of thyroidectomized goats possesses the same qualities, and may be administered by mouth; this has led to quite extensive clinical use of this method of treatment, which has failed to show any regular beneficial effects in the hands of most careful observers. (See Sonne, Zeit. klin. Med., 1914 (80), 229.) Of similar significance are the favorable effects obtained by Beebe (Jour. Amer. Med. Assoc., 1906 (46), 484; 1906 (47), 655) and Rogers (*Ibid.*, 1906 (46) 487; 1906 (47), 661) with a serum made by immunization of animals with the nucleoproteins of the thyroid, which have not been corroborated by others.

[26] The serum of patients with exophthalmic goiter shows by Abderhalden's method a constant power to digest thyroid tissue, and sometimes ovary or testicle (Lampé and Fuchs, Münch. med. Woch., 1913 (60), No. 39).

[27] Thompson, Amer. Jour. Med. Sci., 1906 (132), 835; Cohn and Peiser, Deut. med. Woch., 1912 (38), 60.

and some observers state that the pressor substance (presumably epinephrine) in the blood is much increased in exophthalmic goiter.[28] Although the *thymus* is often found enlarged, sometimes greatly so, in 70 to 80 per cent. of cases of exopthalmic goiter, its relation to the disease is as yet entirely unknown.[29] (See also Parathyroids, relation to Exophthalmic Goiter, p. 704).

THE PARATHYROIDS AND PARATHYROID TETANY[30]

The parathyroids were originally considered as but a form of undeveloped accessory thyroids,[31] but they are now known to be independent organs[32] of great importance. Parathyroids contain no appreciable amounts of iodine (Estes and Cecil),[33] although 14 per cent. of parathyroids obtained at autopsy contain a colloid material (Thompson and Harris).[34] MacCallum found the results of parathyroidectomy in dogs very different from the results of thyroidectomy. The most prominent symptoms are muscular twitchings, gradually passing into tetanic spasms, and due to nervous impulse rather than to muscular changes, since they do not appear in muscles from which the nerve-supply has been cut off. Trismus, protrusion of the eyes, and rapid respiration without cyanosis (*i. e.*, air hunger) are also observed, and death usually results from exhaustion. MacCallum believed these symptoms due to some toxic substance which accumulates on account of the absence of the parathyroids, for it was found that simply diluting the dog's blood by withdrawing part of it, and injecting a corresponding amount of salt solution, caused a temporary cessation of the tetanic symptoms; and injections of emulsions of parathyroid checked the symptoms for some time, presumably through neutralizing the hypothetical poisons. Degenerative changes that were observed in the cerebral ganglion-cells also favor the view that some unneutralized toxin is responsible for the symptoms following parathyroidectomy. On the other hand, profound mental symptoms and insomnia are said to have resulted from feeding parathyroid to man.[35] It is not known whether tetany parathyreopriva and idopathic tetany are either identical or very closely related.[36]

Metabolism after parathyroidectomy may show the following changes: There is a reduction in the assimilation limit for carbohydrates (Hirsch, Underhill[37] and

[28] Bröking and Trendelenburg, Deut. Arch. klin. Med., 1911 (103), 168.
[29] Review by Eddy, Canad. Med. Assoc. Jour., March, 1919 (9), 203, 242.
[30] A review of this subject is given by MacCallum, Ergeb. inn. Med., 1913 (11), 569; Boothby, Endocrinoloy, 1921 (5), 402, Jacobson, Ergebnisse d., Physiol., 1924 (23), 180.
[31] Anatomy of parathyroids, see Bergstrand, Acta Med. Scand., 1920 (52), 791.
[32] See Vincent and Arnason, Endocrinology, 1920 (4), 199; Cameron and Carmichael, Amer. Jour. Physiol., 1921 (58), 1.
[33] Johns Hopkins Hosp. Bull., 1907 (18), 331; also Cameron, Jour. Biol. Chem., 1914 (16), 465.
[34] Amer. Jour. Med. Sci., 1908 (19), 135.
[35] Morris, Jour. Lab. Clin. Med., 1915 (1), 26.
[36] See Paton and Findlay, Quart. Jour. Exp. Physiol., 1917 (10), 203.
[37] Jour. Biol. Chem., 1914 (18), 87.

others). MacCallum and Voegtlin[38] found an increased elimination of calcium and a loss of the same element from the blood and brain, which may be responsible for the increased nervous irritability. The blood calcium usually falls from the normal value of about 11 mg. per 100 cc. to about 5 mg., tetany developing when the value reaches about 7 mg. (Hastings and Murray),[39] but these authors found no evidence of the alkalosis reported by others. According to most observers, nitrogenous metabolism is altered as shown by the increased excretion of nitrogen, and especially of ammonia. Greenwald[40] found increased ammonia less conspicuous than increased undetermined nitrogen and sulfur, and decreased phosphorus excretion. Blood phosphate and magnesium are not much altered.[41] There may occur an increase in the bases of the blood (alkalosis, *q. v.*)[42] which disappears under the acidosis that results from the spasms of tetany,[43] and sometimes under the influence of acid therapy with NH_4Cl or $CaCl_2$.[44] The urea and undetermined blood nitrogen are increased even before the onset of the tetany,[45] which observation supports the view that parathyroid tetany depends on the presence of some toxic agent. W. F. Koch[46] found increased guanidine and methyl guanidine in the urine of dogs deprived of parathyroids. Numerous observers supported these findings and obtained other evidence that guanidine may be responsible for the symptoms of parathyroid tetany, but the studies of Greenwald[47] have rendered doubtful the value of the analytical methods used, and at present the importance of guanidine in parathyroid tetany has become dubious (see p. 669).[48]

Hypocalcemia and Tetany.—Howland and Marriott found a definite decrease in the calcium of the blood in human tetany and in parathyroidectomized dogs, as have many subsequent investigators. Injection of phosphates reduces blood calcium, and when the reduction has reached 6 mg. per 100 cc., symptoms of tetany appear,[49] just as after parathyroidectomy. However, patients with severe nephritis may have blood calcium figures as low, without signs of tetany. MacCallum[50] has found evidence that in parathyroidectomized dogs the blood contains something which greatly increases the irritability of the nerves, possibly by abstracting calcium from the tissues, for removal of calcium from the circulating blood of normal animals by dialysis results in nerve hyperexcitability resembling that seen in tetany. In view of the conflicting facts that have been recorded, the theory that the increased irritability and spasm of tetany result exclusively from hypocalcification of the nerve tissue is at present unproved. Calcium does diminish nervous irritability, as shown by J. Loeb, and hence when administered it may favorably influence the symptoms of tetany parathyreopriva, but this does not establish the theory. The fact that bleeding or injection of NaCl solution reduces the symptoms is against the calcium deprivation theory and supports the intoxication theory.[51] On the other hand, the marked changes in dentition and bone repair observed in parathyroidecomized animals by Erdheim[52] indicate an abnormality in calcium metabolism, which, however, might be

[38] Jour. Exp. Med., 1909 (11), 118; 1913 (18), 618.

[39] Jour. Biol. Chem., 1921 (46), 233.

[40] Amer. Jour. Physiol., 1911 (28), 103; Jour. Biol. Chem., 1913 (14), 363.

[41] Howland and Marriott, Quart. Jour. Med., 1918 (11), 289; Greenwald, Jour. Biol. Chem., 1924 (61), 649.

[42] Bibliography by Tezner, Monats. Kinderheilk., 1924 (28), 97.

[43] Wilson, Stearns and Thurlow, Jour. Biol. Chem., 1915 (23), 89, 123. McCann, *ibid.*, 1918 (35), 553. Togawa, Jour. Lab. Clin. Med., 1920 (5), 299; Cruickshank, Brit. Jour. Exp. Path., 1923 (4), 213; Anderson and Graham, Quart. Jour. Med., 1924 (69), 62.

[44] See György, Klin. Woch., 1922 (1), 1399.

[45] Haden and Orr, Amer. Jour. Med. Sci., 1924 (167), 108.

[46] Jour. Biol. Chem., 1913 (15), 43; Jour. Lab. Clin. Med., 1916 (1), 299.

[47] Jour. Biol. Chem., 1924 (59), 329.

[48] An increase in the choline of the blood was observed in parathyroidectomized dogs by Shanks, Jour. Physiol., 1924 (58), 466.

[49] Binger, Jour. Pharmacol., 1917 (10), 105. See also Salvesen *et al.*, Jour. Biol. Chem., 1924 (60), 311.

[50] Verh. Deut. Path. Ges., 1912 (15), 266; Jour. Exp. Med., 1914 (20), 149.

[51] We may well doubt the finding of Wiener (Pflüger's Arch., 1910 (136), 107) that it is possible to secure an antitoxin for the poison of tetany thyreopriva by immunizing with the serum of animals in tetany.

[52] Frankfurter Zeit. Pathol., 1911 (7), 175.

secondary to an intoxication. Also in rickets,[53] osteomalacia, and osteoporosis, the parathyroids are said to show hyperplasia,[54] and sometimes this is seen in nephritis with abnormal calcium metabolism.[55] Deprivation of calcium is said to cause parathyroid hyperplasia,[56] just as iodine deficiency causes goiter, and taken with all the related evidence it seems difficult to doubt that the parathyroids have an important role in calcium metabolism.[57] The occurrence of tetany in rickets and osteomalacia with hypocalcemia, is also a significant fact. The well established relation of pregnancy and lactation to tetany[58] may well be related to the calcium drain at this time; also the fact that phosphates, which reduce blood calcium, may incite tetany in children[59] as well as in animals.[60] On the other hand, patients exhibiting the Chvostek sign may not show any reduction in blood calcium,[61] but perhaps it is not so much the total blood calcium as the free ionic calcium that is reduced.[62]

Tetany as an Intoxication.—In favor of the view that parathyroid tetany depends on an intoxication are the observations of Luckhardt, Dragstedt, and their colleagues.[63] They have found that if bacterial proteolysis in the intestines is prevented in parathyroidectomized dogs by special diets for 3 to 6 weeks no tetany occurs, and by that time readjustment has developed to such an extent that tetany does not appear thereafter on ordinary diets. However, the resistance of these dogs to a considerable variety of poisons is markedly reduced, and they may be profoundly intoxicated by amounts of toxic chemicals which will not affect normal animals. A number of factors, such as the feeding of large amounts of meat, the occurrence of constipation, exposure to high temperatures, excessive muscular exercise, pregnancy, the occurrence of oestrus, sexual excitement, and infections, may induce tetany or depression in these animals although rarely causing disturbance in normal dogs. These facts suggest that whereas the chief source of the tetany poisons in the blood of the parathyroidectomized animal has been the gastrointestinal tract, under certain special conditions they may come from other places.[64] Although calcium reduces tetany arising under these conditions, perhaps by reducing nerve irritability, the fact that injection of large amounts of physiological NaCl solution has the same effect supports the view that the tetany results from intoxication rather than from calcium deficiency alone.[65] Luckhardt and Compere[66] suggest that an increased permeability of the bowel wall is present in parathyroid tetany, permitting absorption of toxic materials and loss of calcium.

[53] Pappenheimer and Minor, Jour. Med. Res., 1921 (42), 391.
[54] Strauch, Frankfurter Zeit. Pathol., 1922 (28), 319; Klemperer, Surg. Gyn. Obst., 1923 (36), 11.
[55] Bergstrand, Acta Med. Scand., 1921 (54), 539, 791. Full review on anatomy and pathology of parathyroids.
[56] Luce, Jour. Path. Bact., 1923 (26), 200.
[57] See Salvesen, Jour. Biol. Chem., 1923 (56), 443.
[58] Review by Dragstedt *et al.*, Amer. Jour. Physiol., 1924 (69), 477.
[59] Af Klercker, Zeit. f. Kinderheilk., 1921 (28), 71.
[60] Salvesen *et al.*, Jour. Biol. Chem., 1924 (60), 311.
[61] Handovsky, Jahrb. f. Kinderheilk., 1920 (91), 432.
[62] Freudenberg and György, Jahrb. f. Kinderheilk., 1921 (96), 5; Salvesen and Linder, Jour. Biol. Chem., 1923 (58), 635.
[63] Bibliography given in Amer. Jour. Physiol., 1924 (69), 477.
[64] See also Hammett, Amer. Jour. Physiol., 1922 (63), 151.
[65] Why parathyroidectomized animals so often develop cataracts is unknown.
[66] Proc. Soc. Exp. Biol. Med., 1924 (21), 523.

Administration of calcium may have its beneficial effect in part by decreasing the permeability.

Implantation of parathyroid tissue in persons with tetany parathyreopriva has been said to be successful in removing symptoms in a few cases,[67] but the viability of implants is too short to have accomplished the supposed results. The relation of the parathyroids to tetany of infants is not so well established,[68] although several observers have found hemorrhages in the parathyroids in these cases. Some cases of "gastric tetany" are also reported to have improved under parathyroid feeding, which is also said to be beneficial in *paralysis agitans*,[69] although there seems to be no anatomic basis for assuming a parathyroid deficiency in this disease.[70] However, the recent studies by Collip[71] have established that the parathyroid does produce hormones, for he has obtained an active preparation which, when fed or injected into dogs, prevents or controls parathyroid tetany, restoring the calcium content of the blood serum to normal limits, and with overdosage causing a hypercalcemia.[72]

The Relation of the Parathyroids to Exophthalmic Goiter.—This has not yet been definitely established. As nervous manifestations are prominent after parathyroidectomy, it has seemed to some that these organs may be more closely associated with exophthalmic goiter than is the thyroid itself;[73] furthermore, as in tetany, there is a tendency to alkalosis in exophthalmic goiter.[74] Against the hypothesis that exophthalmic goiter is due to parathyroid insufficiency, however, stand the following facts: (1) Removal of one lobe of the thyroid often causes improvement or recovery in this disease, yet with the lobe of the thyroid is often removed the adjacent parathyroid, which would decrease the amount of parathyroid tissue, and make worse any existing parathyroid insufficiency. (2) Therapeutic administration of parathyroid tissue or extract has had no significant effect on the disease. (3) No considerable or characteristic anatomical changes occur in the parathyroids in exophthalmic goiter,[75] while all cases show changes in the thyroid. (4) The parathyroids seem to have but slight influence on metabolism (MacCallum), while metabolic abnormalities are very marked in exophthalmic goiter.[76]

THE ADRENALS AND ADDISON'S DISEASE

Like the hypophysis, the adrenals are essentially double organs, containing nervous and glandular tissues. The medulla is of sympathetic nervous system origin, a part of the chromaffin[77] system, which in most animals is enclosed in a layer of entirely different nature and origin,

[67] Danielsen, Beit. klin. Chir., 1910 (66), 85.
[68] See Haberfeld, Virchow's Arch., 1911 (203), 282.
[69] Berkeley, Med. Record, 1916 (90), 105; Kühl., Münch. med. Woch., 1921 (68), 1083.
[70] Koopman, Frankf. Zeit. Path., 1921 (25), 342.
[71] Jour. Biol. Chem., 1925 (63), 395.
[72] See also Bisgaard, Acta. Med. Scand., 1925 (61) 433.
[73] See Iversen, Arch. Internat. de Chir., 1914 (6), 255.
[74] Hollö and St. Weiss, Klin. Woch., 1924 (3), 1632.
[75] MacCallum, Johns Hopkins Hos. Bull., 1905 (16), 287.
[76] The calcium excretion in exophthalmic goiter parallels the nitrogen (Towles, Amer. Jour. Med. Sci., 1910 (140), 100). The blood calcium may be either above or below normal (Herzfeld and Neuburger, Deut. Med. Woch., 1924 (50), 1324).
[77] The chrome reaction (observed in the adrenal medulla, carotid and coccygeal glands and the sympathetic ganglia), as well as other reactions for these tissues, is based on reduction of chromic acid to chromium dioxide by epinephrine (Ogata, Jour. Exp. Med., 1917 (25), 807; Beitr. path. Anat., 1923 (71), 376).

the cortex being an epithelial structure, derived from the urogenital anlage, and resembling most closely in structure (and perhaps in function) the corpus luteum of the ovary. Indeed, in some marine animals (eels, sharks, etc.), the sympathetic tissue portion and the cortical tissue exist as separate organs.

The adrenal cortex seems to be related especially to the generative system,[78] as shown by the following facts:

1. The embryologic origin in the urogenital anlage, and the histologic structure which is similar to the corpus luteum.

2. In many animals there occurs hypertrophy of the cortex during the breeding season, and there are histological differences in the glands of males and females (Kolmer). Castration causes hypertrophy of the adrenal cortex, while adrenalectomy causes hyperplasia of the interstitial cells of the ovary, but does not affect the testicles.[79]

3. Many cases of sexual precocity have been observed in association with tumors or hypertrophy of the adrenal cortex; and defective sexual development has been found associated with atrophy of this tissue.[80]

4. The medulla increases relatively little in size after birth, while the cortex increases with the development of the individual.

Whether the cortex has other functions or not is not yet known,[81] but Biedl and others have found evidence that cortical substance is essential for life. Animals with accessory adrenals, which contain only cortical substance, withstand ablation of the adrenals proper, presumably because the rest of the chromaffin substance remains to compensate.[82] Chemically the cortex is characterized by containing a very large proportion of lipoids, and by not containing the specific vaso-constrictor principle epinephrine.[83]

The Adrenal Lipins.—In water-free human adrenals (cortex and medulla both included) there was found 36.3 per cent. of ether-soluble material, of which 20.6 per cent. was cholesterol and 33 per cent. was phospholipins.[84] The proportion of fats and lipoids varies greatly during changes of age, disease, and perhaps of function, and there are those who believe the adrenal cortex to be the chief source or controlling agency of the lipoids of the blood, to which much important function is ascribed in the reactions of immunity. (See Lipoids, under Fatty Metamorphosis.) The quantity of choles-

[78] See Kolmer, Pflüger's Arch., 1912 (144), 361; Vincent, Surg. Gyn. Obst., 1917 (25), 299.

[79] Jaffe and Marine, Jour. Exp. Med., 1923 (38), 93.

[80] See Glynn, Quart. Jour. Med., 1912 (5), 157; Jump *et al.*, Amer. Jour. Med. Sci., 1914 (147), 568.

[81] It does not have a marked effect on the development of tadpoles, hence differing from the thyroid and thymus (Gudernatsch). There is some indication that the cortex is related to the pancreas and to sugar metabolism (see Tokumitsu, Mitt. Path. Inst. Univ. Sendai, 1921 (1), 161). Insulin administration causes adrenal enlargement in pigeons (Riddle *et al.*, Amer. Jour. Physiol., 1924 (68), 461). Hewer (Brit. Med. Jour., 1922 (1), 138) found evidence that feeding cortex leads to increased fat deposition and various other effects.

[82] See Fulk and MacLeod (Amer. Jour. Physiol., 1916 (40), 21) who found that the active principle of other chromaffin tissues has the same physiological effect as that of the adrenal medulla.

[83] It is not established that the adrenal cortex may not manufacture epinephrine which is stored in the medulla (see Hartman, Amer. Jour. Physiol., 1923 (65), 623).

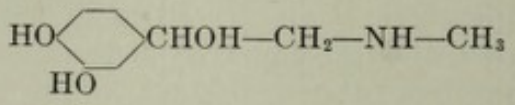
[84] Wells, Jour. Med. Res. 1908 (17), 461.

terol in the blood usually varies directly with the quantity in the adrenals;[85] but removal of a large part of the adrenal tissue in animals results in hypercholesterolemia.[86]

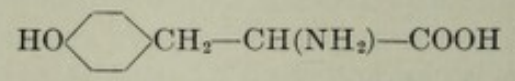
When cholesterol is fed in large amounts some is deposited in the adrenal cortex,[87] while in many diseases, notably delirium tremens (Hirsch)[88] the lipoid content of the adrenals is greatly decreased. In renal and arterial disease the adrenal lipoids have been found increased.[89] Chauffard *et al.*,[90] have assayed the adrenal cholesterol, giving the average normal figures as 20 to 30 parts per 1,000, with low figures in rapid pulmonary tuberculosis (2 to 4), average in fibroid phthisis (28), while in hypertension the figure is high (46 to 82). In uremia, however, there is eventually a loss of both lipoid and epinephrine.[91]

The lipins of the adrenal cortex are said to contain little or no neutral fat,[92] but free fatty acids which may be increased when the cholesterol decreases. Loss of body fats is not accompanied by a loss of adrenal lipoids ordinarily, although they decrease in acute infections, especially pneumonia.[93] A vaso-depressor effect is produced by extracts of adrenal cortex, perhaps caused by choline which has been found in such extracts, or possibly by histamine.

The medulla is characterized, besides by its pigmentary content, by the remarkably active internal secretion, epinephrine,[94] which it always contains in greater or less amount. Presumably epinephrine, of which the formula is



is derived from the aromatic radical of the proteins, its close relationship to tyrosine being seen when the formula of the latter is compared.



That epinephrine is formed from tyrosine directly, is, however, not yet demonstrated. There are also other amines and aromatic compounds which might be formed in the body, that have a pressor effect, and which perhaps are formed, although not yet identified.[95] It is to be borne in mind that the formation of epinephrine is not limited to the adrenals, but that other islands of chromaffin sympathetic tissue can do the same,[96] which explains the observed discrepancies between the anatomic changes in the adrenals and the clinical manifestations of deficiency in epinephrine.[97]

[85] Marino, Arch. farm. sper., 1923 (36), 172.
[86] Joelson and Shorr, Arch. Int. Med., 1924 (24), 841.
[87] Krylov, Beitr. path. Anat., 1914 (58), 434.
[88] Jour. Amer. Med. Assoc., 1914 (63), 2186.
[89] Chauffard, Compt. Rend. Soc. Biol., 1914 (76), 529.
[90] Ann. de Méd., 1920 (8), 149.
[91] Pfeiffer, Zeit. ges. exp. Med., 1919 (10), 1.
[92] Borberg, Skand. Arch. Physiol., 1915 (32), 287.
[93] Elliott, Quart. Jour. Med., 1914 (8), 47; Laignel-Lavastine, Compt. Rend. Soc. Biol., 1918 (81), 324.
[94] This name, given by Abel and Crawford, is to be preferred to the others in common use, especially the most-used term "adrenalin," which has been copy-righted by a manufacturing establishment, so that this name means specifically their product, and not the active principle of the adrenal from whatever source.
[95] See Barger and Dale, Jour. Physiol., 1910 (41), 19.
[96] Vincent, Proc. Roy. Soc., B, 1908 (82), 502.
[97] Neither partial adrenalectomy nor injection of epinephrine affect antibody formation (Frieber, Cent. Bakt., 1921 (86), 247).

According to Goldzieher[98] the normal human adrenals contain together about 4 mg. epinephrine, which may be increased in conditions with high blood pressure, such as arteriosclerosis and nephritis, in which he found an average of 5.8 mg.; and in septic conditions with low pressure he found it reduced to an average of 1.5 mg.[99] Lüksch[1] gives a normal figure of 4 mg. for each gland, also finding the amount lowered in infectious diseases and burns, and increased in nephritis. The human adrenal contains no epinephrine before birth,[2] but Fenger[3] found it present in the adrenal of unborn domestic animals. Autolysis of the adrenal decreases the amount,[4] but not all of the epinephrine is destroyed even several days after death, as shown by Ingier and Schmorl,[5] who, using both morphological and chemical methods, also found a gradual increase in the epinephrine content of normal human glands from birth to the ninth year, after which it remains practically constant at about 4.5 mg. (males 4.4, females 4.71 mg.). They also found a slight increase in arteriosclerosis, more in acute and chronic nephritis and a decrease in diabetes and narcosis, there being practically no epinephrine in the adrenal of Addison's disease. In most of the infectious diseases they found no changes, and in amyloid infiltration the amount was about normal. The amount of chromaffin substance and epinephrine do not always run parallel, although Borberg[6] found a close parallelism; this author also failed to observe any marked decrease of chromaffin substance in narcosis.[7] Elliott[93] found a low epinephrine content in acute infectious diseases, and especially low in acute cardiac failure associated with great mental distress; he did not find any increase in the epinephrine in nephritis or in any other disease. With inanition the size of the adrenals increases while the epinephrine content falls.[8]

The function of the epinephrine is presumably to modify the tone of the non-striated muscle fibers which are under control of the sympathetic nervous system, acting upon some receptive substance present in the muscle, perhaps at the nerve endings. One must agree with Stewart[9] that much of the evidence submitted as to adrenal function is valueless, and he doubts the indispensability of epinephrine although admitting

[98] Wien. klin. Woch., 1910 (23), 809.

[99] See also Reich and Beresnegowski, Beitr. klin. Chir., 1914 (91), 403. Ohno (Verh. Japan, Path. Gesell., 1916 (6), 15) found the normal content to be about 5.6 mg. averaging 8.32 mg. in chronic nephritis, 9.45 in beriberi, and low figures in asthma.

[1] Virch. Arch., 1917 (223), 290.

[2] Moore and Purinton, Amer. Jour. Physiol., 1900 (4), 51; Julian Lewis, Jour. Biol. Chem., 1916 (24), 249.

[3] Jour. Biol. Chem., 1912 (11), 489.

[4] Commessatti, Arch. exp. Path. u. Pharm., 1910 (62), 190.

[5] Deut. Arch. klin. Med., 1911 (104), 125.

[6] Skand. Arch. Physiol., 1912 (27), 341; 1913 (28), 91.

[7] Pitini (Gaz. degli Ospedali, 1920 (41), 415) reports a decrease in epinephrine after chloroform, and Corbett (Jour. Amer. Med. Assoc., 1922 (79), 543), after ether, shock, and many other conditions.

[8] Vincent and Hollenberg, Jour. Physiol., 1921 (54), lxix.

[9] Physiol. Reviews, 1924 (4), 163. Review on effects of adrenalectomy and the relation of adrenals to metabolism.

that the cortical substance is essential for life. Hoskins,[10] agreeing that the cortex is the indispensable part of the gland, grants to the medulla and its production of epinephrine a function to reinforce the sympathetic system in time of stress,[11] and Hartman[12] finds evidence that the adrenal medulla plays a part in preventing fatigue. But it is a fact of much practical importance that administration of epinephrine will not compensate successfully for the loss of the adrenals, whether because the gland secretes other things, or because the intermittent artificial administration of the epinephrine will not compensate for the regulated secretion of the gland under normal conditions, or both. It would seem that the adrenal has an effect on other glands, for injections of epinephrine cause glucosuria in animals, as also does manipulation of the adrenals, or painting the epinephrine on the pancreas. Biedl sums up the evidence with the statement that the internal secretion of the chromaffin system is of importance in the mobilization of the sugar of the blood, and the formation of the glycogen in the tissues. That the adrenal is at all implicated in human diabetes has not been demonstrated. There seems to be a relationship of mutual stimulation between thyroid and adrenal, for thyroid secretion sensitizes the sympathetic nerve endings to epinephrine, and both liberate carbohydrates from the sugar depots.[13]

Acute insufficiency of the adrenals, caused most often by hemorrhagic infarction, but sometimes by other lesions, may cause sudden collapse, asthenia or death.[14] The extent to which the cortex and medulla respectively are responsible is undetermined. The French authors especially lay great weight on adrenal insufficiency as a cause of pathological states.[15] Surgical shock has also been attributed, at least in some cases, to exhaustion of the adrenals, which takes place under the influence of the anesthetic and the stimulation to the nervous system by the operative manipulation, perhaps augmented by concurrent infections.[16] Of all these possibilities, however, but few have been conclusively established.

It is possible that in some cases of trauma to the adrenal, acute hemorrhage, or infection, intoxication from an excess of epinephrine might occur, but it is improbable that fatal results could be produced in this way, for the lethal dose for dogs and rabbits is about 0.1 to 0.25 mg. per kilo, and the two adrenals in man contain in all but about 4 to 5 mg. epinephrine.

In animals, repeated doses of epinephrine produce a decreasing effect, not only on blood pressure but on the glucosuria and other symptoms, indicating an acquirement of tolerance, but, because of its nonprotein nature, epinephrine does not cause the production of antibodies.[17]

[10] Physiol. Reviews, 1922 (2), 343; discusses relation of adrenal to circulation.
[11] See also Trendelenburg, Ergeb. Physiol., 1923 (21, ii), 500.
[12] Amer. Jour. Physiol., 1922 (60), 255.
[13] See Endocrinology, 1917 (1), 404.
[14] Literature by Lavenson, Arch. Int. Med., 1908 (2), 62; Materna, Ziegler's Beitr., 1910 (48), 236.
[15] See Sergent, Presse Méd., 1921 (29), 813; Cowie and Beaven, Arch. Int. Med., 1919 (24), 78; Kay and Brock, Amer. Jour. Med. Sci., 1921 (161), 555.
[16] See Hornowski, Arch. méd. exper., 1909 (21), 702; Virchow's Archiv., 1909 (198), 93.
[17] See Elliott and Durham, Jour. of Physiol., 1906 (34), 430.

Many studies have been directed to determine the relation of the adrenal to hypertrophy of the heart and to interstitial nephritis with high blood pressure. Some have found more or less increase in size in the adrenals under these conditions, chiefly involving the cortex, and a slight increase in the epinephrine content has been reported, but it is very doubtful if these observations are of significance.[18] It has been reported by several investigators that the blood in such conditions contains sufficient epinephrine to permit of its detection and measurement by its physiological effects (dilatation of the frog's iris, contraction of the rabbit uterus or blood vessels, inhibition of contraction of the intestine).[19] The critique of this work by Stewart,[20] however, makes it necessary to discount most of the published results, as being inadequately controlled. He found no epinephrine even in blood coming direct from the adrenal veins, unless the gland had been stimulated or manipulated, and none could be detected in the serum from several patients with high pressure from various causes, or in mental disturbances and exophthalmic goiter. Vaso-constrictor substances may be present in serum, both normal and pathological, which are not epinephrine. His negative results are corroborated by Janeway and Park.[21] Bröking and Trendelenburg,[22] using a perfusion method which they believe to be reliable, found a normal pressor effect from the blood of persons with arteriosclerosis and high blood pressure, a decrease in nephritis with high pressure, a great increase in exophthalmic goiter, and no changes in pregnancy, chlorosis, and diabetes. A few cases of hypertension associated with chromaffin tumors of the adrenal have been reported, and also occasionally with cortex tumors.[23]

Arterial Degeneration from Epinephrine.[24]—An interesting result of repeated injections of epinephrine into animals is the appearance of a marked atheromatous degeneration of the aorta, with calcification. This was first observed by Josué, and since confirmed by many others. These lesions are quite different from those of human aortic arteriosclerosis, the chief change being degeneration of the muscle-cells of the media, without any considerable inflammatory reaction. There is, however, more resemblance to the atheromatous changes seen in the arteries of the extremities. They do not seem to be due to the heightened blood pressure, since simultaneous administration of substances that keep the blood pressure down does not prevent the atheroma from developing (Braun), while other substances that raise blood pressure, such as nicotine (Josué) or pyrocatechin (Loeb and Githens), do not cause atheroma. Presumably, therefore, epinephrine causes the arterial changes by a direct toxic action, but the influence of increased blood pressure cannot be entirely excluded. However, slow injection of epinephrine, so regulated that there is an increase in the blood content without significant rise of pressure, fails to produce arteriosclerosis.[25] Myocardial degeneration is also observed in experimental animals, and later may lead to an interstitial myocarditis (Pearce). These experiments suggest the possibility that oversecretion of epinephrine may be a cause of arteriosclerosis, but there is no evidence that this actually occurs in man.

[18] See Pearce, Jour. Exper. Med., 1908 (10), 735; Thomas, Ziegler's Beitr., 1910 (49), 228.

[19] See Högler, Wien. Arch. inn. Med., 1923 (6), 343.

[20] Jour. Exp. Med., 1911 (14), 377; 1912 (15), 547; also Rogoff, Jour. Lab. Clin. Med., 1918 (3), 209.

[21] Jour. Exp. Med., 1912 (16), 541.

[22] Deut. Arch. klin. Med., 1911 (103), 168.

[23] See review by Oppenheimer and Fishberg, Arch. Int. Med., 1924 (34), 631.

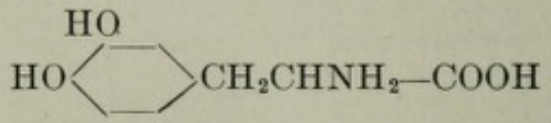
[24] Literature given by Saltykow, Cent. f. Path., 1908 (19), 369.

[25] van Leersum and Rassers, Zeit. exp. path., 1914 (16) 230.

Addison's Disease[26]

As pointed out before, the profound deficiency in the pressor principles evident in the manifestations of Addison's disease implies loss of function, not only of the adrenal medulla, but also of the rest of the chromaffin tissues which produce this same sort of material. Therefore it is possible to have any amount of destruction of the adrenals without Addison's disease, if there is sufficient compensation by the other chromaffin structures, or, conversely, Addison's disease may occur when the adrenals seem morphologically little altered, which occurs in about 10 per cent. of all cases. In typical cases, however, the adrenals have been found entirely devoid of epinephrine,[27] and usually the structural alterations are conspicuous. While it is generally held that destruction of the medulla is an essential feature of Addison's disease, there have been reported several cases in which the cortex alone has been destroyed. I have recently observed such a case in which the only anatomical change was a selective destruction of the cortex leaving an apparently intact medulla in both adrenals, with a typical clinical picture of Addison's disease. It is perhaps of significance that in this case there was not so marked a fall in blood pressure as is commonly seen in this disease, for it remained at 110 or above despite the general weakness and confinement in bed. Furthermore, in two cases of ordinary Addison's disease, Högler[28] found the blood devoid of the epinephrine effect demonstrable in normal blood.

The pigmentation of the skin[29] has not yet been explained, but in view of the fact that oxidizing enzymes readily convert epinephrine, tyrosine, and related aromatic substances into pigments, and that in Addison's disease we have a deficiency in a tissue which is known to be concerned in the metabolism of aromatic compounds, it seems probable that the pigmentation is the result of this defective metabolism of the chromogenic aromatic compounds. In support of this view is the observation of Bittorf[30] that the skin of persons with Addison's disease has an augmented power of oxidizing epinephrine and tyrosine to pigmented substances. Bloch[31] believes the pigmentation to result from the presence of excessive quantities of 3.4-dioxyphenylalanine, which



may be a precursor of epinephrine, and which is oxidized to a melanin by special oxidizing enzymes ("dopaoxidase") present in the skin. Heudorfer[32] looks upon the skin pigmentation as a visible sign of an effort of the skin to compensate for the deficient metabolism of the adrenals.

[26] Literature on Chemistry, by Eiselt, Zeit. klin. Med., 1910 (69), 393.
[27] Ingier and Schmorl, Deut. Arch. klin. Med., 1911 (104), 125.
[28] Wien. Arch. inn. Med., 1923 (6), 343.
[29] According to Straub (Deut. Arch. klin. Med., 1909 (97), 67) pigmentation may occur within 17 days after thrombosis of the adrenal vein.
[30] Arch. exp. Path., 1914 (75), 143; Deut. Arch. klin. Med., 1921 (136), 314.
[31] Zeit. exp. Med., 1917 (5), 179; Arch. f. Dermatol., 1917 (124), h. 2.
[32] Münch. med. Woch., 1921 (68), 266.

Until the pigment of Addison's disease has been isolated and analyzed, however, these hypotheses will probably remain unproved. (See Pigmentation, Chap. XX.) Addison's disease can occur without pigmentation.

That there is a deficiency in the formation of epinephrine is attested by the low blood pressure and general low tone of the unstriated muscle tissue. Carbohydrate metabolism is also altered, hypoglucemia and an increased sugar tolerance often being observed, but not constantly.[33] There is also an increased uric acid excretion.[34] Whether the adrenals exert a detoxicating effect, and the symptoms of the disease are partly the result of an autointoxication of some sort, is at present unknown, although this idea has often been advanced. The general metabolism of Addison's disease shows no very striking or characteristic changes, over and above those associated with the emaciation. Wolf and Thacher[35] found a decrease in endogenous creatine and purine excretion, and some evidences of acidosis towards the end of the disease; deaminizing power and oxidation of cystine sulfur to SO_4 were not impaired. Eiselt believes that there is a toxocogenic loss of tissue. Because of the low blood pressure renal function may be much depressed[36] but there are usually no striking changes in blood chemistry. There seems to be usually little change in blood volume or in the red cells and hemoglobin.[37] Basal metabolism is not much altered.

Administration of adrenal tissue and extracts, or epinephrine, whether by mouth or subcutaneously, is not effective in ameliorating the course of Addison's disease, at least in most cases. Thus, in 97 cases collected by Adams,[38] adrenal treatment caused some improvement in 31, 43 were not benefited, 7 were made worse, while 16 were described as permanently improved. Similar results are described by Rowntree.[39] The symptom most favorably affected is usually the muscular and gastro-intestinal asthenia, while the pigmentation is not usually altered. There is little effect on metabolism.[40]

The Hypophysis and Acromegaly[41]

Although the hypophysis contains in its anterior lobe and in the pars intermedia, a certain number of spaces filled with colloid and resembling the alveoli of the thyroid in appearance,[42] there is no evidence that an

[33] See Rosenow and Jaguttis, Klin. Woch., 1922 (1), 358.
[34] Lescheziner, Virchow's Arch., 1915 (221), 67.
[35] Arch. Int. Med., 1909 (3), 438.
[36] See Müller-Deham, Wien. Arch. inn. Med., 1922 (3), 323.
[37] See Brown and Roth, Amer. Jour. Med. Sci., 1925 (169), 47.
[38] Practitioner, 1903 (71), 472.
[39] Trans. Assoc. Amer. Phys., 1924 (39), 426.
[40] Beutenmüller and Stoltzenberger, Biochem. Zeit., 1910 (28), 138.
[41] Full bibliography in the monograph by Harvey Cushing, "The Pituitary Body and its Disorders," Philadelphia, 1912; also Bassoe, "Endocrinology and Metabolism," 1924 (1), 805.
[42] Composition of hypophysis given by MacArthur, Jour. Amer. Chem. Soc., 1919 (41), 1225.

appreciable amount of iodine is present here except when therapeutically administered.[43] The posterior lobe contains an active diuretic and pressor substance,[44] the exact nature of which is not yet known, although in many respects its action resembles that of epinephrine. It seems less active in producing arteriosclerosis than is epinephrine, and its pressor effects are of longer duration. It seems to stimulate smooth muscle without respect to innervation (thus differing from epinephrine), but with a special potency in stimulating contractions of the uterus; and hence it has a wide clinical use under the name *pituitrin*. The chemical nature of pituitrin is not yet determined, but it seems to be closely related to or identical with β-iminazolylethylamine, the base derived from histidine,[45] which also stimulates uterine contractions, but which differs in causing bronchial spasm and urticarial reactions. Hanke and Koessler,[46] however, have demonstrated the absence of significant quantities of histamine in fresh hypophysis.

Injection of the posterior lobe extract lowers the assimilation limit for carbohydrates and causes glycogenolysis (Cushing),[47] and is a powerful galactagogue (Ott). According to Dudley[48] there seems to be at least three physiologically active principles in the posterior lobe extracts; one having an oxytocic activity about twelve times as great as histamine. Abel,[49] however, believes there is but one specific hormone in the posterior lobe, which he has obtained in a form over a thousand times as active as histamine in stimulating smooth muscle, and which has all the physiological properties of the whole extract. There are also small amounts of non-specific depressor substances in the extract. He believes that the specific hormone has the function of regulating the removal of water by the kidneys; in its absence diabetes insipidus develops, which is relieved by administering pituitrin.

Removal of the anterior lobe of the gland in young animals is followed by marked metabolic and developmental changes, notable being adiposity, nutritional changes in the skin and its appendages, sexual inactivity and underdevelopment, subnormal body temperature and increased carbohydrate tolerance.[50] These manifestations correspond to those observed in certain human conditions (Froehlich's syndrome)[51] associated with defects in the hypophysis. Removal of the posterior lobe does not produce any characteristic and constant effects, although marked polyuria and erotism have resulted. Feeding to young amphib-

[43] Wells, Jour. Biol. Chem., 1910 (7), 259. See also Thomas and Delhounge.[51]

[44] Lewis, Miller and Matthews, Arch. Int. Med., 1911 (7), 785; Herring, Quart. Jour. Exp. Physiol., 1914 (8), 245 and 267.

[45] See Abel and Kubota, Jour. Pharm. Exp. Ther., 1919 (13), 243.

[46] Jour. Biol. Chem., 1920 (43), 557; see also Dale and Dudley, Jour. Pharmacol., 1921 (18), 27.

[47] See also Bull. Johns Hopkins' Hosp., 1913 (24), 40.

[48] Jour. Pharm., 1923 (21), 103.

[49] Review in Bull. Johns Hopkins Hosp., 1924 (35), 305.

[50] Concerning metabolism after hypophysectomy see Benedict and Homans, Jour. Med. Res., 1912 (25), 409.

[51] Metabolism in Froehlich's syndrome has been studied by Rosenbloom (Interstate Med. Jour., 1917 (24), 475) who found a slight loss of sulur and phosphorus.

ians stimulates growth (Smith[52]), but the early reports that anterior lobe fed to young rats has a stimulating effect on growth, and especially on sexual development and activity have not been confirmed by later workers.[53] Robertson describes a modification of growth in mice fed anterior lobe substance, which he attributes to a specific substance, *tethelin*, containing phosphorus and probably an iminazolyl group, and hence related to the active constituent of the posterior lobe, although it has no pressor effect.[54] Feeding of anterior lobe to salamanders will produce gigantism (Uhlenhuth).[55]

Puncture of the hypophysis produces the same effect as puncture of Bernard's diabetic center in the fourth ventricle,[56] and stimulation of the gland has a similar effect, presumably because of the secretion of a glycogenolytic agent. A diminution of posterior lobe secretion occurring in certain conditions of hypopituitarism leads to an acquired high tolerance for sugars, with the resultant accumulation of fat. In hibernating animals, also, the adiposity and lowered temperature are associated with hypoplasia of the anterior lobe of the hypophysis. There also seems to be some relation between the hypophysis and urinary secretion, for extracts of the posterior lobe cause marked polyuria, and in some instances of "diabetes insipidus," lesions have been found in the hypophysis.[57] Like the thyroid, the hypophysis enlarges during pregnancy.[58] Feeding of hypophysis or injection of pituitary extract is said to increase both gaseous and nitrogenous metabolism,[59] and in a case of hypopituitarism the urine has been found to contain a high proportion of undetermined nitrogen and of neutral sulfur.[60] Varying results have been obtained in studies on the basal metabolism of hypopituitarism,[61] but it may be much depressed.[62]

Acromegaly.—The accumulating evidence seems to indicate that acromegaly depends upon a hyperfunctioning of the anterior lobe tissue of the hypophysis, one of the most important facts being the improvement which has followed removal of the hyperplastic tissues in several cases successfully operated. Although there are many cases of tumor of the hypophysis without acromegaly, this is of no significance since it is not to be expected that all tumors will carry on the functions of the tissue in which they arise. Acromegaly without hypophyseal changes is rare, especially if we consider the finer cytological evidence of cellular activity.[63] So far, little of chemical interest has been learned concerning this disease. The metabolism studies generally indicate a retention of nitrogen, phos-

[52] P. E. Smith, Univ. Calif. Publ. (Physiol.), 1918 (5), 11.
[53] Sisson and Broyles, Johns Hopkins Hosp. Bull., 1921 (32), 22.
[54] Jour. Biol. Chem., 1916 (24), 409.
[55] Jour. Gen. Physiol., 1921 (3), 347.
[56] Amer. Jour. Physiol., 1913 (31), xiii.
[57] Simmonds (Münch. med. Woch., 1913 (60), 127) holds that the pars intermedia is responsible.
[58] See Erdheim and Stumme, Ziegler's Beitr., 1909 (46), 1.
[59] See McKinlay, Arch. Int. Med., 1921 (28), 703.
[60] Stetten and Rosenbloom, Proc. Soc. exp. Biol. and Med., 1913 (10), 100.
[61] Means, Jour. Med. Res., 1915 (32), 121.
[62] See Talbot, Amer. Jour. Dis. Chil., 1920 (20), 331.
[63] See Lewis, Bull. Johns Hopkins' Hosp., 1905 (16), 157.

phorus and calcium, because of the overgrowth of bone and soft tissues.[64] According to some observers this retention is decreased, or changed to an excess elimination by administration of hypophyseal substance.[65] The elimination of endogenous uric acid is said to be greatly increased in acromegaly, and decreased in cases with hypofunction of the gland.[66] A considerable excretion of creatine was observed by Ellis.[67] In the early stages of acromegaly there is a definite increase in metabolism, which rarely exceeds +20; whereas in cases of hypophyseal insufficiency there is a corresponding decrease (Cushing).[68]

Glucosuria and actual diabetes are frequently present in acromegaly (40 per cent. of the cases collected by Borchardt),[69] presumably from interference with the regulating function of the hypophysis, but this assumption has been questioned because of the fact that lesions in this location might also produce glucosuria by affecting the "diabetic center." However, since puncture of the hypophysis causes glucosuria, while injection of posterior lobe extract produces glucosuria dependent upon hyperglucemia (Cushing), and in view of the fact brought out by Borchardt that in cases of tumor of the hypophysis without acromegaly, glucosuria has never been observed, there is much probability that in many if not all of the cases of glucosuria with acromegaly, it is the hypophysis itself that is concerned, and that both the acromegaly and the glucosuria are caused by hyperactivity of the gland. In later stages of acromegaly there may develop a hypoactivity because of pressure upon the posterior lobe or infundibular stalk, whereupon the sugar disappears and is replaced by an increased tolerance for sugar.[70]

Thymus[71] and Other Ductless Glands

From the chemical standpoint little of interest is known concerning this organ.[72] It is frequently used as a source of nucleic acids, in which it is rich, but there is no study of its chemical changes that is of interest in pathology. Numerous reports have indicated that removal of the thymus causes marked changes in ossification and development, but the more recent studies do not indicate that the thymus is essential for life or growth (Park and McClure[71]). Gudernatsch[73] found that feeding thymus to tadpoles causes a great increase in the rate of growth, and decreases or suppresses the developmental changes, having exactly the opposite effect from thyroid feeding, and Abderhalden[74] has found that this property persists after digestion of the thymus

[64] See Bergeim, Stewart and Hawk, Jour. Exp. Med., 1914 (20), 218.

[65] See Rubinraut, Dissert., Zurich. Gebr. Leeman, 1912; Medigreceanu and Kristeller, Jour. Biol. Chem., 1911 (9), 109.

[66] Falta and Nowaczynski, Berl. klin. Woch., 1912 (49), 1781; Thannhauser, Deut. Arch. klin. Med., 1924 (143), 287.

[67] Jour. Amer. Med. Assoc., 1911 (56), 1870.

[68] Jour. Amer. Med. Assoc., 1921 (76), 1721.

[69] Zeit. klin. Med., 1908 (66), 332.

[70] Full discussion in Johns Hopkins Hosp. Bull., 1911 (22), 165; 1913 (24), 40.

[71] In addition to Biedl's "Innere Sekretion," see Wiesel, Ergebnisse Physiol., 1911 (XV (1)), 416; Park and McClure, Amer. Jour. Dis. Chil., 1919 (18), 317.

[72] A full discussion of the composition of the thymus is given by Zunz (Arch. internat. physiol., 1920 (25), 459), who shows that involution does not take place before 35 in healthy men. Enzymes of thymus are discussed by Widmark, (Zeit. physiol. Chem., 1924 (135), 122).

[73] Arch. Entwicklgs., 1912 (35), 457; Amer. Jour. Anat., 1914 (15), 431.

[74] Arch. ges. Physiol., 1915 (162), 99.

tissue. The failure of metamorphosis on thymus diet presumably depends on a lack of some substance rather than on the presence of a specific agent inhibiting metamorphosis.[75] As yet no substance has been isolated which can be considered as a specific internal secretion of the thymus, although the frequent concurrence of abnormal conditions in the thyroid and thymus, in the adrenals and thymus, in the hypophysis and thymus, together with the frequency of polyglandular conditions, leaves no question that the thymus is to be considered with the other members of this system,[76] however different its histological structure may be. Thymus administration by mouth is said to counteract the effect of thyroid feeding in stimulating metabolism.[77] The enlargement of the thymus that occurs in most cases of exophthalmic goiter is accompanied at times by symptoms that suggest an intoxication from this source.[78] Uhlenhuth[79] considers the thymus to be antagonistic to the parathyroids and responsible for tetany parathyreopriva. Riddle[80] has found evidence that in birds the thymus is indispensable for the production of the enveloping materials of the eggs (shell and egg white), through the agency of a hormone designated as "thymovidin;" perhaps in mammals the thymus is only a developmental residue. The chemistry of the PINEAL GLAND[81] can be dismissed practically without consideration, since no positive facts have been brought to light.[82] Extracts from the organ show no distinct physiological effects.[83] Tumors of the pineal gland have been found associated with adiposity and with precocious sexual development, but whether from the action of the gland itself or from the pressure on the brain, cannot be said.[84] Extirpation of the pineal seems to have no noticeable effects of any sort (Dandy[85]) although McCord[86] reports increased growth and early sexual maturity in animals fed pineal substance, which was not corroborated by Sisson and Finney.[87] Izawa,[88] however, finds that removal of the pineal in young animals permits premature development of the sex organs of both sexes.

The CAROTID GLAND is more directly related to the adrenal medulla, in that it contains chromaffin tissue. It should, presumably, contain a pressor principle, as Moulon found, but Gomez[89] obtained only lowering of blood pressure from extracts of this gland, and bilateral removal causes no characteristic effects.[90] No epinephrine could be found by Aszódi and Paunz.[91]

The Internal Secretions of the REPRODUCTIVE ORGANS.—Too little is known as to the chemical character of these secretions to warrant their discussion in this place. Our information concerning their behavior is reviewed in articles in Barker and Mosenthal's "Endocrinology and Metabolism," and by Marshall.[92]

[75] Uhlenhuth, Endocrinology, 1919 (3), 284.
[76] See Marine *et al.*, Jour. Exp. Med., 1924 (40), 429.
[77] Halverson *et al.*, Arch. Int. Med., 1916 (18), 800.
[78] See review by Halsted, Bull. Johns Hopkins Hosp., 1914 (25), 223; Eddy, Canad. Med. Assoc. Jour. March, 1919.
[79] Jour. Gen. Physiol., 1918 (1), 23; Endocrinology, 1919 (3), 285.
[80] Amer. Jour. Physiol., 1924 (68), 557.
[81] Concerning the composition of the pineal gland see Fenger, Jour. Amer. Med. Assoc., 1916 (67), 1836.
[82] Bibliography by McCord, Endocrinology and Metabolism, Vol. 2.
[83] Jordan and Eyster, Amer. Jour. Physiol., 1911 (29), 115; Dixon and Halliburton, Quart. Jour. Exper. Physiol., 1909 (2), 283. Dana and Berkeley, Med. Record, 1913 (83), No. 19.
[84] See Pappenheimer, Virchow's Arch., 1910 (200), 122; Krable, Endocrinology, 1923 (7), 379.
[85] Jour. Exp. Med., 1915 (22), 237. Also Camus and Roussy, Endocrinology, 1920 (4), 507; and Kolmer and Löwy, Arch. ges. Physiol., 1922 (196), 1.
[86] Jour. Amer. Med. Assoc., 1914 (63), 232 and 517.
[87] Jour. Exp. Med., 1919 (31), 335.
[88] Amer. Jour. Med. Sci., 1923 (2), 166.
[89] Amer. Jour. Med. Sci., July, 1908.
[90] Massaglia, Frankf. Zeit. Path., 1916 (18), 333.
[91] Biochem. Zeit., 1923 (136), 159.
[92] Physiol. Reviews, 1923 (3), 335.

CHAPTER XXV

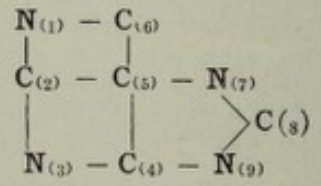
URIC-ACID METABOLISM AND GOUT[1]

THESE subjects have been the object of such a prodigious amount of research that it is far beyond the scope of this work to review the history and the details of the investigations. Such a review is also particularly unnecessary, since it can be found in the works on physiological chemistry and various treatises on metabolism. Consequently the attempt will be made in this chapter merely to give, as briefly as possible, the views now most generally accepted concerning the nature and metabolism of uric acid, and its relation to pathological processes. For the historical discussion, indicating by what devious steps we have reached our present understanding concerning this long-disputed subject, the reader is referred to the articles mentioned below, upon which I have drawn freely.[1]

THE CHEMISTRY OF URIC ACID

It is the very great service of Emil Fischer to have shown us the structure of the uric-acid molecule, the empirical formula of which, $C_5H_4N_4O_3$, had long been known. He demonstrated that it is a member of a group of substances, which are all characterized by being built up about a certain nucleus, C_5H_4. Because the simplest member of the group is a synthetically formed body, *purine*, the nucleus is called the "*purine nucleus.*" The structural relations of the better-known "*purine bodies*" to this purine nucleus and to each other are clearly shown by their structural formulæ, as given below:

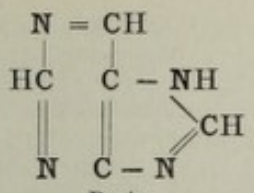
The atoms in the "purine nucleus" are arranged as follows:



To each atom has been given a number, as shown, for the purpose of facilitating reference to the location of various atoms and groups that are attached to this nucleus. The structure of purine itself is as shown below.[2]

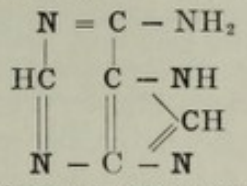
[1] Complete reviews are given by F. H. McCrudden, "Uric Acid," New York, 1906; Brugsch and Schittenhelm, "Die Nukleinstoffwechsel und seine Störungen," Jena, 1910; Walter Jones, "Nucleic Acids," Monographs on Biochemistry, 2nd ed., 1920; Pratt, "Endocrinology and Metabolism," 1924 (4), 411; Summaries of recent work are given by Benedict, Jour. Lab. Clin. Med., 1916 (2), 1; Rose, Physiol. Reviews, 1923 (3), 544; Folin, Berglund and Derick, Jour. Biol. Chem., 1924 (60), 361.

[2] In these formulæ the symbols of the atoms forming the purine nucleus are in heavy type.



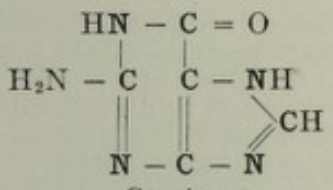
Purine

The derivatives of purine are described by stating to which atom of the purine nucleus the combining groups are attached. Thus, adenine is referred to as 6-amino-purine, and therefore has the following formula:

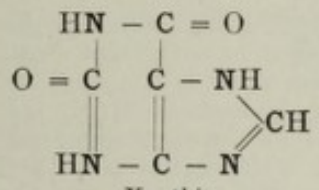


Adenine (6-amino-purine)

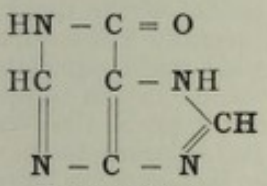
Other important members of this group of "purine bodies," (also called *xanthine* bodies, *alloxuric* bodies, and *nuclein* bodies) are built up about the purine nucleus as shown below:



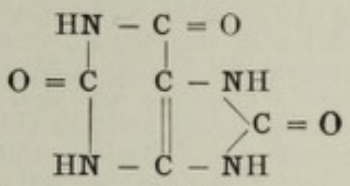
Guanine
(2-amino-6-oxypurine)



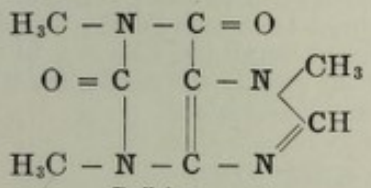
Xanthine
(2, 6-dioxypurine)



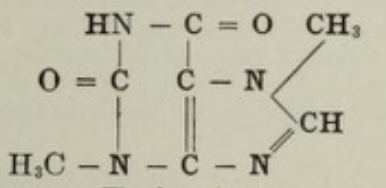
Hypoxanthine
(6-oxypurine)



Uric acid
(2-6-8-trioxypurine)

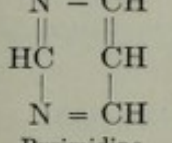


Caffeine
(1-3-7 trimethyl-2-6 dioxypurine)

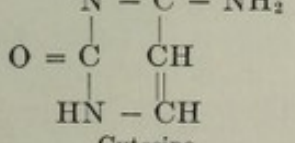


Theobromine
(3-7-dimethyl, 2-6 dioxypurine)

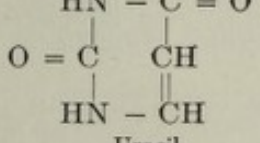
As shown by their structural formulæ, the *pyrimidines* present in the nucleic acids are also closely related to the purines, viz:



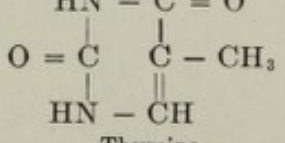
Pyrimidine



Cytosine
(2-oxy, 6-amino-pyrimidine)



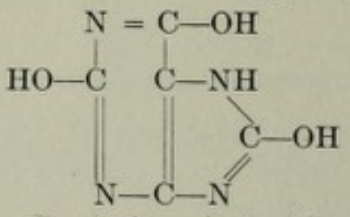
Uracil
(2-6-dioxy-pyrimidine)



Thymine
(5-methyl, 2-6-dioxy-pyrimidine)

Properties of Uric Acid.—Uric acid, when pure, is white, and crystallizes in rhombic tablets. Its solubility is very slight; at room temperature (18°) one part dissolves

in about 40,000 parts of water, so that a saturated solution contains only 0.0253 gram to the liter. It is much more soluble in blood-serum, dissolving in 1,000 parts,[3] probably held in some complex combination, or, as the studies on the physico-chemical behavior of uric acid by Schade[4] indicate, it may be held in supersaturation by a colloidal phase that forms at near the neutral point when uric acid begins to come out of solution.[5] His and Paul have shown that in a saturated solution only 9.5 per cent. of the molecules are dissociated, this occurring in two steps; the first and chief dissociation is into H and $C_5H_3N_4O_3$, which then undergoes further cleavage into H and $C_5H_2N_4O_3$, the latter dissociation being very slight. If any other acid is present in the solution, its dissociation and liberation of free hydrogen ions interferes with the dissociation of the uric acid, and as the undissociated uric acid is extremely insoluble, the amount dissolved in an acid solution is much less than in a neutral solution.[6] Gudzent[7] found that saturated solutions of urates gradually precipitate out the salts because of a transformation of part of the uric acid into what he believes to be a *lactim* form. (The lactim form is shown in the following formula, as compared with the isomeric lactam form shown above, in which uric acid is supposed to exist ordinarily.)



(Lactim form of uric acid)

Uric acid yields two series of salts with alkalies, corresponding to the two steps in dissociation: One, in which one atom of the base enters, is called the *biurate* or *monobasic* urate; the other is the so-called "neutral" or *bibasic* urate.[8] Of the two, the latter is much the more soluble. The monosodium urate forms colloidal solutions in water, from which the crystalline salt gradually falls out. The quadriurate, of which much was said in the earlier literature, probably does not exist (Kohler).[9]

In the urine the uric acid and the urates are kept in solution by the phosphates, the disodium phosphate preventing the decomposition of the urates into uric acid by the acid salts of the urine. Possibly other constituents of the urine, especially the pigments and NaCl, also aid in its solution. Urine may form quite stable supersaturated solutions and Kohler states that the urine is a truly supersaturated solution of sodium urate. How the uric acid is kept in solution in the blood is not exactly understood, but Gudzent believes that uric acid can exist in the blood only as the monosodium urate, and in the less soluble but more stable lactim form, which is soluble only to the extent of 8.3 mg. per 100 c.c. serum (the lactam form being soluble up to 18 mg.). However, amounts over 20 mg. per 100 c.c. have been detected in the blood of nephritics; here solution may have been aided by the other retained metabolites. Schade,[4] Bechhold[10] and others have maintained that urates may be present in the blood in a colloidal state which cannot pass out through the kidneys, but this view is contested by Gudzent[11] who believes that it is all readily diffusible, for when injected into the blood it rapidly diffuses into the tissues and secretions.

FORMATION OF URIC ACID

The origin of uric acid is chiefly, although not exclusively, from the nucleoproteins, and it is customary to refer to uric acid formed from

[3] Taylor, Jour. Biol. Chem., 1906 (1), 177.

[4] Zeit. klin. Med., 1922 (93), 1.

[5] See also Benoit, Compt. Rend. Soc. Biol., 1919 (82), 1052.

[6] Concerning the solubility of uric acid in urine see Haskins, Jour. Biol. Chem., 1916 (26), 205.

[7] Zeit. physiol. Chem., 1909 (60), 38.

[8] As a matter of fact, both salts give a slightly alkaline reaction when dissolved in water (Taylor).

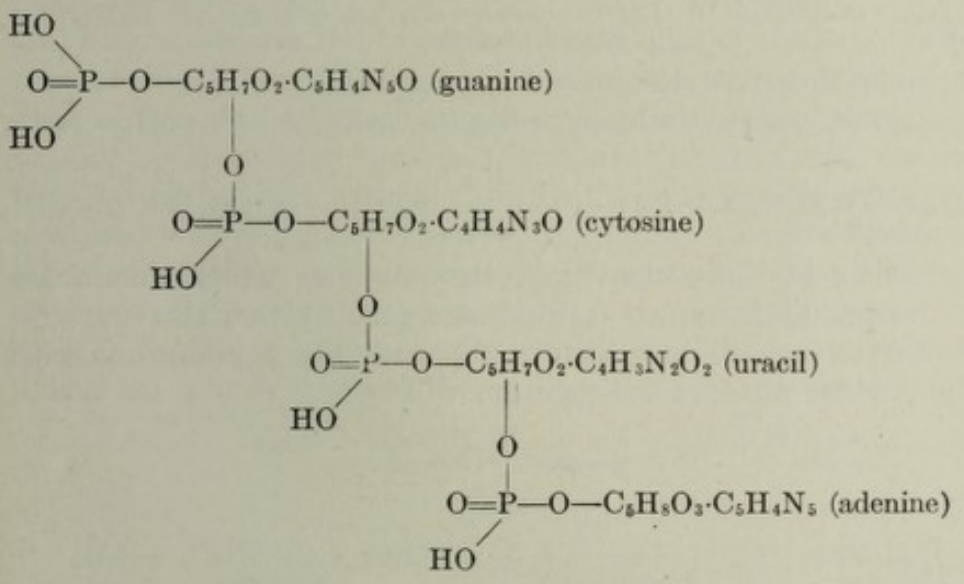
[9] Zeit. physiol. Chem., 1911 (72), 169; 1913 (78), 205; Zeit. klin. Med., 1919 (87), 338.

[10] Biochem. Zeit., 1914 (64), 471.

[11] Zeit. klin. Med., 1923 (99), 20.

the nucleoproteins of the foods as "*exogenous*" uric acid, in contrast to the "*endogenous*" uric acid that is formed from the nucleo-proteins of the body cells during their catabolism. This may be readily explained by a brief consideration of the composition of the nucleo-proteins. The nucleoproteins may be looked upon as salts formed through combination of proteins with nucleic acid. Nucleic acid, in turn is a compound of phosphoric acid with purine bases, pyrimidine bases, and carbohydrate radicals, constituting a complex sort of glucoside.

A long series of careful analytical studies has at last shown us that nucleic acids, are, whatever the source, quite similar in composition, consisting always of a complex containing phosphoric acid, the two amino purines (adenine and guanine), two pyrimidines (either cytosine and uracil or cytosine and thymine), and a carbohydrate, which may be either a pentose or a hexose. Apparently there are two sorts of nucleic acids, one from plants, which contains always uracil and pentose, and one from animal tissues, containing instead thymine and a hexose. So constant are the findings in regard to these compounds that it has seemed feasible to consider their manner of union in the intact nucleic acid molecule, and Levene[12] has proposed as the structure of yeast nucleic acid the following arrangement:[13]



It will be seen that this proposed formula postulates in the nucleic acid molecule, one radical of each of the two purines and pyrimidines, each of these being united by a carbohydrate radical to a phosphoric acid radical. Recognizing that this must be looked upon as a provisional formula, it will serve as a base of departure from which to consider the metabolism of nucleic acid.

The grouping of carbohydrate + purine or carbohydrate + pyrimidine is referred to as a "nucleoside," analogous in terminology to "glucoside." The same groupings plus the phosphoric acid radical constitute

[12] Jour. Biol. Chem., 1921 (48), 119.

[13] Jones (Jour. Biol. Chem., 1923 (55), 557) has suggested a different arrangement.

the "nucleotids," nucleic acid thus being made up of four nucleotids. Emil Fischer reported[14] the synthetic production of a nucleotid, composed of phosphoric acid united to a glucoside of theophyllin, this really constituting the long-sought synthesis of a nucleic acid, even though the artificial product is not the same as any known to occur in nature.

With these facts before us we may consider the manner in which nucleic acids are disintegrated in the animal body. So large a molecule can conceivably be disintegrated in many different ways; that is, the lines of cleavage might pass through several different points and in many different orders, but there is evidence available which causes us to believe that the process is quite constant in animal metabolism. Jones considers it probable that the first step is a decomposition of the tetranucleotid into dinucleotids, and that these are in turn split into mononucleotids. Little is known about the subsequent career of the two pyrimidine nucleotids, but we have an abundance of information concerning the nucleotids containing the purines, and it is in these our present interest lies. Each nucleotid has two points at which it might be split, and we have reason to believe that there exist in animal tissues enzymes which may specifically attack each bond. One enzyme separates the phosphoric acid radical from the nucleoside, thus:

$$\underset{\text{guanylic acid}}{H_2PO_4 - C_5H_8O_3 - C_5H_4N_5O} + H_2O \xrightarrow[\text{phospho-nuclease}]{} H_3PO_4 + \underset{\text{guanosine}}{C_5H_9O_4 - C_5H_4N_5O}$$

and this enzyme is therefore designated as *phospho-nuclease.*

Another enzyme, *purine nuclease*, splits off, instead, the purine radical, thus:

$$\underset{\text{guanylic-acid}}{H_2PO_4 - C_5H_8O_3 - C_5H_4N_5O} + H_2O \xrightarrow[\text{purine-nuclease}]{} H_2PO_4 - C_5H_9O_4 + \underset{\text{guanine}}{C_5H_5N_5O}$$

Following either of these cleavages, the enzymes which deaminize purines begin to act, and there is formed as a result either the free oxypurines or the oxypurine still bound in the glucoside-like combination with sugar. If the purines are free the reaction will be:

$$\underset{\text{guanine}}{C_5H_5N_5O} + H_2O \xrightarrow[\text{guanase}]{} \underset{\text{xanthine}}{C_5H_4N_4O_2} + NH_3$$

or, in case the guanine glucoside is present:

$$\underset{\text{guanosine}}{C_5H_9O_4 - C_5H_4N_5O} + H_2O \xrightarrow[\text{guanosine—deaminase}]{} C_5H_9O_4 - \underset{\text{xanthosine}}{C_5H_3N_4O_2} + NH_3$$

In the latter case a hydrolytic enzyme, xanthosine-hydrolase, then splits off the xanthine, so that by either route the end result is the same.[15] By a similar series of changes the adenine radical is converted into hypoxanthine, either directly by adenase:

$$\underset{\text{adenine}}{C_5H_5N_5} + H_2O \xrightarrow[\text{adenase}]{} \underset{\text{hypoxanthine}}{C_5H_4N_4O} + NH_3$$

or by adenosine-deaminase the hypoxanthine-glucoside (inosine) is formed, and later the hypoxanthine is split off.

[14] Sitzungsber. k. Akad. Wissensch., Berlin, 1914 (33), 905.

[15] Concerning properties of nucleosidases see Levene *et al.*, Jour. Biol., Chem., 1924 (60), 693.

We now have hypoxanthine and xanthine, which. in the presence of oxygen, are oxidized to form uric acid, thus:

$$\underset{\text{hypoxanthine}}{C_5H_4N_4O} + O \xrightarrow[\text{hypoxanthine-oxidase}]{} \underset{\text{xanthine}}{C_5H_4N_4O_2}$$

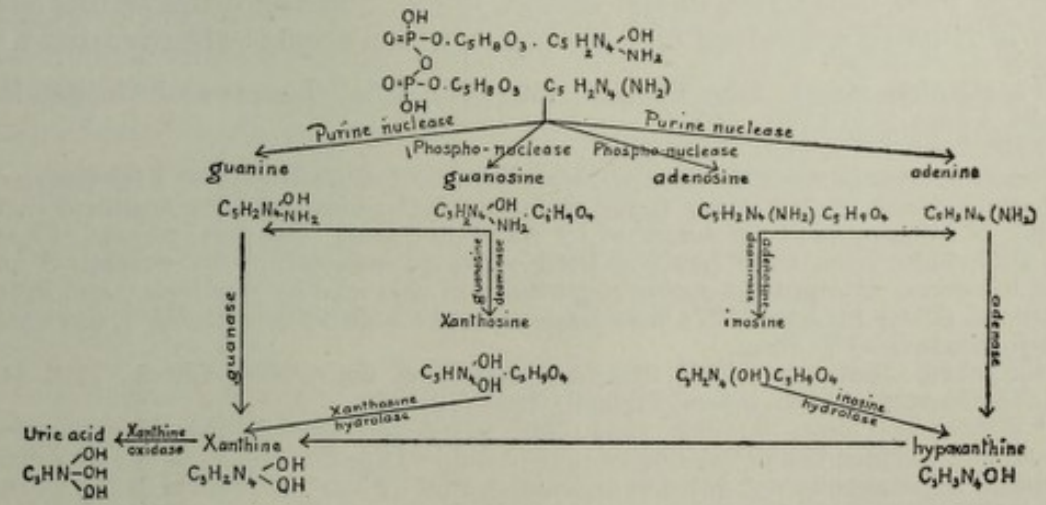
$$\underset{\text{xanthine}}{C_5H_4N_4O_2} + O \xrightarrow[\text{xanthine-oxidase}]{} \underset{\text{uric acid}}{C_5H_4N_4O_3}$$

Further oxidation of the uric acid causes its conversion into the much more soluble allantoin, thus:

$$\underset{\text{uric acid}}{C_5H_4N_4O_3} + O + H_2O \xrightarrow[\text{uricase}]{} \underset{\text{allanitoin}}{C_4H_6N_4O_3} + CO_2$$

It is thus evident that the steps of the disintegration of nucleic acid are numerous, but that each separate process is a simple one; and also, that it has been possible to follow out and distinguish the several steps and to establish the fact that each depends on a distinct and specific enzyme. Not every tissue possesses all the enzymes of purine destruction, and in different species of animals the distribution of the enzymes is different. For example, the enzyme xanthine-oxidase, which oxidizes xanthine into uric acid, is found in man only in the liver, and also in other animals it is of limited distribution, being found usually only in the liver or in the liver and kidney, but in the dog it seems to be present in several tissues. The deaminizing enzymes, adenase and guanase, are much more widely distributed, but by no means universally. Adenase, for example, is not present in the tissues of the rat, and not in the tissues of adult human beings.[16] Guanase is absent from the spleen and liver of the pig and from human spleen, although present in most other tissues. Uricase, the enzyme which destroys uric acid, also has peculiarities of distribution, being seldom found in any other tissue than the liver or kidney and being absent entirely from the tissues of man, and from the birds and reptiles so far examined. The significance of this distribution of uricase will be discussed at greater length a little later.

The following graphic expression of the series of steps leading to the formation of uric acid has been presented by Amberg and Jones;[17]



[16] There have been some reports indicating the presence of adenase in fetal human tissues (Long, Jour. Biol. Chem., 1913 (15), 449).

[17] Zeit. physiol. Chem., 1911 (73), 407.

Another possible source of uric acid is through synthesis. In birds, which eliminate most of their nitrogen in the form of uric acid, synthesis of uric acid undoubtedly occurs. It must also be considered that young mammals can synthesize the purines necessary for their growth from foods which contain no purines.[18] It would seem possible, therefore, for synthesis of uric acid to occur in adult mammals, and Benedict[1] has observed the excretion of over 100 grams of uric acid by a dog kept on a purine-free diet, while Kollmann[19] has reported a similar phenomenon in a human subject.[20] As pointed out by Ackroyd and Hopkins,[21] purines might readily be synthesized from arginine and histidine.

It should also be mentioned that not all of the purine bases of the body is bound in the form of nucleic acid. A considerable amount is present in a free condition, or at least not bound in nucleic acid, especially in muscle tissue.[22] Uric acid can be formed as well from the free purine bases as from purine bases liberated from nucleic acid—indeed, evidence has been brought forward indicating that a large proportion of the uric acid arising during metabolism (endogenous) comes from the free hypoxanthine of the muscles.

As to the place where uric acid is formed, it seems probable that in different animals different organs are chiefly concerned, for it has been found that the distribution of the enzymes mentioned above varies greatly in the various organs and tissues of different species.[23] In most animals the xanthine oxidase, which forms uric acid from xanthine, is localized chiefly or solely in the liver, and this is the case in man; therefore it is presumable that uric acid is formed chiefly in the liver from purines by the steps described above. That there may be other methods of forming uric acid is possible.

DESTRUCTION OF URIC ACID[24]

With most mammals but little of the total amount of purine bases taken as food or set free in the tissues, appears in the urine as uric acid, most of it being converted into allantoin, which seems to be excreted with

[18] McCollum, Amer. Jour. Physiol., 1909 (25), 120. Osborne and Mendel, Zeit. physiol. Chem., 1912 (80), 307.

[19] Biochem. Zeit., 1921 (123), 235.

[20] An apparently reversed reaction, whereby uric acid destroyed by liver tissue can be resynthesized by the same tissue acting upon the disintegration products in the absence of oxygen, has been described by Ascoli and Izar. See Zeit. physiol. Chem., 1910 (65), 78. Their work has not been repeated successfully by others. I have failed in several attempts to secure resynthesis of uric acid by dog livers, and Spiers, (Biochem. Jour., 1915 (9), 337) who made a more extensive investigation, was unable to corroborate their findings.

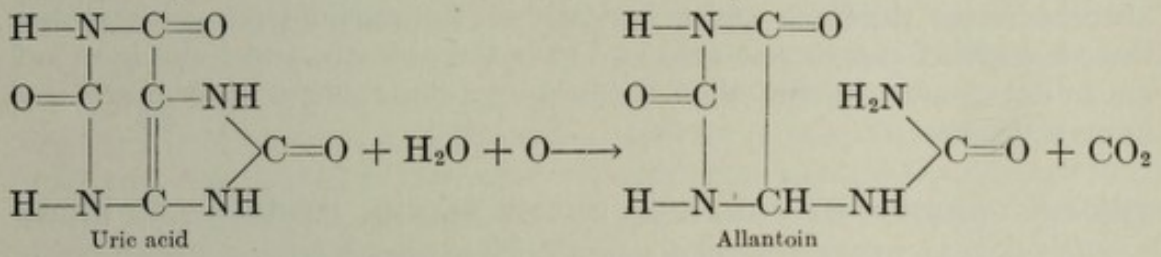
[21] Biochem. Jour., 1916 (10), 551; see also Rose, Jour. Biol. Chem., 1921 (48), 563, 575; Clementi, Arch. intern. Physiol., 1921 (16), 100.

[22] Whiteside (Anat. Record, 1923 (26), 91) found that in the liver, pancreas, thyroid and thymus the proportion of nucleic acid varies directly with the proportion of nuclear substance to cell volume, indicating that at least in these cells the purines must be chiefly in the nuclei.

[23] A compilation of this distribution is given by Wells, Jour. Biol. Chem., 1910 (7), 171.

[24] See discussion by Wells, Jour. Lab. Clin. Med., 1915 (1), 164.

little or no loss.[25] Thus, when dogs, pigs, or rabbits are fed nucleic acid, about 93 to 95 per cent. can be recovered as allantoin, 3 to 6 per cent. as uric acid, and 1 to 2 per cent. as purine bases (Schittenhelm). It would seem that practically all the purines can be accounted for in these three forms combined, the proportions varying in different species. In man alone, except for the chimpanzee[26] and orang-utan, does a considerable proportion escape as uric acid,[27] a fact in complete harmony with repeated observation that the tissues of man have no power whatever to destroy uric acid *in vitro;* the earlier reports of positive uricolysis by human tissues *in vitro* undoubtedy being erroneous. Even the monkey has active uricolytic enzymes in its liver, and therefore excretes its purines chiefly as allantoin. With mammals as a whole, therefore, uric acid is destroyed to the extent of being converted into allantoin,[28] the close relationship of which to uric acid is shown by the structural formula:



With most mammals the oxidation of uric acid takes place chiefly in the liver, but in some of the herbivora the kidneys are more active, as far as experiments *in vitro* can show.

Whether man can destroy uric acid at all has been a matter of dispute. It was found by Wiechowski and others that uric acid injected subcutaneously is excreted almost quantitatively and unchanged in the urine, but more recent investigators have been able to recover but 50 to 80 per cent. To be sure, human urine does contain a very little allantoin, 7 to 14 mg. per day, but this amount is too small to be of much significance, for it is possibly all derived from the food, as the human organism cannot destroy allantoin.[29] On the other hand, it has been found repeatedly that nucleic acid or purines given by mouth are by no means quantitatively excreted in the urine, even when uric acid, allantoin, and purine bases are added together. Apparently a considerable proportion of the purine nitrogen fed, about half in most experiments, is excreted as urea.[30] As allantoin seems not to be at all disintegrated in the human

[25] Allantoin may be found in the blood of other mammals but not in man Hunter, Jour. Biol. Chem., 1917 (28), 369).

[26] Wiechowski, Prager med. Woch., 1912 (37), 275; Wells and Caldwell, Jour. Biol. Chem., 1914 (18), 157.

[27] An interesting exception is furnished by the Dalmatian coach hound, which excretes large amounts of uric acid, but also much allantoin (Benedict;[1] Wells, Jour. Biol. Chem., 1918 (35), 221; Onslow, Biochem. Jour., 1923 (17), 334, 564).

[28] See Hunter and Givens, Jour. Biol. Chem., 1914 (18), 403.

[29] See Ackroyd, Biochem. Jour., 1911 (5), 217, 400, 442.

[30] See Taylor, Jour. Biol. Chem., 1913 (14), 419; Givens and Hunter, *ibid.*, 1915 (23), 299. About one-tenth as much uric acid is excreted in the sweat as in the urine, sweat containing 0.1 mg. per c.c. (Adler, Deut. Arch. klin. Med., 1916 (119), 548.)

body it would seem probable that if purines are destroyed, as these experiments indicate, they pass through some other route than allantoin, and possibly that part of the purines which is destroyed does not pass through the stage of uric acid. Experiments show that outside the body uric acid can be destroyed by other routes than through allantoin; thus, it can be disintegrated into glycine, ammonia, and CO_2; or by another method of destruction it yields first alloxan ($C_4H_2N_2O_4$), then parabanic acid ($C_3H_2N_2O_3$) which in turn yields oxalic acid and urea. There is no evidence, however, that any of these alternative routes is ever followed in the animal body. It is probable that the failure to find all the purines of the food as uric acid in the urine depends on their partial destruction in the intestine by bacteria,[31] and the loss of uric acid injected intravenously may likewise occur through excretion into the intestine. It is highly probable, in view of all available evidence, that in man most of the purine absorbed from the food, and practically all the purine from cell metabolism, is converted into uric acid and excreted as such, and there is as yet no actual demonstration that there is any destruction of uric acid by human tissues.

THE OCCURRENCE OF URIC ACID IN THE BLOOD, TISSUES, AND URINE

As can be seen from the foregoing discussion, the amount of uric acid that appears in the urine depends upon a number of factors, which may be enumerated as follows:[32] (1) The amount of purine bodies taken in the food, upon which, chiefly, depends the amount of exogenous uric acid. (2) The extent to which food purines are destroyed in the alimentary canal by the intestinal bacteria.[33] (3) The amount of destruction of tissue nucleo-proteins. (4) The amount of purine bases formed in the muscle tissue. (5) The amount of conversion of purine bases into uric acid. (6) The amount of destruction of uric acid, if any, occurring in the body. (7) Possibly upon the capacity of the tissues to synthesize uric acid; and in case such power to synthesize uric acid exists, upon the presence of the precursors of uric acid in the body. (8) The retention of uric acid in the blood and tissues. (9) The power of the kidneys to excrete uric acid.[34]

If we also take into account the fact that the solubility of uric acid in the urine depends chiefly upon the amount of neutral phosphates present and also upon the temperature, reaction, and concentration of the urine, it becomes apparent how totally devoid of significance is the presence of crystals of uric acid and urates in the urine, and how fallacious is

[31] See Siven, Arch. ges. Physiol., 1914 (157), 582; Thannhauser and Dorfmüller, Zeit. physiol. Chem., 1918 (102), 148; Morris and Ecker, Jour. Infect. Dis., 1924 (34), 592.

[32] Review on uric acid metabolism and many data given by Höst, Nord. Mag. Laev., 1917 (78 Suppl.). See also Jour. Biol. Chem., 1919 (38), 17.

[33] Steudel (Zeit. physiol. Chem., 1923 (127), 291) says that when there is marked intestinal fermentaion on a carbohydrate diet, the uric acid excretion may be greatly reduced because of the fermentative destruction of its precursors in the intestine.

[34] There seems to be no definite threshold for excretion of uric acid as with sugar (see Ambard and Wolf, Compt. Rend. Soc. Biol., 1924 (90), 786).

any theorization based upon the excretion of considerable quantities of uric acid when all the above-mentioned factors, especially the diet, are not controlled and taken into consideration. Yet on just such an inadequate basis was once constructed an enormous amount of theorization as to "uric-acid diathesis," "uric-acid intoxication," "lithemia," etc., until it came to be popularly believed that a large share of the minor ailments of humanity, and in particular all non-infectious diseases of the joints and muscles, are dependent upon the presence of excessive quantities of uric acid or urates in the blood. But it may safely be stated that at the present time there exists no good evidence which makes it probable that uric acid is responsible for any pathological conditions whatever, except uric-acid calculi, "uric-acid infarcts" in the kidneys, and certain manifestations of gout. Uric acid is possessed of but a very slight degree of toxicity, and the body is able to get rid of it in such large measure that an actual intoxication with uric acid probably never occurs.[35]

The amount present in the urine may be very considerably increased by eating food rich in purines, of which sweet-breads, liver, and kidney are the best examples; and also coffee with its caffeine (trimethyl purine), may give rise to a little uric acid, although the methylated purines seem to be destroyed in large part, or eliminated as something else than uric acid. Large quantities of meat will also increase the uric acid, because of the free purines contained in muscle; and even a diet rich in proteins free from purine will increase the uric acid excretion over that of a low protein diet.[36] On a purine-free diet the excretion of endogenous uric acid is increased by increasing even the non-protein calories (Höst).[32] However, the amount of uric acid in the blood is not correspondingly raised by purine-rich diets,[37] this being regulated by the binding function of the tissues and by excretion through the kidneys.[38]

Whenever much destruction of the nucleoproteins of the tissues is occurring in the body, the elimination of endogenous uric acid becomes abnormally raised, the best examples being the resolution of pneumonic exudates, and leukemia, especially leukemia under *x*-ray treatment (*q. v.*). In neither of these conditions, however, do any symptoms or tissue changes arise that can be referred to the excessive uric acid. Folin and Denis[39] originally reported that human blood normally contains 1.5–2.5 mgs. uric acid in 100 c.c., and the amount bears no fixed relation to the amount of urea and total non-protein nitrogen of the blood.[40] Improvements in methods have somewhat raised these figures, the

[35] The significance of the high blood uric acid in eczema is unknown, but it may possibly have some etiological relation to the skin lesion (Schamberg and Brown, Arch. Int. Med., 1923 (8), 801).

[36] Taylor and Rose, Jour. Biol. Chem., 1914 (18), 519; Lewis and Doisy, *ibid.*, 1918 (36), 1.

[37] See Denis, Jour. Biol. Chem., 1915 (23), 147.

[38] Stocker found uric acid in saliva, increased in all conditions associated with uricemia (Inaug. Dissert., Zurich, 1913).

[39] Jour. Biol. Chem., 1913 (14), 29; Arch. Int. Med., 1915 (16), 33.

[40] In infants the amount is slightly lower, about 1.3 to 1.7 mg. Liefmann, Zeit. Kinderheilk., 1915 (12), 227.

Folin-Wu method giving the upper normal figure as 3.0 to 3.5 mg. Still later modifications added slightly to these figures, and Lennox and O'Connor[41] give 2.5 to 4.5 as the normal amount, and slightly higher values are found after 12 hours fasting. Consequently, in evaluating the figures on blood uric acid in the literature it is necessary to take into account the normal standard for the method of analysis employed. Furthermore, our data are complicated by the fact that not all the uric acid of the blood is in a free state. It has been shown by Benedict that there is a considerable amount of uric acid in the corpuscles of ox blood in a combined form (*d*-ribose uric acid) which cannot be detected until the compound is hydrolyzed, but human blood contains little if any of this combined uric acid.[42] However, Morris and Macleod[43] find that in human blood there is present in plasma as well as in corpuscles, two forms of uric acid, only one of which is measured by the Folin-Wu method which has furnished most of the figures on blood uric acid now in the literature.[44]

Any difficulty in renal elimination is usually accompanied by an increase in the amount of uric acid in the blood, in uremia as much as 15 to 20 mg. or even more being sometimes found per 100 c.c.[45] In early interstitial nephritis there may be 4 to 8 mg. of uric acid per 100 c.c. blood without a corresponding increase in urea and creatinine, which suggests that uric acid may be less easily excreted by the diseased kidney than the other chief nitrogenous constituents of the urine.[46]

In normal individuals there seems to be little uric acid present in the tissues. By using Folin's method, Fine[47] found that various tissues contain quantities comparable to that in the blood of the same person, whether this is normal or increased in amount. Gudzent and Keeser[48] find that tissues from cadavers, and also surgically removed tissues, contain varying amounts of uric acid, their figures per 100 gms. of tissue being; in muscle, 3–4 mg.; liver, 8–16 mg.; kidneys, 4–8 mg.; brain, 5–11 mg.; pancreas, 12–6 mg.; thyroid, lung, and testicles, 2–6 mg. Ordinarily these quantities are not sufficient to permit readily of isolation of the uric acid in a pure state, but in the tissues of a young woman who died after complete suppression of urine for nine days following poisoning with $HgCl_2$, I found considerable amounts.[49]

Folin[1] says that the injection of 20 mg. per kilo of uric acid into the blood of human subjects is often accompanied by fairly severe, although transient, subjective symptoms. Headache, nausea, and extreme weakness are the main symptoms. The level of uric acid in the blood rises to

[41] Jour. Lab. Clin. Med., 1924 (10), 99.
[42] See Newton and Davis, Jour. Biol. Chem., 1922 (54), 603.
[43] Jour. Biol. Chem., 1922 (50), 65.
[44] See also Weil and Guillaumin, Paris Méd., 1922 (45), 585; Bornstein and Griesbach, Biochem. Zeit., 1920 (106), 190.
[45] See Folin and Denis, Arch. Int. Med., 1915 (16), 33; Meyers and Fine, *ibid.*, 1916 (17), 570; Baumann *et al.*, *ibid.*, 1919 (24), 70.
[46] See Myers, Jour. Lab. Clin. Med., 1920 (5), 490.
[47] Jour. Biol. Chem., 1915 (23), 473.
[48] Zeit. klin. Med., 1922 (94), 1.
[49] Jour. Biol. Chem., 1916 (26), 319.

very high figures—over 20 mg. per 100 c.c.,—and falls very slowly, reaching in one experiment 11.8 mg. after 3 hours, and after 27 hours it was at 8.7 mg. From 30 to 90 per cent. of the uric acid injected into man is excreted with the urine in from 2 to 4 days after the injection. He believes that in man, as he found in dogs, the injected uric acid appears not to pass from the blood into the general body tissues, although in the dog a part of the uric acid is removed from the blood by some tissue or tissues, probably the kidney, with extreme rapidity. After injections of 20 mg. per kilo of body weight into human subjects, the uric acid remains at unmistakably high levels in the blood for from 2 to 3 days—these higher levels corresponding in general to the length of time that extra uric acid is excreted with the urine. On the average, 40 to 60 per cent. of the administered uric acid disappears. He deduced that the injected uric acid is destroyed within the blood or the body tissues, but it may be suggested that this is impossible to prove, since it is highly probable that excessive uric acid will be excreted in greater or less amounts into the bowel[50] where it will be disintegrated by the intestinal bacteria.[31] Furthermore, other observers have recovered in the urine virtually all the uric acid injected,[51] although total recovery has not been accomplished by most recent investigators.

Folin[1] found that in dogs most of the uric acid injected intravenously is taken up and in large part held for some time in the kidneys, little being taken out of the blood by other tissues. He believes that the process in man is similar, except that dogs have a much greater power to destroy uric acid in the blood. The human characteristic of a comparatively high uric acid level in the blood he attributes to the marked inability of the human kidney to store and to excrete uric acid. He also found that injected uric acid is far from non-toxic in man, causing much prostration and increased protein catabolism, especially in those with the least rapid elimination. There is a marked variability in the permeability of the red corpuscles by uric acid (Theis and Benedict),[52] which may be of influence in the retention and excretion of uric acid, although as yet the relation of this fact to gout has not been determined.

GOUT

After adjusting the many contradictory statements of earlier investigators, the present status of our conception of uric-acid metabolism in gout may be briefly summarized as follows: The excretion of uric acid in patients with *chronic gout*, when kept upon a definite diet, does not differ greatly from the excretion of normal individuals on the same diet. Normally the elimination of uric acid varies within rather wide limits, even on a constant diet, but the excretion in chronic gout tends to stand

[50] There may be a significant excretion of uric acid in the bile, for Brugsch and Rother have found as high as 50 mg. in 100 c.c. of bile. (Klin. Woch., 1923 (2), 1209.)

[51] See Chantraine, Biochem. Zeit., 1922 (133), 613.

[52] Jour. Lab. Clin. Med., 1921 (6), 680; see also Chauffard *et al.*, Compt. Rend. Soc. Biol., 1922 (86), 31.

at or slightly below the lower normal limits.[53] As a rule, gouty patients on a purine-free diet excrete less endogenous uric acid than normal persons, and when given purines in the food the rate of excretion of these exogenous purines is slower than normal. There seems to be no particular relation between the amount of uric acid in the blood and the occurrence or severity of attacks.[54]

McClure and Pratt[55] have corroborated the statement of others that if abundant purines are fed to gouty patients they may be found to have less than normal capacity to excrete the excess,[56] which may be explained, on the ground of renal inefficiency, or as the result of a greater capacity of the tissues to hold urates.

In the intervals between the attacks of *acute gout* the elimination of uric acid is said to remain within the normal limits; however, for a period of one to three days before each acute attack the amount of uric acid is usually decreased considerably. With the onset of the attack the amount of uric acid excreted becomes increased, and for a few days remains above the average, then subsides to about the normal. Of these two features, the increased output of uric acid during the attack seems to be more constant than the reduced output preceding it, but cases occur in which the uric acid excretion shows no variation from that of normal persons. In certain cases of rheumatoid arthritis the behavior of the purine metabolism resembles that of gout.[57]

As yet we have no definite information either as to the cause of this behavior of the uric acid during the paroxysms of acute gout, or as to its part in causing the paroxysm. However, in view of the fact that monosodium urate is found in the joints during the attacks, it seems most probable that for some as yet unknown reason there occurs a precipitation or anchoring of the urates in the tissues, which is associated with the attacks of pain and swelling. We do not know, however, that it is the deposition of urates that causes the attacks. Indeed, the fact that uric-acid retention precedes the attack, rather than accompanies it, seems to suggest that it is the absorption of the urate, rather than its deposition in the joints, that is responsible for the local disturbances. It is also possible that during the period of retention the uric acid is held in the blood in some form that cannot be eliminated by the kidney, and that its deposition in the joints in an absorbable form occurs simultaneously with the attack. The failure of recent studies on the enzymatic transformation of purines to locate anywhere in the human body an enzyme destroying uric acid, makes hazardous the attempt to explain gouty

[53] The drug atophan (phenylcinchonic acid) causes a marked increase in the uric acid excretion in both normal and gouty subjects, often a greater excess of excretion occurring than the total amount of free uric acid in the blood, which is normally about 100 mg. Apparently atophan mobilizes the uric acid in the tissues.

[54] Pratt, Amer. Jour. Med. Sci., 1916 (151), 92; Bass and Herzberg, Deut. Arch. klin. Med., 1916 (119), 482.

[55] Arch. Int. Med., 1917 (20,) 481- bibliography.

[56] Rosenbloom, Jour. Amer. Med. Assoc., 1918 (70), 285.

[57] W. J. Mallory, Jour. Path. and Bact., 1910 (15), 207; Ljungdahl, Zeit. klin. Med., 1914 (49), 177.

metabolism as a result of enzymatic abnormalities. However, there can be little doubt that the fundamental reason for the existence of uric acid gout in man lies in the inability of the human organism to destroy uric acid. Because man cannot destroy uric acid rapidly by oxidation, as can all other mammals, he is always a potential victim of uric acid retention and deposition.

Analyses of the blood in 120 cases of gout by Gettler and St. George[58] led them to the following conclusions: Cases of gout as a general rule show some increase in the uric acid content of the blood, though some of the chronic cases were within normal limits from this standpoint. The increase is more marked in the acute type of the disease. The uric acid content of the blood in cases of gout is abnormally high without a corresponding increase in the nonprotein nitrogen products of the blood; but the majority of cases show a slight but constant increase in the nonprotein nitrogen and creatinine. An increase of uric acid in the blood, with the patient on a purin-free diet, may be a symptom, but is not diagnostic of gout. Such a condition is usual in leukemia, and is often found in polycythemia, metallic poisoning, diabetes, and other conditions.[59]

Folin and his colleagues[1] examined 15 patients with gout, only one of whom failed to show a high level of blood uric acid, although such patients often have at least a normal capacity to excrete injected uric acid, and so they suggest that the capacity of the kidneys to store uric acid may be defective. They found that there are almost always distinct evidences of renal disturbances in gouty subjects, so that high protein diets commonly cause an abnormally marked increase in the non-protein N of the blood. Normally the joint fluids contain about the same amount of uric acid as the blood, but in gout larger amounts may be present, *e. g.*, 19 to 20 mg. per 100 c.c.[60]

It should be mentioned in addition that it is not the uric-acid metabolism alone that is altered in gout. Irregular periods of nitrogen retention and nitrogen loss are quite constant features. The cause of this variability, and the form in which the nitrogen is retained, are quite unknown, although there is some evidence that the retained nitrogen is in the form of purine bodies (Vogt). Most of the excessive loss occurs during the acute attacks,[61] and the retention of nitrogen between attacks may be partly to repair the loss; against this, however, is the fact that there is not sufficient gain in weight to account for all of the nitrogen retention. Associated with the delayed excretion of ingested purines is also a delayed excretion of the other nitrogenous products of protein food.[62] The proportion of purine bases to uric acid, is not altered in gouty urine.[63] The statements in regard to phosphoric acid elimina-

[58] Jour. Amer. Med. Assoc., 1918 (71), 2033.
[59] See Kraus, Deut. Arch. klin. Med., 1922 (138), 340; Feinblatt, Arch. Int. Med., 1923 (31), 758; Fishberg, *ibid.*, 1924 (34), 503.
[60] Bass and Herzberg, Deut. Arch. klin. Med., 1916 (19), 482.
[61] Brugsch, Zeit. exp. Path. u. Ther., 1906 (2), 619.
[62] Levene and Kristeller, Jour. Exp. Med., 1912 (16), 303.
[63] Heffter, Deut. Arch. klin. Med., 1913 (109), 322.

tion, which depends largely on decomposition of nucleins, are contradictory, but it seems probable that it shows no characteristic alteration in gout. Amino acids, especially glycine, are said to be excreted in excess,[64] but the analytical evidence for this statement is not entirely satisfactory. There is no significant change in the basal metabolism.[65] There is often a hydercholesterolemia in gout,[66] and blood calcium is often excessively high.[67] During attacks of acute gout the blood viscosity is low because of hydremia.[68]

It may be seen from the foregoing discussion that we neither understand fully the intricacies of metabolism in gout, nor know whether uric acid is responsible for either the acute painful attacks or for the anatomical alterations in the kidneys, heart, and blood vessels. Indeed McCrudden[69] and others have shown that it is possible for gouty patients to have a persistently low content of uric acid in the blood, below the average normal quantity, and to have typical acute attacks without change in either the uric acid content of the blood or its excretion; attacks were even observed to occur when the blood uric acid was at a subnormal figure from administration of atophan, which increases its elimination. Furthermore, Bass and Herzberg[70] found that uric acid can be injected into the blood of gouty subjects until the blood contains as much as 10 mg. per 100 c.c. without causing any joint symptoms, and Folin[1] had a similar experience with even larger doses. They also found that intravenous uric acid injection caused less uricæmia in the gouty, in spite of reduced renal excretion, and hence they conclude that in gout the tissues have an increased capacity for taking up uric acid.

Gudzent says that gouty persons differ from normal in a selective tissue affinity for uric acid, for when urates are injected into the blood in normal persons they first diffuse out into the tissues and then are rapidly eliminated by the kidneys, but in gouty subjects the injected uric acid after diffusing into the tissues is not readily diffused back into the blood to be eliminated by the kidneys.[71]

Folin[1] found that intravenous injection of 20 mg. of uric acid per kilo does not produce attacks in gouty subjects, although it may produce enough renal injury to cause an increase in the blood non-protein-N. The characteristically deficient quality of human kidneys to store uric acid, which the dog kidney does with remarkable speed, is apparently exaggerated in gout, and Folin believes there is no reason to accept Gudzent's view that there is a historetention of uric acid in gout. The ability of the kidney to excrete uric acid in gout does not seem to be so much lowered as the high blood level would suggest, for injected uric acid

[64] Bürger and Schweriner, Arch. exp. Path., 1913 (74), 353.
[65] Wentworth and McClure, Arch. Int. Med., 1918 (21), 84.
[66] Chauffard and Troisier, Ann de Méd., 1921 (9), 149.
[67] Coates and Raimont, Biochem. Jour., 1924 (18), 921.
[68] Laporte and Rouzaud, Bull. Soc. Med. Hôp., 1924 (48), 70.
[69] Arch. Int. Med., 1915 (15), 1046.
[70] Deut. Arch. klin. Med., 1916 (119), 482.
[71] See Gudzent *et al.*, Zeit. klin. Med., 1920 (90), 147.

is readily excreted, and the concentration of uric acid in the urine may be quite as high as in normal persons.[72]

It is very possible that some entirely different product of metabolism than uric acid is responsible for most of the changes and symptoms of gout[73]—indeed, this would seem to be the case were it not for the great frequency of the deposition of monosodium urate in the joints and cartilages, both during the acute attacks and in chronic gout. This indicates that there is surely something abnormal in the conditions of uric-acid solution and circulation. Why the urate is precipitated in these definite places is another of the many unsolved problems of gout. The local nature of the deposition indicates that it must depend upon local changes; but the hypothesis that there occur first degenerative changes in the tissues which determine the precipitation of the urate, seems to have been disproved by the demonstration that the deposition of the urates precedes the necrosis. The fact that the presence of other sodium salts in a solution decreases the solubility of urates in that solution, and the fact that cartilage and tendons are richer in sodium salts than the blood, may possibly have something to do with the fact that the urates are precipitated in these particular tissues. Possibly the deposition of urates in cartilages and tendons depends entirely on physical conditions, since other substances present in the blood at a point near saturation, such as calcium and pigments, also tend to be thrown out in these avascular tissues (see Calcification, Ochronosis). On the other hand is the fact that in leukemia and nephritis we may find a higher concentration of uric acid in the blood than in gout, and this uricæmia may be protracted, without gouty deposits or joint symptoms.

The histology of urate deposits, both experimental and gouty, has been carefully studied by Freudweiler,[74] His,[75] Krause,[76] and Rosenbach.[77] Their results all indicate that uric acid and urates excite some slight inflammatory reaction, cause a slight local necrosis, and seem to act as weak tissue poisons (His). However, they may be deposited without causing necrosis (Rosenbach). Possibly part of the material observed in areas of urate deposition, and generally considered as necrotic tissue, merely represents the framework of the crystalline deposit (Krause). When experimentally injected, the urates are absorbed slowly by phagocytic leucocytes and giant cells. Why the gouty tophi can be deposited in the chronic process and cause no pain or inflammation, while in acute gout deposition of urates seems to cause such marked symptoms, is also an unanswered question, unless we accept the explanation that the slower rate of deposition and the lack of dissolved urates account for the absence of symptoms with the tophi.[78] Magnus-Levy holds with Pfeiffer, that the local inflammatory processes must be ascribed to dissolved urates, since they often extend for some distance about the joints, and hence the attack is ascribable to the solution rather than the formation of the deposits, a fact in harmony with the known increased elimination of uric acid during the attack.

[72] See Loewenhardt, Klin. Woch., 1922 (1), 2319.

[73] In swine a "guanine gout" occurs; see Schittenhelm and Bendix, Zeit. physiol. Chem., 1906 (48), 140.

[74] Deut. Arch. klin. Med., 1899 (63), 266.

[75] *Ibid.*, 1900 (67), 81.

[76] Zeit. klin. Med., 1903 (50), 136.

[77] Virchow's Arch., 1905 (179), 359.

[78] Almagia (Hofmeister's Beitr., 1905 (7), 466) has found that joint cartilage placed in urate solutions becomes filled with crystals, which infiltration does not occur with cartilage of any other origin, or with tendons.

That urates may cause necrosis of the tissues has been definitely established, and this may lead to connective-tissue formation and contraction.[79] But the actual increase of uric acid in the blood and tissues in gout is so slight that we are not warranted in saying that the usual tendency to sclerosis in all the organs in gout is due to the action of uric acid, rather than to some other unknown agent or agents. Excess of uric acid in the blood is by no means pathognomonic of gout, for we may have relatively great excesses of uric acid in the blood in leukemia, in some cases of nephritis, and after eating large amounts of nucleoproteins, without a symptom of gout. Furthermore, it is quite possible that the precursors of uric acid, the purine bases, are responsible for more harm than the uric acid itself. Thus, administration of adenine to dogs and rabbits will produce degenerative changes in the kidneys, associated with the deposition of substances resembling uric acid and urates in the renal tissue; and Mandel[80] states that purine bases may cause fever, independent of infection.

Many have looked upon renal alterations, leading to failure of excretion of uric acid, as the primary cause of gout; but the evidence in favor of this is faulty, because frequently renal changes are slight or entirely absent in gout, whereas marked nephritis of all forms may exist without the coexistence of gout, and, as mentioned above, the kidney in gout may show no lack of ability to excrete uric acid injected into the tissues. Magnus-Levy, however, seems to believe that a renal retention of uric acid is of importance, and that it may occur without morphological changes in the kidneys; but as a matter of fact, in gout a chronic glomerulo-nephritis is generally present,[81] and Folin found that intravenous injections of uric acid into gouty subjects caused enough kidney injury to lead to an increase in non-protein N, which did not happen with normal subjects.

The newer methods of blood analysis have given support to this view, and, as pointed out in preceding pages, Fine[82] and numerous others have called attention to the fact that in early interstitial nephritis the blood shows a greater increase in uric acid than in urea or creatinine, as if the diseased kidney found more difficulty in excreting uric acid than the other substances.[83] As a result the blood in early nephritis may show quite the same figures for uric acid, urea and creatinine as are found characteristically in gout. Myers and Fine[84] state that even normal kidneys can concentrate uric acid only 20 times, whereas urea is concentrated 80 and creatinine 100 times. However, experimental injection of uric acid into gouty subjects usually shows that the kidneys have a normal ability to excrete this excess. Furthermore, very few of the many persons with mild chronic nephritis and uricemia ever develop gout. Lennox[85] has observed a marked increase of blood uric acid to

[79] Because the gouty tophi do not suppurate, even when ulcerated through the skin, it has been suggested that the urates have antiseptic properties. Bendix (Zeit. klin. Med., 1902 (44), 165), however, could not demonstrate such antiseptic properties experimentally. The tophi do not always consist solely or even largely of urates, for these may be replaced by calcium salts (Kahn, Arch. Int. Med., 1913 (11), 92). They may also contain cholesterol (Chauffard *et al.*, Presse Méd., 1920 (28), 905; 1922 (30), 253).

[80] Amer. Jour. Physiol., 1904 (10), 452; 1907 (20), 439.

[81] See Brogsitter and Wodarz, Deut. Arch. klin. Med., 1922 (139), 129.

[82] Jour. Amer. Med. Assoc., 1916 (66), 2051.

[83] See also Denis, Jour. Biol. Chem., 1915 (23), 147.

[84] Jour. Biol. Chem., 1919 (37), 239.

[85] Jour. Amer. Med. Assoc., 1924 (82), 602.

be a constant finding in starvation, in persons whose kidneys were normal according to the usual tests, and therefore doubts the significance of the uric acid level as an indication of renal impairment. He found increases in fasting to be ordinarily from 100 to 240 per cent, the figures (ordinarily 10 to 12 mg. per 100 cc.) being quite as high as those usually obtained in gout.

Hence we are still confronted with the question whether gout is anything more than a form of nephritis in which chiefly uric acid excretion is impaired, or whether there does exist a special disease, gout, which causes uric-acidemia more or less independently of renal abnormalities.[86] Recent observations on the occurrence of more than one form of uric acid in the blood suggest the possibility that some abnormality in this distribution may play a role, as yet undetected, in gout. It is also possible that different conditions are grouped under the term gout, there being reason to suspect that chronic gouty arthritis is a primary joint disease with deposition of urates in the diseased tissues, despite normal excretion, whereas constitutional gout may depend on defective urate elimination.[87]

URIC-ACID INFARCTS[88]

Uric-acid infarcts, as the deposits of urates and uric acid observed in the kidneys of at least half of all children dying within the first two weeks of life are called, give evidence of the slightness of the toxic effects of these substances upon the tissues. Usually little or no change occurs in the renal tubules as a result of these depositions, except such as can be attributed to their mechanical effect,[89] but they may serve as the starting point of calculi. The reason for the formation of these infarcts is not at all understood. Spiegelberg[90] found it possible to cause them experimentally in young dogs, in which they do not occur naturally, by injection of 0.25 gram of uric acid per kilo. He was unable to explain why this deposition should occur in young animals but not in old, for he could not find evidence of lessened oxidative power on the part of young animals, and the solvent power of infants' urine was found equal to or greater than that of adults. Other authors, however, have found a lower oxidative power in young animals, and Mendel and Mitchell[91] have found that in the embryo pig, uricolytic enzymes do not appear until just at or just after the time of birth. As human tissues have no demonstrable power to oxidize uric acid, however, these animal experiments cannot be applied to the uric acid infarcts in human infants. Possibly the uric-acid infarcts of infants are the result of the great destruction of nucleoproteins that results from the change of the nucleated fetal red corpuscles to the non-nucleated adult form, or from a destruction of leucocytes which is said to take place at the time of birth. Flensberg believes that a hyaline substance is secreted in the urine of new-born infants which acts as a matrix for urate deposition. McCrudden considers the high concentration of infants' urine an important factor. Minkowski[92] observed that administration of adenine to dogs led to a deposition of uric acid or some

[86] See McClure, Arch. Int. Med., 1917 (20), 641.

[87] See Thannhauser, Deut. Arch. klin. Med., 1921 (135), 224; 1922 (139), 100.

[88] See discussion by Wells and Corper, Jour. Biol. Chem., 1909 (6), 321.

[89] I have observed a case of fatal *hematuria neonatorum*, associated with most extensive hemorrhagic infarction of both kidneys. In the bloody urine, *B. coli* was found in large numbers. From the anatomical findings and history it seemed quite possible that the injury of the kidneys by uric-acid infarcts might have determined the localization of the bacteria in these organs, with resulting hemorrhages. (Trans. Chicago Path. Soc., 1909 (7), 242.)

[90] Arch. exp. Path. u. Pharm., 1898 (41), 428.

[91] Amer. Jour. Physiol., 1907 (20), 97.

[92] Arch. exp. Path. u. Pharm., 1898 (41), 375.

similar substance in the kidneys. Schittenhelm[93] found the same deposits in the kidneys of rabbits fed adenine, but not when they were fed guanine. According to Nicolaier,[94] the crystals thus deposited are not uric acid or urates, but 6-amino-2-8-dioxypurine, derived from the adenine (6-amino-purine) by direct but incomplete oxidation. He could not find this substance in either human urine or in a uric acid calculus. Eckert[95] obtained urate deposits by intravenous injection into rabbits of at least 0.08 gm. per kilo, but subcutaneous injections were ineffective; injury to the renal epithelium by whatever cause interferes with this deposition of urates. These experimental infarctions are undoubtedly related to the human form, and indicate that the latter depend upon the presence of an excessive amount of uric acid in the infants' urine, in which a ratio of uric acid to urea of 7.9 to 74.9, as against the adult ratio of about 2 to 85, was found by Sjoquist. According to Niemann,[96] in the first few days of life the infant excretes from 80 to 100 mg. of uric acid daily, while after the fifth day the amount falls to 30 to 40 mg. daily. Similar figures were obtained by Schloss and Crawford,[97] who also found a corresponding increase in the phosphoric acid, showing that the uric acid must originate from nucleoproteins. The blood of fetus and mother have the same uric acid content,[98] but after birth the infant's blood has more during the first three or four days, paralleling the high excretion.[99]

Adult kidneys may also show uric acid deposits in the tubules of the papillæ, independent of gout. They occur as a result of cell decomposition, according to M. B. Schmidt,[1] who found them especially in pneumonia, leukemia, and sarcoma, but not in carcinoma.[2]

[93] *Ibid.*, 1902 (47), 432.
[94] Zeit. klin. Med., 1902 (45), 359.
[95] Arch. exp. Path., u. Pharm., 1913 (74), 244.
[96] Jahrb. f. Kinderheilk., 1910 (71), 286.
[97] Amer. Jour. Dis. Children, 1911 (1), 203.
[98] Slemons and Bogert, Jour. Biol. Chem., 1917 (32), 63.
[99] Kingsbury and Sedgewick, *ibid.*, 1917 (31), 261.
[1] Verh. deut. Path. Ges., 1913 (16), 329.
[2] Abderhalden and Kankeleit (Zeit. exp. Med., 1916 (5), 172), have produced renal deposits by feeding large amounts of tyrosine, glycine, and leucinimid to rabbits. These deposits consisted of the amino acid as fed, and caused suppression of urine by blocking up the tubules. They also caused necrosis and inflammatory reactions.

CHAPTER XXVI

DIABETES

Introduction.—Although the preparation of an active agent from the pancreas, insulin, has done much for the treatment of diabetics, and has answered some of the questions concerning the relation of the pancreas to diabetes and carbohydrate metabolism, nevertheless most of the fundamental problems in this field remain unsolved, and we find that the discussion of the chemistry of diabetes and carbohydrate metabolism as formerly prepared for this book by R. T. Woodyatt can be used with little change as representing our present knowledge. Therefore we are using this material as the basis of our discussion, with such modification as is required to bring the subject to date. It is impossible in this place to review the entire literature and history of the subject, which will be found in the works of the writers cited below.[1] This chapter will be devoted

[1] *Older Literature:*
Bouchardat—De la glycosurie ou diabète sucré, Paris, 1875.
Külz—Beiträge zur Path. und Ther. der Diabetes Melitus, Marburg, 1874–5.
Bernard—Leçons sur le Diabète et la Glycogenèse Animale, Paris, 1877; Vorlesungen über Diabetes, Berlin, 1878.
Cantani—Diabetes Melitus (German translation by Kahn), Berlin, 1880.
Frerichs—Ueber den Diabetes, Berlin, 1884.
Larger Works:
Naunyn—Der Diabetes Melitus, Berlin, 1906. Diabetes Melitus; in Nothnagel's Handbuch (2nd), Vienna, 1906.
Lépine—Le diabète sucré, Paris, 1909.
von Noorden—(a) Die Zuckerkrankheit (6th), Berlin, 1912. (b) New Aspects of Diabetes, New York, 1913.
Pavy—Carbohydrate Metabolism and Diabetes, London, 1906.
Macleod—Diabetes (Longmans, Green), 1913.
Cammidge and Howard—New Views on Diabetes Melitus, Oxford Univ. Press, 1924.
Allen—Glycosuria and Diabetes, Boston, 1913.
Foster—Diabetes Mellitus, Philadelphia, 1915.
Joslin—Treatment of Diabetes Melitus, New York, 1923.
Monographs, etc.:
Magnus-Levy—Diabetes Melitus; in Kraus and Brugsch, Spezielle Path. u. Ther. innerer Krankheiten, Berlin, 1913.
Gigon—Neuere Diabetes Forschungen, Ergebnisse der inneren Medizin, 1912, IX, p. 206.
von Mering—Behandlung der Diabetes melitus; in Penzoldt and Stinzing's Handbuch der Spezielle Therapie (2nd), 1912.
Lusk—Elements of the Science of Nutrition (3rd), New York, 1917.
Benedict and Joslin—Metabolism in Diabetes Melitus, Carnegie Institution, Washington, 1910.
Benedict and Joslin—A Study of Metabolism in Severe Diabetes, *ibid.*, 1912.
Allen, Stillman and Fitz—Total Dietary Regulation in the Treatment of Diabetes. Monograph of the Rockefeller Institute for Medical Research, New York, 1919.
Woodyatt—Pathological Metabolism of Diabetes Melitus; Barker's "Endocrinology and Metabolism," 1924 (4), 263.

only to an outline of the chief established facts, with an indication of the main lines along which the thought of leading students has been directed. It will involve a brief discussion of the problems of carbohydrate physiology, but only in so far as they are contingent upon the main topic—while for a discussion of that anomaly of the metabolism which leads to the excretion of acetone, aceto-acetic acid and β-hydroxybuytric acid in the urine, the reader is referred to the section on acidosis. (Chapter XXII.)

Whereas the normal urine at all times contains reducing substances and substances which are optically active, which yield crystalline compounds with the hydrazines and respond to other so-called sugar tests, these substances are not all sugars, nor are all the sugars glucose. The quantity of fermentable reducing substance in normal urine averages about 4 parts in 10,000 (0.04 per cent.) according to Lavesson.[2] If the total quantity of urine were 1500 c.c. this would imply a daily excretion of about 0.6 gm. Bang and Bohmannson[3] estimated the total reducing substance in the urine of normal adults as between 0.21 and 0.24 per cent., of which about 18 per cent. was fermentable (0.038 to 0.043 per cent. of fermentable reducing substances in the urine). This is doubtless subject to change depending on the diet and other factors. Benedict, Osterberg and Neuwirth[4] found an excretion of fermentable reducing substance ranging between 0.903 and 1.161 gm. in twenty-four hours in the case of a normal adult on an ordinary mixed diet. During a fast it fell to zero, according to these writers, and on a diet low in carbohydrate it averaged 0.75 gm. while with a high carbohydrate diet it rose to 1.5 gm. Felsher and Woodyatt[5] have shown that both in the normal animal and in the human diabetic there is a definite point of saturation with glucose, which, when overstepped, is followed by a sharp increase in the urinary excretion of sugar.

When an abnormal amount of sugar occurs in the urine, regardless of the kind, the condition may be called *melituria*. When the sugar is glucose (dextrose), the term *glucosuria* is applied; when levulose, *levulosuria*, and so on. Other known forms of melituria are lactosuria, galactosuria, fructosuria, pentosuria, etc. All these are but symptoms, many of them being caused by a variety of mechanisms, which will be discussed presently.

The term diabetes is often loosely used to cover any variety of melituria, but it is preferably limited to certain forms; namely, to the *glucosurias* (or the mixed meliturias in which d-glucose is the predominating sugar), and further than this, to those particular *glucosurias which continue* even after the glycogen reserves of the body have become depleted and when the diet is free of carbohydrate; or, to those transient glucosurias whose nature by one means or another can be proved to be identical with the continuous forms (latent or mild diabetes). Over against these

[2] Biochem. Zeit., 1907 (4), 40.
[3] Zeit. physiol. Chem., 1909 (63), 443.
[4] Jour. Biol. Chem., 1918 (34), 217.
[5] Jour. Biol. Chem., 1922 (50), 121; 1924 (60), 737.

are the *meliturias in which other sugars than glucose play the chief rôle, and glucosurias which are essentially transient* because they depend solely on the ingestion or administration of excessive quantities of glucose or the sudden liberation into the blood of glucose derived from preformed glycogen or other fixed compound of sugar.

Thus, the glucosuria which follows puncture of the floor of the fourth ventricle (Claude Bernard's piqûre) does not occur in animals which contain little glycogen. The same applies to the adrenal, thyroid and hypophysis glucosurias. But after complete pancreas extirpation (pancreas diabetes) and in the spontaneous human disease (diabetes melitus) or its counterpart in animals, and during the continuous administration of phlorhizin, the glycogen may be nearly or quite exhausted and the diet consist solely of meat and fat and still the glucosuria will continue. On the other hand a partial pancreas extirpation, a mild diabetes melitus, or an interrupted phlorhizinization may give rise to transient glucosuria, the diagnosis of which may be difficult. In general, experience teaches that all persistent glucosurias prove to be diabetic and that, except in phlorhizin poisoning, every genuine diabetes implies a disturbed function of the pancreas. In forming a judgment of the value of any experimental work on diabetes (histological, chemical or clinical), the student will do well to examine critically the records of quantitative food and urinary analyses offered by the investigator, to show what type and what grade of diabetes is under consideration.

CARBOHYDRATE PHYSIOLOGY [6]

Certain facts concerning the physiology of the carbohydrates may be briefly recalled before entering into the discussion of the individual meliturias.

The appearance of sugar in the urine implies a source or sources of sugar and the existence of a kidney membrane of such a physical character that molecules of sugar may migrate through it with a certain degree of facility. The factors which may influence the purely physical penetrability of the kidney membrane to sugar molecules are those involved in a discussion of kidney function and secretion in general and need not be elaborated here.

Assuming that the physical penetrability of the kidney membrane to sugar molecules is normal and that it varies only within constant limits, there are then two basic moments which determine how much sugar will pass into the urine. These are: 1. The rate at which sugar molecules enter the cells constituting the kidney membrane. 2. The rate at which these molecules of sugar undergo chemical change into something else within the membrane.

These same factors of supply and utilization determine the elimination of sugar from any cell or tissue or the organism as a whole, but

[6] For review on carbohydrate metabolism see Magnus-Levy, Handbuch d. Biochemie, 1925 (8), 338; Shaffer, Physiol. Reviews, 1923 (3), 394.

in the case of internally situated cells the elimination is directly or indirectly into the blood, whereas, in the case of the kidney cells sugar may pass into the urine as well as into the blood and thus leave the body permanently.

Sugar may pass out of a cell unchanged when the rate at which it enters the cell (from internal and external sources) exceeds the rate at which it undergoes change into something else within the cell, the same holding in the case of the cells forming the kidney membrane. *Thus sugar passes from the kidney membrane into the urine when the rate at which sugar molecules enter the kidney membrane exceeds the rate at which they are denatured or utilized within it.* In order to understand the various ways in which melituria may be produced it is necessary to analyze further these factors of *supply* and *utilization.*

The Sugar Supply to the Kidneys is chiefly via the blood, although some may be liberated within the kidney cells themselves from glycogen deposits or from the transformation of protein during metabolism. The factors which influence the rate at which sugar molecules enter the kidney membrane from the blood, are *immediate* and *remote.*

The more *remote factors* are those which determine the quantity of sugar in general circulation, and the distribution of blood to the kidneys. The relative quantity of sugar circulating in the blood will depend on the combined rates at which sugar enters the blood from the outside and from all the organs, and on the rate at which sugar passes out of the blood into the cells. The absolute value of these factors, supply and depletion, and their ratio, will determine fluctuations of the total blood sugar. The supply to the kidneys is accordingly influenced by the balance of supply and depletion elsewhere.

The *immediate factors* which determine the rate at which sugar is brought to the kidney membrane will include all of those which may influence the state of the blood as to viscosity etc., and the rate at which sugar actually enters the kidney membrane will be influenced by changes in the state of that membrane, but apart from those things there are two factors of major importance in determining the supply of sugar to the kidney membrane at any instant, namely, the *concentration of sugar in the blood plasma of the kidney capillaries* and the *extent of the surface of contact between the blood plasma and the kidney membrane.* In accordance with a method followed by M. H. Fischer it is convenient to think of the kidney membrane simply as including all that lies between the blood plasma on the one hand and the urine on the other. So considered, the surface of contact between the blood plasma and the kidney membrane is the internal surface of all the capillaries of the cortex, in so far as these are filled with circulating blood. The kidney membrane might be conceived otherwise and be made to include only the layer of epithelial cells, in which case the surface of contact might be considered as the surface of those cells in so far as they are bathed in plasma; or the matter might be carried within the cells, in which case the problem of surface would become one of surfaces between phases of an heterogeneous system in the chemical sense, and so on.

For present purposes it is convenient to mass all such intermediate factors and deal with the sum of their effects, *i. e.*, to think of this surface as the internal surface of the active capillaries. The surface of contact between the blood plasma and kidney membrane, so considered, is clearly subject to variations, for as more or less blood is forced into the capillaries of the cortex there must be changes in the diameter, length or number of capillaries containing circulatory blood, or in all three. Any or all of these changes imply changes of capillary surface. It therefore becomes apparent that other factors remaining the same, changing volumes of blood in the cortical capillaries would imply changing rates of sugar diffusion from the blood into the kidney cells, even though the concentration of sugar in the blood should remain unchanged. And it is also apparent that if the blood should be diluted with water in such a way as to double its volume and halve the blood sugar percentage this would not of necessity change the rate at which sugar was passing from the blood into the kidney membrane, provided the extra volume of blood developed for itself a pro-

portional amount of extra capillary surface.[7] Thus it has been shown by Epstein[8] that the rate at which sugar is excreted in diabetes is not always proportional to the concentration of sugar in the blood, but is more nearly proportional to the product obtained by multiplying the blood sugar percentage by a number representing the approximate blood volume, and other experiments to be described later point in the same direction.

The Utilization of Sugar may be considered for present purposes as the sum of the processes by which a sugar such as glucose is converted into something else within the cells. In the case of glucose it includes *oxidation* to yield finally CO_2 and water; *polymerization* to yield a series of substances, chief of which is glycogen; *reduction* to fat; *transformation*, as to lactic acid; *combination*, etc. The rate of utilization[9] by all of these methods taken collectively is influenced in the first place by the rate at which glucose molecules enter the cells and secondly by the reaction conditions encountered within it. The utilization rises with an increasing supply of glucose. As to the factors which enter into the reaction conditions found within the cell there is little definite knowledge. With a constant glucose supply the rate of utilization may fall as the result of a deficiency of that hypothetical substance derived from the pancreas. It is well known that acid may retard glycogen formation and hasten glycogen hydrolysis. It would appear from the work of Murlin and Kramer[10] that alkali may increase glucose utilization. The rate of actual oxidation is influenced by the supply of oxygen, etc. The following may serve to suggest other factors.

It might be conceived that the cell contained molecules of a glucolytic catalyst or enzyme similar in its effects to metallic hydroxides, that glucose molecules as fast as they entered the cells would come into collision with catalyst molecules, perhaps combining with them, and that as a result of the encounter the glucose molecules would be dissociated into unsaturated fragments or ions. From the moment of union or dissociation they would cease to behave as glucose molecules. The unsaturated fragments might subsequently suffer various fates, depending upon the character and quantities of various substances in the cell. Thus, some might combine with oxygen to yield, finally, carbon dioxide and water. Others might com-

[7] This raises the question of the geometrical form of capillaries and the mechanism by which an organ or a tissue accommodates varying volumes of blood. It is interesting to note that in capillary systems, notably that into which the efferent artery from the glomerulus breaks up, the capillary strands may vary in calibre, which would lead one to expect that a pressure just sufficient to force blood through the larger tubes would not suffice to inject the smaller, while a rising pressure should throw into action erstwhile empty collaterals and vice versa. A system of collateral spillways would limit the distension of already filled capillaries and make the accommodations of varying blood volume largely a matter of throwing in and cutting out capillary cylinders. Such a method if followed exclusively would make the surface rise faster than the volume, since the new channels would be of somewhat smaller diameter. On the other hand, if a capillary were cylindrical, increasing its volume by increasing its diameter would lessen the ratio of surface to volume. Increasing the volume of a cylinder by increasing its length would increase lateral surface in proportion to volume. Possibly in health, and within certain limits, variation of the blood volume may cause proportional changes of capillary surface.

[8] Jour. Biol. Chem., 1914 (18), 21; Proc. Soc. Exp. Biol., 1916 (13), 67; also "Studies in Hyperglycemia in Relation to Glycosuria," Albert A. Epstein, N. Y., 1916.

[9] It might not seem desirable to include such processes as temporary storage in the form of glycogen under the heading of utilization. The term is used for convenience.

[10] Jour. Biol. Chem., 1916 (27), 499.

bine with each other to form polymers like glycogen, others again undergo reduction to fat or molecular rearrangement to give lactic acid. The relative quantities undergoing those several changes, would depend upon the relative quantities of H and OH ions, of available oxygen, salts, etc., found in the various phases of the cell. This conception is based on that used by Nef to explain the behavior of sugars in alkaline solutions. For a concrete conception of the dynamics of a reaction between an organic substrate and catalyst the reader is referred to Van Slyke's study of the enzyme urease.[11]

The general principles outlined above may be illustrated by experiments with timed intravenous injections of glucose. It has long been known that if a comparatively large dose of glucose is injected rapidly into a peripheral vein a marked glucosuria usually results. Pavy, however, emphasized the fact that a material fraction of a dose so given fails to be excreted and appears to be utilized. Doyon and Du Fourt demonstrated that with a standard dose of glucose the percentages excreted and utilized respectively are influenced by the time consumed in injection, the slower rates of injection causing lower percentage excretions and vice versa. Blumenthal chose a standard injection time of about 10 seconds and varied the weight of sugar given in that time. He found that a certain dose of glucose might be injected into the ear vein of a rabbit without causing any glucosuria at all. However, the maximum dose which could be so injected once could not be repeated 15 minutes later without causing glucosuria. He assumed from this that the first dose "saturated" the tissues and that fifteen minutes later the utilization of sugar had only resulted in a partial desaturation. He, therefore, determined the dose of glucose which might be injected repeatedly at 15 minute intervals for as long as 3 hours without ever causing glucosuria. His figures varied between 0.6 and 1.3 gm. per kg. of body weight per hour. This he termed the "utilization limit," whereas the largest dose which could be given within 10 seconds *once* without causing glucosuria he called the "saturation limit." The latter he placed at 0.8 gm. per kg. but R. M. Wilder has been unable to confirm this latter observation. Woodyatt, Sansum and Wilder[12] made continued intravenous injections of glucose at uniform rates by means of a motor driven pump for 2 to 17 hours with the following findings:

If chemically pure glucose in aqueous solution is injected continuously into the peripheral venous blood of a normal resting man, dog or rabbit at the rate of 0.8 gm. per kg. of body weight per hour, or at any slower rate, the injection may be sustained in most cases, hour after hour for 7 hours and probably longer without producing any glucosuria in the usual sense of the word. If the rate is advanced to 0.9 gm. of glucose per kg. of body weight per hour, while all other conditions remain the same, the injection may be sustained for a short time without causing glucosuria, but in nearly all cases abnormal quantities of glucose begin to appear in the urine after 5 to 30 minutes of injection. Once established, the glucosuria then tends to proceed at a uniform rate as long as the rate of injection and other conditions remain fixed. However, if the injection rate is again reduced to 0.8 gm. per kg., glucosuria promptly ceases. Thus during the continuance of an injection at the latter (0.8 gm.) rate there can be no continued accumulation of unchanged glucose in the body, but the

[11] Jour. Biol. Chem., 1914 (19), 141.

[12] Jour. Amer. Med. Assoc., 1915 (65), 2067 (preliminary report); Woodyatt, Harvey Society Lectures, 1916; Wilder and Sansum, Arch. Int. Med., 1917 (19), 311; Woodyatt and Sansum, Jour. Biol. Chem., 1917 (30), 155.

rate of injection is equalled by the rate of utilization if we leave out of consideration the trace of sugar which can be detected in the urine by refined methods. It is important to note that it makes no appreciable difference whether one uses an 18 or a 72 per cent. glucose solution for injection. The tolerance limit for glucose may be demonstrated at the same point regardless of wide variation in the quantity of water administered with the glucose, even though the blood volume and the blood sugar percentages may be influenced by variation of the water supply. Also, if glucose is injected continuously and uniformly at a rate productive of some glucosuria, the glucose excretion may proceed at a constant rate in spite of marked variation in the water supply during successive hours. A certain dog receiving by vein 20 gm. of glucose per 10 kg. per hour for 8 hours, excreted every hour close to 0.42 gm. of sugar per 10 kg. of body weight. Yet during the experiment water was injected at varying rates into the same vein with the glucose, so that the hourly volume of urine varied between 6 c.c. and 128 c.c. and the percentages of sugar in the urine varied between 0.35 and 4.9. This emphasizes the fundamental importance of the *rate* at which sugar is supplied to the organism in determining the occurrence or non-occurrence of glucosuria and in fixing the rate of excretion when the latter occurs.

In view of the above generalities, several specific mechanisms suggest themselves by which glucosuria might be produced:

(1) An increased supply of preformed glucose to the whole organism from without (alimentary glucosuria).

(2) A decreased utilization in the organism as a whole (pancreatic diabetes).

(3) An increased supply to the kidneys resulting from the liberation into the blood of sugar previously stored or combined in other organs. Thus, the rapid hydrolysis of glycogen following puncture of the floor of the fourth ventricle and analogous nerve stimulations, and occurring in the acid, asphyxial, narcotic, thyroid, epinephrine, and hypophysis glucosurias. In an analogous manner lactose may enter the circulating blood from the mammary gland, and pentose from unknown sources.

(4) An increased supply to the kidneys due to decreased utilization in other organs. The breaking down of glycogen mentioned in (3) might be so interpreted and one might think of the possibility that in various diseases the ability of a part to utilize sugar may be altered.

(5) Decreased utilization in the kidney itself.

(6) Increased physical penetrability of the kidney membrane to glucose.

Both (5) and (6) are hypothetical conditions, the latter having been proposed as the basis of so called *kidney diabetes*, a state in which glucosuria occurs with a normal or subnormal *percentage* of sugar in the blood, in which the rate of sugar excretion is, in comparison with other forms of glucosuria, little influenced by the diet,[13] and which seems not inconsistent with good health.

THE BLOOD SUGAR[14]

The normal blood sugar concentration is found to average 0.10 per cent., but, as statistics show, it may vary at least between 0.06 and

[13] Cf. Epstein;[5] Strouse, Jour. Amer. Med. Assoc., 1914 (62), 1301; Arch. Int. Med., 1920 (26), 768; Lewis and Mosenthal, Johns Hopkins Hosp. Bull., 1916 (27), 133; Lewis, Arch. Int. Med., 1922 (29), 418; March, Arch. Int. Med., 1921 (28), 54.

[14] See review by Macleod, Physiol. Reviews, 1921 (1), 208.

0.11 per cent. The literature contains numerous references to that blood sugar concentration which if exceeded leads to glucosuria ("threshold" value). In accordance with the general principles above discussed we should expect this value to vary. It has been placed at 0.147 to 0.164 per cent. by Foster, between 0.17 and 0.18 per cent. by Haman and Hirschman, at about 0.20 per cent. by Pavy, and other writers have reported greater variations, due in part doubtless to differences in the analytical methods used. How widely the threshold blood sugar percentage may be varied by extreme variations of the blood volume and other factors has not been settled. Following the ingestion of free glucose the blood sugar percentage ordinarily rises, and in a similar way, but more slowly, after feeding of starch. Fisher and Wishart gave 50 gm. of glucose in 150 c.c. of water by stomach to dogs weighing 8 to 9 kg. and found in the first hour blood sugar percentages of 0.16 and 0.13. In succeeding hours there was little variation from 0.11 per cent. In harmony with the previous work of Gilbert and Baudoin and the more recent studies of others on man, these experiments showed that the blood sugar percentage rises during the first hour, then falls and thereafter remains normal. There was no increase of the blood volume during the first hour, the hemoglobin percentage remaining unchanged, probably because the large quantity of glucose in the bowel held water there. But in the second hour the blood volume became large and the hemoglobin showed the effects of dilution. In this same hour the sugar percentage returned to normal. But the absorption of glucose was only completed in the fourth hour and calorimetric observations by Lusk showed that the metabolism also ran at a uniform rate 20 per cent. above the basal level into the fourth hour. Accordingly the observed blood sugar percentages first rose as the rate of sugar supply was increased, but fell again *during the maintenance of the increased supply and while the metabolism was constant*, owing to the shifting of water.

When concentrated (54 to 72 per cent.) glucose solutions are injected continuously into the blood at rates of 0.4 to 0.8 gm. per kg. per hour, there is at first a steep rise of the blood sugar percentage, followed by a fall coincident with an increased hydremia, after which a new equilibrium is established and the blood sugar percentage may become constant at a "normal" level exactly as in the above. By injecting glucose at the same rates in sufficiently dilute solutions this initial rise may be very much reduced and the blood sugar percentage established in later hours may even be lower than that observed before injection began. On the other hand, if glucose is injected at rates above 0.9 gm. per kg. per hour, glucosuria begins, and if the rate of injection is rapid enough may be made intense. As glucose passes through the kidney membrane, water tends to accumulate with the glucose on the urinary side of the membrane (increased diuresis, polyuria). In the same way that glucose in the bowel lumen may tend to withhold water from the blood, so a sufficient quantity of glucose in the urinary tubules may manifest the same tendency in this locality. Whether the glucose in the urinary tubules will have the effect

of concentrating the blood or vice versa will depend on the quantitative distribution of free sugar between these two fluids, and the quantity of water available for distribution between the blood sugar and the urinary sugar. During continuous intravenous injections of glucose at rates from 2.7 gm. per kg. per hour upward, 30 to 40 per cent. of the glucose injected may be excreted and there is a strong tendency toward dehydration of the whole body. This may be neutralized by supplying water with the sugar as fast as it flows away in the urine, provided the rate of injection is not so great that the necessary traffic in water overtaxes the cardio-renal mechanism. By employing these high rates of injections and maintaining the water balance at as low levels as compatible with life and recovery, it is possible to produce and maintain for hours blood sugar concentrations as high as 2.38 per cent. Joslin observed 1.49 per cent. of sugar in the blood of a fatal case of diabetes with nephritis. The blood sugar of diabetics passing sugar in the urine is as a rule higher than normal, but not necessarily so, much depending on the water balance. Joslin's statistics show a range of 0.07 to 0.43 per cent. Several investigators have maintained that the blood has a considerable glucolytic power, which is defective in diabetics, but this has failed of confirmation by others.[15]

THE STATE OF THE SUGAR IN THE BLOOD

It has long been believed that the sugar circulating in the blood exists in two physical states, a diffusible and a non-diffusible, *i. e.*, as (*a*) Free glucose in a state comparable to that of glucose dissolved in water, *sucré actuelle* of Lépine. (*b*) Sugar in a colloid state, *sucré virtuelle*, Lépine. By the former term a very specific idea is conveyed. One might think for instance of single molecules or clusters of two or three, each holding in its sphere of influence a certain number of water molecules like suns in solar systems. Such small masses move at high velocities, "diffuse" readily and create high "osmotic pressures." By the latter term is meant sugar in the blood which does not behave physically like glucose in aqueous solution nor respond to the ordinary chemical tests for sugar, but from which free glucose may be reliberated by such simple procedures as boiling with dilute acids. Such sugar is supposed to exist as a component of particles having the larger dimensions which characterize colloids (non-diffusoids). But as to the actual chemical nature of these a great variety of proposals have been made. Thus Pavy proposed glucose molecules held entire to the colloids of the blood in a state of simple adsorption (comparable to the state of molecules of a dye electrically bound to particles of a colloid clearing agent). He also proposed glucose chemically incorporated in the structure of the protein molecule, and between these extremes by the same author a score of suppositions have been made by others, among which Drechsel's jecorin, a lecithin-sugar compound, is a notable example. Another worthy of serious considera-

[15] Tolstoi, Jour. Biol. Chem., 1924 (60), 69; Bürger, Zeit. ges. exp. Med., 1923 (31), 98.

tion is that of glucose built up into polymers intermediate between disaccharides and glycogen. The basis for assuming the existence of combined sugar in the blood lies chiefly in the observation that following glucose administrations the increase in the reducing power of the blood which results from heating the blood with dilute acid is greater than the increase resulting from the same process before sugar administration (see Pavy, Lépine, Loewi). Also, if glucose is added to fresh blood and the mixture placed in the incubator, the reducing power falls but may be in part rehabilitated by boiling with dilute acid.[16] The blood corpuscles are impermeable to glucose, but there is more sugar in the blood than is found free in the serum (van Crefeld).[17]

As to the sugar which is determined by the ordinary methods of blood analysis, it would appear that we are dealing almost exclusively with free glucose. As yet no one has succeeded in proving the existence in blood of a combined sugar capable of spontaneous conversion into free sugar. Michaelis and Rona dialyzed separate portions of the same blood against isotonic salt solutions containing graduated quantities of sugar. A sugar solution which neither lost nor gained sugar during the dialysis they regarded as having an amount of free sugar equal to that in the blood. Titration of the blood sugar and of the sugar in such a solution gave almost identical figures. They accordingly concluded that all of the reducing sugar in this blood must have been as free to diffuse as was that in the simple salt solution. But this ingenious experiment of Michaelis and Rona does not show conclusively that in circulating blood there is no sugar in a state of colloidal adsorption, because drawn blood rapidly undergoes survival changes (*e. g.*, lactic acid formation) which might influence the affinity of its colloids for sugar. However, McGuigan and Hess[18] led the blood of living animals through collodion tubes enclosed in jackets filled with isotonic salt solutions and found that when equilibrium was established the concentration of reducing sugar in the salt solution and in the plasma was the same, proving that even in life all of the titrable plasma sugar is in a state of subdivision which lets it migrate through the interstices of a collodion membrane. This would make the adsorption idea seem untenable.

Closely related to the question of the state of the sugar in the blood is that of its state in the cells. Palmer[19] has studied the percentages of sugar found in the various tissues in relationship to the plasma sugar concentration. The titrable sugar of the tissues was found below that of the blood in all organs except the liver. Of course, owing to the large quantities of glycogen which occur in that organ and the rapidity with which it breaks down into glucose, liver tissue would naturally analyze high for sugar. In the muscles the titrable sugar was found at 0.04 and

[16] A critical review of the literature to 1912 will be found in the books by Macleod and Allen. Compare also the article by Levene and the recent studies of Lombroso favoring the polymerization idea.

[17] Nederl. Tijd. Geneesk., 1921 (2), 779.

[18] Jour. Pharm. and Exp. Ther., 1914 (6), 45.

[19] Jour. Biol. Chem., 1917 (30), 79.

0.041 per cent. with blood sugar at 0.10 and 0.105 per cent. On the other hand the tissues generally when boiled with dilute acid show a higher content of "combined" sugar than the blood. This is most striking in the case of the liver and due by common consent to the polymers of glucose in that organ.

It serves a useful purpose to consider the body as a whole as an heterogeneous system made up of phases, and to assume that glucose on entering the body distributes itself between these phases as acetic acid may distribute itself between the fat droplets and the aqueous part of milk; that glucose in a certain type of phase behaves as though in water and in another type of phase rapidly undergoes chemical changes. The blood is a tissue in which the dominant phase is in the nature of a physical solvent for glucose, like water. In the cells the dominant phases are of such a character that glucose on entering them rapidly undergoes chemical change. But both types of phase are present in both blood and cells although in different proportions. The blood is therefore the phase par excellence in which to study the "sucré actuelle" and the tissues the place to study the "sucré virtuelle." According to this conception "sucré virtuelle" would be glucose in process of utilization or storage and not beyond recall, hence chiefly glucose polymers.

DIOSE[20]

Diose, glycollic aldehyde, $CH_2OH—COH$, the simplest sugar, of which there is but one possible form, is highly sensitive to oxidative influences and, *in vitro*, readily condenses with alkali to yield a complex mixture of higher sugars and saccharinic acids in a manner analogous to that manifested by the trioses. Notwithstanding its instability and sensitiveness to oxidative changes in the test tube, it would appear that glycollic aldehyde is insusceptible of direct oxidation in the body but that it may be converted into glucose, like other sugars, and then utilized. When given intravenously at the rate of only 0.1 gm. per kg. per hour, unchanged diose appears in the urine after the first few minutes of injection (author). P. Mayer reported glucosuria and death following administration of impure glycollic aldehyde to rabbits. Parnas and Baer saw an increase of glycogen in tortoise livers perfused with glycollic aldehyde. This is confirmed by Barrenscheen. Smedley noted the rapid disappearance of diose added to liver emulsions. Sansum and Woodyatt, and also Greenwald observed slight increases of the glucosuria following parenteral administrations of diose in phlorhizinized but not completely deglycogenized dogs. The extra sugar could have come from glycogen in these experiments. A final proof of the conversion of diose into glucose in the living body has not been brought. The relationship of this substance to glycine, $CH_2NH_2—COOH$; glycollic acid, $CH_2OH—COOH$; and ethyl alcohol, $CH_3—CH_2OH$; is close. Lusk states that glycine is capable of conversion into glucose in the body. However, glycollic acid and alcohol are apparently not sugar formers.

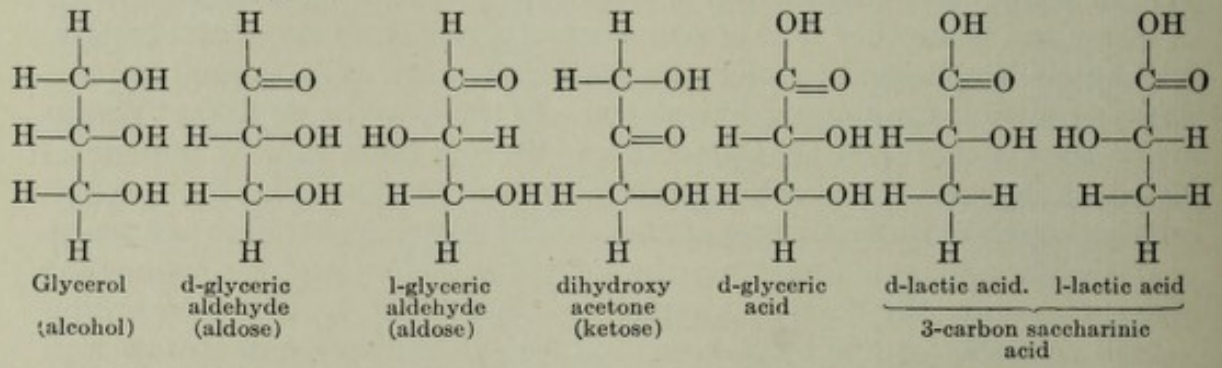
TRIOSES[21]

There are three possible trioses, d- and l-glyceric aldehyde and the ketotriose dihydroxyacetone. The optically inactive d, l-glyceric aldehyde has been prepared

[20] Literature on diose: Mayer, P., Zeit. f. physiol. Chem., 1903 (38), 135; Woodyatt, R. T., Jour. Amer. Med. Assoc., 1910 (55), 2109; Parnas and Baer, Biochem. Zeit., 1912 (41), 386; Smedley, Ida, Jour. Physiol., 1912 (44), 203; Sansum, W. D. and Woodyatt, R. T., Jour. Biol. Chem., 1914 (17), 521.

[21] Literature on Trioses: The chemical literature is reviewed and an improved method of preparing glyceric aldehyde described by Witzemann, E. J., Jour. Am. Chem. Soc., 1914 (36), 1908, and *ibid.*, p. 2223. The biological literature is reviewed by Sansum, W. D. and Woodyatt, R. T., Jour. Biol. Chem., 1916 (24), 327.

and recently the d- and l-forms. The preparation is still tedious and expensive. Dihydroxy-acetone is somewhat easier to prepare. Both trioses are unstable, easily oxidized and very prone to undergo rearrangements and condensation with even traces of alkali. Under the influence of alkali they yield complex mixtures of hexoses, chiefly 3-ketohexoses, formerly known as α and β-acrose, from which Schmitz[22] has isolated d,l-fructose and d,l-sorbose. If oxygen is available as well as alkali, they burn. If the alkali is strong and oxygen lacking, much lactic acid is formed together with certain rearranged tetrose, pentose and hexose molecules, known as saccharinic acids (or "saccharines," of Kiliani). The same phenomena occur when the alkali is dilute, but more slowly. The structural formulae of the trioses and their relationship to glycerol, glyceric acid and lactic acids (the latter of which might be regarded as a 3-carbon saccharinic acid) may be seen from the following chart:



Neuberg fed animals and men considerable doses of impure d,l-glyceric aldehyde (glycerose) and saw its apparently complete utilization. Parnas demonstrated increased glycogen in tortoise livers perfused with d,l-glyceric aldehyde. Smedley noted the rapid disappearance of glyceric aldehyde added to liver emulsions. Sansum and Woodyatt fed pure crystalline d,l-glyceric aldehyde to rabbits and guinea pigs in doses as high as 2.8 gm. per kg. with no apparent ill effects. A dose of 5 gm. per kg. in a rabbit caused diarrhea with unchanged triose in the passages. There was marked diminution of urine with albuminuria, which then persisted for 10 days. A dose of 6.8 gm. per kg. killed in 4 hours. In no case was there an alimentary triosuria. The average lethal dose by the subcutaneous route was 2.2 gm. per kg. as compared with 18 gm. of glucose per kg. in the same set of animals. Suppression of urine is a regular manifestation, but the visceral changes at autopsy are slight. When d,l-glyceric aldehyde is injected intravenously at the rate of only 0.15 gm. per kg. per hour, and possibly at slower rates, unchanged glyceric aldehyde appears in the urine, but no glucose. (It will be recalled that glucose may be injected continuously at the rate of 0.8 gm. to 0.9 gm. per kg. per hour without causing glucosuria.) When administered to diabetic individuals d,l-glyceric aldehyde may increase glucosuria. When given to completely phlorhizinized and glycogen-free dogs it is possible to demonstrate a quantitative conversion of the triose into glucose, the increase in glucosuria corresponding exactly with the weight of glyceric aldehyde given. However, owing to the toxic effects of glyceric aldehyde on the kidneys there may be an incomplete excretion of all the sugar formed. The suppression of urine has in the past been mistaken for a beneficial effect, since it may lead to diminished excretions of sugar, acetone, aceto-acetic and β-hydroxybutyric acids.

Embden and his coworkers demonstrated the formation of lactic acid from glyceric aldehyde added to washed blood corpuscles. The keto-triose, dihydroxyacetone, was observed to produce less lactic acid, but otherwise it is not improbable that the behavior of the ketotriose is analogous to that of the aldo forms. Thus Mostowski found dihydroxyacetone to be a glycogen former, and Ringer[23] reported its complete transformation into glucose in the fully phlorhizinized dog.

The complete conversion of d,l-glyceric aldehyde into glucose in phlorhizinized dogs—its transformation into glycogen in the perfused liver, its disappearance as such when added to liver emulsions, all indicate

[22] Ber. Deut. Chem. Ges., 1914 (46), 2327.
[23] Ringer and Frankel, Jour. Biol. Chem., 1914 (18), 233.

that glyceric aldehyde (like diose and other sugars in general) is converted into glucose in the body as a preliminary step in utilization. The fact that large doses may be given by the alimentary route without causing melituria or death, whereas much smaller doses given subcutaneously may prove lethal, together with the very low rate at which glyceric aldehyde has to be given by vein in order not to produce melituria, all point to the liver (and bowel wall) as the chief sites of its conversion. Glyceric aldehyde has figured prominently in theories of the normal catabolism of glucose, and on the basis of his observations concerning the formation of lactic acid from this triose by blood corpuscles, Embden regards it as a chief normal intermediate substance in the oxidation of glucose in the cells. Now glucose may be oxidized in the body at the rate of 0.6 gm. per kg. per hour under suitable circumstances, and if every molecule of glucose oxidized were first split to give two molecules of glyceric aldehyde, as the Embden hypothesis would demand, then glyceric aldehyde would be formed in the body at the rate of 0.6 gm. per kg. per hour, and the place of formation would be within the cells of the body at large, the muscles representing the most important sites of oxidation. However, if glyceric aldehyde is introduced into the systemic blood at only one-fourth of this rate, unchanged triose appears in the urine and may be demonstrated in the blood. But glyceric aldehyde has never been found in the blood, urine or tissues under any other circumstances. Glyceric aldehyde may of course enter the body via the portal route at faster rates without causing triosuria, but then, as stated, it would appear not to be oxidized directly but first assimilated, *i. e.*, transformed into glucose. Recently, for other reasons, von Fürth[24] has also questioned the tenability of Embden's hypothesis.

Lactic acid from triose: When alkali acts on glucose (or hexoses in general) in the absence of oxygen, lactic acid is formed in amounts as high as 40 to 60 per cent. of the weight of the sugar used, provided the conditions are properly controlled. But in the presence of sufficient oxygen no lactic acid is formed. Still, preformed lactic acid will not be destroyed if it is added to this latter mixture. So it is clear that lactic acid is not an intermediate in the oxidative breakdown of sugars in the alkaline solution. Meisenheimer accordingly suggested the obvious probability that there was some labile precursor of lactic acid which burned in the presence of oxygen; in the absence of oxygen, rearranged to give lactic acid. He proposed glyceric aldehyde as such a body. Nef, however, held that the immediate precursor of lactic acid is methyl glyoxal (CH_3—CO—COH), which forms lactic acid by undergoing what is known to chemists as a "benzilic acid rearrangement." These phenomena are remarkably similar to those that occur in the body.

One other important point should be emphasized in this place. The trioses condense in the presence of alkali to yield among other things certain hexoses, and, as described under *hexoses*—any sugar of that type will in the presence of alkali enter into a complex equilibrium with several other hexoses. Any of these may again split into 3-carbon compounds such as the trioses and then again condense, and so on, as long as they do not become converted into lactic acid or the saccharinic acids—substances which are not reconvertible into sugar. Nef formulated the view that—were it not for the occurrence of these irreversible reactions—*any sugar in the presence of alkali would come finally to represent an equilibrium of every possible sugar of* 2 *to* 6 *carbon atoms* (*i. e.*, 56) together with all of the myriad intermediate forms. In the body, however, lactic acid can be converted into sugar. So this bar to the great

[24] Biochem. Zeit., 1916 (69), 199.

equilibrium is there nonexistent, and it is conceivable that in the body there actually exists an equilibrium of this sort.

In all of Embden's experiments there was perhaps a lack of oxygen, so that the phenomena *in vivo* and in alkaline solution *in vitro* are strikingly parallel. Embden, on the basis of these experiments, regards sarcolactic acid (*i. e.*, d-lactic) as a *chief normal* breakdown product of glucose in the body over the glyceric aldehyde route. But it is hard to see why this assumption is more rational than it would be to say that lactic acid is an intermediate in the oxidative breakdown of sugars in the alkaline solution outside the body, which it certainly is not. Although lactic acid will disappear from a surviving asphyxiated muscle if oxygen be resupplied to it (Fletcher)[25] and although this disappearance will not occur in a simple alkaline peroxide solution it may nevertheless be effected by the addition of a second catalyst, and still we know that the lactic acid was not an intermediate in the original solution until the oxygen supply became deficient. Lactic acid is probably an intermediate in the sugar catabolism only during relative asphyxia.

All the substances whose formulæ are given above have been shown to be capable of conversion into glucose in the body. The details of the steps involved have not been established, but, in conformity with the chemical theories developed by Nef and discussed under hexoses, the transformation of these substances into glucose, as well as their occurrence in the course of its breakdown, are best explained on the basis that all of them participate in the same great chemical equilibrium with the sugars, and that this participation depends upon their dissociation into unsaturated residues. These residues are in dynamic chemical equilibrium with the molecules from which they are derived and with those derived from sugars. When there is a rapid loss of glucose from the body these substances tend to become glucose, in accordance with the laws of chemical equilibrium.

TETROSES

There are six possible tetroses (4 aldo- and 2 keto-). The entire subject of their physiology, which has undoubtedly considerable biologic importance, has been little studied. They have never been found in the urine, since there are at present no established methods for their detection, and efforts have been lacking.

PENTOSES[25a]

Chemical theory demands the existence of fourteen pentoses, *i. e.*, six aldopentoses, four 2-keto-pentoses and four 3-keto-pentoses. Only those better known to chemists have received biological study, *e. g.*, arabinose and xylose.[26] Of these the optically inactive or d- l-arabinose, the l-arabinose and l-xylose are the best known. When even small quantities of pentose gain access to the circulating blood, pentose is excreted in the urine. Ebstein[27] reports the appearance of traces in the urine of a man (in which none had been previously demonstrated), after the administration of

[25] Jour. of Physiol., 1907 (35), 247.

[25a] See review by Bach, Münch. Med. Woch., 1917 (64), 105.

[26] Rhamnose is a methyl pentose, representing a class of substances closely related to the pentoses.

[27] Virchow's Archiv., 1892 (129), 401.

so little as 0.25 gram of l-arabinose by mouth. Bergell[28] found reactions for pentose in the urine seven to ten minutes after ingestion of the same sugar, and when given subcutaneously. Fr. Voit[29] saw about 50 per cent. excreted. Neuberg and Wohlgemuth[30] gave a normal man 15 grams of d-l-arabinose and recovered 4.5 grams of d-, and only 1.04 grams of l-arabinose in the urine. On the other hand l-arabinose becomes converted in part into the dextro-form, since both forms appear in the urine when only one is given. Xylose has been found to behave in general like arabinose.[31]

Since all writers agree that 10 to 50 per cent. of administered pentoses are excreted in the urine even when given per os in very small quantities, and since pentoses occur in many foods (plums, cherries, apples, etc.) or result from the bacterial decomposition of other carbohydrates, it is inevitable that *alimentary pentosurias* should occasionally occur in nearly every normal individual, and, as a matter of fact, most normal urines give reactions which indicate the presence of some pentose (Cremer, Funaro, Cominotti). Vice versa, one may conclude that very little pentose normally occurs in the blood, since otherwise more of it would appear in the normal urine than does; and finally, that pentoses must play but a minor rôle in the general metabolism of the carbohydrates. Therefore it is highly improbable that during the breakdown or synthesis of glucose in the body the hexoses split to any extent into a pentose and formaldehyde. The same holds good for the behavior of hexoses in the presence of alkali. They split almost exclusively into chains of 2, 3 and 4 carbon atoms (Nef).

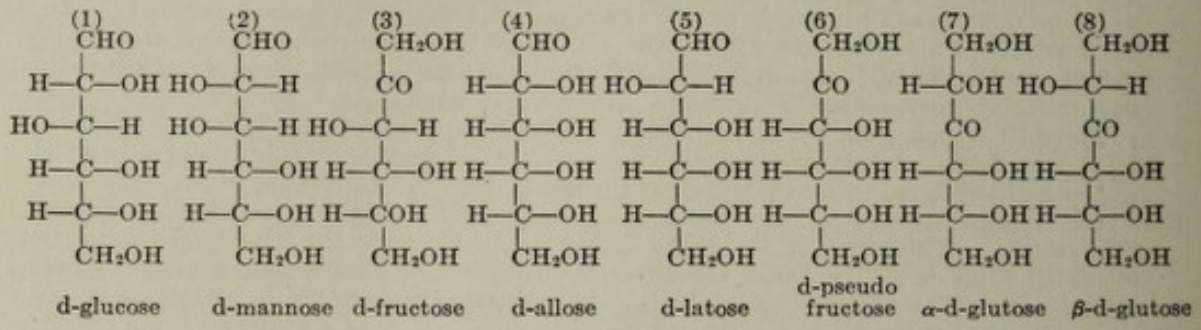
Chronic Pentosuria[32]

The literature contains reports of perhaps 50 cases in which considerable quantities of pentose have been excreted steadily in the urine regardless of the character or quantity of the food. Even during a fast the quantity excreted has remained virtually constant in some cases. Outputs as high as 36 grams per day have been recorded. Such quantities of pentose could not be introduced into the body from without by any known means without causing pentosuria of marked degree. Accordingly, in some cases there is either an overproduction of endogeneous pentose, or an abnormal entry into the blood stream of pentose which is normally bound in the tissues. The process would then be analogous to that in which lactose from the mammary glands occasionally gains access to the general circulation and appears in the urine. This conclusion is confirmed by the work of Bial, Blumenthal and Tintemann, who found that certain pentosurics displayed no lessened tolerance for administered pentose. The origin of the pentose is unknown. Nucleo-protein of cell nuclei, and galactose have been suggested as possible sources. The disease has been found in different members of the same family and appears to be a harmless anomaly. The pentose found in the urine in cases of all types has sometimes been reported as optically inactive, sometimes as dextro- or levorotatory; l-arabinose (dextro-rotatory), d-xylose and d-xyloketose appear to have been identified.[33]

[28] Festchr. f. E. v. Leyden, 1902 (2), 401.
[29] Deut. Arch. f. klin. Med., 1897 (58), 523.
[30] Zeit. f. physiol. Chem., 1902 (35), 41.
[31] For literature, see Neuberg, "Der Harn sowie die übrigen Ausscheidungen, etc." (Springer, Berlin, 1911), I, p. 370.
[32] See Garrod, "Inborn Errors of Metabolism," Oxford Med. Publ., 1909; Lancet, July, 1909.
[33] For literature see Hiller, Jour. Biol. Chem., 1917 (30), 129; Wrzesnewski, Biochem. Zeit., 1922 (132), 135; Cammidge and Howard, Brit. Med. Jour., 1920 (2), 777.

HEXOSES

Chemical Introduction.—Structural theory demands the existence of 32 isomeric hexose sugars of the formula $C_6H_{12}O_6$. The behavior of the hexoses when dissolved in very dilute alkali makes it convenient to consider them in four natural series of eight members each. Thus one series comprises the 8 hexoses whose structural formulæ appear below. This may be called the d-glucose series.



There is also an l-glucose series in which the members are the mirror images of the above. There is a third series comprising d-galactose, d-talose, d-tagatose, l-sorbose, l-idose, l-gulose and alpha and beta d-galtose; and a fourth series whose relationship to the d-galactose series is the same as that of the l-glucose to the d-glucose series. Consideration of the d-glucose series will bring out the principles common to all. Examination of the 8 formulæ shows that numbers 1, 2, 4 and 5 have aldehyde groups (H—C=O) at the end of the chain and are hence aldohexoses. Members 3 and 6 have ketone (C=O) groups, at the second carbon atom, and are therefore 2-keto-hexoses, while numbers 7 and 8 having ketone groups at the third carbon atom are 3-keto-hexoses. Since each series of 8 sugars has a like number of the different types there are in all 16 aldohexoses, eight 2-keto- and eight 3-keto-hexoses.

It had long been known that if a solution of any optically active sugar, such as d-glucose, was alkalinized the solution gradually lost its optical activity. It was later shown by Lobry de Bruyn and van Ekenstein that a solution of d-glucose in very dilute alkali comes to contain a group of 4 hexoses in dynamic chemical equilibrium.[34] Nef[35] held that in such solutions there is an equilibrium of at least eight hexoses as above depicted. Any one of these sugars when placed in alkali reproduces the other seven, since the members of the series are reciprocally convertible one into another. The same holds good for the members of the d-galactose series and for the l-galactose and l-glucose series. But the reciprocal transformation of the members of one series, such as d-glucose, into a hexose of another series, such as d-galactose, occurs, if at all, only to a minute degree, because such transformation involves the breaking of the hexose chain into 2, 3 and 4 carbon fragments with subsequent recombinations, and when this occurs irreversible reactions are prone to intervene. The formation of lactic acid and the saccharines are representative of these irreversible reactions. (In the body, however, glucose may be converted into lactic acid in the muscles and elsewhere, whereas in diabetes lactic acid can be converted easily back into glucose; in diabetes galactose is convertible into glucose, etc., so that in the body the transformation of hexoses of different groups one into another offers no difficulty.)

In order to explain the effects of dilute alkali on hexoses just described, some conception of labile intermediate products is a logical necessity, for when levulose changes into glucose there is necessarily some intermediate phase. The nature of these phases has been the subject of study by many chemists, and this study involves always the question of *sugar dissociation*.

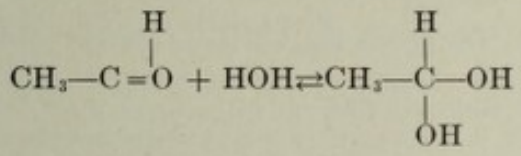
Sugars are weak acids. They form salts with metals, and Cohen[36] and later Michaelis and Rona[37] have determined by physico-chemical methods the ionization constants for glucose and other sugars. Sugars are also polyatomic alcohols, and

[34] Rec. trav. chim. de Pays Bas (14), 158 and 203; (15), 92; (16), 257; (19), 1 and 10.
[35] Liebig's Annalen, 1907 (357), 294; 1910 (376), 1.
[36] Zeit. f. physikal. Chem., 1901 (36), 69.
[37] Biochem. Zeit., 1912 (47), 447.

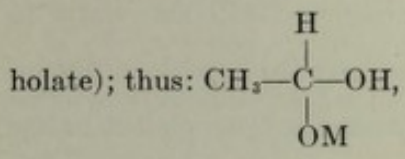
either aldehydes or ketones. Sugar chemistry reverts to the chemistry of these three classes of compounds.

A. P. Mathews[38] and Michaelis have suggested that the effect of alkali on a sugar such as glucose is to increase enormously the concentration of the glucose anion, *i. e.*, KOH leads to the formation of K-glucosate, which, being the combination of a powerful base with a very weak acid, has a high electrolytic dissociation constant. These anions according to this view are subject to cleavages and intramolecular rearrangements. Nef also holds that the first effect of the alkali (*e. g.*, KOH) is to form a salt, but his far more detailed conception of the subsequent changes which lead to reciprocal transformations of hexose sugars one into another, involves other principles which represent the outgrowth of his earlier work on the properties of simpler aldehydes and ketones.

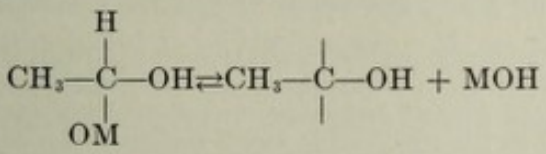
These reciprocal transformations are dependent, according to Nef, upon the aldehydic or ketonic character of the sugar, whereas the oxidative phenomena and the saccharinic acid formation to be described presently, depend upon the alcohol groups. The principles involved can best be understood if we first consider the behavior of a simple aldehyde (acetaldehyde) and a simple ketone (acetone). Acetaldehyde in the presence of water forms a hydrate (comparable to chloral hydrate).



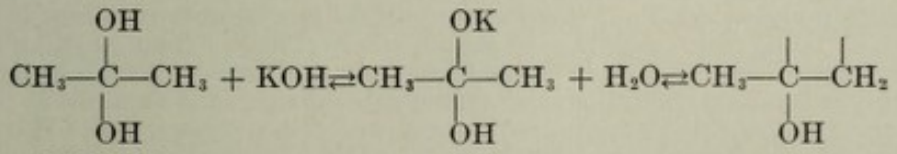
This hydrate possesses ionizable hydrogen in its OH groups, and in the presence of a metallic hydroxide, MOH, can accordingly form a salt (comparable to an alcoholate); thus:



and this salt being highly dissociable *falls apart into MOH and a "methylene enol"* (in this case hydroxyethylidene):



(Herein lies the point of departure of Nef's view from the foregoing.) This methylene enol then rearranges to form the "olefine dienol" $CH_2 = CHOH$ (in this case vinyl alcohol). Ketones, on the other hand, form the olefine dienol directly without forming the methylene enol. In the case of KOH and acetone (dimethyl ketone) there is the same formation of the hydrate followed by salt formation and the loss of KOH, but the latter does not all come from one C atom as it does when split out of aldehydes, thus:

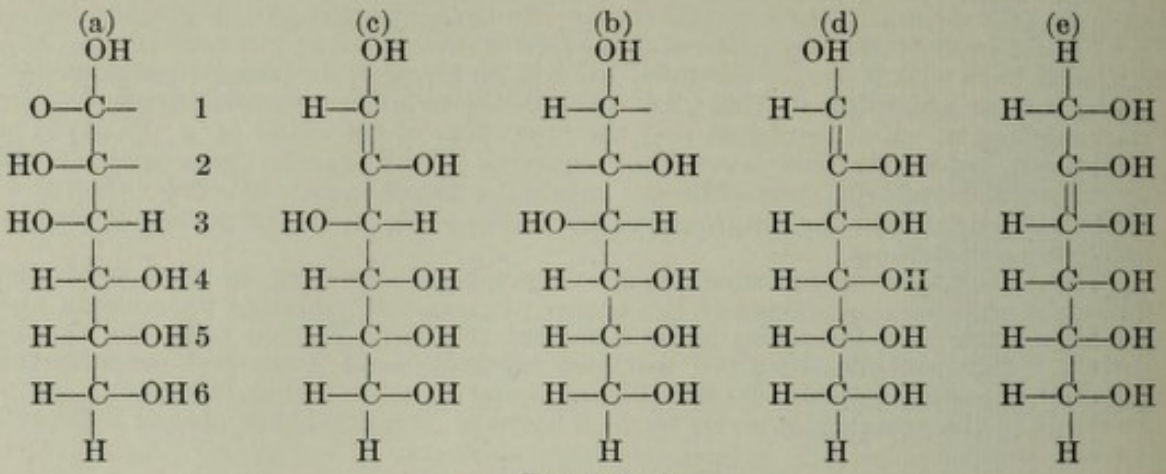


(hydrate of acetone)

In a manner entirely analogous to what occurs in the simple aldehydes and ketones, the two aldohexoses, glucose and mannose, and the ketohexose levulose, can form one

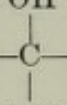
[38] Jour. Biol. Chem., 1909 (6), 1.

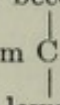
and the same enol molecule. And vice versa this enol molecule may open its double bond in two ways as shown below ((a), (b) and (c)) and the dissociated molecules.



Enol molecule
(*a*, 1, 2 d-glucose olefine dienol)

(a) and (b) will be in dynamic equilibrium with the enol (c). Now if H and OH are again taken on by (a) and (b) this assumption of the elements of water can take place in three different ways, to regenerate d-levulose, d-mannose and d-glucose. Thus if in the case of (a), OH is added to carbon atom number one this will form the group



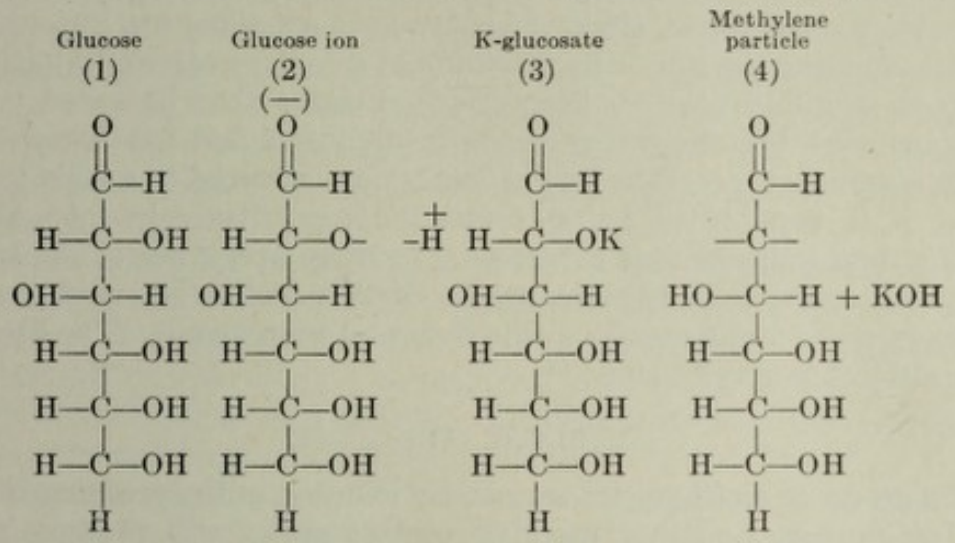
which represents a hydrated aldehyde group and will lose water to become CHO. Then H going to the second C atom completes the formula of d-mannose. In a similar way (b) can form d-glucose. But if the OH went to the second carbon atom this group would thereby become a hydrated ketone similar to the hydrate of acetone and lose water to form  while H going to the end carbon atom would complete the formula of d-levulose. In a manner entirely analogous there is an enol molecule which is common to d-allose, d-lactose and d-pseudo fructose (see (d) above).

It will be noticed that each of these enols is in equilibrium with a 2-keto-hexose and two aldo-hexoses. Now these 2-keto-hexoses, d-fructose and d-pseudo fructose, in accordance with general ketone behavior, are capable of yielding another common enol, *i. e.*, a 2–3 enol (with the double bond between the second and third carbon atoms) as represented at (e) and this 2-3 enol (by a process like that just detailed for the 1–2 enols) can account for the formation of the two 3-keto-hexoses whose formulæ are given above. The same general principles hold for each of the series of hexoses. (For further elaboration of the theory see Nef's original papers.) A similar use of enol molecules in this connection is made by Neuberg.

It remains now to point out that if to a simple aqueous solution of sugar, oxygen be supplied in the form of air or H_2O_2, no oxidation occurs. But if the solution be alkalinized then the sugar is readily burned. In the absence of oxygen and the presence of alkali somewhat stronger than that found most favorable for the reciprocal transformation above detailed, there occur certain irreversible reactions such as the formation of lactic acid and the so-called saccharines. When an alkaline sugar solution is treated with oxygen it yields CO_2, H_2O, and formic, glycollic, glyceric and certain trihydroxy-butyric and hexonic acids, depending on the sugar used. Without oxygen, or with too little oxygen, lactic acid and the saccharinic acids make their appearance (cf. the formation of lactic acid). The explanation of these phenomena rests in the conception that alkali increases the dissociation of sugar, and that the dissociated fragments burn or rearrange depending upon the conditions of the experiment.

In this connection, according to Nef, we are dealing with the alcohol groups of the sugars and may advantageously turn for a moment to the properties of methyl alcohol. This substance consists under ordinary circumstances of a great prepon-

derance of undissociated molecules in dynamic equilibrium, with a very minute quantity of dissociated methylene, *i. e.*, $CH_3OH \rightleftarrows CH_2 + H_2O$. The primary effect of alkali (KOH) is to form a salt, $CH_3—OK$ (or K-methylate), which being highly dissociable breaks down to give CH_2 and KOH. The proportion of free methylene is thereby enormously increased. What then befalls the methylene will depend on the amount of oxygen present and on the various other factors which enter into the conditions of the experiment. These general principles are applicable directly to the polyatomic alcohols—the hexoses and other sugars—as shown on a preceding page.



In the presence of sufficient oxygen the methylene particle takes on oxygen to form first an osone. In the absence of oxygen it undergoes intramolecular rearrangements, the details of which need not here be entered into. It is these which gives rise to the 6-carbon acids known as the saccharines or saccharinic acids.

GALACTOSE

A normal individual weighing 75 kilos may eat about 50 grams of galactose and show but a trace of melituria. More than this is likely to cause the presence of measurable amounts of galactose in the urine, the alimentary tolerance limit for this sugar being therefore about 0.6 to 0.8 grams per kilogram of body weight. We have no direct data concerning the time within which 50 grams of galactose are absorbed by a man of average weight. When given intravenously at uniform rates, unchanged galactose appears in the urine of dogs receiving slightly more than 0.1 gm. per kilo per hour. The tolerance for galactose appears to be lessened in phosphorus poisoning and in many other conditions which cause apparent parenchymatous changes in the liver, so that after administration of 50 grams of galactose by mouth, as much as 10 to 12 grams may be excreted in the urine (Bauer). On the other hand, ligation of the common duct does not lessen the tolerance for galactose in rabbits (Reiss and Jehn, Hierose) so that the lowered tolerance following phosphorus administration appears to be independent of the disturbed biliary function. Infants suffering from gastro-enteritis may show alimentary lactosuria, and along with the lactose some of its constituent galactose may appear in the urine. The question thus naturally arises as to whether the lowered tolerance for galactose in phosphorus poisoning and

other liver diseases may not be due to an increased permeability of the intestinal wall, or to changes elsewhere in the body besides the liver. Wörner found that galactose injected directly into the portal vein was handled by healthy and phosphorized rabbits in the same relative proportions as when given to these animals by mouth, thus apparently excluding the bowel as a contributor to the decreased tolerance. It is unlikely also that the kidneys in phosphorized animals were rendered abnormally permeable for galactose, since when the kidneys alone are phosphorized without affecting the liver, the excretion of galactose after administration by mouth or into a vein has been retarded rather than hastened. These principles have become incorporated in a clinical test for disease of the hepatic parenchyma. When galactose is administered to a fully diabetic animal it is capable of being converted quantitatively into glucose. Existing data indicate that galactose, like diose and glyceric aldehyde, is chiefly converted into glucose before further utilization, and that this process is carried out mainly in the liver and bowel wall. The literature of the subject is given below. [39]

LEVULOSE (FRUCTOSE)

The group of eight sugars formed by levulose in the presence of alkali includes glucose, and any member of this group will produce all the others. Then, in cases of glucosuria with alkaline urine (whether physiological or due to medication or to bacterial decomposition), levulose might be expected to occur along with glucose. May and Koenigsfeld have reported instances of this "urinogenous levulosuria." Magnus-Levy doubts the correctness of these observations.

Alimentary Levulosuria.—The tolerance of a normal body for levulose given per os is variable. Doses of 50 to 70 gm. in man cause as a rule no levulosuria, but more is likely to do so. Animals in which the liver parenchyma has been damaged by phosphorus are said to have a lower tolerance. In many other diseases of the liver the same holds true, and H. Strauss believed this fact could be made the basis of a clinical test for liver function. Naunyn, however, emphasizes the fact that in certain cases of cirrhosis of the liver with collateral anastomoses between the portal vein and vena cava, there is also a lessened tolerance for levulose given by mouth, owing to the fact that levulose then enters the general circulation without having entered the liver. As far back as 1871 Eichhorst showed that levulose introduced per rectum, *i. e.*, where it will presumably enter an hemorrhoidal vein after resorption, is more likely to cause alimentary levulosuria than when swallowed, because of the extra-hepatic anastomoses between the hemorrhoidal veins and the vena cava. As in the case of diose, glyceric aldehyde and galactose, intravenous injection of levulose produces levulosuria in dogs when the

[39] Bauer, Deut. med. Woch., 1908 (35), 1505; Reiss u. Jehn. Deut. Arch. f. klin. Med., 1912 (108), 187; Roubitscheck, Deut. Arch. f. klin. Med., 1912 (108), 225; Naunyn, "Beiträge zur Lehre von Ikterus, etc.," Reichert-Dubrissches Archiv. für Anatomie, 1869, p. 579; Schöpffer, Arch. f. exp. Path. u. Pharm., 1873 (1), 73; Rowe, Arch. Int. Med., 1924 (34), 388.

rate of injection is between 0.1 and 0.2 gram per kilo per hour. Jacobson[40] found that Eck fistula animals have a more marked lowering of tolerance for levulose than for glucose. These facts support the belief that the liver plays the same important part in "assimilating" levulose as with other sugars.

Spontaneous Alimentary Levulosuria, i. e., the appearance of levulose in the urine from such small quantities of levulose as occur naturally in the food, had been demonstrated in but eight cases up to 1920. In five of these levulose appears to have been the only sugar present. These persons showed a decreased tolerance for ingested levulose and ceased passing the sugar when the diet was carbohydrate-free. The tendency of thought would be to look for the cause of such phenomena in a disturbed hepatic function.[41]

It is interesting to note that of the above-mentioned five cases of pure levulosuria, two showed a lessened tolerance for glucose, and one symptoms of dispituitarism, one developed during the puerperium, and one had an endocarditis; *i. e.,* four out of five had evidence of derangements of the endocrinous glands. The literature has been reviewed and a case reported by Strouse and Friedman.[42]

Mixed Levulosuria, or the occurrence of levulose along with glucose in severe cases of diabetes, is said by some to be a common event. In view of the great frequency of combined liver and pancreatic changes found at autopsy in diabetic cases, and in view also of the frequent occurrence in diabetes of signs which point to disturbances of other glands with internal secretion besides the pancreas, this would harmonize well with the view just given.

Spontaneous or Idiopathic Levulosuria, having a character similar to chronic pentosuria, and running a steady course uninfluenced by diet, has been reported in one case by Rosin. In this instance the tolerance for glucose was also diminished.

POLYSACCHARIDES

Closely related to these meliturias are the forms in which the polysaccharides,—lactose, maltose and saccharose,—are the sugars concerned.

Lactosuria.—When 2 to 3 grams of lactose per kilogram of body weight are given in pure form by mouth to a healthy adult dog or man—*alimentary lactosuria* generally occurs. Another form of lactosuria is that seen in *lactating women.* In these cases the lactose gains access to the general circulation from milk stasis in the breast. Yet another form, the *lactosuria in children* having gastro-intestinal diseases, has its origin in the lactose of the milk or artificial food. In these cases lactosuria may develop after the ingestion of lactose, in quantity and form[43] incapable of causing it in a healthy child. The tolerance for lactose is most strikingly

[40] Amer. Jour. Physiol., 1920 (52), 233.
[41] See Barrenscheen, Biochem. Zeit., 1922 (127), 222.
[42] Arch. Int. Med., 1912 (9), 99.
[43] Pure aqueous solutions of sugar differ in the rate of absorption from those in which the sugar is incorporated in heterogeneous mixtures.

decreased in so-called "*intoxication*" (Finkelstein) in which lactosuria may follow ingestion of 0.4–0.5 g. per kilo of body weight. (Grósz places the assimilation limit for healthy sucklings at 8.6 g. per kilo.) This might be explained in two or more ways. The lactase in the bowel might be deficient and permit unhydrolyzed sugar of milk to accumulate in abnormal concentration in the lower bowel, and then be absorbed unsplit; or, as seems more probable, the bowel wall—because of ulcers or simple inflammatory changes—might become abnormally permeable. The intravenous tolerance limit for lactose approaches zero. During prolonged intravenous injections of lactose into dogs at the rate of 2 gm. per kilo per hour, lactose was excreted at the rate of injection during the fourth hour and the following four hours.[44]

Leopold and Reuss reported that when 1 gram of lactose was injected subcutaneously into a dog or infant, exactly 1 gram reappeared in the urine; but that if the injections were made daily, the quantity excreted fell little by little and finally became zero. Helmholz and Woodyatt have repeated this experiment in dogs, and found that at first the gram injected might reappear in the urine as stated. Sometimes, however, the occurrence of an increase in the reducing power of the urine above the figure representing 1 gram of lactose was noted. This suggested a splitting of the lactose into glucose and galactose. Nor could they obtain more than a temporary disappearance of the sugar following subsequent injections, even when carried on for weeks—such as Leopold and Reuss reported. The point of chief interest in these experiments is that the apparently increased hydrolysis of lactose developing with successive doses resembles a reaction of immunity, with a substance of known chemical composition as the antigen. But it is possible that the successive injections simply result in a lessened excretion of the lactose by the kidneys. Abderhalden and his co-workers reported that the serum of animals similarly treated develops an increased power to split the disaccharide employed, as determined by means of the polariscope. These experiments were paralleled with cane sugar (saccharose) and with di-, tri-, and higher peptids. Other observers have failed to corroborate these findings.

Saccharosuria (cane sugar in the urine) occurs under conditions quite similar to those mentioned for lactose, except that there is no saccharosuria corresponding to the lactosuria of women.

Maltosuria has often been reported, but the chemical detection of this sugar is uncertain.

Other polysaccharoses, such as isomaltose, glycogen, etc., have been thought by some writers, to occur in the urine.

GLUCOSURIAS

Glucose is the sugar which enters into the normal glycogen and forms the bulk of the body sugar. Glucosurias are naturally the most important of the meliturias.

[44] Unpublished experiments by W. D. Sansum.

(1) *Alimentary glucosuria, e saccharo.* Not infrequently it is impossible to make a healthy man eat and retain sufficient glucose to cause glucosuria, and it would be hard to define an increased glucose tolerance. This statement is corroborated by the studies of Taylor and Hulton[45] on man. As indicated before, an increased supply of glucose to the body may increase the urinary sugar and as Benedict *et al.* have stated, there may be no sharp line of division between these changes and a gross glucosuria. In dogs weighing 10 kilos the maximum rate of glucose absorption is apparently reached with doses of 50 grams and perhaps less. Larger doses do not further increase the rate of absorption. This rate may be 1.8 gram per kilo per hour. If with this rate of absorption the rate of utilization in the bowel wall and liver is 0.9 gram per kilo per hour, or less, glucose will enter the systemic blood at the rate of 0.9 gm. per kilo per hour, or more, and this will normally cause a gross glucosuria. The physiological state of the liver and the rate of sugar absorption are factors of importance in determining alimentary glucosuria. Glucosuria following the ingestion of starch alone—*alimentary glucosuria ex amylo* was said not to occur in healthy individuals, but the feeding of large quantities of starch increases the urinary sugar and if the tests used for its detection are of sufficient delicacy the increase is measurable. With a sufficiently small urinary volume ordinary tests may detect the extra sugar.

(2) *Glucosurias which depend upon the discharge of sugar from stored glycogen.* These may be due to the action of (a) nerves, (b) drugs, (c) the so-called internal secretions.

(a) Claude Bernard's piqûre, or puncture of the floor of the fourth ventricle between the points of origin of the eighth and tenth pairs of nerves, causes a glucosuria which ceases when the glycogen of the liver is reduced to a low percentage. Following this operation the blood is found to contain an excess of sugar (hyperglucemia) to which the glucosuria is immediately due. If the vagus nerve is cut stimulation of the central end has a similar effect, so that the vagus is said to carry the afferent impulse to the center in the *calamus scriptorius.* By severing different portions of the nervous system and stimulating the cut surfaces, the path of the efferent impulse has been traced from the glycogenic center through the cord to the upper thoracic spinal roots, by the *rami communicantes* to the inferior cervical and superior thoracic ganglion, thence via the splanchnic nerves to the liver. This center and nervous arc form probably an important link in the mechanism for regulating the quantity of sugar in the blood. Nervous glucosurias having the same mechanism as "la piqûre" occur in a great variety of conditions associated with insult to the nervous system, *e. g.*, commotio cerebri, brain tumor, tabes, meningitis, severe mental shock, etc. How a splanchnic impulse operates to cause increased hydrolysis of glycogen is unsettled. Glycogen hydrolyzes outside the body under the influence of acids, *i. e.*, of H ions or plus charges of electricity, and a nerve impulse might theoretically operate

[45] Jour. Biol. Chem., 1916 (25), 173.

directly, or, as Macleod has suggested, through an increase of glycogenase in the liver; or as held by the von Noorden school, by causing an increased section of epinephrine—since piqûre glucosuria is said not to occur in animals deprived of the adrenals (Mayer, Kahn, Nishi) or after section of the left splanchnic nerve, which supplies both adrenals. (b) Similar phenomena occur in asphyxia (carbonic and lactic acid accumulation), and when acids are directly administered; also after the administration of certain drugs whose effects, including lactic and carbonic acid accumulation in the body fluids, closely parallel those of a deficient oxygen supply (phosphorus, carbon monoxide, chloroform, hydrazine, arsenic, etc.). Certain other drugs, such as curare, strychnia, etc., may interfere with respiratory movements and so cause glucosuria by secondary asphyxia; although other drugs, of which there are many, may operate to cause glucosuria in any of the ways by which glucosuria can be produced.[46]

(c) The ductless gland extracts which produce glucosuria include those of the adrenal, thyroid and hypophysis. Epinephrine has been discussed in another place, and the reasons are there developed for the belief that the glucosuria it causes is due to a mobilization of sugar from glycogen, which leads to hyperglucemia. Ringer[47] showed that when an animal is fully phlorhizinized the subcutaneous injection of epinephrine causes no additional output of sugar nor alteration of the G:N ratio, a fact which has been confirmed by Sansum and Woodyatt—thus proving that epinephrine has no power to intensify a diabetes which is already at the point which is called complete. Lusk[48] also showed by respiration experiments the correctness of this interpretation. Eppinger, Falta and Rudinger[49] stated that epinephrine intensifies pancreas diabetes, and used this observation in support of their idea that epinephrine, like thyroid extract, exerts in the liver a sugar-mobilizing and sugar-building effect, antagonistic to the action of the pancreas, which, according to the doctrine of the von Noorden school, checks the formation of sugar from glycogen and also from protein and fat. But in their work there has been no adequate proof that before giving the epinephrine the pancreas diabetes was as complete as a pancreas diabetes can be, or that the increased intensity of the diabetes was any greater than could have been explained by a discharge of sugar from glycogen. The power of pituitary extracts to produce glucosuria is likewise ascribable to their effects on glycogen.

PHLORHIZIN DIABETES[50]

Phlorhizin was obtained by alcoholic extraction of the bark and roots of apple, pear, plum and cherry trees by L. de Koninck in 1835.

[46] The production of glucosuria by a given drug should not be confused with excretion of paired glycuronic acid compounds, such as occurs after the administration of many aldehydes, ketones, alcohols and phenols. The reducing power in these cases is not due to glucose but to its oxidation product, COOH—$(CHOH)_4$—COH.

[47] Jour. Exper. Med., 1910 (12), 105.

[48] Arch. Int. Med., 1914 (13), 673.

[49] Zeit. f. klin. Med., 1908 (66), 1; 1909 (67), 380.

[50] For a treatise of the whole subject of phlorhizin glucosuria, with bibliography, see the monograph by Lusk (Phlorhizin Glykosurie, Ergeb. der Physiol., 1912 (13), 315), free use of which has been made in the following.

Its glucosidic character was established by Stas, who found that it could be split into glucose ("phlorose") and a substance (phloretin) which by acid hydrolysis yielded phloroglucin and an acid (phloretinic acid). It was not until 1886 that von Mering published his first experiments upon its physiologic action.

While phlorhizin causes glucosuria when taken by mouth, its greatest effect is obtained by subcutaneous injection. One gram of phlorhizin triturated in 5 to 15 c.c. of olive oil, or in 20 per cent. alcohol, and injected subcutaneously once every 24 hours, will maintain the maximum glucosuria which can be produced in a dog of 10 kilogrammes. Phlorhizin is mostly (80–90 per cent.) excreted in the urine. It is soluble in ether, optically active, and gives a garnet coloration with ferric chloride, so that it interferes with the polariscopic tests for β-hydroxybutyric acid in the urine, and masks the Gerhardt reaction for aceto-acetic acid.

Phlorhizin causes glucosuria in frogs and other cold-blooded animals, as well as in warm-blooded forms in general, including birds. That geese show glucosuria with phlorhizin (von Mering, Thiel) is important, because birds do not pass sugar in the urine when operations are performed upon them which do cause a definite excess of blood sugar (pancreatectomy, Minkowski). *Phlorhizin causes glucosuria in birds—hyperglucemia does not. Hence phlorhizin does not cause glucosuria by producing hyperglucemia.* In harmony with this syllogism are the data obtained by Minkowski, Levene, von Czylharz and Schlesinger, Lewandowsky, Lêpine, Porcher, Junkersdorf, Erlandsen, Frank and Isaac—all of whom have found the blood sugar concentration in phlorhizinized animals low (0.065 per cent.; 0.072 per cent.; 0.012 per cent., etc.). Conflicting results have also been published, but the methods employed in these instances have not usually been beyond criticism (Pavy, Biedle and Kolisch). Even after ligation of the renal vessels or bilateral nephrectomy, no hyperglucemia has been demonstrated in phlorizinized animals, whereas if phlorhizin acted by liberating sugar from glycogen reserves in the liver and elsewhere, or from any source distant from the kidneys, hyperglucemia might be expected. In view of these facts von Mering himself interpreted the action of phlorhizin as a *kidney* diabetes.

Zuntz injected phlorhizin directly into one renal artery and collected the urine from each kidney separately. The kidney on the injected side almost at once secreted saccharine urine, and the other kidney secreted sugar only after the lapse of minutes. This experiment has been successfully repeated by others, and seems to prove that phlorhizin can cause glucosuria by acting directly on the kidneys. The many experiments which have been made to determine the relative blood sugar content of the renal artery and vein during phlorhizin glucosuria, add little to this subject.

The questions arise: *Are the kidney cells the only structures which are directly affected by phlorhizin?* and, *What is the exact nature of the phlorhizin effect?*

Levene collected the bile of phlorhizinized dogs and found that it exhibited reducing power after the phlorhizin injection, but not before.

Ray, McDermott, and Lusk failed to find similar properties in vomited bile from phlorhizinized dogs. Brauer repeated Levene's work—using a different method in that he cleared the bile with lead acetate prior to making the sugar tests, and then found no reducing substance. Woodyatt obtained results like Levene's but found later no reaction for sugar when the bile was cleared in the way Brauer recommended. Still, in the native state it yields characteristic crystals of an osazon, and ferments with yeast after, but not before phlorhizinization. Karl Grube perfused tortoise livers with salt solution containing phlorhizin and was able to cause more rapid deglycogenation than when the same salt solution minus phlorhizin was used in control experiments. Now Ray, McDermott and Lusk, in working with bile which had been in the alimentary tract, used material that had had time to lose its sugar by resorption. Brauer's clearing method may take out a trace of sugar even if present originally, and it must be said that there is some evidence favoring the idea that phlorhizin acts in the liver, although much less strongly than in the kidneys. Attempts have also been made to demonstrate a direct action of phlorhizin on the mammary (Cornevins) and sweat glands (Delmare). Cornevins' positive findings were not confirmed by Cremer and Porcher, whereas Delmare's work has not been repeated. But R. Pearce, working with a blood-sugar method, found an increase of sugar in the pancreatic juice, and Underhill has brought further evidence in support of a general action. M. H. Fischer had some nephrectomized frogs, which are able to live indefinitely in water because they excrete through the skin. With the writer, some of these frogs were injected with phlorhizin into the dorsal lymph sac, and sugar was found next day in the water in which the frogs were, but not in the water occupied by control frogs. The possible origin of this sugar in the slime makes it desirable to repeat this crucial experiment. Although the view most commonly held is that phlorhizin acts specifically and exclusively on the kidney cells this has never been proved, and there is much to suggest a general cell effect exhibited most strikingly in the kidney.

Regarding the fundamental nature of the action of phlorhizin, nothing satisfactory has been evolved. Minkowski suggested that phloretin and sugar are split apart in the kidney epithelium, and that the sugar is then excreted while the phloretin is retained in the body. The retained phloretin then takes up a new molecule of glucose from the blood to reform phlorhizin,—which in the kidney is again split, etc. (vehicle theory). Zuntz has determined with a given minute dose of phlorhizin how much sugar can be eliminated in a given time by one kidney; then, figuring what weight of phlorhizin is in the kidney, and how much sugar comes out of the kidney, he reckons how frequently the synthesis and hydrolysis of phlorhizin would have to occur. He makes it 26 times per minute, which he deems too fast to be probable, but in view of the work which can be accomplished by traces of organic and inorganic carriers (catalyzers, enzymes), this criticism is not convincing.

Whatever the action of phlorhizin may prove ultimately to be, this action finds its chief or final expression in the cells of the kidney, and there leads to a disturbance of equilibrium, whereby the relative blood sugar and urinary sugar concentrations are altered in favor of the urine. The blood sugar must be in equilibrium with the sugar content of the various cells, and this with the sources (glycogen and protein) from which the sugar comes. The sugar of the entire body may be conceived of as a gas exerting its partial pressure in every cell and body fluid,—here more dense, there less so, depending upon local physico-chemical conditions, but nevertheless everywhere in communication. Phlorhizin acting in the kidneys, and regardless of a possible action elsewhere, creates a void into which the blood sugar flows, and into which secondarily, as into a vortex, sugar flows from all the sources of the body.

Metabolic Phenomena.—When a fasting dog is kept continuously under the maximum effects of phlorhizin, there is at first a very great glucosuria while the urinary nitrogen remains low. The ratio of the urinary glucose to the urinary nitrogen (G : N ratio) may be as high as 10 or 15 to 1, or higher. If such a dog is killed the liver is found to have a normal appearance and to contain glycogen. As time goes on the rate of glucose excretion falls and the nitrogen tends to increase, until after two or three days the G : N ratio is about 3.65 to 1, as shown by Lusk. Then for 12 to 24 hours it may remain constant. It sometimes happens that the ratio falls to 2.8 or some point between 3.65 and 2.8 before constancy is established. It then proceeds at this lower level instead of 3.65. If a dog is killed at about the time constancy is attained, or somewhat sooner, the liver may be found in a state of fatty infiltration with the glycogen low but not absent. In later stages the excessive fat in the liver again disappears. There is then first a rapid loss of glucose and a simultaneous melting away of glycogen. To compensate for the falling out of the carbohydrate from the metabolism there is an increased breakdown of protein and a rapid mobilization of fat, finding temporary expression in a fatty infiltration of the liver. But as the fat reserves run low the fat deposited in the liver is utilized. Coincident with the partial exhaustion of the carbohydrate reserves of the body and the increased fat and protein metabolism, acetoacetic and β hydroxy butyric acids being to appear in the urine, and since they are excreted partly in the form of the ammonium salts the urinary ammonia is also increased. These acids arise from lower fatty acids having an even number of carbon atoms in the chain, and from certain amino-acids, whenever the mixture of fatty acids and glucose actually metabolizing is too rich in the former in comparison with the latter.

However, such animals are not free of glycogen. If they are subjected to some treatment which has a strong glycogen mobilizing effect the glucosuria may be made to rise temporarily, just as though the dog had been given a dose of sugar. Thus, exposure to cold sufficient to cause shivering, the administration of epinephrine, or an ether or nitrous oxide narcosis, injection of acid (and various other toxic sub-

stances capable of producing tissue asphyxia and acidosis), all may increase the urinary glucose without increasing the nitrogen, and thus cause an increased G : N ratio. But if the exposure to cold is long and intense enough a time comes when it ceases to have this effect, and if epinephrine is given subcutaneously in the dosage of about 0.4 mg. per kg. of body weight once every three hours there is for a time a heavy increase of the glucose output, but this becomes less and less until after 6 or 8 doses the ratio becomes constant again, regardless of whether epinephrine is given or not. In such dogs neither cold nor narcosis nor other toxic effects will increase the output of glucose, and analyses of the liver and muscles reveal no glycogen.[51] In a long series of dogs so treated Sansum and the writer have not encountered ratios above 3.2 to 1, and the 2.8 ratio recurs frequently.

Since the glycogen is gone and the dog is fasting, the sugar which continues to appear in the urine must have its origin in body fat or protein, or both.

Sugar from Fat.—If such a dog be given large quantities of fat in the diet no change occurs in the G : N ratio, nor any increase in the glucosuria, except such as may be ascribed to the glycerol of the fat (Lusk). On the other hand, propionic acid, according to Ringer, may cause a rise in the sugar excretion and a corresponding rise in the G : N ratio.[52] From this it is concluded that the fats of the food do not as a rule form sugar in the body, although sugar formation from at least one lower fatty acid is possible in view of Ringer's experiment.

Von Noorden and Falta and their associates have regarded sugar formation from fat as a regular normal phenomenon, because in diabetes melitus they believe that high ratios occur which make this view necessary.[53]

Sugar from Protein.—If instead of fat, protein be given to the dog above mentioned, there occurs an absolute rise in the sugar of the urine and a corresponding rise in the nitrogen, *but the G : N ratio remains constant.* Following a meat feeding there may be fluctuations of the ratio during short periods, but this statement generally holds if the time of observation is 12 to 24 hours. These facts have led Lusk to the conclusion that when in a fasting, fully phlorhizinized animal, or one fed on meat and fat alone, a constant G : N ratio of 3.65 : 1 is seen; this means that the glucose and the nitrogen are coming from one and the same source, viz., protein. A gram of nitrogen corresponds to 6.25 grams protein, and if for each 6.25 grams protein metabolized as indicated by the N in the urine, 3.65 grams glucose are excreted, then 58 per cent. of the protein metabolized is converted into glucose and so excreted. In like manner the 2.8 : 1 ratio would indicate a 45 per cent. conversion. A percentage above 58 has not been satisfactorily proved to occur. If to the fully

[51] Administration of insulin reduces only slightly the excretion of glucose (Colwell, Jour. Biol. Chem., 1924 (61), 289).

[52] The dogs used by Ringer were not free of glycogen and possibly the extra sugar did not arise from the acid given.

[53] See review by Geelmuyden, Ergebnisse Physiol., 1923 (21_1), 274.

phlorhizinized dog a definite quantity of glucose, galactose, starch or other assimilable form of carbohydrate is given, this may under favorable circumstances be excreted quantitatively in the urine as glucose, and the ratio of G : N will rise. The sugar which appears in the urine under such circumstances over and above that represented by N × G : N has been called "extra sugar" by Lusk.

If all the carbon contained in protein were converted into glucose, and all this excreted together with the nitrogen, the G : N ratio would be 8.25 : 1. A higher ratio than this would necessarily mean that sugar was coming from some source other than protein, or that all of the N was not appearing in the urine, some being retained in the body. If the liver were free from glycogen and no carbohydrate were eaten, such a high ratio would speak in favor of sugar formation from fat. Falta reports having seen cases of diabetes in which this occurred, but in human cases it is difficult to be sure of the absence of glycogen and food carbohydrate; moreover, such high ratios, unless too long continued, might imply retention of nitrogen.

Sugar from Other Substances.—A large number of other substances when administered to phlorhizinized dogs are capable of increasing the output of sugar. Of importance in this connection are certain of the amino acids, viz.; glycine, alanine, aspartic and glutamic acids, and arginine. Others, such as leucine, tryosine and phenyl alanine do not form sugar in the body but increase the output of the acetone substances. The sugar-forming power of protein is doubtless due to its content of the former group of amino acids.[54] Lactic acid and glycerol are also among the sugar formers.

The chief interest in phlorhizin diabetes lies in the opportunities it offers of studying the character of the intermediate metabolism minus that of sugar, and so of studying sugar metabolism. Another interest might be found were the physiologic effects of this glucoside in animals interpreted with relationship to its normal rôle in plant physiology.

PANCREAS, DIABETES AND DIABETES MELITUS

Historical.—In 1788 Cawley reported atrophy and stone of the pancreas in a case of diabetes. The coincidence of diabetic symptoms and lesions of the pancreas was further studied by Bright, Lloyd and Elliotson (1833). It was Bouchardat[55] who first definitely formulated the belief that pancreatic disease was the cause of diabetes melitus, but his views were uncongenial to the clinicians of his time and it remained for von Mering and Minkowski[56] (1889) to prove that *complete* pancreatectomy leads invariably to the development of a severe diabetes. This applies not only to dogs but to cats, rabbits, pigs (Minkowski), tortoises,[57]

[54] See Dakin, Jour. Biol. Chem., 1913 (14), 155.

[55] "De la Glycosurie, etc.," II edit., Paris, 1883. Cited from Naunyn.

[56] Arch. für exp. Path. u. Pharm., 1889 (26), 371; 1893 (31), 85.

[57] Aldehoff, Zeit. f. Biol., 1891–2 (28), 293; Velich, Wien. Med. Zeitung. 1895 (40), 502; Marcuse, W., Zeit. f. klin. Med., 1894 (26), 225.

frogs,[57] eels,[58] and other animals, and appears sooner in old than in young animals.[59]

Effects of Pancreas Extirpation.—The glucosuria begins soon after the operation and increases in intensity. It persists in spite of a non-carbohydrate diet long after the glycogen reservoirs in the liver and muscles have become greatly impoverished (to 0.1–0.2 per cent. in the liver), but like the human disease, it usually ceases during a fast or may disappear just before death.[60] The glucosuria may be accompanied by an excretion of the acetone bodies,—acetone, aceto-acetic and β-hydroxy-butyric acids. In fact, the metabolic changes secondary to this operation closely parallel those found in the human disease, with certain differences which perhaps are ascribable to species or to the fact that in the experimental diabetes digestion is altered by absence of the pancreatic juice, etc. Although Minkowski's work was assailed from many quarters, the following points have become firmly established by frequent repetition. (1) *Complete* removal of the pancreas causes a true diabetes (as above); (2) Ligation or obliteration of the duct (or ducts) of Wirsung, no matter how scrupulously carried out, has no such effect; (3) If about one-fifth of the pancreas with its arterial supply be separated from the rest of the gland, this fifth may be implanted extraperitoneally at a distance from the original site. No diabetes results from this operation, or at most only a transient glucosuria.[61] Now if the main body of the pancreas be fully extirpated with ducts, nerves and bloodvessels, still only a transient glucosuria or none at all develops. At this stage all possible damage to nerves and external secretion has been inflicted and proved incapable of causing diabetes. (4) In the course of weeks the graft atrophies (Sandmeyer's experiment), and then a persistent glucosuria supervenes; or the encapsulated fragment which has been placed in an accessible place under the skin may be extirpated, in which case within a few hours a severe diabetes ensues. (5) There is no other organ in the body extirpation of which has any similar effect, nor (except for phlorhizinization), is there any known means of experimentally producing a *true* diabetes without injury to the pancreas. (6) No toxic substance derived from the body of diabetic individuals, man or animal, has been found which is capable of causing diabetes in a second animal. *These facts lead to the conclusion (reached by Minkowksi) that pancreatic tissue provides "a something," separate from the pancreatic juice (internal secretion of the pancreas), the lack of which is responsible for the symptoms of diabetes.*

[58] Capparelli, Biol. Zentralbl., 1893 (13), 495.

[59] See Langfeldt, Acta med. Scand., 1920 (53), 1.

[60] This statement, based on experimental work, appears in the 2d (1914) edition of this book.

[61] Allen (Amer. Jour. Med. Sci., 1920 (160), 781) points out that while diabetes may be prevented by retention of one-eighth of the pancreas, the removal of but one-third or one-fourth permits alimentary glucosuria, indicating that the pancreas has but little margin of safety in respect to normal carbohydrate utilization. When only one-eighth to one-tenth of the pancreas remains there is a persistent glucosuria if enough carbohydrates are fed, and hopeless diabetes uncontrollable by fasting results when but one-twentieth of the pancreas is left.

Islet Theory.—Morphologically the pancreas may be regarded as stroma, ducts, acini and islands of Langerhans. It has been proposed, notably by Opie[62] in this country, that the antidiabetic internal secretion of the pancreas is elaborated by islet cells. This view finds support in the following facts: (1) In diabetes melitus the islets are frequently found in a state of hydropic or hyaline degeneration, while the remaining organ may appear normal.[63] (2) Cancer, pancreatitis and the experimental injection of caustics into the ducts very frequently spare the islets and fail to cause diabetes. (3) It is claimed that in pancreatic grafts, such as described above, islet cells predominate, while acinus cells and ducts disappear.

Grafts of this kind consist of much connective tissue, generally more or less infiltrated with round cells, and collections of epithelium. Concerning the latter, remains of ducts and acini are usually present in some proportion, and there are also epithelial cell masses regarded as islets on morphological grounds. Differences of opinion still exist as to the relative proportion of the different epithelial elements. Lombroso,[64] whose exhaustive monograph reviews the literature to 1910, concludes that the internal function of the pancreas is not monopolized by islet cells. Bensley[65] developed intra-vital staining methods which, for the first time, made possible the sure differentiation of islet cells from duct or acinus epithelium without reference to form or arrangement, and appears to have proved that these cells are regenerated from duct epithelium. He also showed the great normal variations in size and number of islets in different individuals (guinea-pigs). His study explains certain of the discrepancies which occur in the literature, especially in the estimation of the quantity of islet tissue in pancreatic rests, grafts, etc. Allen[66] has reported that when proper sized fragments of pancreas, in connection with the ducts, are left *in situ*, and the remainder of the gland is removed, the subsequent development of severe diabetes may be coincident with disappearance of islet tissue while acinus cells and ducts are unaffected. This operation, according to Allen, is eminently satisfactory for producing experimental diabetes without infection and without loss of the external secretions.

Despite the abundant evidence of the relation of the islets to diabetes, it is well established that no constant pathological changes are found in the islets of persons dying with diabetes. For examples, in 26 cases studied by Warren and Root,[67] only 13 showed hyaline changes in the islets, and these were mostly in persons over 45 years of age; in five no changes at all were found, the other 8 showing slight sclerosis or lymphocytic infiltration. Furthermore, some normal islets seem to be present in all cases, no matter how great the changes may be in the others.

[62] "Diseases of the Pancreas," Lippincott & Co., 1910.
[63] See Homans, Jour. Med. Res., 1914 (30), 49.
[64] Ergeb. der Physiol., 1910 (10), 1.
[65] Am. Jour. of Anat., 1911 (12), 297.
[66] Glycosuria and Diabetes, Boston, 1913.
[67] Amer. Jour. Path., 1925 (1), 415.

There is a possibility that regeneration of islets takes place, especially under insulin treatment.[68]

Any doubt that previously existed as to the significance of the islets as sources of a specific hormone controlling carbohydrate metabolism, has been removed by the studies on

The Nature of the Internal Secretion of the Pancreas. Insulin.—The abundant work that has been done on this subject has been fully reviewed by Macleod,[69] from whose summary we have drawn extensively. It is the outcome of the pioneer observations of Mering and Minkowski on the diabetes that follows pancreas extirpation, and which made probable the existence of a pancreatic hormone, the final demonstration of which was accomplished in 1922 in Macleod's laboratory, by Banting, Best and Collip. Their success was foreshadowed by several observers, particularly Zuelzer (1907), E. L. Scott (1911), Knowlton and Starling (1912), Clarke (1917), Murlin (1913), Kleiner (1919), and Paulesco (1921), who succeeded occasionally in demonstrating the existence of substances capable either of diminishing one or other of the symptoms of diabetes in laboratory animals, or of increasing the rate of consumption of sugar by the perfused heart, but their observations were never carried far enough to carry conviction that a hormone capable of controlling all the symptoms of diabetes had been demonstrated.

The achievement of the Canadian investigators consisted in their removing, by fractional alcoholic precipitation, much of the extraneous and toxic materials in the extracts of the pancreas, so that the resulting product, "insulin," was sufficiently non-toxic to permit of its clinical and experimental use. This preparation, although undoubtedly far from a pure preparation of the active hormone, is tremendously active, so that the crude picrate in 0.1 mg. doses will cause the combustion of one to five grams of glucose, and insulin has been obtained containing but 0.002 mg. of nitrogen per unit (Clowes). Insulin behaves like a catalyst of the second order, *i. e.*, one which activates another catalyzer, which in this case accomplishes the oxidation of glucose.

Since pure insulin has not yet been isolated, its chemical nature is unknown. The general properties of the active preparations now in general use, are described by Macleod as follows:

> The solution can be evaporated to dryness *in vacuo* without loss of potency, and the powder contains about 14 per cent. of nitrogen, is free from phosphorus, but contains sulfur. A solution gives a distinct biuret test, but only faintly those of Millon and Hopkins-Cole. It gives the Pauly reaction (iminazole ring). The insulin is readily absorbed by kaolin and charcoal, and considerable losses are apt to occur in passing it through a porcelain filter. It does not dialyze through membranes or collodion sacs; it is precipitated by half-saturation with ammonium sulfate, by barium salts of weak acids, by picric acid and by uranium acetate. The solubility in water and alcohol is determined by the H-ion concentration, the iso-electric point being at a *p*H below 7. The insulin is relatively stable to mild oxidizing and reducing agents. Insulin is also soluble in phenol and the cresols, glacial acetic acid, and formamide.

[68] See Boyd and Robinson, Amer. Jour. Path., 1925 (1), 135.

[69] Physiol. Reviews, 1924 (4), 21. Also Campbell and Macleod, Medicine, 1924 (2), 195.

It is insoluble in most other organic solvents. These reactions, and its destruction by pepsin and trypsin, suggest that insulin is probably a substance having a very complex structure and protein-like in its nature (Dudley), but it is possible that it is really only adherent to this protein, and is itself of much simpler chemical structure. This view is supported by the fact that it is sometimes impossible to detect any biuret reaction with highly potent insulin prepared from the pancreas of the skate or to obtain from this source precipitates containing it by means of high concentrations of alcohol, or by adjustment of the iso-electric point. Whether it is really a protein or not, it is significant that all the methods now used for its purification are based on reactions that are characteristic of colloids. Thus, precipitation at the iso-electric point, or by picric acid followed by the regeneration from the picrate as a hydrochloride, or by benzoic acid, or by half saturation with ammonium sulfate.

Piper, Allen and Murlin[70] report that insulin is best preserved in solution when the *p*H is below 7 and above 5.7, and it seems not to lose potency when bacteria and yeasts grow in the solution. Although it somewhat resembles Hopkins glutathione it contains no sulfur in the sulfhydryl form and hence cannot be related to this oxidizing agent. Many observers believe that insulin is a polypeptid of considerable complexity,[71] and perhaps it is truly a protein complex.[72] The fact that consistent values for the glucose equivalent of insulin are readily obtained, supports the assumption that insulin must have a direct chemical action on glucose (Wilder).[73] Abel and Geiling[73a] found that phosphorus is not a constituent of insulin, but that it does contain sulfur in a labile form with which the insulin activity is apparently related.

Macleod says that typical insulin should exhibit the following physiological properties:

1. When given to normal animals (rabbits), it should cause immediate reduction of the blood sugar, and when hypoglucemic symptoms supervene these should be immediately and permanently removable by injections of glucose.

2. When given to diabetic (depancreatized) animals the symptoms (hyperglucemia, glucosuria, acetonuria) should immediately disappear and the general condition greatly improve; and when carbohydrate is also given the respiratory quotient should rise decidedly and glycogen be deposited in the liver.

Insulin is found, not only in the intact pancreas, but also in the pancreas in which the acinar tissue has been largely atrophied with persistence of the islets. This, and its isolation from the islet tissue of certain fish in which the islets occur independent of the acinar elements, conclusively establish the islets as the chief source of the active hormone, and probably the so-called β-cells of the islets are the ones that manufacture insulin. Best[74] and Scott have also succeeded in preparing insulin, or insulin-like substances, from other sources, including various organs and tissues both of normal and diabetic animals. It would appear,

[70] Jour. Biol. Chem., 1923 (58), 321.
[71] See Shonle and Waldo, Jour. Biol. Chem., 1924 (58), 731.
[72] Somogyi, Doisy and Shaffer, Jour. Biol. Chem., 1924 (60), 31.
[73] Endocrinology, 1924 (8), 630.
[73a] Jour. Pharm. Exp. Ther., 1925 (25), 423.
[74] See Amer. Jour. Physiol., 1924 (68), 161.

therefore, that the distribution of hormones capable of accelerating the disappearance of glucose from the blood is widespread, not only in the animal kingdom but in the vegetable as well. It is possible, however, that these substances merely stimulate the secretion of insulin from the pancreas of the animal into which they are injected, or serve as precursors for its production.

When insulin is injected into diabetics the effects are marked and definite, although transitory. The hyperglucemia, the glucosuria, and the ketonuria decrease promptly, the liver begins to store up glycogen, the respiratory quotient rises as the burning of sugar is resumed and the body energy begins to be derived from carbohydrates instead of only from fats and proteins. But how the insulin acts is still an unsettled problem. We know that it increases the storage of glycogen in animals given an abundance of glucose, and that it prevents the glucosuria that is produced by adrenalin, asphyxia, narcosis and piqûre. If insulin is added to a perfusion fluid passed through the heart, the amount of glucose removed from the fluid is greatly increased (from 0.3 to 8 mg.), but insulin added to a glucose solution *in vitro* has no recognizable effect. The effect of insulin is specific for glucose, for the hypoglucemia that results from injection of insulin can be completely relieved by no other sugar. As far as the evidence now available indicates, it is the intact glucose molecule, not any particular group of the molecule, that is essential for the inhibiting effect.[75]

Through what mechanism the insulin acts is unknown. It does not seem to affect the rate of glucolysis in solutions of glucose incubated with blood or tissues. Winter and Smith[76] have suggested that insulin causes a change from the more stable α and β varieties, as which it exists when in simple solution, to a more labile form called γ glucose, and that only this gamma glucose is utilizable in metabolism. This attractive hypothesis has not yet been confirmed,[76a] although there is evidence that in some way, as yet unknown, insulin does make glucose more susceptible to oxidation.[77] Macleod says that by exclusion we are forced to conclude that insulin, after entering the blood, must pass into the tissues, and set up in them some process which leads to a reduction in the amount of free glucose, thereby creating, as it were, a vacuum for this sugar, with the result that glucose is removed from the blood. He believes that insulin causes the sugar of the blood (and tissues) to diminish both in the normal and diabetic animal, partly because of an increase in the relative amount of carbohydrate that is metabolized and partly because some of the glucose is converted into non-saccharine material, which is mainly glycogen in diabetes, but is some other substance in the normal animal. In the latter case insulin actually causes the glycogen stores to become depleted because

[75] Insulin does not increase the oxidation of alcohol or acetone. (Hirschfelder and Maxwell, Amer. Jour. Physiol. 1924 (70), 520).

[76] Jour. Physiol., 1923 (62), 100.

[76a] See Denis and Hume, Jour. Biol. Chem., 1924 (60), 603.

[77] Neuberg *et al.* (Deut. med. Woch., 1923 (49), 1407) report that insulin increases the amount of acetaldehyde that is formed when liver tissue acts on glucose.

of the demand for glucose which it sets up, partly to form this unknown substance and partly to be oxidized. We fail to identify the substance because it has lost the reducing properties upon which the identification of sugars depends and, at the same time, it is not precipitated by alcohol as glycogen after treatment of the tissue with caustic alkali. Winter and Smith have found that, running parallel with the diminution in the concentration of sugar, the concentration of the inorganic phosphates also becomes reduced in the blood,[78] which would seem to indicate that the new substance is a compound containing a phosphoric acid group. These difficulties in accounting for the sugar which disappears, as a result of insulin, recall the equally mysterious disappearance of carbohydrate observed to occur when sugar in varying quantities is injected into normal animals. Even when the entire body is examined for sugar and glycogen in a few minutes following the injection, it is impossible to account at the most for more than about 70 per cent. of the injected carbohydrate.

Whatever the process may be, it is certain from the sudden rise in the respiratory quotient and the decrease in the ketonuria, that in some way insulin makes it possible for the diabetic organism to burn sugar as a normal organism does, and these facts establish the validity of the islet theory of diabetes, however inconstant the anatomical changes may be in the pancreas of diabetics. Anatomical changes do not necessarily correspond to functional defects and especially the functional results of impaired circulation may conceivably be much greater than the anatomical alterations. Therefore, some of the clinical diabetes, especially the milder forms in arteriosclerotic elderly patients, may rest more on a functional than on an anatomic basis. As Woodyatt points out,[79] in such cases the individual does have the power to produce insulin in increased amounts to permit of oxidation of a considerable part of any added glucose in his diet, *e. g.*, when with a glucose supply as high as 400 grams per day, all but 4 to 6 grams are utilized day after day, the individual must be able to produce enough insulin to effect the utilization of 394 to 396 grams of glucose. At the empirical figure of 1 unit of insulin to 2 grams of sugar this would imply the power to produce at least 197 units of insulin per day, and whatever else might be wrong with the patient's body it would seem certain that he does not lack insulin-producing cells in quantity sufficient to produce this large amount of insulin when called on to do so. On the other hand, the patient with serious anatomical injury to his islets excretes most or all the sugar given above the tolerance limit, as if he had little or no capacity to increase the insulin formation on demand. It is of course also possible that some forms of diabetes may depend not on absence of insulin but upon the presence of agents destroying or inhibiting the insulin, or preventing the tissue oxidation of sugar despite the presence of insulin. In this connection may be mentioned the augmentation of diabetes that is produced by intercurrent

[78] See also Staub *et al.*, Klin. Woch., 1923 (2), 2337.
[79] Southern Med. Jour., 1924 (17), 145.

infections or intoxications.[80] It has even been suggested that the trypsin may attack the insulin in the pancreas capillaries and so cause diabetes.[81]

Metabolism of Diabetes.—The general metabolism of the diabetic approximates the normal in mild and moderate types, but in severe diabetes it is low (Joslin).[82] The freezing point of the blood is much lowered in diabetes ($\Delta = -0.59$ to -0.62), not parallel to the hyperglucemia, suggesting that other unknown crystalloids are also increased (Lippmann).[83] In addition to the ketone bodies of diabetic acidosis, aldehydes have been found in the blood (Stepp).[84]

In the severest cases of diabetes, *i. e.*, "*complete diabetes*," glucosuria persists even when the individual subsists on a fat-protein diet, and after the glycogen in the body has been reduced to a mere trace. When this stage has been reached, and provided no carbohydrate food is eaten, it is found that the total glucose in the urine bears from day to day a constant ratio to the total nitrogen in the urine as already described for phlorhizin diabetes. This "G : N ratio" is not always the same. In depancreatized dogs nourished solely on fat and protein, it is often found, as Minkowski first recognized, at 2.8 : 1, and in human diabetes the same value for G : N is sometimes seen. But, as in phlorhizinized dogs, higher ratios may occur in the human disease.

If in such a case of diabetes as this we give by mouth 40 grams of glucose there may appear in the urine close to 40 grams of extra sugar. Plainly such extra sugar has escaped utilization of any kind. It cannot have been oxidized or converted into fat, since these processes are irreversible, although it might have existed momentarily in the body as glycogen or other isomer of glucose. What phase in the utilization of this glucose is primarily disturbed is another question. To say that 40 grams of ingested glucose causes the appearance of 40 grams of extra sugar in the urine does not prove that the diabetic body is inherently incapable of using any sugar or every carbohydrate. It might still be capable of using a two, three, or four carbon atom sugar, some other member of the group of 32 hexoses, or, as some have it (von Noorden), sugar which has first been built up into glycogen, etc., provided these substances could be kept from undergoing transformations into the non-utilizable glucose. As a rule, however, when other sugars are fed to complete diabetics, they are transformed into glucose and appear as such in the urine. This phenomenon has much of significance for the general theory of sugar metabolism and is an indication of the nature of the primary disturbance in diabetes, as will now be shown.

[80] No decrease in blood complement was found in diabetics to account for their known susceptibility to infection, by Rockwood and Beeler, Jour. Infect. Dis., 1924 (34), 625.

[81] Epstein *et al.* Amer. Jour. Physiol., 1925 (71), 316.

[82] "Diabetic Metabolism with High and Low Diets," E. P. Joslin, Carnegie Inst. Publn., 1923. See also Wilder, Boothby and Beeler, Jour. Biol. Chem., 1922 (51), 311.

[83] Zent. inn. Med., 1920 (41), 41.

[84] Biochem. Zeit., 1920 (107), 60.

Theory of Diabetes.—What sort of a chemical process is involved when levulose, for example, is converted in the body into glucose? As already stated in the chemical introduction, the reciprocal transformations of hexoses one into another in the alkaline solution *in vitro* depend upon a preliminary ionization of the sugars followed by salt formation, the salts then undergoing dissociation which, according to Mathews and Michaelis, is still purely electrolytic with rearrangements of the anion; but which, according to Nef, is a non-electrolytic dissociation of the type which he calls methylene dissociation. *Some form of dissociation must be a prelude also to these transformations in the body.* This view is logically just as necessary as it has been found to be for the organic chemist, and, it may be added, that for the oxidation of sugars as well as for their polymerization a preliminary dissociation is essential. Now since the diabetic body can transpose other sugars into glucose, it must be able at least to dissociate the former sugars deeply enough for this process. These transpositions are accomplished chiefly in the portal system and perhaps in other places too, but certainly levulose and many other substances can be made in the liver into glycogen, whose hydrolysis then yields glucose.

The degree or character of the dissociation necessary for reciprocal transformations differs from that which is a necessary prelude to destructive reactions such as oxidation. A very weak alkali suffices *in vitro* for the former, while for the latter it is necessary to use a somewhat stronger alkali concentration.[85] The diabetic body therefore behaves as though it were weakened with respect to the alkali concentrations which it can bring to bear on sugars.

As far back as 1871, Schultzen suggested that the error in diabetes might be found in the disability of the body to dissociate the glucose molecule into two 3-carbon substances.[86] Baumgarten[87] also supported the idea of a "fermentative splitting" which precedes oxidation, because he found a greater percentage utilization of certain substances closely allied to glucose (such as gluconic acid, saccharic acid, mucic acid, etc.), than of glucose itself; whereas gluconic acid and glucose, for example, differ only in that the sugar has an aldehyde group where the acid has carboxyl. Similar general ideas have been expressed from time to time by others. Woodyatt has urged in place of the vaguer terms, the adoption of chemical "dissociation" in the sense in which it is used in the field of pure organic chemistry, notably for the explanation of the behavior of aldehydes, ketones and alcohols.[88] There can be no doubt that the dissociation of glucose in the body is a normal occurrence. This is directly and conclusively shown whenever muscles make lactic acid ($C_3H_6O_3$) out of glucose ($C_6H_{12}O_6$) since in this process no chemical phenomenon is involved save cleavage of the hexose and intramolecular

[85] See Woodyatt, Jour. Biol. Chem., 1915 (20), 129.

[86] Glyceric aldehyde and glycerol, according to Schultzen.

[87] Zeit. f. exp. Path. u. Pharm., 1905 (2), 53.

[88] Cf. Nef, *loc. cit.*, and Stieglitz, Qualitative Chemical Analysis, New York, 1912, I, pp. 289–292.

rearrangement. The polymerization of sugar into glycogen might be similarly interpreted. Direct proof of a failure of glucose dissociation in diabetes has not yet been brought, although its absence would explain all the metabolic phenomena more directly and simply than any other single physiologic error which has been hypothecated.[89] It is, moreover, a tangible chemical conception, whereas to say that the body loses its power to oxidize sugar or to "fix" it as glycogen is merely to name effects in physiologic terms.

It might be assumed that all sugars upon entering certain phases of the cells (phases especially well represented in liver cells), meet conditions which are equivalent to those met in a weakly alkaline solution, favoring reciprocal transformations, and, as A. P. Mathews points out, polymerization; but not conditions conducive to the deeper destructive reactions. That is, especially in the liver, there may be *the equivalent* of dilute alkali for all sugars. Glucose, being the least dissociable, represents the form into which all other sugars tend to accumulate. But in the normal body a special glucolytic enzyme (alkali carrier or intensifier?) destroys glucose selectively. All other sugars must become glucose before destruction. In diabetes the condition necessary for the deep dissociation of glucose is lacking or inactive. The studies of Murlin, Kramer, Sweet and Karver, show that alkali administration (Na_2CO_3) may increase glucose utilization, especially when introduced into the duodenum where it may neutralize acid entering the bowel from the stomach and thus spare the liver and pancreas from the effects of absorbed acid. Underhill's experiments[90] with bicarbonate feeding in diabetes confirm these observations. Insulin, therefore, seems to behave like an alkali in facilitating the activation of sugar for oxidation, but since the chemical nature of insulin is unknown, we have no direct evidence that it has the properties of an alkali.

One difference between diabetes melitus and phlorhizin diabetes is that in the former the glucosuria is due to hyperglucemia, the sugar loss being an overflow like water escaping from an overfilled tank; whereas in phlorhizin poisoning there is apparently an hypoglucemia—the loss resulting in this case, to carry out the simile, from a leak in the bottom of the tank which keeps the water at a lower level. But the results are the same. Moreover, if in diabetes melitus we could measure only the chemically active or dissociated blood sugar, it is possible we should again find for this kind of sugar an hypoglucemia comparable to that of phlorhizin diabetes. This conception coincides with the doctrine that in diabetes melitus there is a *primary underconsumption of sugar* as opposed to the idea of *a primary overproduction.*

Overproduction vs. Underconsumption.—The chief exponents of overproduction have been the followers of Kraus, and of von Noorden in

[89] Cases of diabetes have been described associated with excretion of large amounts of *glycuronic acid*, which is a possible intermediate in glucose catabolism. (See Weber, Paris Méd., 1920 (10), 472.)

[90] Jour. Amer. Med. Assoc., 1917 (68), 497.

whose books "Die Zuckerkrankheit" and "New Aspects of Diabetes" will be found the arguments favoring this idea. A translation of Minkowksi's criticism of the latter has been made by Lusk.[91] In this place it may be briefly recalled that the chief arguments favoring underconsumption in addition to what has already been said are the following: (1) The respiratory quotient in diabetes is frequently found to be low, and when carbohydrate food is administered this quotient rises but little, whereas in health the administration of carbohydrate food results in a greater rise.[92] After insulin administration together with glucose, there is also a sharp rise in the respiratory quotient, together with a corresponding improvement in the condition of the patient in other respects. (2) The acetone bodies (acetone, aceto-acetic acid and beta-hydroxybutryic acid) appear in the urine when for any reason the quantity of sugar burning in the body falls below a certain minimum, as in starvation, or when a person accustomed to a mixed diet is suddenly switched to a full calory diet composed exclusively of fat, or of fat and carbohydrates, with the carbohydrate calories representing less than 10 per cent. and the fat calories more than 90 per cent. of the total (Zeller[93]). In these cases the restoration of sugar to the diet abruptly and permanently stops the output of acetone bodies. But in severe diabetes the excretion of acetone bodies is less affected by the giving of sugar unless insulin is also given. Following single large doses of glucose alone there may indeed be a temporary fall in the acidosis, but this is never permanently attainable. One interpretation made of these facts is as follows. In diabetes there is an acetone body output because sugar, although brought to the cells, fails to take part in certain chemical reactions which normally occur between sugars and certain of the breakdown products of butyric acid and which normally prevent the diabetic acidosis. Hence the bringing of more sugar has little effect. And here again one might suggest that in diabetes glucose fails to interact with the products mentioned because the glucose is not sufficiently dissociated or prepared by the insulin. Another interpretation has been to the effect that the sugar simply causes a compensatory decrease of the fat metabolism, *i. e.*, spares fat, thereby decreasing the formation of the acidosis bodies. The mechanism of the process is is in any case still a theme for research.

There are numerous other theories of diabetes, for the presentation of which the reader is referred to the larger works, and which are now largely of historic interest since the observations with insulin have given so much new information. Lépine has long stood for the view that the pancreas secretes a glucolytic oxidizing ferment. Naunyn's theory pays particular regard to the ability of the body to "fix" glycogen, while glycogen formation is held to be a necessary preliminary step in the utilization of sugar. The failure to fix glycogen he calls "*diszoamylie*," and the other metabolic

[91] Medical Record, Feb. 1, 1913.

[92] For the literature of respiration studies in diabetes see Joslin, Treatment of Diabetes Melitus, New York, 1916; and Diabetic Metabolism with High and Low Diets, Washington, 1923. Also Du Bois, Harvey Society Lectures, 1916; and "Studies from the Department of Physiology of Cornell University," 1915 *et seq.*; published in the Archives of Internal Medicine and reprinted as Bulletins.

[93] Arch. f. Physiol., 1914, p. 213.

disturbances he regards as sequences. The complex development of this same general idea by von Noorden, with the added element of primary sugar overproduction, has already been alluded to. Pavy saw in the diabetic a failure to *assimilate sugar;* that is, a failure of the body to incorporate sugar in a colloidal combination which would at once permit of its transportation to the points of utilization, and prevent its premature excretion. The assimilation he held occurred in the villi of the intestines, and the lymphocytes he regarded as the morphologic elements which carry the sugar. Cohnheim's theory that the muscle formed glucolytic enzymes, for which the pancreas supplies an essential activator, is without any substantial experimental support at the present writing. Allen proposed that the pancreas supplies an "amboceptor" which is essential for the proper colloidal blood sugar combination. The formation of hexosephosphates as a step in the preparation of glucose for combustion has been looked on by some as an important factor in diabetes, and McCann and Hannon[94] have found that some types of diabetes are characterized by a more ready oxidation of calcium hexosephosphate than of glucose. Brugsch[95] found evidence that insulin increases the formation of hexosephosphoric acid, and muscular activity increases phosphorus excretion.[96]

Bronzed diabetes, the name given to that form of hemochromatosis in which, along with the hepatic cirrhosis, there is an associated fibrosis of the pancreas, and, as a result of this, the symptoms of pancreatic diabetes, will be found discussed under the heading "hemochromatosis," Chapter XX.

Diabetic coma is discussed under "acid intoxication," Chapter XXII.

Lipemia, which is observed frequently and most severely in diabetes, is discussed in Chapter XVIII.

Glycogen in pathological processes is discussed in Chapter XVIII.

Xanthoma diabetica[97] is discussed under cholesterol in Chapter VIII.

[94] Bull. Johns Hopkins Hosp., 1923 (34), 73.
[95] Biochem. Zeit., 1924 (147), 112.
[96] Blatherwick *et al.*, Jour. Biol. Chem., 1924 (61), 241.
[97] See Fahr, Deut. Med. Woch., 1919 (45), 1373.

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