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INTRODUCTION TO
PHYSIOLOGICAL CHEMISTRY

BY MEYER BODANSKY

AND

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INTRODUCTION TO PHYSIOLOGICAL CHEMISTRY

BY

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SECOND EDITION

REWRITTEN AND RESET

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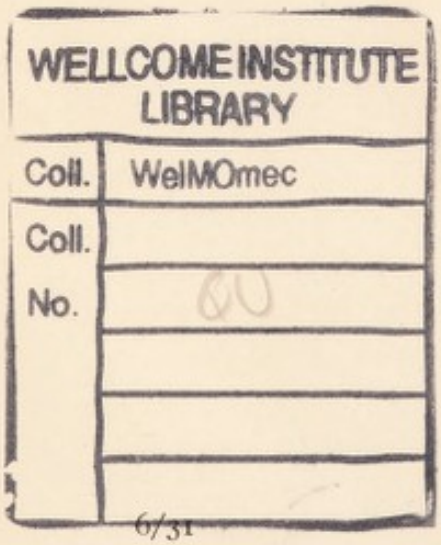

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PREFACE TO THE SECOND EDITION

THE privilege of revising this textbook is one which the author has sincerely appreciated, as it has given him the opportunity of incorporating many of the more recent contributions in physiological chemistry. In reviewing these contributions he has abstained from conveying an impression of finality and has attempted to show the changing aspects of the subject. The experience of the last few years has proven that even long cherished conceptions and apparently well established facts may at any time undergo drastic revision. The same may be expected of the future. With this subject in such a state of flux and rapid development it is not likely that even a frequently rewritten textbook will quite keep abreast of the times and it is therefore especially desirable that the student of biochemistry should be introduced to the literature as early as possible. For this reason, the author has continued the practice of referring directly to specific investigations and has included as abundant a bibliography as is consistent with moderate brevity.

In preparing this, the second edition, it seemed appropriate to add two chapters, one dealing with the composition of foodstuffs and the other devoted to a brief consideration of the composition of milk and of certain tissues, including bone, cartilage and muscle. Otherwise, the scope of the book, though somewhat extended, has not been changed materially.

The author takes this opportunity to thank Professor John J. Abel for the micro-photograph of insulin crystals, reproduced on page 296, and Dr. W. Robson for permission to use the diagram on page 335. Acknowledgment is also due to Dr. John Pryde and Messrs. J. and A. Churchill for permission to reproduce, with slight modifications, the diagram on page 207 from Pryde's "Recent Advances in Biochemistry," second edition.

To his colleagues and many friends in other institutions the author is deeply indebted for the warm reception accorded the first edition and for much valuable advice and criticism. It is also a pleasure to acknowledge his obligations to his associates at the University of Texas for their suggestions and to his publishers for the care which they have exercised in printing this work.

MEYER BODANSKY.

GALVESTON, TEXAS,
April, 1930.

PREFACE TO FIRST EDITION

In aiding the student to correlate physiological chemistry with allied sciences and to define its scope, a textbook fulfils a very useful purpose. A small book, if it is sufficiently coherent and comprehensive, is likely to serve this purpose better than a large one, valuable as the latter may be as a source of reference. It was this idea that stimulated the author to write the present book. He has aimed to make it brief enough for use as an introductory volume and yet to give it sufficient scope to cover the field comprehensively. Laboratory methods and the description of tests have been omitted intentionally, since they are to be found in laboratory manuals devoted to the subject. The main aspects of physiological chemistry have been developed in relation to recent advances in the science. It is hoped that in this way the student will be afforded not only a knowledge of fundamental principles but also a realization of the developmental state of the subject.

It is obvious that a certain amount of condensation has been necessary, but the author hopes that he has not condensed the material at the expense of vital information. Wherever he has felt that collateral reading would be desirable, he has referred the student to easily accessible sources, such as journal articles, reviews, monographs, and other works. The student who enters upon the study of physiological chemistry is, strictly speaking, not a beginner. He is not unfamiliar with the principles of inorganic and organic chemistry, and in many cases he has received some training in physico-chemical concepts. He has therefore attained sufficient maturity to profit by collateral reading.

For a considerable amount of the material the author was dependent upon various books and journal articles. To the authors of these he takes this opportunity to acknowledge his debt of gratitude. *Physiological Reviews*, edited for the American Physiological Society and published by the Williams and Wilkins Co., Baltimore, Md., and the "Monographs on Biochemistry," edited by R. H. A. Plimmer and F. G. Hopkins and published by Messrs. Longmans, Green and Co., have been of especial value to the author in helping him to correlate and synthesize the vast literature on the subject of physiological chemistry. He also takes this opportunity to express his gratitude to Dr. Graham Lusk and

Dr. D. D. Van Slyke for their kind permission to reproduce certain material from their works. The author also wishes to thank P. Blakiston's Son and Co., for their kindness in permitting him to reproduce, from Hawk's "Practical Physiological Chemistry," the absorption spectra given on page 157 of this book.

In the preparation of the book, much discerning advice and criticism was received from Dr. C. L. Alsberg, director of the Carnegie Food Research Institute at Stanford University. The author takes this occasion to express his sincere gratitude for Dr. Alsberg's unfailing interest and almost daily encouragement.

Some of the points of view developed in the present volume the author has derived from his teacher and friend, Dr. William C. Rose of the University of Illinois. He wishes to acknowledge at this time his debt to Dr. Rose, as well as a similar debt to Dr. Byron M. Hendrix of the University of Texas.

Important suggestions were also received from the author's colleagues at Stanford University, among whom are included Professors L. B. Becking, George S. Parks, and Robert E. Swain.

For reading the proof and for valuable criticism, the author is indebted to Dr. B. M. Hendrix and Dr. Marion Fay of the University of Texas. He also wishes to thank Miss Elizabeth D. Runge, librarian of the University of Texas School of Medicine, for her assistance in verifying the references.

The author will at all times welcome suggestions and criticism.

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December 8, 1926.

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INTRODUCTION TO PHYSIOLOGICAL CHEMISTRY

CHAPTER I

INTRODUCTION

THE borders of a science are not easily defined. This is particularly true of physiological chemistry, or biochemistry, a subject which is primarily concerned with the chemical phases of life. As a science it touches all the biological sciences and is of especial application in the field of medicine.

Much of the modern work in immunology and bacteriology, for example, has a chemical basis. The bacteriologist who wishes to study the growth of bacteria usually becomes involved in problems pertaining to their food, the acidity or alkalinity of their environment, the nature of their metabolic products, and many similar questions which require biochemical methods for accurate study. Most pathologists agree that biochemistry is playing, and will continue to play, a very important part in the development of pathology and medicine. This view is based partly on the rapid progress that has been made in recent years in the study of metabolic disorders and other diseases. It is safe to say that the biochemist who wishes to contribute to medical knowledge can do no better than to ally himself with a capable pathologist.

It is frequently difficult to determine where physiology ends and biochemistry begins, assuming that each has a beginning and an end. The two subjects are so closely interwoven that in many of the modern textbooks of physiology much space and emphasis are given to purely biochemical or even physico-chemical considerations. Similar tendencies are likewise exhibited by modern pharmacologists. In short, the biochemist is close kin to the physiologist, pharmacologist, and pathologist, and, in order to work in cooperation with them, he should learn something of their methods and viewpoints.

The student of biochemistry should be equipped with a knowledge

of fundamental chemical principles, as he must learn to apply these principles to physiological processes. He also requires technique in quantitative chemical analysis. Equipped with these tools, he may proceed to study the composition of tissues, the chemical constitution of foodstuffs, the fate of these in digestion and metabolism, the data of animal calorimetry, and similar problems. It should always be borne in mind, however, that in the nature of things he will be limited in certain respects. A tissue subjected to chemical manipulation is no longer a living tissue; the process of analysis frequently involves its destruction. One of the student's tasks, therefore, is to learn to correlate analytical data with function. As biochemists, we are especially interested in the numerous changes or reactions that occur in every living cell of the living organism.

Scope.—What, then, is to be the scope of our subject? It should obviously include a consideration of the composition and chemical properties of protoplasm. Protoplasm is the main constituent of the living cell, whether plant or animal, and is regarded by most biologists as the physical basis of life. In appearance it is slimy or jelly-like; its main constituents are present in finely divided aggregates or in a state of emulsion. Being a heterogeneous mixture, protoplasm exhibits the properties of substances in the colloidal state. Physico-chemical methods of study and physico-chemical concepts have therefore been most useful in contributing to our meager understanding of the properties and behavior of protoplasm.

We shall consider in this chapter, in a preliminary way, certain of the constituents that are found in living tissues. This will afford us a knowledge of what the body requires for the purposes of growth and maintenance, and will serve as an introduction to the study of the three main classes of foodstuffs—the carbohydrates, fats, and proteins. The chemical processes that render the food we eat assimilable are included under the head of digestion. In the chapter devoted to this topic, the progress of the foodstuffs in the alimentary tract will be followed, and a study will be made of the changes that take place under the influence of the saliva, the gastric juice, and the secretions of the pancreas and small intestine. The mechanism whereby the products of digestion pass through the intestinal wall into the blood and lymph will then be discussed. After absorption, the food material is carried to the tissues where it is used either for tissue formation or for energy production. The higher animals possess a very elaborate transport system, the blood and lymph. This system is also concerned with the transportation of oxygen to the tissues and of carbon dioxide from them.

In the tissues, substances undergo many chemical transformations. These changes will then be considered in the chapters devoted to tissue oxidations and intermediary metabolism. The end-products of metabolism are taken up by the blood and carried to the various organs of excretion. A study of the urine will be undertaken in this connection. In metabolism, as a result of the oxidative changes which the foodstuffs undergo, energy is produced in the form of heat, work; in certain cases, light, as in the fire-fly, and electricity, as in the electric eel and torpedo ray.

Within the past two decades, much has been accomplished also in the field of nutrition. It is now well understood not only that there must be an adequate supply of carbohydrate, fat, and protein in the diet, but also that the character of these substances, particularly that of the proteins, is an important factor in determining their usefulness to the organism. Thus, proteins lacking certain amino acids, such as tryptophane, lysine, tyrosine, cystine, histidine and arginine, are of limited value to the higher organism in the repair of worn-out tissues. This is because these organisms, including man, are unable to synthesize the necessary amino acids from other substances. Certain inorganic or mineral constituents are equally essential to life. Among these may be mentioned iron, calcium, sodium, potassium, phosphorus, iodine, and chlorine. Finally, in the absence of certain recently discovered but as yet chemically undefined food factors, called vitamins, normal growth, maintenance, and reproduction are impossible. All of these topics will be considered in the chapter devoted to nutrition.

At times, normal physiological processes become deranged. In diabetes the human organism loses the power of properly utilizing carbohydrates. Injury to the kidney may be accompanied by changes in the composition of the blood. The study of these and other pathological conditions lies, likewise, within the province of biochemistry. Biochemical methods of diagnosis have become exceedingly valuable to the physician and surgeon.

General Properties of Living Matter.—Certain properties are usually recognized as distinguishing the living from the lifeless. These are motility, irritability, reproduction, nutrition, respiration, metabolism, and growth. J. Loeb (Dynamics of Living Matter, 1906, p. 1) has defined living organisms as chemical machines consisting chiefly of colloidal material and possessing the peculiarity of preserving and reproducing themselves. Elsewhere (The Organism as a Whole, 1916, p. 23) Loeb states that the essential difference between living and non-living matter consists in this: the living cell synthesizes its own complicated specific material from indifferent or non-specific simple com-

pounds of the surrounding medium, while a non-living substance, like a crystal, simply adds the molecules found in its supersaturated solution. He believes that the synthetic power of transforming small "building stones" into complicated compounds specific for each organism is one of the secrets of life. It would appear, therefore, that the function of enzymes (see Chapter VI) is of fundamental importance. The orderliness and interdependence of the reactions of the living organism have also been contrasted with the apparently uncorrelated, unordered reactions that follow shortly upon death.

Other manifestations that may be associated with death are the changes in the hydrogen-ion concentration of the tissues, and the alterations in their oxidation-reduction potentials, i.e., the capacity of the tissues to oxidize and reduce other substances. On close examination, it will be seen that autolytic changes in tissues, changes in hydrogen-ion concentration, and changes in oxidation potential are all interdependent. As has been essentially pointed out by Lotka,[1] it is the study of the manifestations associated with the phenomena of life, rather than its definition, that is the province of science to-day.

Composition of Protoplasm.—Among the essential constituents of protoplasm are proteins, lipids (fats and fat-like substances), probably a small amount of carbohydrate, inorganic salts, carbon dioxide, and water. Quantitatively, water is the most important constituent, most tissues containing between 75 and 90 per cent. In young animals or in actively growing tissues, there is a greater proportion of water than in older animals or in less active tissues. Many of the properties of protoplasm are dependent on its water content, any variation from the normal affecting cell permeability and the distribution of electrolytes inside and outside the cell membrane.

Elementary Composition of Tissues.—Of the elements, oxygen is the most abundant. The human body is more than 60 per cent oxygen. Combined with hydrogen, it is present as water; together with hydrogen and carbon, it forms the carbohydrates. Carbon is second in order of abundance. Oxygen, hydrogen, carbon, nitrogen, and sulfur enter into the composition of proteins. Phosphorus is present in combination in phosphoproteins, phospholipids, nucleic acids, nucleoproteins, and in inorganic combinations particularly in blood and bone. Sulfur is likewise present in a variety of combinations but chiefly in the amino acid cystine, which is a constituent of nearly all proteins. It is especially abundant in the proteins of hair, nails, horns, feathers, wool, and other sclerous tissues.

[1] A. J. Lotka, Elements of Physical Biology, Williams and Wilkins, Baltimore (1925), p. 18.

Sodium and potassium are widely distributed in plants and animals and are very important physiologically. Potassium is more abundant than sodium in plants and is believed to take part in certain synthetic reactions, such as the photosynthesis of protein. The rhythmic contraction of muscle, especially of heart muscle, has been attributed to the radioactive properties of potassium. In the blood plasma, sodium is more abundant than potassium, whereas the reverse holds in the red corpuscles. The presence of lithium in lung tissue has likewise been reported.

Calcium is especially abundant in the skeletal structures of vertebrates and marine algæ. Magnesium is also widely distributed, having been found in liver, kidney, brain, heart, muscle, bone, etc. It is a component of the chlorophyl molecule, chlorophyl being the green coloring matter of plants and a very important factor in plant economy. Certain forms of algæ contain large amounts of magnesium.

Iron is an essential constituent of plant and animal protoplasm. It is a constituent of hemoglobin, which is a protein present in red blood corpuscles. Hemoglobin has the property of readily combining with oxygen to form oxyhemoglobin. The latter in turn is dissociated or reduced at low oxygen tensions. In this way, hemoglobin takes part in respiration, by acting as a carrier of oxygen from the lungs, where the oxygen tension is high (about 100 mm. Hg), to the tissues, where the tension is low (5–10 mm. Hg). In certain of the lower animals, the Mollusca and Crustacea, copper-protein compounds (hemocyanins) are present and are said to play a rôle similar to that of hemoglobin. It has also been shown that copper, zinc, manganese, aluminum, and even nickel and cobalt are normal constituents of plant and animal organisms. Of these, copper and possibly manganese stimulate the formation of red blood corpuscles under certain conditions.

Iodine is a constituent of brain and liver, but is especially important because of its presence in thyroxin, the physiologically active constituent of the thyroid gland. Many marine plants contain iodine as well as bromine. Animal tissues are likewise known to contain small amounts of bromine. Quantitatively, chlorine is the most important of the halogens, being present in combination with sodium and potassium in all body fluids and contributing in that way to the maintenance of the osmotic relations of the tissues. Fluorine occurs in plants and is also an important constituent of bones, teeth, and the shells of molluscs.

Silicon is found in plants and in many marine organisms, such as diatoms and sponges. In the higher animals, it has been found in hair, skin, thymus, and muscle, and in the lens of the eye, where it is present to the extent of about 0.05 per cent.

Among the more rarely occurring elements may be mentioned arsenic (found in minute quantities in the thyroid, brain, liver, hair, etc.), boron, lead (present in certain corals), and vanadium (found in the blood of Ascidia). Still other elements, including cerium, barium, radium, strontium, and even gold, have been reported as present in living tissues, but whether these are normal constituents of protoplasm or whether their occurrence is merely adventitious, it is impossible to say.

To summarize, the following elements are known to be more or less widely distributed in animal tissues: hydrogen, carbon, oxygen, nitrogen, sulfur, phosphorus, potassium, sodium, lithium, calcium, magnesium, iron, manganese, silicon, chlorine, bromine, iodine, fluorine, aluminum, arsenic, cobalt, copper, nickel, zinc, vanadium.

Relation between the Composition of the Earth's Crust and that of the Human Body.—A general idea of the relative amounts of the elements that go to make up the human organism may be obtained from Table I, compiled by Lotka. The earthly origin of man and the relation of the chemical composition of his body to that of the earth's crust

TABLE I

AVERAGE COMPOSITION OF THE HUMAN BODY*

	Pounds	Per cent
Oxygen	97.20	63.03
Carbon	31.10	20.20
Hydrogen	15.20	9.90
Nitrogen	3.80	2.50
Calcium	3.80	2.50
Phosphorus	1.75	1.14
Chlorine	0.25	0.16
Fluorine	0.22	0.14
Sulfur	0.22	0.14
Potassium	0.18	0.11
Sodium	0.16	0.10
Magnesium	0.11	0.07
Iron	0.01	0.01
Total	154.00	100.00

* From A. J. Lotka, Elements of Physical Biology, Williams and Wilkins Co., 1925, p. 197.

have always aroused much interest and speculation. Despite the apparent abundance of plant life, the carbon content of the earth's crust is only about 0.18 per cent. The carbon content of the human body

is about 20.2 per cent. Next to oxygen, silicon is the most abundant element in nature; yet in our bodies it is present in an almost negligible amount. Aluminum is likewise plentiful in the earth's crust, but is present in exceedingly small amounts in the human body.

Relation to Composition of Sea Water.—Comparative studies of the mineral constituents of tissue fluids have brought out the highly interesting fact that there is a remarkable uniformity of composition in different animals. A particularly extensive study has been made with respect to the elements sodium, potassium, calcium, and magnesium. In the following table are given the results obtained by Macallum with

TABLE II

COMPOSITION OF SERA OF ANIMALS AS RELATED TO THAT OF SEA WATER*

Species	Na	K	Ca	Mg
Dogfish (*Acanthias vulgaris*)	100	4.6	2.7	2.5
Cod (*Gadus callarias*)	100	9.5	3.9	1.4
Pollock (*Pallachinus virens*)	100	4.3	3.1	1.5
Dog	100	6.9	2.5	0.8
Man	100	6.1	2.7	0.9
Crab (*Homarus americanus*)	100	3.7	4.9	1.7
Limulus polyphemus	100	5.6	4.1	11.2
Jellyfish (*Aurelia*)—tissue fluid	100	5.2	4.1	11.4
Ocean water	100	3.6	3.9	12.1

* After Macallum, Trans. Coll. Phys. of Philadelphia, **39**, 289, 1917.

the blood sera of various animals, the results being calculated on the basis of 100 for the percentage concentration of sodium in any given species. In the table are also included analyses of ocean water. What is the significance of these observations? In the first place, it is to be noted that the proportion of calcium to sodium in sea water and in sera is nearly the same. The correspondence is not so close with potassium, but nearly so. On the other hand, there is considerable variation in the case of magnesium. Macallum and, somewhat earlier, the German physiologist, Bunge, have suggested that the high content of sodium chloride in the blood of vertebrates may be an inheritance from our remote ancestors who lived in the sea. Supposing that these animals took to the land after the development of a closed circulatory system, it might follow that the composition of the sea, as it was at that time, has persisted in their blood to the present day. How, then, are we to account for the divergences in potassium and magnesium? It has

been suggested that since the Cambrian period less potassium has been supplied to the sea than prior to that time, because so much of this element has been required by plant life, which has been more profuse since the Cambrian era. On the other hand, the magnesium content of the ocean and the proportion of magnesium to sodium have been steadily increasing since pre-Cambrian time, the concentration found at present in the higher animals corresponding presumably to the low magnesium content of the sea at the time the animals in question acquired a terrestrial habitat. The calcium content has been increasing but slowly, owing perhaps to the utilization of calcium in the building of corals and the bones and shells of other marine organisms. To sum up, it may be supposed that the blood serum of mammals resembles, except for the difference in its magnesium content, diluted sea water of our own day.[2]

The body fluids hold in solution a variety of substances. It is a curious fact, and one with which the student is doubtless familiar, that substances in solution behave very much like gases. For this reason, a brief review of the gas laws is appropriate in this connection.

Gas Laws.—The relations governing the behavior of gases were first discovered empirically and, as later found, only approximately. Boyle's law (1662) states that when the temperature of a gas is held constant, the volume varies inversely as the pressure:

$$\text{Volume} = k_1 \frac{1}{\text{Pressure}}, \text{ or } V = k_1 \frac{1}{P}.$$

Gay-Lussac's law (1801) states that when the pressure is held constant, the volume varies directly as the absolute temperature:

$$V = k_2 T$$

Combining these two equations,[3] we obtain:

$$PV = kT,$$

[2] For further details the student is referred to A. B. Macallum, The Paleochemistry of the Body Fluids and Tissues, Physiol. Reviews, **6**, 316 (1926); see also F. W. Clarke, The Data of Geochemistry, 4th edition, United States Geological Survey, Bulletin 695.

[3] The steps in the derivation follow:

$$V = \frac{k_1}{P} \text{ (Boyle's law)}; \quad V = k_2 T \text{ (Guy-Lussac's law)}; \quad P = k_3 T.$$

(The last expression is a corollary of the first two laws; it states that at constant volume, the pressure of a gas is proportional to its absolute temperature.)

The value of k may be found by substituting for P the standard pressure of 1 atmosphere; for V, the volume of the molecular weight of the gas in grams; and for T, the standard temperature, 0° C., or 273° Absolute. Using the proper units, k, which we will call the *molecular gas constant* and designate by R, becomes 0.08204 liter-atmosphere per degree, or 8.31×10^7 ergs per degree.[4] Since, by Avogadro's Law, the molecular weight of all gases occupies the same volume at a given temperature and pressure, the relation $PV = RT$ will hold for all gases. The equation naturally applies to quantities of a mol when the proper factor is introduced. Hence, dropping subscripts and introducing this factor, we obtain the general formulation of the three gas laws:

$$PV = nRT$$

when n denotes the number of mols of gas present.

The above relation, $PV = nRT$, is approximate, holding for certain gases only within certain temperature and pressure ranges. When it is attempted to give this relation a theoretical background, as in the kinetic molecular theory, it is necessary to assume (1) that the molecules of a gas are so far apart that they exert no attraction upon each other and (2) that the space which they themselves occupy is negligibly small in comparison with the volume of the containing vessel. These are conditions attained by no real gas and hence may be taken as properties of the ideal or perfect gas. In chemical thermodynamics compliance (1) with the relation $PV = nRT$ and (2) with the relation that the energy is a function of the temperature alone is taken as the definition of a perfect gas.

Multiplying these together, $V^2P = k_1k_2k_3 \frac{T^2}{P}$;

$$V^2P^2 = k_1k_2k_3T^2;$$

or

$$PV = \sqrt{k_1k_2k_3T^2} = kT.$$

[4] $R = \frac{(1 \text{ atmosphere})(22.4 \text{ liters})}{273} = 0.08204$ liter-atmosphere, per degree.

In the c.g.s. system of units, $R = \frac{(76 \text{ cm. Hg pressure})\ (22{,}400 \text{ cc.})}{273°}$. A pressure of 76 cm. Hg is equal to a force of $76 \times 13.6 \times 980$, or 1.013×10^6 dynes per $\overline{\text{cm.}}^2$ Therefore,

$$R = \frac{\left(\frac{1.013 \times 10^6 \text{ dynes}}{\overline{\text{cm.}}^2}\right)(22{,}400\ \overline{\text{cm.}}^3)}{273°} = 8.31 \times 10^7 \text{ ergs, per degree.}$$

Laws of Solution, Osmotic Pressure.—Of the many properties which substances in solution exhibit, one of the most interesting is that of osmotic pressure. The classical experiments of Pfeffer may be used to illustrate the phenomenon. The accompanying sketch (Fig. 1) shows the form of Pfeffer's apparatus. A precipitate of copper ferrocyanide is deposited in the walls of a porous cup *A*. *B* is a mercury manometer. The cup is filled with the solution to be tested, the surface of the latter being made even with the surface of the mercury in *C*. Tube *D* is then sealed off and the whole apparatus immersed in a bath of distilled water kept at constant temperature. The water at first passes through the membrane, increasing the pressure inside the apparatus and forcing the mercury up the manometer tube. This continues until a point is reached when there is no further increase in pressure in the cup. The reading of the manometer is then taken as the osmotic pressure of the solution.

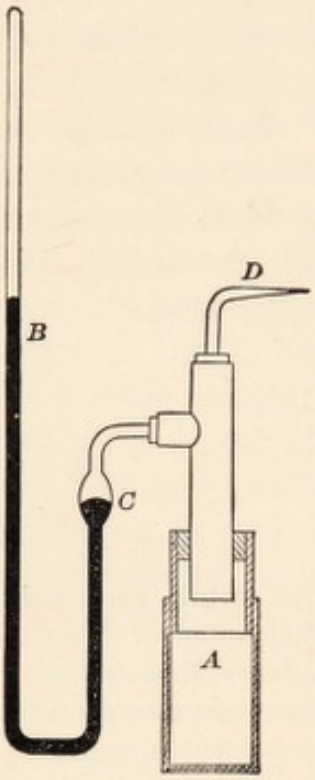


FIG. 1.

Before considering the mechanism of this phenomenon, let us see what relation it bears to the concentration of the solute and the temperature of the solution. The following data from Pfeffer show that the osmotic pressure is directly proportional to the concentration.

TABLE III

RELATION OF OSMOTIC PRESSURE TO CONCENTRATION

C Per cent of cane sugar	P cm. Hg	$\frac{P}{C}$
1	53.5	53.5
2	101.6	50.8
4	208.2	52.1
6	307.5	51.3

This relation, the importance of which was first pointed out by van't Hoff, may be put into a slightly different form. In making up solutions of a definite amount of material, the concentration of the solution varies inversely as the volume of the solution. For instance,

2 gms. dissolved in 100 cc. gives twice as strong a solution as the same amount dissolved in a volume twice as great, 200 cc. Hence, if the osmotic pressure of a solution is directly proportional to the concentration of the solute, it is inversely proportional to the volume.

$$\text{Volume} = k_1 \frac{1}{\text{Pressure}}.$$

This relation is analogous to Boyle's law for gases.

Similarly, as the following table indicates, the relation between osmotic pressure and temperature is analagous to Gay-Lussac's Law, the pressure varying directly with the temperature.

TABLE IV

VARIATION OF OSMOTIC PRESSURE WITH TEMPERATURE

Temperature	T Absolute Temperature $273° + °C.$	Osmotic Pressure in cm. Hg	$\frac{\text{Osmotic Pressure}}{T} = k_2$
6.8° C.	279.8°	50.5	0.181
13.2° C.	286.2°	52.1	0.182
14.2° C.	287.2°	53.1	0.185
22.0° C.	295.0°	54.8	0.186
36.0° C.	309.0°	56.7	0.183

The question that next presents itself is whether Avogadro's hypothesis applies to solutions. The significance of Pfeffer's work was not appreciated until van't Hoff recalculated the data in order to bring out the existing relations between pressure, volume and temperature. In studying the effect of temperature on osmotic pressure, Pfeffer used a 1 per cent solution of cane sugar (1 gram in 100.6 cc. of solution) or one containing 0.02906 mol of sucrose per liter. From the gas laws, van't Hoff calculated the gas pressure of 0.02906 mol of hydrogen at 0° C. and found it to be 0.649 atmosphere. Since according to the formula, $P = k_3T$, the pressure of the gas would increase $\frac{1}{273}$, or 0.00367 for each degree above 0° C., or 273° A., then for any other temperature, the pressure would be

$$0.649\ (1 + 0.00367t)$$

where t is the temperature in Centigrade. Van't Hoff now recalculated Pfeffer's data on this basis, comparing the observed results of osmotic

pressure with the values computed for the various temperatures from the gas laws. These data are tabulated below:

TABLE V

Temperature	Observed pressure in atmospheres	Calculated gas pressure in atmospheres
6.8° C.	0.664	0.665
13.7° C.	0.691	0.681
15.5° C.	0.684	0.686
22.0° C.	0.721	0.701
32.0° C.	0.716	0.725
36.0° C.	0.746	0.735

In the case of gases, R, the molecular (or molar) gas constant, in the equation $PV = RT$ is equivalent to 0.08204 liter-atmosphere. Taking 0.649 as the osmotic pressure of 1.0 per cent sucrose at 0° C. and 760 mm. Hg pressure, and 34.2 liters as the volume occupied by one mol of the solute, van't Hoff obtained 0.0813 as the value for R when the gas equations were applied to the solution. These results led him to the important conclusion that "*the osmotic pressure exerted by any substance in solution is the same as it would exert if it were a gas in the same volume as that occupied by the solution.*" All this holds in dilute solutions where the volume of the solute is small as compared with the volume of the solvent.

The formula $PV = nRT$, known as the van't Hoff equation, applies only to certain substances, such as urea, glucose, sucrose. Van't Hoff pointed out that the osmotic pressure of salts of strong acids and strong bases was greater than would be expected from this formula. How was this to be explained? In 1887 Arrhenius advanced the hypothesis that the molecules of certain substances when brought into solution dissociate into electrically charged particles or ions. This is known as the theory of electrolytic dissociation. It will be recalled that, many years before, Faraday classified substances as electrolytes and non-electrolytes; the former, he believed, were broken up by the passage of the electric current. What really happens, however, is that the molecules dissociate spontaneously and the ions carry the electric charge. The osmotic effect of a salt that dissociates almost completely is nearly twice as great as that produced by an equimolecular quantity of a non-electrolyte like urea or cane sugar. This is so because each molecule of NaCl, for example, may yield two particles or ions.

The laws of dilute solution which we have outlined above are not detached pieces of information. They can be shown to follow rigorously, i.e., mathematically, from the First and Second Laws of Thermodynamics. We shall not attempt to show this mathematical development here, but a clearer idea of the mechanism underlying the laws of dilute solutions, as well as various other phenomena to be discussed later, such as osmotic pressure and Donnan's theory of membrane equilibria, may perhaps be obtained by considering some of the simpler concepts of thermodynamics.[5]

The First Law is familiar to us as the Law of Conservation of Energy. Whenever a system undergoes a change in energy, the change is evidenced in the heat absorbed from or given off to its environment and the work done by or on the system.

Increase in energy of a system as it changes from a state A to a state B	is equal to	the heat absorbed	minus	the work done by the system on its surroundings.	
$E_B - E_A$ or ΔE	=	q	−	w	(1)

The Second Law, known as the Law of Entropy, is more difficult to understand. Ordinary observation shows us that every system, left to itself, changes in such a way as to approach a definite final state of equilibrium. Substances diffuse from concentrated solutions to dilute ones, heat passes from hot bodies to cold, clocks run down. In short, systems lose their capacity for spontaneous change. And they can lose this capacity without losing any energy. For instance, let us conceive of a system in which there is a hot body and a cold one. The heat passes from the former to the latter, until finally the two are at the same temperature. No energy has been lost, but the capacity for spontaneous change has vanished. The more a system lacks the capacity for spontaneous change, the more entropy it is said to have. It is not possible to calculate the absolute entropy of a system, but changes in entropy may be expressed as follows:

Increase in entropy of a system as it changes from a state A to a state B	is equal to	$\frac{\text{Heat absorbed}}{\text{Absolute Temperature of system}}$	
$S_B - S_A$, or ΔS	=	$\frac{q}{T}$	(2)

A system in changing from one state to another at constant temperature bears the potentiality of doing a certain maximum amount of work. Actually this potentiality is never realized; friction and other sources of degradation interfere. But it is sometimes approached. For instance, zinc dropped into a beaker of sulfuric acid forms zinc sulfate and hydrogen. There is not much work done; the hydrogen evolved does a little against the atmosphere. But the same zinc can be rigged up as one electrode of a galvanic cell; connected together with another of hydrogen in

[5] For a more detailed discussion the student is referred to G. N. Lewis and M. Randall, Thermodynamics, McGraw-Hill Co., New York, 1923.

contact with a platinized electrode to a motor, it forms an arrangement in which much more work is obtained. Theoretically, eliminating sources of degradation, a process can be obtained realizing the entire potentiality of maximum work.

In such a theoretical, reversible process the First Law would naturally also hold

$$\Delta E = q - w, \text{ or transposing, } q = w + \Delta E.$$

According to the Second Law:

$$\Delta S = \frac{q}{T} \text{ or } q = T\Delta S.$$

Substituting for q,

$$w = T\Delta S - \Delta E.$$

This equation expresses the work done in such a process. Every time the system does an amount of work, w, the maximum amount of work (we shall designate this as A), which it is capable of doing, is decreased by just that amount. $-\Delta A$, then, or the change in A equals w.

$$-\Delta A = w = T\Delta S - \Delta E$$

or

$$\Delta A = \Delta E - T\Delta S.$$

It must be realized that not all of this work which is done is available for external utilization. For instance, in the galvanic cell, some of the work is electrical; some of it is done against the constant pressure of the atmosphere. Only the former is available. It is *net* work. It is free,—free for useful purposes. For this reason it is known as *free energy*.

Change in maximum work = Net work or Free Energy + Unavailable Work.

If a system can do no net work; if, in short, it shows no change in free energy, then quite obviously it is in equilibrium. In this way, then, the state of equilibrium is defined by the equation

$$\textit{Change in Free Energy} = 0, \text{ or } dF = 0.$$

Another way in which the state of equilibrium can be viewed is through the concept of "escaping tendency." Material substances of a system tend to escape from one part to another: molecules of water pass from a solution to the air above; molecules of sucrose leave concentrated regions for more dilute ones. At equilibrium, the escaping tendency of each substance will be constant throughout the system. For most of us a very concrete representation of escaping tendency exists in the notion of the pressure of a gas: the greater the pressure, the greater the tendency for the molecules to escape.

The exact relation between the free energy and the pressure can be derived from the two fundamental laws and the definitions already laid down.

$$\begin{array}{lcccccl} \text{Change in free energy of a system as it changes from state } A \text{ to state } B & = & \text{Gas constant} & \times & \text{Absolute temperature} & \times & \log \dfrac{\text{Pressure in State } B}{\text{Pressure in State } A} \\ F_B - F_A & = & R & \times & T & \times & ln \dfrac{P_B}{P_A} \end{array}$$

Due to the assumptions which the derivation uses, this equation applies only to systems which are perfect gases. In a perfect gas, the pressure is identical with escaping tendency. But in other cases this is not so. A term, "fugacity," has therefore been conceived which may be substituted for pressure. When this is done in the above equation, we obtain the relation between the change in free energy and the fugacity, or escaping tendency, of any type of substance.

$$F_B - F_A = RT\ln\frac{\text{Fugacity } B}{\text{Fugacity } A}$$

$$= RT\ln\frac{f_B}{f_A}$$

In studying systems which are solutions, we are interested in knowing just how much of the change in free energy or, more generally, in any property is affected by the change in the amount of the components. This is expressed by the term, *partial molal quantity*. For instance, in a solution containing n_1 mols of solute and n_2 mols of solvent, the partial molal free energy of the solute equals the rate of change of the free energy of the solution as the amount of solute is altered.

$$\overline{F}_1 \text{ (partial molal free energy of solute)} = \frac{\delta F}{\delta n_1}$$

As was stated above, the laws of dilute solutions can all be deduced mathematically from the two fundamental laws. The meaning of the equation so obtained, in the case of osmotic pressure, can now be illustrated.

Suppose we have a cell with two compartments, each containing distilled water and separated by a membrane MM' through which the water

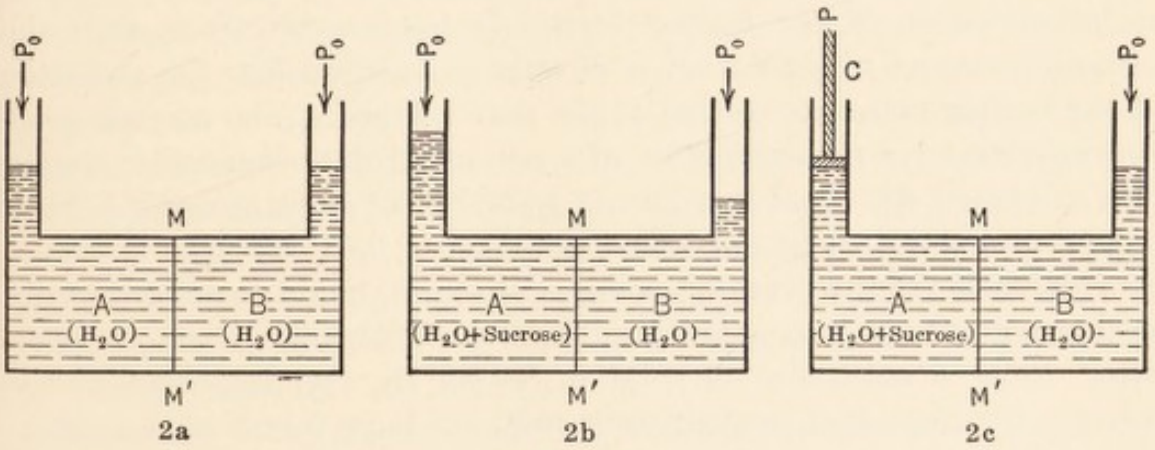


FIG. 2.

can pass (Fig. 2a). In this case the tendency of the water molecules to escape from A into B will be equal to the tendency for them to pass from B into A. As a result, no water passes either way. Suppose, now,

that some soluble substance such as sodium chloride or sucrose which does not pass through the membrane is added to A. The addition of a solute lowers the escaping tendency of the solvent. Hence the escaping tendency of the water in A is now lowered whereas that in B, remaining the same, exceeds it. Water, therefore, tends to pass into compartment A and the level in A tends to rise. However, a sufficient pressure, P, exerted on the solution in A by means of piston C will prevent an actual passage of water This pressure, P, *minus* the original (usually atmospheric) pressure, P_0, on the pure solvent is known as the osmotic pressure. The equation relating the osmotic pressure to other variables is:

$$\frac{d(P - P_0)}{dN_2} = \frac{RT}{\overline{v_1}}$$

where N_2 is the mol fraction of the solute, $\overline{v_1}$, the partial mol volume of solvent, R, T, P, P_0, the meanings we have already assigned. In very dilute solutions this equation reduces to van't Hoff's familiar $(P - P_0) = nRT$, or $PV = nRT$ (p. 9).

Elevation of the Boiling-point.—The vapor pressure of a liquid increases with temperature. When the vapor pressure of the liquid equals the pressure of the atmosphere above it, the liquid boils. If a non-volatile substance is added, the vapor pressure is decreased. Hence it takes a higher temperature to produce a vapor pressure equal to the atmospheric pressure, that is, to make the liquid boil. The elevation in boiling-point produced by dissolving one mol (the molecular weight in grams) of a substance in 1000 grams of solvent may be termed the molar elevation of the boiling-point. It follows, therefore, that the osmotic pressure of a solution is directly proportional to the elevation of the boiling-point above that of the pure solvent. The osmotic pressure exerted by a molar solution of a non-electrolyte (molecular weight of a substance dissolved in a liter of water) is 22.4 atmospheres. Such a solution exhibits a boiling-point elevation of 0.54° C.

Depression of the Freezing-point.—Biological fluids do not ordinarily lend themselves to boiling-point methods for determining osmotic pressure. Since a substance in solution lowers the freezing-point of the solvent, freezing-point or cryoscopic methods have found wide application. Water freezes at 0° C. A molar solution of a non-electrolyte in water lowers the freezing-point of the water to − 1.86° C. Illustrative data of freezing-point measurements of the blood sera of various mammals are given below:[6]

[6] From Robertson's "Principles of Biochemistry," 1924 edition, p. 283.

TABLE VI

Species	Lowering of freezing-point or Δ	Species	Lowering of freezing-point or Δ
Man	0.526°	Rabbit	0.592°
Ox	0.585°	Dog	0.571°
Horse	0.564°	Cat	0.638°
Pig	0.615°	Sheep	0.619°

These data show, in the first place, that the sera of widely differing species of animals exhibit remarkable similarity of osmotic pressure. In the second place, since the freezing-point depression (Δ) is about $\frac{1}{3}$ that of a molar solution of a non-electrolyte the osmotic pressure of the blood is about 7.5 atmospheres. Urine usually has a much higher osmotic pressure, the value of Δ being frequently greater than 2.0°. In severe nephritis, abnormally high values for Δ of the blood serum have been observed.

Osmotic Pressure Phenomena in the Organism.—The osmotic pressure of solutions depends on the concentration of solute and the nature of the membrane. In the animal organism one of the most important membranes is the capillary endothelium. Under normal conditions, it will allow everything but proteins to pass from the circulatory system to the tissue spaces. The proteins, then, depress the escaping tendency of the water on the circulatory side of the capillary endothelium and offer resistance to the capillary blood pressure. Where the other side contains no protein, as is the case in Bowman's space in the glomeruli of the kidney, this depression of the escaping tendency will amount to about 30 to 40 mm. Hg, as was first shown by Starling,[7] and the filtration of water through the capillaries of the glomeruli will encounter that much resistance. The fluid of the tissue spaces, on the other hand, has about half the protein content of the blood. Hence the resistance which water will encounter in filtering from capillary to tissue space should only amount to 15 to 20 mm. Hg.

In certain pathological conditions, the protein content of the blood is lowered or else changes so that it is not as effective as normally in depressing the escaping tendency of water. Filtration into the tissue spaces becomes easier and edema results. This topic will be considered again in other connections.

[7] J. Physiol., **19**, 312 (1896).

De Vries[8] made the first attempts to measure osmotic pressure in living cells, using the epidermal cells of certain plants for this purpose. The outer wall of most plant cells consists of a framework or skeleton of cellulose. In the normal state, the protoplasm within the cells presses closely against this framework. Placing a plant cell in solutions of higher osmotic pressure than that of the cell sap results in loss of water from the cell; the protoplasm contracts and draws away from the outer membrane as shown in Fig. 3*d*. Owing to its rigidity, the cellulose wall can withstand considerable changes of internal pressure. The phenomenon described is called plasmolysis. The degree of plasmolysis is determined by the concentration of the outer fluid. If the plas-

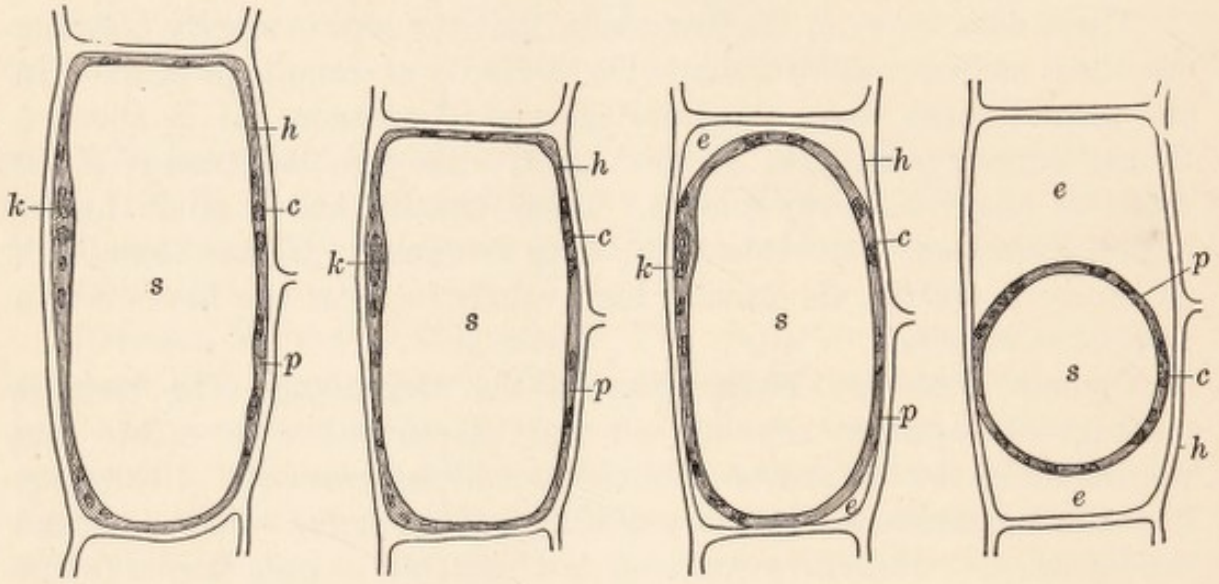


Fig. 3*a*. Fig. 3*b*. Fig. 3*c*. Fig. 3*d*.

Fig. 3*a*.—Young, partly-grown cell of the cortical parenchyma of the peduncle of *Cephalaria leucantha*. Fig. 3*b*.—The same cell in 4 per cent KNO_3. Fig. 3*c*.—The same cell in 6 per cent solution. Fig. 3*d*.—The same cell in 10 per cent solution. Figures 3*a* and 3*d* drawn from nature. Figures 3*b* and 3*c* schematic. All in longitudinal optical section. *h*, cell wall; *p*, parietal protoplasm; *k*, nucleus; *c*, chloroplasts; *s*, cell sap (in vacuole); *e*, intruded salt solution. (After Hugo de Vries, Opera e Periodicis Collata, vol. **1**, 296.)

molyzed cells are placed in solutions of lower osmotic pressure than that of the cells themselves (i.e., in hypotonic solutions), they will regain their former appearance and turgor. In a solution of equivalent osmotic pressure to the cell contents (isotonic solution), plasmolysis does not occur. The osmotic pressure of cells of unknown tonicity can be determined by suspending them in a series of solutions of known concentration and observing the concentration of the solution that just fails to

[8] Jahrbuch für wissenschaftliche Botanik, **14**, 427 (1884); Z. physikal. Chem., **2**, 415 (1888).

cause plasmolysis. Conversely, cells of known tonicity may be employed in determining the osmotic pressure of unknown solutions.

The membrane surrounding red blood corpuscles cannot withstand great changes of internal pressure. For this reason, when red corpuscles are immersed in hypotonic solutions, they swell, and, if the swelling is sufficient, the cells may burst. The process is called hemolysis and consists in the liberation from the corpuscles of hemoglobin, the red coloring matter of the blood. On the other hand, red corpuscles contract when placed in solutions of higher osmotic pressure (hypertonic solutions), the membrane acquiring a characteristic irregular or crenated appearance. The behavior of red corpuscles toward solutions of varying concentration has been made the basis of a method for determining indirectly the osmotic pressure of solutions. It involves the use of the hematocrite, which is a graduated capillary tube of small diameter into which blood may be drawn. On centrifuging at high speed, the corpuscles separate from the serum and collect at one end of the tube, the volume occupied by the corpuscles being read off from the graduations on the tube. When immersed in an isotonic solution, the corpuscles do not change in volume; but when suspended in hypotonic or hypertonic solutions, they increase or diminish in volume, as the case may be.

Electrolytic Dissociation.—Measurement of the degree of dissociation of electrolytes in solution is made by determining the electrical conductivity of the solution. For description of methods, the student is referred to modern textbooks on physical chemistry.[9] Acids, bases, and salts are electrolytes and in water dissociate into cations (positively charged ions) and anions (negatively charged ions).

Dissociation of Acids.—An acid, HA, will dissociate as follows:

$$HA \rightleftarrows H^+ + A^-$$

Applying the mass-law equation,

$$\frac{[H^+] \times [A^-]}{[HA]} = K_a$$

K_a is the dissociation constant of the acid in question; $[H^+]$, the concentration of hydrogen ions; $[A^-]$, the concentration of the negative ions, and [HA], the concentration of the undissociated acid.

[9] W. C. McC. Lewis, A System of Physical Chemistry, Longmans, Green & Co., 1925. H. S. Taylor, A Treatise on Physical Chemistry, Van Nostrand, 1924. F. H. Getman, Outlines of Theoretical Chemistry, John Wiley & Sons, Inc., New York, 1926 edition.

Dibasic acids dissociate in two steps; for each step there is a different constant, as follows:

$$H_2A \rightleftarrows H^+ + HA^-$$
$$HA^- \rightleftarrows H^+ + A^=$$

Applying the mass law to this acid,

$$\frac{[H^+] \times [HA^-]}{[H_2A]} = K_1; \quad \frac{[H^+] \times [A^=]}{[HA^-]} = K_2$$

For oxalic acid, $K_1 = 3.8 \times 10^{-2}$, $K_2 = 4.9 \times 10^{-5}$; for tartaric acid, $K_1 = 9.7 \times 10^{-4}$, $K_2 = 4.5 \times 10^{-5}$.

Phosphoric acid is tribasic and ionizes in three steps:

$$H_3PO_4 \rightleftarrows H^+ + H_2PO_4^-$$
$$H^+ + HPO_4^=$$
$$H^+ + PO_4^{\equiv}$$

The three constants are:

$$K_1 = 9 \times 10^{-3}; \quad K_2 = 8.8 \times 10^{-8}; \quad K_3 = 3.6 \times 10^{-13}.$$

Acids that are highly dissociated (strong acids) will give, according to the mass-law equation, a high value for the dissociation constant K. Weakly dissociated acids will give low values. Acid solutions of equivalent normality have the same amount of replaceable hydrogen, but the concentration of hydrogen ions in an acid solution depends on the degree of dissociation of the acid. Thus, 0.1N hydrochloric acid contains the same amount of replaceable hydrogen as 0.1N acetic acid, but the concentration of hydrogen ions in the former is approximately 65 times greater than in the latter. Physiologically, the effect of acids frequently depends on the concentration of hydrogen ions.

Dissociation of Bases.—Bases ionize according to the equation:

$$BOH \rightleftarrows B^+ + OH^-$$

where B is any basic radical. The dissociation constant is derived from the relation:

$$\frac{[B^+] \times [OH^-]}{[BOH]} = K_b$$

Ionization of Water.—In view of the preceding discussion it is natural to inquire regarding the behavior of water. Water dissociates according to the equation:

$$HOH \rightleftarrows H^+ + OH^-$$

From the mass law, it follows that

$$\frac{[H^+] \times [OH^-]}{[H_2O]} = K_W$$

However, the degree of dissociation is so slight that the concentration of the undissociated portion is very nearly the same as the total concentration. Accordingly, it is permissible to simplify the equation to

$$[H] \times [OH] = K_W.$$

The dissociation constant for water has been measured by many investigators, the value generally accepted being $K_w = 1.012 \times 10^{-14}$ at 25° C. Since on dissociation a molecule of water yields one hydrogen ion and one hydroxyl ion,

$$[H] = [OH] = \sqrt{K_W} = 1.006 \times 10^{-7}$$

or approximately 1×10^{-7}. This means that 1 liter of water contains one ten-millionth of a gram of hydrogen ions, or that there is one gram of hydrogen ions in 10 million liters of water. A tenth-normal solution of hydrochloric acid contains one gram of hydrogen ions in 10 liters of water.

***p*H.**—For several reasons it is usually more convenient to express hydrogen-ion concentration in simplified form. In 1909, Sørensen pointed out that there were advantages in designating hydrogen-ion concentration in terms of the logarithm (to the base 10) of its reciprocal. This suggestion has since been accepted universally. Sørensen gave to $\log \frac{1}{[H]}$ the symbol P_H, but for typographical reasons it has been found more convenient to use the form *p*H. Thus, a neutral solution has a hydrogen-ion concentration (C_H) or $[H^+]$ value of 1×10^{-7}. The *p*H value of such a solution is therefore $\log \frac{1}{1 \times 10^{-7}}$, or $\log 10^7$, or 7.

If the hydrogen-ion concentration is given, the corresponding *p*H may be calculated as follows:

$$[H^+] = 2 \times 10^{-6}$$

$$pH = \log \frac{1}{[H]}; \quad \therefore pH = \log \frac{1}{2 \times 10^{-6}}$$

$$= \log 1 - \log (2 \times 10^{-6})$$

Since

$$\log 1 = 0, \; p\text{H} = -\log (2 \times 10^{-6})$$
$$= -\log 2 - \log 10^{-6}$$
$$= -.301 + 6$$
$$p\text{H} = 5.699, \text{ or approximately } 5.7$$

pH values may be converted into hydrogen-ion concentration as follows:

$$p\text{H} = 2.3$$
$$= 3 - .7$$
$$= 3 - \log 5.01$$
$$= \log \frac{1}{5.01 \times 10^{-3}}$$
$$[\text{H}^+] = 5.01 \times 10^{-3}.$$

Acid solutions have a pH range below 7, whereas the range of alkaline solutions is above 7. The following table of figures, showing approximately the relation of normality of HCl and NaOH solutions to pH, will make this point clear. For this purpose the assumption is made that the acid and alkali are completely ionized.

TABLE VII

Normality	Concentration of H ions	Concentration of OH ions	pH	pOH
N HCl	1	10^{-14}	0	14
0.1HCl	10^{-1}	10^{-13}	1	13
0.01HCl	10^{-2}	10^{-12}	2	12
0.001HCl	10^{-3}	10^{-11}	3	11
0.0001HCl	10^{-4}	10^{-10}	4	10
0.00001HCl	10^{-5}	10^{-9}	5	9
0.000001HCl	10^{-6}	10^{-8}	6	8
0.0000001HCl	10^{-7}	10^{-7}	7	7
Neutrality	10^{-7}	10^{-7}	7	7
0.0000001NaOH	10^{-7}	10^{-7}	7	7
0.000001NaOH	10^{-8}	10^{-6}	8	6
0.00001NaOH	10^{-9}	10^{-5}	9	5
0.0001NaOH	10^{-10}	10^{-4}	10	4
0.001NaOH	10^{-11}	10^{-3}	11	3
0.01NaOH	10^{-12}	10^{-2}	12	2
0.1NaOH	10^{-13}	10^{-1}	13	1
N NaOH	10^{-14}	1	14	0

As stated, these values are approximate, since neither HCl or NaOH are 100 per cent ionized. Closer approximations are given in the following table, which includes data for acetic acid and ammonium hydroxide, a weak acid and base, respectively.[10]

TABLE VIII

Acid	Normality	$[H^+]$	pH
HCl	1.0	8.0×10^{-1}	0.1
	0.1	8.4×10^{-2}	1.071
	0.01	9.5×10^{-3}	2.022
	0.001	9.7×10^{-4}	3.013
	0.0001	9.8×10^{-5}	4.009
NaOH	1.0	0.89×10^{-14}	14.05
	0.1	0.85×10^{-13}	13.07
	0.01	0.76×10^{-12}	12.12
	0.001	0.74×10^{-11}	11.13
CH_3COOH	1.0	4.3×10^{-3}	2.366
	0.1	1.36×10^{-3}	2.866
	0.01	4.3×10^{-4}	3.366
	0.001	1.36×10^{-4}	3.866
NH_4OH	1.0	1.7×10^{-12}	11.77
	0.1	5.4×10^{-12}	11.27
	0.01	1.7×10^{-11}	10.77
	0.001	5.4×10^{-11}	10.27

The Determination of Hydrogen Ions.—In the present volume it is possible to refer only very briefly to the two methods that are in common use for the determination of hydrogen ions. The first is an electrometric method; the second involves the use of indicators. Very valuable treatises on this subject have been prepared by W. M. Clark[11] and Michaelis. Another valuable source of information is Kolthoff and Furman's book[12] on potentiometric titrations.

[10] Temperature exerts an appreciable effect on the pH of bases, but with acids, the pH is practically independent of the temperature. The data for sodium and ammonium hydroxide are for 18° C. In all cases, the pH values are those given by Michaelis in Die Wasserstoffionen-konzentration, 2d edition, Berlin, 1922, p. 21; also in Michaelis, L., Hydrogen Ion Concentration, translated by W. A. Perlzweig, Baltimore, 1926, p. 24. The data for the hydrogen-ion concentration have been corrected in several instances.

[11] W. M. Clark, The Determination of Hydrogen Ions, 3d edition, Baltimore, 1928.

[12] I. M. Kolthoff and N. H. Furman, Potentiometric Titrations, John Wiley & Sons, Inc., New York, 1926.

If a stick of metal, such as zinc, is placed in water, some of the metal dissolves, producing positively charged ions. This leaves the metal negatively charged. The metal then attracts the positively charged ions; a difference of potential thus results. In general the magnitude of the potential difference depends upon two factors—the tendency of the metal or electrode to dissolve in pure water and the concentration of the metallic ions in solution. If the two are equal there is no difference of potential. The larger the difference between the two, the greater is the difference in electrical potential. This principle is the basis of the electrometric method. Certain metals, such as platinum and palladium, have the power of adsorbing large quantities of hydrogen gas, thus forming a hydrogen electrode. There is no satisfactory method for measuring the potential of one electrode; but it is possible to combine the single electrode with a standard electrode of known potential, thereby obtaining a voltaic cell, the electromotive force (e.m.f.) of which may be measured. For the standard electrode, one may use a hydrogen electrode dipping into acid of known hydrogen-ion concentration.

Other forms of electrodes have been devised and have found wide application in biological studies. These electrodes are adequately described in the works mentioned above.

Indicator Method.—Organic indicators are dyes which are essentially weak acids or weak bases and give rise to color changes in varying degrees of acidity and alkalinity. A familiar example of an acid indicator is phenolphthalein. This compound exists in two tautomeric forms; in acid solution it is in the colorless form, whereas when added to an alkaline solution it acquires a magenta color. Methyl orange is a weak base which in acid solution is pink and, in basic solution, yellow. A number of indicators exhibit gradations in color within certain ranges of hydrogen-ion concentration and have therefore found extensive application in biochemistry.

The color that develops when a given indicator is added to a solution of unknown *p*H may be compared with the color given by the same indicator in solutions of known hydrogen-ion concentration. These standard solutions are usually prepared from mixtures of highly purified electrolytes which act as buffers, a buffer solution being one that does not readily change its hydrogen-ion concentration upon the addition of small amounts of acid or alkali. The buffer solutions used frequently as standards contain the following constituents.[13]

[13] Details as to the quantities to be used and as to methods of preparation are to be found in the book by Clark, previously cited.

Constituents	For *p*H range
KCl—HCl	1.2– 2.2
KH Phthalate—HCl	2.2– 3.8
KH Phthalate—NaOH	4.0– 6.2
KH_2PO_4—NaOH	5.8– 8.0
KCl—NaOH	7.8–10.0

Buffer standards may be prepared for a wide range of *p*H values. A large number of dyes are now also available for the determination of hydrogen-ion concentration. Among these are the following indicators:

Indicator	*p*H range	Indicator	*p*H range
Thymol blue	1.2–2.8	Bromthymol blue	6.0–7.6
Bromphenol blue	3.0–4.6	Phenol red	6.6–8.2
Methyl red	4.4–6.0	Cresol red	7.2–8.8
Bromcresol purple	5.4–7.0	Thymol blue	8.2–9.8

As we proceed from one phase of biochemistry to another, we shall see the important bearing of hydrogen ions upon physiological phenomena.

Colloids and Crystalloids.—The student is no doubt familiar with the observations of Thomas Graham,[14] who, in 1861, found that certain substances,—urea, sodium chloride, sucrose, etc.,—readily diffused through parchment membranes, but that other substances,—gelatin, egg albumin, starch, etc.,—failed to do so. To distinguish the two classes of substances, Graham called the first group crystalloids, and the non-diffusible substances, because of their glue-like character, colloids. Aqueous colloidal solutions Graham called hydrosols. When sufficiently concentrated, these set to a gel, hence the name hydrogel.

A substance may, however, exhibit, under one set of conditions, the properties of a crystalloid, whereas, under different conditions, it may behave as a colloid. For example, sodium chloride dissolved in water is a crystalloid; in benzene it forms a colloidal solution. It is more correct, therefore, to speak of a substance as being either in the colloid or the crystalloid state.

[14] Liebig's Annalen, **121**, 1 (1862).

Homogeneous and Heterogeneous Systems.—Colloids are usually, though not always, amorphous, and in water frequently form viscous solutions. However, the real criterion of the colloidal state is that the particles are so much larger than molecules that they possess surface, and yet not so large as to settle out easily by the action of gravity. In true solution, a substance exists either as ions, molecules, or small aggregates of molecules. The individual particles cannot be distinguished even with the aid of the ultramicroscope. Solutions of this type are said to be homogeneous; there is but one " phase," a term first employed in this sense by the distinguished American scientist, J. Willard Gibbs. Colloidal systems, on the other hand, are made up of more than one phase, and are therefore heterogeneous. Suppose a solid mass, such as a bar of gold, were placed in water. This would constitute a two-phase system, the solid mass being one phase; the water, the other. There would still be two phases even though the gold bar were subdivided into smaller and smaller particles until particles of colloidal dimension were obtained. In a colloidal system, the suspended particles constitute the dispersed or internal phase (also dispersoid); the other phase is the dispersion medium or external phase (also continuous phase).

Size of Colloidal Particles.—The limit of vision with the aid of the microscope is about 0.0001 mm. or 0.1μ.[15] Colloidal particles range in diameter between $1\mu\mu$ and $100\mu\mu$ ($1\mu = 0.001$ mm.; $1\mu\mu = 0.001\mu$) and therefore are below the range of microscopic visibility. It is possible, however, to detect particles of colloidal dimension by their diffraction images. Faraday, and later Tyndall, observed that when a beam of light is sent through a clear solution of finely divided gold, some of the light is diffracted by the solid particles. The effect is the same when a beam of sunlight passes into a darkened room through a small opening. The light is made visible because of the scattering of a portion of it by the particles of dust in its path. In turn, the motion of these particles is made visible in the diffused light. Even distilled water may contain particles which exhibit a similar effect, but the phenomenon is much more apparent in colloidal suspensions. This is known as the Tyndall or Faraday-Tyndall phenomenon and is of importance in this connection because upon this principle is based the ultramicroscope.

The Ultramicroscope.—This instrument was devised by Siedentopf and Zsigmondy.[16] It may be so arranged that a beam of light can be passed horizontally into a liquid or colloidal solution at right angles to the line of vision through a microscope. The presence of

[15] μ = micron; $\mu\mu$ = milli-micron.

[16] R. Zsigmondy, Colloids and the Ultramicroscope. Trans. by Alexander. John Wiley & Sons, Inc. Also, R. Zsigmondy, Kolloidchemie, Leipzig, 1921.

colloidal particles in the liquid is made evident by small points of light in more or less rapid motion against a dark background. This is brought about by the diffraction of rays of light by the colloidal particles. Exceedingly minute particles do not give rise to individual points of light but rather to areas of diffuse light. Diffraction images can also be obtained by having the light enter the solution from the side. This is accomplished with the aid of a condenser which does not permit direct illumination to reach the eye of the observer.

This apparatus has been modified by Zsigmondy. The objectives of two compound microscopes are so cut away that when the microscopes are placed at right angles they may be brought very close together (Fig. 4). The liquid to be examined is introduced in the space between the two objectives, where it is held by capillary attraction, thus obviating the necessity for a cell and large amounts of material. With this arrangement, it is possible to discern particles whose diameters range from 1 to 2 milli-microns. Further details are beyond the scope of the present work and are to be found in treatises devoted to colloid chemistry.

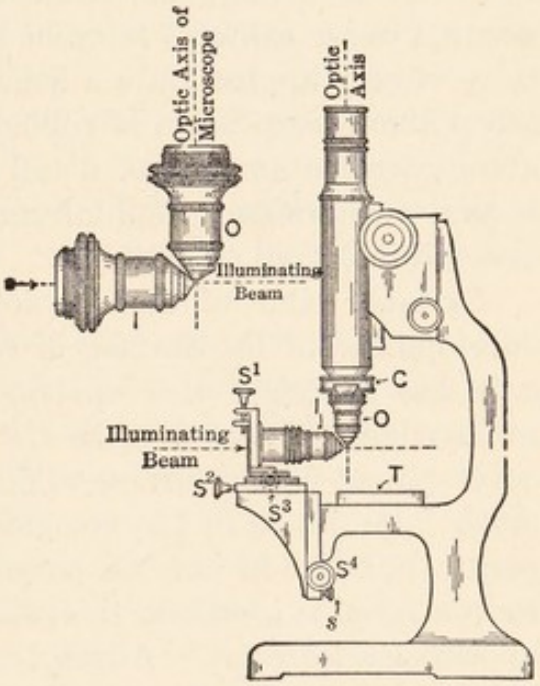


FIG. 4.

Brownian Movement.—The English botanist, Robert Brown, observed in 1827 that, in water, particles of plant pollen were in continual motion. This property is quite general with all suspensions and emulsions where the particles are not too large. The phenomenon has been given the name Brownian movement and is due to the bombardment of the colloidal particles by molecules and ions. By the law of probabilities, the number of impacts on one side of a colloidal particle at any one moment may be expected to be greater than at other points. This will result in motion in the direction of most impacts. Brownian movement is more marked in the case of smaller colloidal particles than in that of larger ones.

Suspensoids and Emulsoids.—The division of colloids into two classes is based on the ability of some colloids to take up water. Kaolin, platinum, gold, arsenious sulfide, etc., when in the colloidal state consist of pure solid and are hence classified as suspensoids (also lyophobic,

or hydrophobic, literally "water-hating," colloids). Other colloids in aqueous solution have so marked an attraction for water that the dispersoid may contain large quantities of water. These are the emulsoids (also hydrophilic or lyophilic, literally "water-loving," colloids). The latter group is of primary importance physiologically, protoplasm being essentially an emulsoid type of colloid. Familiar examples are starch, soap, egg white and gelatin. A certain amount of confusion is due to the fact that colloidal systems in which both phases are liquid are usually classified as emulsions. According to Bayliss,[17] it would be better to confine the name emulsoid to those cases in which the internal phase contains more or less water, although it may sometimes more closely approximate a solid. It would be impossible to give an acceptable classification of colloids without bringing into the discussion a considerable amount of detail and the conflicting points of view of several authorities. This information belongs more properly to works devoted to colloid chemistry.

Surface.—One of the characteristics of a dispersed system is the development of the surface of contact between the phases. A 1-cm. cube has a surface of 6 sq. cm. Subdivision of this into exceedingly small cubes, having 0.1μ as the side dimension, would result in the development of the surface to 60 sq. m. The question of surface is of much importance in physiological phenomena in which surface forces play a part, as in enzyme action. Many substances tend to become concentrated at a surface, this phenomenon of surface condensation being known as adsorption. Adsorption is an important property of colloids for the reason that these have a large surface as compared with their mass. Adsorption is doubtless a factor in the staining reactions of tissues, the action of drugs, and many other phenomena.

Electric Charge.—Colloidal particles usually carry either a positive or a negative electric charge and are attracted to poles of opposite sign. The neutralization of the charge of colloidal particles causes them to be precipitated. The movement of electrically charged particles toward an oppositely charged electrode is known as cataphoresis. Hemoglobin, ferric hydroxide, and aluminum hydroxide are electropositive; gold, silver, platinum, arsenious sulfide, kaolin, and charcoal are examples of electro-negative colloids.

Viscosity.—Viscosity is due to the internal friction of the molecules of a liquid. Solutions are almost always more viscous than the pure solvents. The viscosity of suspensoids in water is not much greater than that of the water, but in the case of emulsoids the viscosity is very markedly increased. Thus, the viscosity of 1 per cent agar is several

[17] Bayliss, The Colloidal State in its Medical and Physiological Aspects, p. 13.

thousand times that of water. Increasing the concentration of the dispersed phase, particularly in the case of emulsoids, increases the viscosity of colloidal systems.

Imbibition and Swelling.—When gelatin takes up water, the total volume is less than the sum of the volumes of the two constituents, indicating that the water in the gelatin is under great pressure. Similar observations have been recorded with other colloids. Bayliss refers to an experiment of Hatschek in which the compression of water imbibed by a preparation of gum tragacanth was equivalent to 400 atmospheres.

Northrop and Kunitz [18] have contributed considerably towards the elucidation of the phenomenon of swelling. They list three types: (1) the swelling of proteins in acid and basic regions, studied by Procter and Wilson [19] and by Jacques Loeb,[20] (2) the initial swelling of dry gelatin, studied by Katz,[21] and (3) the swelling of gelatin solutions of concentrations from 50 down to 5 per cent, studied by Northrop and Kunitz themselves.

In the first type of swelling, the absorption of water is assumed to be due to an osmotic effect. In the case of gelatin, for example, we may look upon the dispersed phase as a salt of gelatin enclosed within a gelatin membrane. The gelatin salt ionizes; but, as the ions cannot leave the membrane, water passes in, by osmosis, and swelling occurs. Acids and alkalies increase the swelling of colloids, but on the addition of neutral salts the swelling due to acid is greatly diminished. In the case of proteins, the addition of neutral salts would have the effect of making the concentration of the salt outside the protein particles greater than its concentration inside, in accordance with the Donnan equilibrium, with the result that the osmotic pressure of the protein would decrease relatively, and the swelling would decrease, as, in fact, it does.

The initial swelling of dry gelatin in water is evidently not connected with the Donnan equilibrium. Katz has shown that the heat effects, volume, pressure and vapor pressure changes are strictly analogous to those observed in the formation of concentrated solutions of many substances. For details, the original paper must be consulted.

As for the third type, Northrop and Kunitz have shown that solutions of gelatin consist of at least two fractions: one insoluble in cold water, the other soluble. Gelatin in concentrated solutions, then, is assumed to consist of an insoluble mesh which holds within it a solution capable of

[18] J. Gen. Physiol., **10**, 161, 893, 905 (1926–27).
[19] J. Chem. Soc., **109**, 307 (1916).
[20] J. Loeb, Proteins and the Theory of Colloidal Behavior, 1924, New York.
[21] Kolloidchem. Beihefte, **9**, 1 (1917–18).

exerting osmotic pressure. Swelling is due to the passage of water into the interstices containing the solution. Quantitative expressions for the kinetics and equilibrium conditions of this swelling have been developed on the basis of this mechanism.

Donnan's Theory of Membrane Equilibria.—The application of Donnan's theory [22] of membrane equilibria to physiological problems has received much attention during the past few years. Briefly stated, the theory defines the relations which will exist between the ions of a solution of electrolytes separated by a membrane which is impermeable to one of the ions. Let us consider, as an illustration, two electrolytes, NaR and NaCl, on opposite sides of a membrane, represented below by a vertical line.

(1)	(2)
Na^+	Na^+
R^-	Cl^-

Of these ions R^- cannot diffuse through the membrane. At equilibrium therefore, the following condition will exist.

(1)	(2)
Na^+	Na^+
R^-	
Cl^-	Cl^-

We can now use the concepts described earlier in the chapter to show how the relation between these ions is obtained. The change in free energy is expressed by the following equation (p. 15).

$$dF = RTln \frac{\text{Fugacity } B}{\text{Fugacity } A}$$

At equilibrium, a transfer, made reversibly, of δn mols of Na^+ and Cl^- from (2) to (1) will not cause any change in the value of the free energy. Using as an approximation, concentrations instead of fugacities, the following expression is obtained:

$$dF = O = \delta n \cdot RT \log_e \frac{[Na^+]_2}{[Na^+]_1} + \delta n\ RT \log_e \frac{[Cl^-]_2}{[Cl^-]_1}$$

Transposing and cancelling:

$$\log_e \frac{[Na^+]_2}{[Na^+]_1} = -\log_e \frac{[Cl^-]_2}{[Cl^-]_1}$$

$$\log_e \frac{[Na^+]_2}{[Na^+]_1} = \log_e \frac{[Cl^-]_1}{[Cl^-]_2}$$

[22] Donnan, Chem. Reviews, **1**, 73 (1924).

or

$$\frac{[Na^+]_2}{[Na^+]_1} = \frac{[Cl^-]_1}{[Cl^-]_2}$$

or

$$[Na^+]_1 \times [Cl^-]_1 = [Na^+]_2 \times [Cl^-]_2.$$

Remembering that on one side of the membrane there is a non-diffusible ion, R^-, the very fact that the product of the concentrations of the diffusible Na^+ and Cl^- ions on one side of the membrane is equal to their product on the other shows that the concentration of either ion on one side is different from its concentration on the other. On the same side containing the non-diffusible ion R^-, the concentration of the cation Na^+ is the sum of the cations combined with the non-diffusible anion, R^-, plus the cations in combination with Cl^-. But on the other side of the membrane the concentration of the Na^+ ions is only that of Na^+ combined with Cl^- and equal to the concentration of Cl^-. Therefore to fulfill Donnan's equation which is

$$[Na^+]_1 \times [Cl^-]_1 = [Na^+]_2 \times [Cl^-]_2$$

the following conditions must exist:

$$[Na]_1 > [Na]_2$$

and

$$[Cl]_1 < [Cl]_2$$

The above is a simple illustration of Donnan's theory. The situation is more complex in dealing with a large number of ions.

This inequality of distribution of ions on the opposite sides of a membrane is frequently encountered in biological systems. On the basis of this relation, Loeb has also accounted for the influence of electrolytes on many of the properties of colloids, such as viscosity, swelling and osmotic pressure.

CHAPTER II

THE CARBOHYDRATES [1]

CONCERNING the mode of formation of carbohydrates in the plant, there is much difference of opinion, although it has been recognized for many years that chlorophyll, the green coloring matter of plants, is in some way concerned with the synthesis and that the carbon is derived from carbon dioxide. The theory which first gained wide acceptance was proposed by Baeyer[2] in 1870. According to Baeyer's hypothesis, the first step in carbohydrate synthesis in the plant is a reduction of carbon dioxide to carbon monoxide, which is then further reduced to formaldehyde.

$$CO_2 \rightarrow CO + O$$

$$CO + H_2 = HCHO.$$

This is followed, according to the theory, by condensation of the formaldehyde, yielding sugars and polysaccharides, with the possible intermediate formation of glycolic or glyceric aldehyde.

In more recent times, the study of the gaseous interchange of plants has led to certain interesting observations. In the process of photosynthesis, which occurs in the light, carbon dioxide is taken up by the plant and oxygen is given off. At the same time respiration also occurs, this being an oxidative process in which oxygen is used and carbon dioxide formed. We are thus dealing with two reactions which may be regarded as occurring in opposite directions. The gaseous exchange due to photosynthesis alone may be determined by comparing the interchange of oxygen and carbon dioxide in the light and in the dark.[3] This has been

[1] The International Union of Pure and Applied Chemistry has proposed the term "glucides" to embrace the carbohydrates and glucosides.

[2] Ber., **3**, 63 (1870).

[3] In the dark, photosynthesis does not occur. The gaseous interchange is due to respiration only. If it is assumed that respiration occurs at approximately the same rate in the light as in the dark, it is possible to calculate the exchange of carbon dioxide and oxygen due to photosynthesis alone. Thus, if for a given time, O is the volume of oxygen absorbed in the dark and C is the volume of carbon dioxide formed, C/O is the respiratory quotient. If in the same time C_1 is the volume of carbon dioxide absorbed in the light and O_1 is the volume of oxygen given off, the carbon

done by a number of investigators, notably Willstätter and Stoll,[4] who have shown that in the process of photosynthesis the volume of carbon dioxide consumed is equal to the volume of oxygen liberated. This has been accepted by some as evidence of the direct conversion of carbon dioxide into formaldehyde. It is to be realized, however, that the same ratio of carbon dioxide consumed to oxgyen produced would be obtained if glycolaldehyde or any sugar of the general formula $C_nH_{2n}O_n$ were the first product formed.

Chlorophyll exists in two forms, designated chlorophyll *a* and chlorophyll *b*, to which the following formulas have been ascribed by Willstätter and Stoll.

Chlorophyll *a* $C_{32}H_{30}ON_4Mg\begin{cases}COO\cdot CH_3\\COO\cdot C_{20}H_{39}\end{cases}$

Chlorophyll *b* $C_{32}H_{28}O_2N_4Mg\begin{cases}COO\cdot CH_3\\COO\cdot C_{20}H_{39}\end{cases}$

These investigators have suggested that chlorophyll enters into combination with carbonic acid and that, by a series of changes, the latter is reduced to formaldehyde, in which form it is liberated from its union with the chlorophyll.[5]

That carbon dioxide may be photochemically reduced to formaldehyde was reported by Baly and his students [6] in 1921. Aqueous solutions of carbon dioxide, contained in quartz vessels were exposed to light of short wave length (200 $\mu\mu$), obtained from a quartz mercury vapor

dioxide assimilated in photosynthesis is $C + C_1$ and the total oxygen produced is $O + O_1$. The ratio $C + C_1/O + O_1$ has been termed the " photosynthetic quotient." For further details consult H. A. Spoehr's monograph on " Photosynthesis," Chemical Catalog Co., New York, 1926.

[4] R. Willstätter and A. Stoll, Untersuchungen über die Assimilation der Kohlensäure, Berlin, 1918.

[5] E. Q. Adams [J. Am. Chem. Soc., **48**, 292 (1926)] has suggested that the formula of chlorophyll *a* is $C_{55}H_{72}O_5N_4Mg$ and of chlorophyll *b* $C_{56}H_{72}O_6N_4Mg$. He has pictured the chlorophyll as going through a cycle of four reactions, two of them associated with the absorption of two quanta each of radiation, and two follow-reactions requiring water and carbon dioxide but not light:

[ ] represents $C_{55}H_{72}O_4N_4Mg$

[ ]—O is *a*-chlorophyll and [ ]—CO_2 is *b*-chlorophyll.

(1) [ ]—O + H_2O + 2 Quanta λ666 mμ → [ ] + H_2O_2

(2) [ ] + CO_2 → [ ]—CO_2

(3) [ ]—CO_2 + H_2O + 2 Quanta λ640 mμ → [ ]—CO + H_2O_2

(4) [ ]—CO + H_2O → [ ]—O + HCHO

[6] J. Chem. Soc., **119**, 1025 (1921); **121**, 1078 (1922).

lamp. As a result, these workers were able to detect the presence of formaldehyde in their solutions. It was suggested by Baly that the action of the ultraviolet light on carbonic acid resulted first in the formation of " activated " formaldehyde, H—C(—OH)—, which then lost energy and appeared as ordinary formaldehyde, H—C(=O)—H. Reactivation of this by ultraviolet light of somewhat longer wave length (290 $\mu\mu$) was reported to yield reducing sugars. These experiments were repeated by Porter and Rampsperger,[7] who took extreme precautions in avoiding contamination of their carbonic acid solutions and who, being unable to demonstrate the production of formaldehyde, concluded that Baly's results may have been due to the presence of some organic impurities. This objection and a more extended study of the problem have led Baly[8] to revise his original view. He now holds that the " activated " formaldehyde produced from carbonic acid by the action of ultraviolet light is directly polymerized to reducing sugars and that formaldehyde, as such, is not produced. However, the sugar thus formed may be photochemically decomposed to carbonic acid and, in the presence of oxidizable impurities, a small amount of the sugar will be chemically decomposed to formaldehyde.

Certain observations had led Baly to suspect that surface was an important factor in the photosynthesis of carbohydrate. To secure an increased surface, suspensions of pure aluminum or of aluminum hydroxide, maintained by a stream of carbon dioxide, were exposed to ultraviolet light. This resulted in much larger yields of carbohydrate than were obtained in the absence of suspended particles. A similar effect was obtained with the carbonates of aluminum, zinc and magnesium.

In using a suspension of nickel carbonate, Baly, Stephen and Hood[8] made the remarkable discovery that carbohydrate synthesis would occur on exposure to visible light, such as that from an ordinary tungsten filament lamp. Although the color was apparently the factor which made possible the utilization of the visible light, it was not by virtue of the green, as shown by the fact that equally good results were obtained with pink cobalt carbonate.

The importance of these studies is apparent, especially as there are several points of resemblance between the photosynthesis as it occurs in the laboratory and in the living plant. Should Baly's work be confirmed and extended it will undoubtedly illuminate a field of inquiry

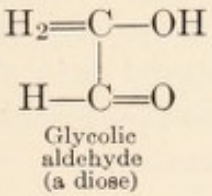
[7] J. Am. Chem. Soc., **47**, 79 (1925).

[8] Proc. Roy. Soc., (A) **116**, 197, 212 (1927); Science, **68**, 364 (1928).

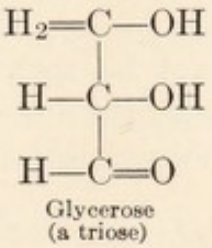
which has long remained dark. It may be hoped that it will give a clear insight into the mechanism by which the green plant acts as a converter of solar energy and into the chemical processes by which the plant achieves the synthesis of complex organic compounds.

Classification of the Carbohydrates

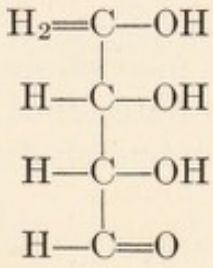
Glycolic aldehyde, containing one aldehyde and one primary alcohol group ($CH_2OH \cdot CHO$), is the simplest aldehyde having the properties commonly associated with the sugars. It is sweet to the taste, crystalline, and readily soluble in water. The following compounds are usually grouped with the simple carbohydrates:



Glycolic aldehyde (a diose)



Glycerose (a triose)



Erythrose (a tetrose)

Sugars having an aldehyde group are termed aldoses; those with a ketone ($\overset{|}{C}{=}O$) group are classified as ketoses. Of the latter, the lowest member is dioxyacetone ($CH_2OH \cdot CO \cdot CH_2OH$).

Except for the methyl pentoses, the general formula $C_m(H_2O)_m$ may be applied to the *simple sugars, or monosaccharides*. These may be classified as follows:

The Monosaccharides [9]

$(C_m(H_2O)_m)$

Biose (diose)—($C_2H_4O_2$)—glycolaldehyde (glycolose).
Trioses ($C_3H_6O_3$) —Aldose—glycerose.
Ketose—dioxyacetone.
Tetroses ($C_4H_8O_4$) —Aldoses—erythrose, threose.
Ketose—erythrulose.
Pentoses ($C_5H_{10}O_5$) —Aldoses—arabinose, xylose, lyxose, ribose.
Ketose—araboketose, xyloketose.
[Methyl pentoses ($C_6H_{10}O_5$)—rhamnose, fucose.]

[9] The classification given here is based on that of Sherman in Chemistry of Foods and Nutrition, Macmillan, 1928 edition, p. 10.

Hexoses ($C_6H_{12}O_6$) —Aldoses—glucose, galactose, mannose, gulose, idose, talose, allose, etc.
Ketoses—fructose, sorbose, tagatose.
Heptoses ($C_7H_{14}O_7$) —Aldoses—mannoheptose, etc.
Ketoses—sedoheptose, etc.

Relatively few of the monosaccharides occur free in nature. Those occurring naturally are fructose, glucose, sedoheptose, and mannoheptose. Arabinose, xylose, galactose, mannose, and other monosaccharides may be obtained by fermentation or hydrolysis of naturally occurring substances. Others are merely laboratory products. Among these may be mentioned erythrose, lyxose, and gulose. Three octoses ($C_8H_{16}O_8$), two nonoses ($C_9H_{18}O_9$) and one decose ($C_{10}H_{20}O_{10}$) have been prepared in the laboratory.

The monosaccharides cannot be split or hydrolyzed into simpler carbohydrates, thus differing from the disaccharides and polysaccharides which on hydrolysis yield monosaccharides. For example,

$$C_{12}H_{22}O_{11} + H_2O \rightleftarrows 2C_6H_{12}O_6.$$

The disaccharides may therefore be looked upon as being made up of two molecules of the same or of different monosaccharides from which one molecule of water has been abstracted. The general formula for these compounds would therefore be $C_{2m}(H_2O)_{2m-1}$.

The Disaccharides
($C_{12}H_{22}O_{11}$)

1. Anhydrides of fructose and glucose: the best-known example is sucrose.

2. Anhydrides of glucose and galactose: e.g., lactose.

3. Anhydrides of glucose and glucose: maltose, isomaltose, trehalose, etc.

A variety of other disaccharides have been described. These include a dipentose saccharide, diarabinose, $C_{10}H_{18}O_9$, and several pentose-hexose saccharides.

By the elimination of two molecules of water from three monosaccharide molecules, we obtain the empirical formula for the trisaccharides [$C_{3m}(H_2O)_{3m-2}$] of which the following are the most familiar compounds:

The Trisaccharides
($C_{18}H_{32}O_{16}$)

1. Anhydride of fructose + glucose + galactose: raffinose. Raffinose occurs in the sugar beet, cottonseed meal, etc.

2. Anhydride of glucose + fructose + glucose: melicitose (melezitose). This is found in the twigs of the larch and of the Douglas fir, and elsewhere.

The tetrasaccharides may be regarded as resulting from the condensation of four monosaccharide molecules with the loss of three molecules of water, $C_{4m}(H_2O)_{4m-3}$. The best-known example is stachyose ($C_{24}H_{42}O_{21}$) which on hydrolysis yields two molecules of galactose and one molecule each of fructose and glucose. It occurs in peas, ash manna, and the twigs of the white jasmine.

The Polysaccharides

The polysaccharides yield on complete hydrolysis either pentoses or hexoses and are obviously the polymerides of these molecular units.

I. Pentosans

$(C_5H_8O_4)_x$

The pentosans are the chief constituents of gums and mucilages.

(*a*) Xylans (anhydrides of xylose). These are found in straw, oat hulls, corn cobs, and most woods.

(*b*) Arabans (anhydrides of arabinose). The arabans are found chiefly in cherry gum or gum arabic.

II. Hexosans

$(C_6H_{10}O_5)_x$

(*a*) Glucosans (anhydrides of glucose). These are the most abundant of polysaccharides. Examples: cellulose, starch, dextrin, glycogen.

(*b*) Mannans or mannosans (anhydrides of mannose). These are found in the ivory nut, from which buttons are made, and in various legumes.

(*c*) Galactans (anhydrides of galactose). Many gums, agar, algæ, lichens, and mosses contain galactans. Fruit pectins likewise contain galactans.

(*d*) Fructosans (anhydrides of fructose). Inulin, present in the tubers of the Jerusalem artichoke, is the most familiar example. It is also present in the bulbs of the onion and garlic. On hydrolysis, inulin yields fructose.

Optical Activity.—A beam of light may be polarized, i.e., caused to vibrate in one plane, by passing through a Nicol prism. This consists of a specially constructed rhomb of Iceland spar or calcite (pure $CaCO_3$). When a beam of polarized light is passed through certain

substances in solution, its plane may be turned either to the right or to the left. Such substances are said to be optically active; those that turn the plane of polarized light to the right are dextro-rotatory, whereas those that turn the beam to the left are levo-rotatory. The polarimeter is an instrument used in measuring the angle through which the plane of polarized light is turned.[10]

Pasteur [11] was among the first to appreciate that a relation existed between optical activity and chemical constitution. In his epoch-making investigations on tartaric acid, he discovered that the so-called racemic acid, which was itself optically inactive, could be separated into two crystalline forms, one being the mirror image of the other. When separated, one form was dextro-rotatory and the other levo-rotatory.

The problem was further elucidated by the work of Le Bel [12] and van't Hoff [13] who independently established the relationship between optical activity in organic compounds and the presence in them of asymmetric carbon atoms. An asymmetric carbon atom is one united to four different atoms or groups of atoms. Lactic acid contains one asymmetric carbon atom. The spatial arrangement of the atoms in lactic acid may be represented as follows:

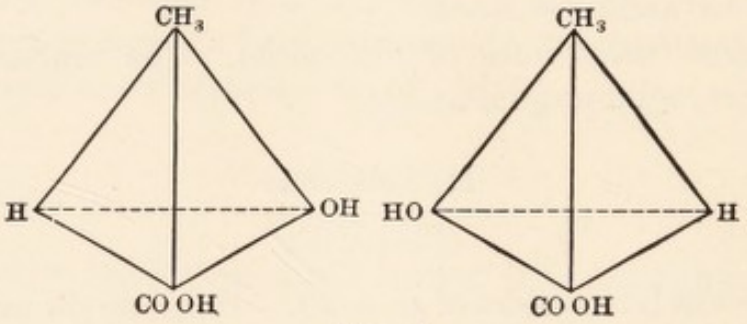


FIG. 5.

Lactic acid, therefore occurs in two optically active forms. Inactive, or racemic, lactic acid is a mixture of the two active forms, in equal proportions. It is of importance to note here that in laboratory syntheses of compounds containing asymmetric carbon atoms the products are optically inactive, since by the law of probabilities an equal number of molecules of the levo- and dextro-rotatory forms would be obtained. Natural syntheses result in the formation of substances which may and frequently do show optical activity.

[10] For a description of the polariscope and its uses, the student is referred to special treatises on the subject, such as Landolt's book "The Optical Rotatory Power of Organic Substances and its Practical Applications" (trans. by Long). See also Getman's "Outlines of Theoretical Chemistry," Chap. V, and "Allen's Commercial Organic Analysis," **1**, p. 41.

[11] Ann. chim. phys. **24**, 442 (1848); **28**, 56 (1850); **31**, 67 (1851).

[12] Bull. soc. chim., **22**, 337 (1874).

[13] *Ibid.*, **23**, 295 (1875).

Tartaric acid contains two asymmetric carbon atoms and exists in four forms, namely as (*a*) inactive racemic acid which may be separated into (*b*) dextro-tartaric and (*c*) levo-tartaric acids; and (*d*) mesotartaric acid which is inactive because of " internal compensation " within the molecule. These compounds may be represented structually as follows:

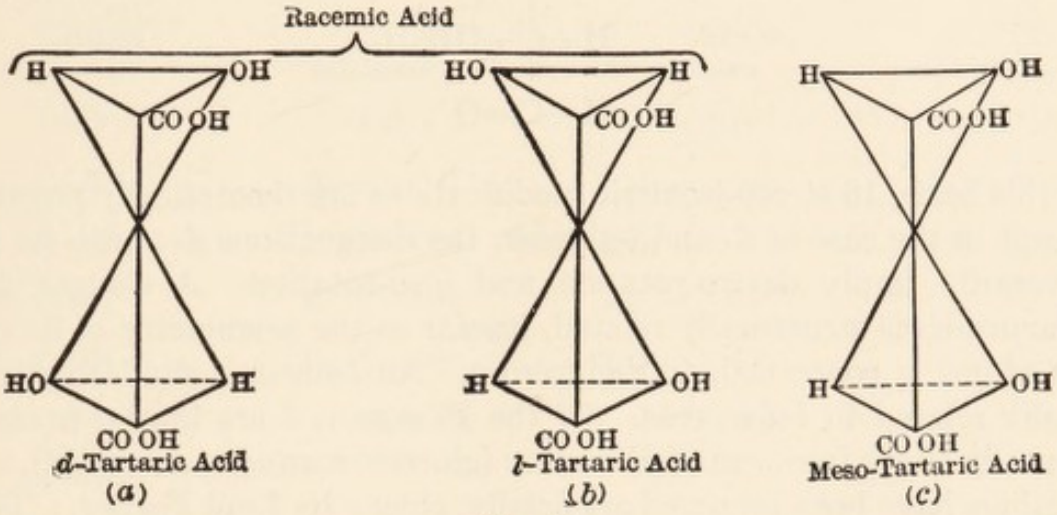
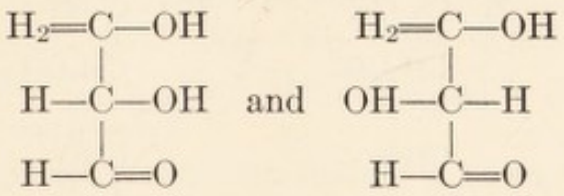
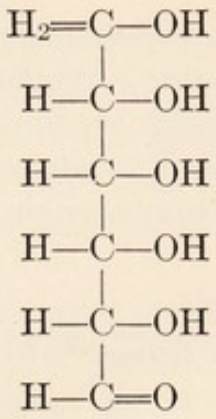


FIG. 6.

Stereo-isomerism and the Constitution of the Monosaccharides.— If the structural formula of glycerose is examined (page 35), it will be seen that the middle carbon atom is asymmetric, since it is united to four different atoms or groups of atoms. Accordingly, there must exist a dextro-rotatory and a levo-rotatory form of glycerose in addition to the inactive or racemic form, which is a mixture in equal proportions of the two optically active varieties. The optically active forms of glycerose may be represented as follows:

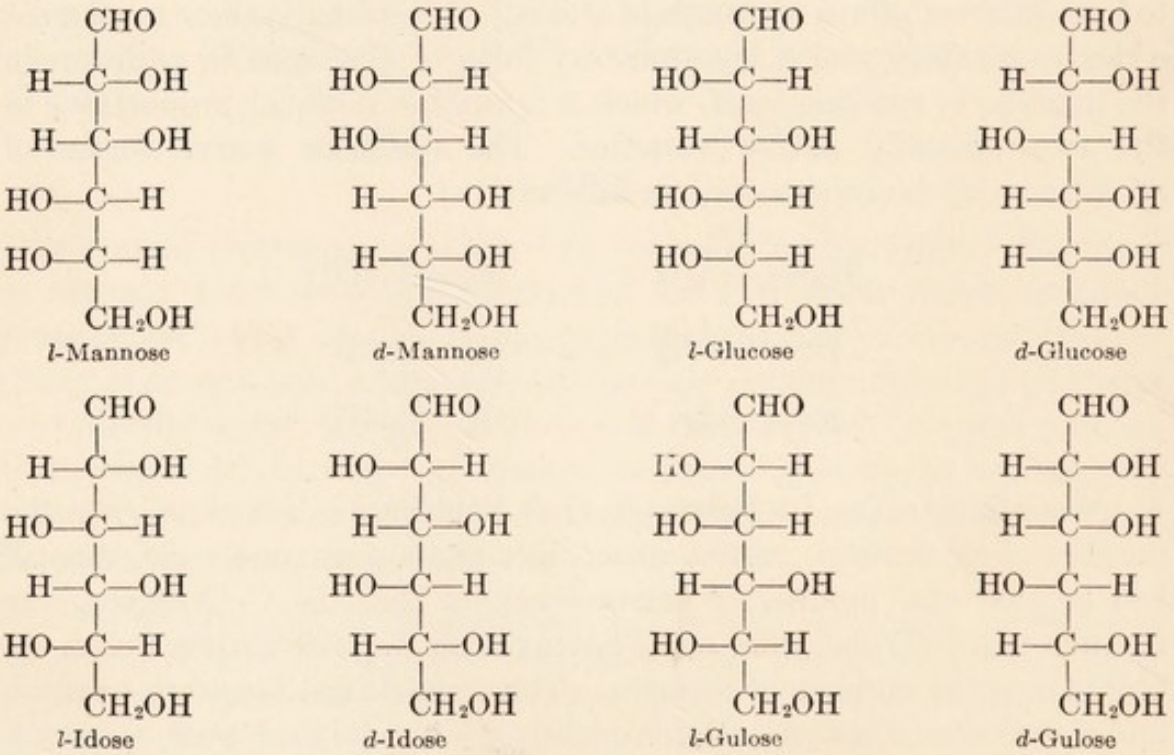


According to the Le Bel-van't Hoff hypothesis, if n represents the number of asymmetric carbon atoms in a given sugar molecule, then 2^n will be the total number of active forms of the sugar. Applying the formula to a 4-carbon-atom sugar having 2 asymmetric carbon atoms, we obtain 4 as the number of isomers. These are *d*- and *l*-erythrose and *d*- and *l*-threose. Similarly, 8 aldo-pentoses, *d*- and *l*-arabinose, *d*- and *l*-xylose, *d*- and *l*-ribose and *d*- and *l*-lyxose, are theoretically possible. In the case of the aldo-hexose sugars, the empirical formula indicates the presence of 4 asymmetric carbon atoms.

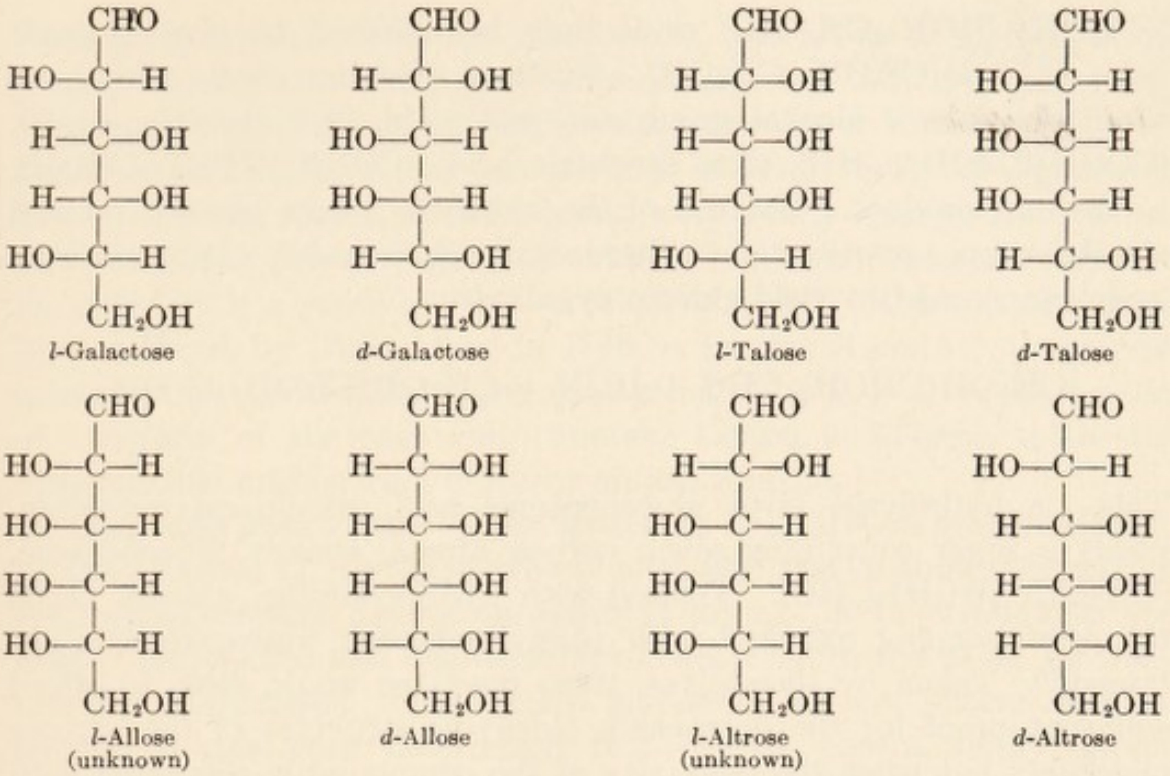


On this basis, 16 stereo-isomeric modifications are theoretically possible. Except in the case of *d*- and *l*-glucose, the designations *d*- and *l*- do not necessarily imply dextro-rotation and levo-rotation. A *d*-sugar is a sugar which is structurally related, insofar as the asymmetry of its carbon atoms is concerned, to *d*-glycerose. An *l*-sugar is similarly structurally related to *l*-glycerose. Of the 16 sugars, 3 are known to occur naturally either free or in combination (glucose, mannose, galactose), and 11 others have been prepared artificially, chiefly by Emil Fischer. Two, *l*-allose and *l*-altrose have not been described. The molecular structures of the 16 isomeric aldo-hexoses may be represented in their simplest form as follows:

THE ALDOHEXOSES [14]



[14] Based on table in E. F. Armstrong's Simple Carbohydrates and Glucosides, Longmans, Green and Co., 1924, p. 34.



The number of aldo-hexose isomerides would be limited to 16 on the assumption that the structure of the aldo-hexose molecule is that of an open-chain aldehyde. The structural formula that the organic chemist assigns to a given organic compound is determined by what is known concerning its physical and chemical properties. If, from this standpoint, the best known of the aldo-hexoses, namely *d*-glucose, is subjected to a critical examination, it is discovered that the straight-chain aldehyde formula, as given above, does not fully account for all the chemical and physical properties of this compound.

Reactions.—On heating glucose with a concentrated solution of hydriodic acid, the oxygen is removed and it is converted to *n*-secondary-hexyliodide, [$CH_3 \cdot CH_2 \cdot CHI \cdot CH_2 \cdot CH_2 \cdot CH_3$], which is a derivative of *n*-hexane, [$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3$]. This proves that glucose is a straight-chain compound.

The hydroxyl groups react with metals to form compounds resembling the "alcoholates." When glucose is treated with acids, acid anhydrides, and acid chlorides, esters are formed in which 5 hydrogen atoms of the hydroxyl groups are replaced by acid radicals. An example of such an ester is glucose pentacetate [$C_6H_7O(O \cdot CO \cdot CH_3)_5$]. This is evidence that there are five hydroxyl groups, and, because of the stability of glucose, it is to be assumed that each hydroxyl group is associated with a different carbon atom.

The aldehyde group of glucose may be reduced with sodium amalgam to an alcohol group, yielding a hexahydric alcohol, sorbitol,

$[CH_2OH(CHOH)_4CH_2OH]$, or it may be oxidized to give gluconic acid, $[COOH(CHOH)_4CH_2OH]$. Further oxidation will affect the terminal primary alcohol group and will yield, first glucuronic acid, $[COOH(CHOH)_4CHO]$, then saccharic acid, $[COOH(CHOH)_4COOH]$, as the end product. Because of the aldehyde group, glucose reduces alkaline copper solutions and other metallic hydroxides. It reacts with hydrocyanic acid to yield glucose cyanhydrin.

$$CH_2OH(CHOH)_4CHO + HCN = CH_2OH(CHOH)_4C(H)(OH)(CN)$$

This, on hydrolysis, gives glucoheptonic acid, which, on reduction, yields a sugar containing seven carbon atoms, namely, glucoheptose, $[CH_2OH(CHOH)_5CHO]$. Treated with hydroxylamine, glucose yields the corresponding oxime;[15] with phenylhydrazine, glucosazone is obtained.[16] Taken by themselves, these reactions would seem to afford sufficient proof for the open-chain aldehydic structure of the glucose molecule; but when the reactivity of the compound is compared with that of other hydroxy-aldehydes, it becomes apparent that glucose is not as reactive as might be expected from the simple aldehyde formula. Indeed, if the structure of glucose were as represented by the open-chain aldehyde formula, it would be impossible to explain certain of its physical properties.

Mutarotation.—It has been observed that the optical rotation of a freshly prepared aqueous solution of glucose or of any other sugar containing a free aldehyde group changes on standing. The initial specific rotations of *d*-glucose prepared in different ways may be totally different.

[15] $CHO + H_2N\cdot OH$ (Hydroxylamine) $= CH:N\cdot OH + H_2O$

$(CHOH)_4$ $\quad$ $(CHOH)_4$

CH_2OH (Glucose) $\quad$ CH_2OH (Glucose-oxime)

[16] $CHO + C_6H_5\cdot NH\cdot NH_2$ (Phenylhydrazine) $= CH:N\cdot NH\cdot C_6H_5 + H_2O$

$CHOH$ $\quad$ $CHOH$

$(CHOH)_3$ $\quad$ $(CHOH)_3$

CH_2OH (Glucose) $\quad$ CH_2OH (Phenylhydrazone of glucose)

$CH:N\cdot NH\cdot C_6H_5 + 2C_6H_5NH\cdot NH_2 = CH:N\cdot NH\cdot C_6H_5 + C_6H_5NH_2 + NH_3 + H_2O$

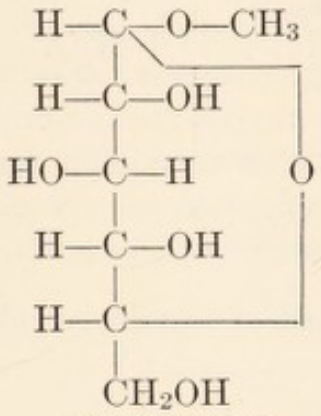
$CHOH$ $\quad$ $C:N\cdot NH\cdot C_6H_5$

$(CHOH)_3$ $\quad$ $(CHOH)_3$

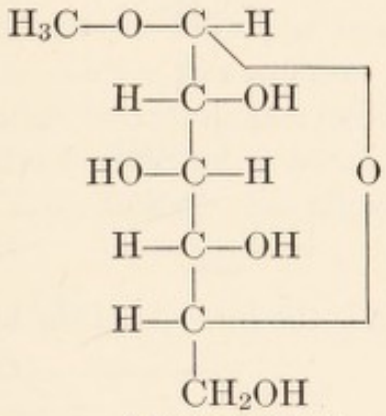
CH_2OH $\quad$ CH_2OH (Glucosazone)

Thus glucose prepared by recrystallization from acetic acid gives, when freshly dissolved in water, a rotation of about + 110° (see page 59). Prepared by crystallization from an aqueous solution above 98° C., the *d*-glucose exhibits an initial rotation $[\alpha]_D$ of about + 19° (*β*-*d*-glucose). Either solution, when allowed to stand, very slowly changes its rotation until a value of $[\alpha]_D$ of + 52.5° is obtained. The change occurs almost immediately if a small amount of alkali is added. This phenomenon, first observed by Dubrunfaut in 1846, is known as mutarotation (also birotation or multirotation) and is believed to be due to the conversion of one form of the sugar into another having a different molecular configuration and hence a different optical rotation.

Glucoside Formation.—When glucose is treated with methyl alcohol in the presence of dry hyhrochloric acid gas, two distinct compounds are formed, neither having an aldehyde group. Both compounds are methyl glucosides, and one may be separated from the other by fractional crystallization. The methyl glucosides do not behave as aldehydes. It has been determined that in each case a methyl group replaces a hydrogen atom belonging to an hydroxyl group attached to the carbon atom which is supposedly part of the aldehyde group of the glucose molecule. The two methyl glucosides have been distinguished by the prefixes *α* and *β*, and for reasons to be considered presently have been represented structurally by the following formulas:



α-Methyl glucoside



β-Methyl glucoside

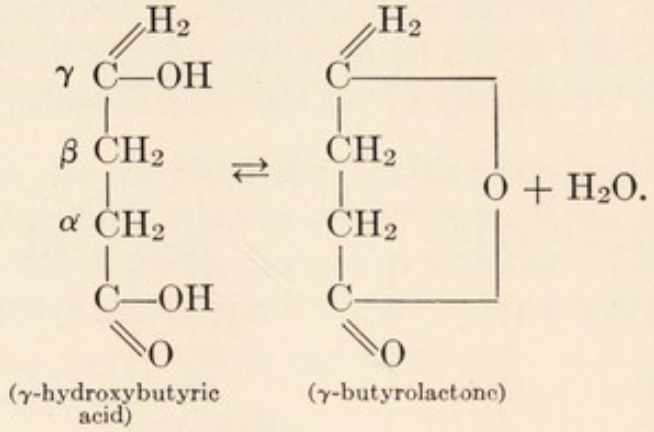
The two compounds differ in solubility, melting point, rotatory power, and crystalline form, the *α*-glucoside crystallizing in long needles, and the *β* form in rectangular prisms.

	Melting Point	Rotatory Power
α-Methyl glucoside	165°	+157°
β-Methyl glucoside	104°	− 33°

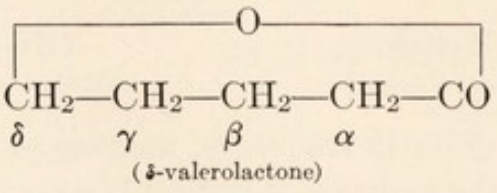
The methyl glucosides also differ in their reactions toward enzymes. Maltase acts only on the α glucoside, having no effect on the β form. The latter is acted on by the enzyme emulsin. The pure β form may be prepared by incubating the mixture of the two glucosides with ordinary baker's yeast. The maltase contained in the yeast hydrolyzes the α-methyl glucoside, and the zymase, which is also present, ferments the resulting glucose to carbon dioxide and ethyl alcohol. The β-methyl glucoside remains unaffected.

The behavior of ordinary glucose in forming two methyl glucosides suggests the probability that an hydroxyl (OH) group is available at the terminal (aldehyde) carbon atom. It also indicates that two distinct forms of glucose are acted upon to yield the two methyl glucosides.

Lactones and Lactals.—A distinguishing feature of the γ- and δ-hydroxyacids is that they are capable of forming cyclic esters, when the carboxyl group enters into reaction with the hydroxyl group. The formation of these cyclic compounds is accelerated by mineral acids, whereas cleavage of the lactone linkage may be accomplished through the agency of alkali hydroxides and carbonates. As an illustration of a γ-lactone, we have:



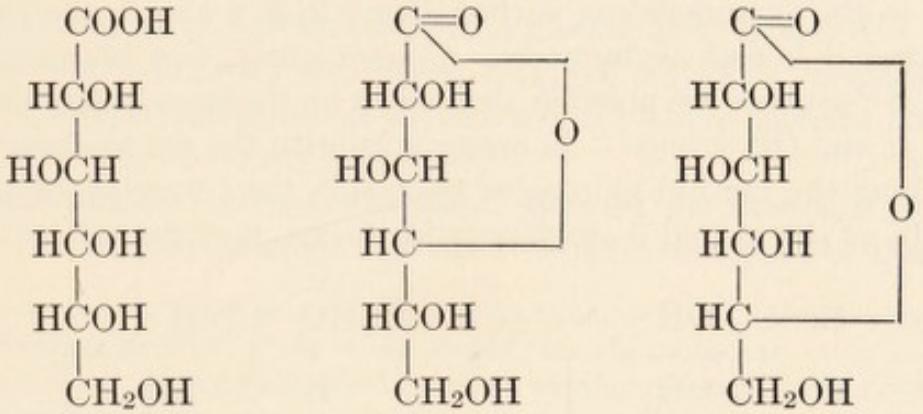
The following is the formula of the lactone of δ-hydroxy valeric acid:



The sugar acids, which may be formed by the oxidation of the aldehyde group to a carboxyl group, show the same tendency to form lactones. In a careful study of the relation between the chemical constitution and the optical rotatory power of 24 different sugar-acid actones, Hudson [17] advanced the hypothesis, now usually referred to as Hudson's "lactone rule," that lactones which are dextrorotatory have the lactone ring on one side, represented on the right side of

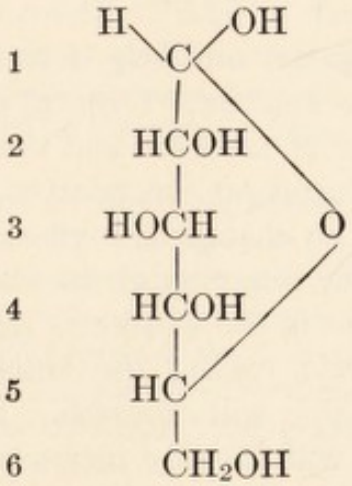
[17] J. Am. Chem. Soc., **32**, 338 (1910).

the structure, whereas lactones which are levorotatory have it on the other side, and that the position of the ring shows the former position of the OH group on the γ-carbon atom. Hudson concluded that the sugar acids form γ-lactones. While this configuration is undoubtedly the predominant one, evidence has however accumulated [18] pointing to the existence of δ-lactones of sugar acids. Thus, gluconic acid is said to yield at least two lactones, namely a γ- and a δ-lactone.



The lactones of the sugar acids are relatively stable and some have been prepared in crystalline form. Levene [19] is of the opinion that in a solution of sugar acids, all theoretically possible lactones are formed. In a freshly prepared solution the unstable lactones are said to predominate, but after a short time only the stable forms are present in measurable quantities.

Analogous to the formation of lactones from the sugar acids are the intramolecular rearrangements which the sugars themselves exhibit. Here also, an oxygen bridge is introduced which may be supposed to link carbon atoms 1 and 4 (γ-linkage), 1 and 5 (δ-linkage), etc. Assuming for the present a <1,5> linkage, the glucose molecule may be represented by the formula:



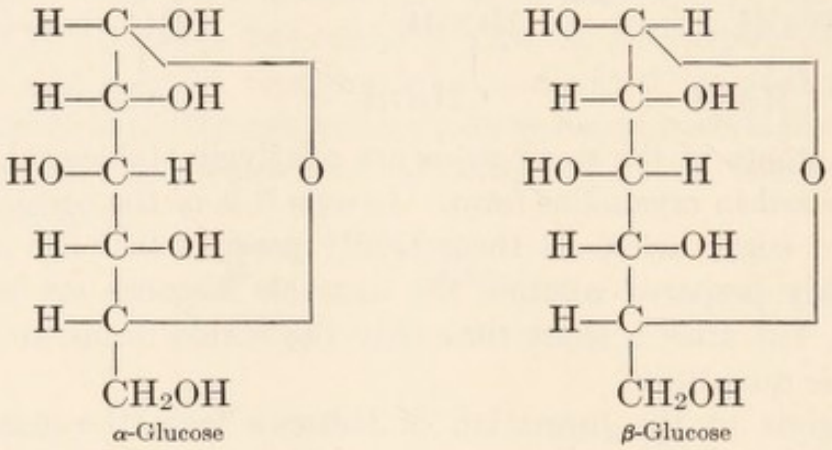
[18] J. U. Nef, Ann. d. Chem., **403**, 204 (1914); Haworth, W. N., and Nicholson, V. S., J. Chem. Soc., London, **129**, 1899 (1926).

[19] P. A. Levene, Chem. Reviews, **5**, 1 (1928).

Levene refers to compounds of this type as " lactals," a term which has been suggested by Helferich and Fries.[20]

Structure of the Glucose Molecule.—We may now consider whether the lactal structure of the glucose molecule offers an adequate explanation for certain physical and chemical properties of glucose which we are unable to account for on the basis of the open-chain aldehyde structure.

It will be observed that in the lactal formula for glucose just given, in addition to the asymmetry of carbon atoms 2, 3, 4 and 5, the terminal carbon atom 1 is also asymmetric. Consequently, two isomeric modifications of *d*-glucose are possible, depending on the space relations of the terminal H and OH groups. In accordance with the nomenclature used in describing the methyl glucosides (page 43), the formulas for glucose, referred to as the α- and β-amylene-oxide forms, may be written:



The existence of two forms of *d*-glucose was demonstrated by Tanret [21] in 1896. He described an " α " glucose ($[\alpha]_D = +110°$) which on standing changed its specific optical rotation to the equilibrium point ($[\alpha]_D = +52.5°$). Another sugar of rotation ($[\alpha]_D = +19°$) increased its rotatory power to 52.5°. These observations have been confirmed by other investigators and help to explain the phenomenon of mutarotation. Both the α- and the β- forms of glucose are present when ordinary anhydrous glucose is dissolved and the initial optical rotation, in any given case, will depend on the relative proportions of the two isomeric modifications. The change of α-glucose to β-glucose is reversible and occurs readily, the direction of the change depending on how much of each form is present in the solution. If, on standing, the change α-glucose $\rightarrow$ β-glucose should exceed the transformation β-glucose $\rightarrow$ α-glucose, the optical rotation will diminish. If the reverse should be true, the optical rotation will steadily increase. When equilibrium is

[20] Ber., **58**, 1246 (1925).

[21] Bull. soc. chim., **15**, 195, 349 (1896).

reached, i.e., when the specific optical rotation is $[\alpha]_D = +52.5°$, it is found that approximately one-third of the glucose is present as α-glucose and two-thirds as β-glucose.

The α- and β-methyl glucosides are clearly derivatives of the α- and β-forms of glucose, respectively. It is interesting to note that the average specific optical rotation of the glucosides is $+62°$, and of the glucoses, $+63.5°$, a physical relationship that has been ascribed to their structural similarity. When α-methyl glucose is hydrolyzed it yields a sugar having a high initial optical rotation, obviously α-glucose, and when the β-methyl glucoside is hydrolyzed, it yields a sugar of low initial optical rotation, obviously β-glucose.[22]

In addition to the cyclic forms of glucose, the existence of a noncyclic form must be assumed if we are to explain its reactions as an open-

[22] The molecular rotation of a sugar is the product of its molecular weight and specific rotation (p. 59). Hudson has shown that the molecular rotation depends on two factors: (1) the optical effect of the end asymmetric carbon atom of the sugar and (2) the optical effect of the remaining asymmetric carbon atoms. If the rotation due to the terminal asymmetric carbon atom of α-*d*-glucose is represented by A and the rotation of the remaining four asymmetric carbon atoms by B, the molecular rotation of the whole molecule is thus $A + B$. The molecular rotation of the other isomer, β-*d*-glucose, will then be $-A + B$. These facts may be summarized as follows:

Molecular rotation of α-*d*-glucose $= 180 \times +110° = 19{,}800 = A + B$
Molecular rotation of β-*d*-glucose $= 180 \times +\ 19° = \ 3{,}420 = -A + B$

Accordingly, the difference in molecular rotation $(2A) = 16{,}380$ and the sum $(2B) = 23{,}220$.

Hudson has developed the following generalizations:

(1) The difference between the molecular rotations of the α and β forms of all the aldehyde sugars and all their derivatives in which the added substance is not joined directly to the end asymmetric carbon atom is a nearly constant quantity (about 16,200).

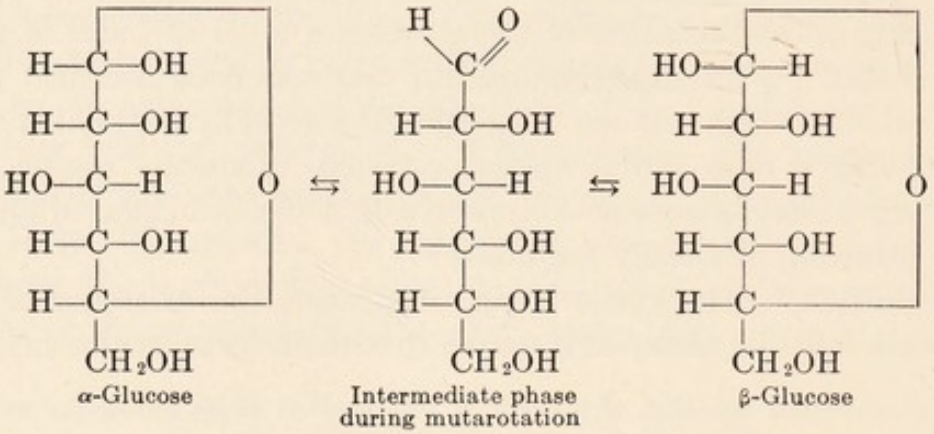
(2) The α and β forms of those derivatives (e.g., glucosides, etc.) of any aldose sugar in which only the asymmetric carbon atom is affected have molecular rotations whose sum is equal to the sum for the α and β forms of the aldose (approximately 23,000 to 24,000).

(3) The names of the α and β forms of the sugars should be so selected that for all sugars which are genetically related to *d*-glucose the subtraction of the rotation of of the β form from that of the α form gives a positive difference, and for all sugars which are genetically related to *l*-glucose a negative difference.

(4) The names of the α and β forms of the derivative of any sugar should be so selected that the difference of their molecular rotations is equal to and of the same sign as the similar difference for the forms of that glucose (*d*- or *l*-) to which the first sugar is genetically related.

These rules have proven to be very useful in the study of the molecular configuration of the sugars and their derivatives. C. S. Hudson, J. Am. Chem. Soc., **31**, 66 (1909).

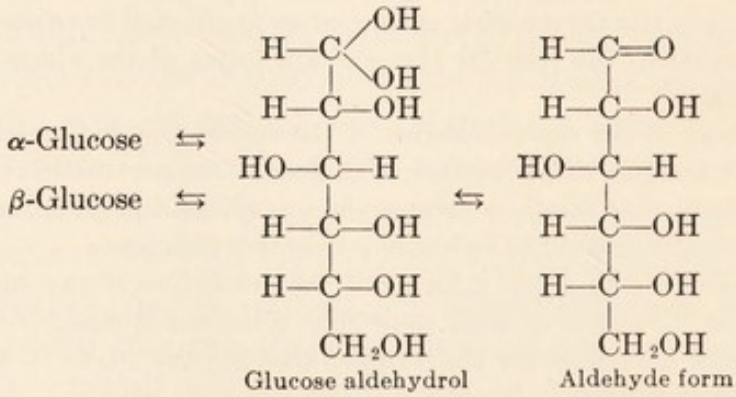
chain aldehyde. The opening of the cyclic structure with the formation of the aldehydic form has for many years been associated with the change of one isomeric form of glucose to the other (α-glucose $\rightleftarrows$ β-glucose) which underlies the phenomenon of mutarotation.



The presence of the transitional aldehyde form is taken as an indication that the sugar is undergoing or has undergone mutarotation. In fact, it has been pointed out (Levene and others) that the sugars or their derivatives which show a higher velocity of mutarotation, are also those in which the change to the aldehyde form proceeds with higher velocity.

It was formerly believed by Lowry that the cyclic isomers take up a molecule of water and pass through a transitory aldehydrol stage to the aldehyde form.[23] This view, however, has been challenged by Armstrong [24] on the ground that there is an increase in the conductivity of sugar solutions during mutarotation, a fact which is inconsistent with the formation of an aldehydrol. The chemical mechanism involved in

[23] If this view were correct, the formation of the aldehyde form of glucose could be represented by the following formulas:



[24] J. Chem. Soc., **83**, 1305 (1903). See also E. F. Armstrong's "The Carbohydrates and the Glucosides," London, 1924 edition, p. 47.

the process of mutarotation has been the subject of intensive study during the last few years, and it seems not unlikely that the phenomenon is purely a tautomeric change and does not depend on the intervention of water. Some of this work has been reviewed by Lowry.[25]

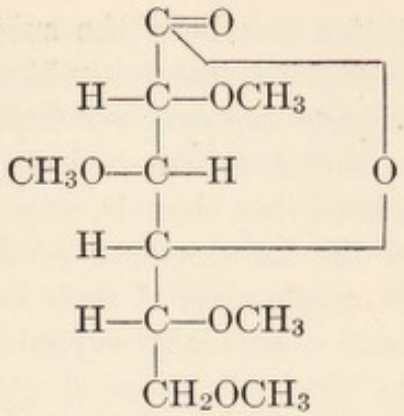
While it is to be admitted that there is, as yet, no general agreement regarding the nature of the intermediate products which accompany mutarotation, nor of the mechanism of their formation, it is probably safe to assume, on the basis of available evidence, that at some stage or another, the open-chain aldehyde form of glucose is present. This variety of *d*-glucose is in equilibrium with the α- and β-isomers of the amylene-oxide forms and possibly with other cyclic isomeric modifications. When glucose is treated with a reducing or oxidizing agent, with phenylhydrazine, hydrocyanic acid or any reagent which acts on the free aldehyde group, the equilibrium relations are disturbed by the removal of the aldehyde form from the reacting system. As a result, the α- and β- forms are converted to the aldehyde, and, as the reaction proceeds, more and more of the cyclic forms are changed to the aldehyde form. On the assumption that at any given moment during a reaction only a small amount of the free aldehyde is present, rests the explanation, which some have urged, for the fact that the sugars are more slowly reactive than are hydroxyaldehydes which do not have a cyclic configuration.

Proof of the Amylene-oxide Formula of Glucose.—In describing the cyclic forms of glucose (p. 46) and the corresponding methyl glucosides (p. 43), the δ- or amylene-oxide, structure was assumed. It remains to present some of the evidence upon which this is based.[26]

(1) 2 : 3 : 6 trimethyl glucose gives on oxidation 2 : 3 : 6 trimethyl gluconic acid, which readily forms a lactone. This has been identified as a γ-lactone. If this compound is methylated, it yields 2 : 3 : 5 : 6 tetramethyl gluconolactone. This is a crystalline solid.

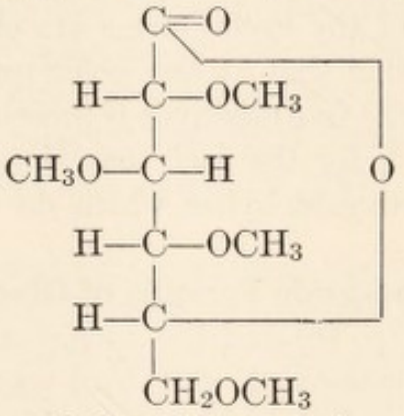
[25] Z. physikal. Chem., **130**, 125 (1927).

[26] The chemical constitution of the carbohydrates has engaged the attention of numerous investigators for over a generation. Numbered among the pioneer workers in this field were Emil Fischer, J. U. Nef and E. F. Armstrong, who laid the foundation for the more recent work of J. C. Irvine, C. S. Hudson, P. A. Levene, W. N. Haworth, J. Pryde, their associates and others, which has resulted in an almost complete revision of our knowledge of the stereochemistry of the sugars. This subject is a very difficult one and the methods of investigation are very complex. In gaining an appreciation of the present status of the subject, the student will be aided by the following references: (1) Progress in the Structural Study of Carbohydrates, J. C. Irvine, Chemical Reviews, **4**, 203 (1927); (2) Active Glucose, P. A. Levene, *ibid.*, **5**, 1 (1928); (3) W. Charlton, W. N. Haworth, and S. Peat, J. Chem. Soc., **129**, 89 (1926); W. N. Haworth and G. C. Westgarth, *ibid.*, p. 880; W. N. Haworth, The Constitution of Sugars, London, 1929.



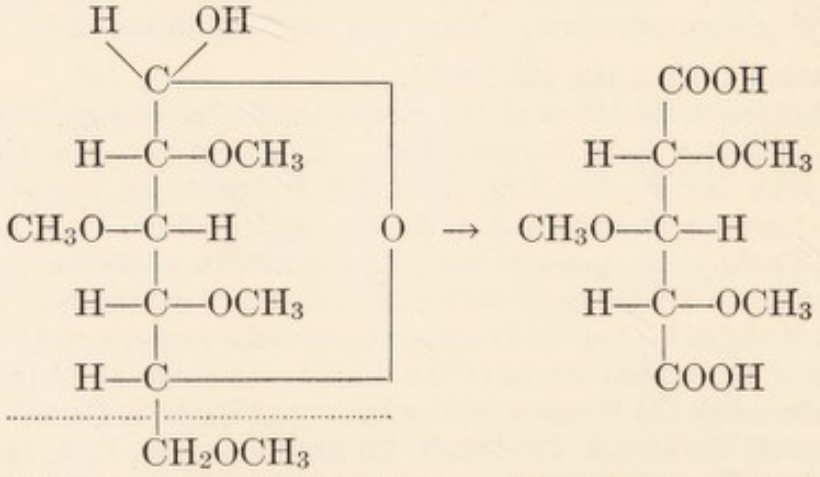
2 : 3 : 5 : 6 Tetramethyl gluconolactone

2 : 3 : 6 trimethyl glucose on methylation forms a tetramethyl glucose. When this is oxidized it yields a tetramethyl gluconolactone, which is a liquid and which in its physical and chemical properties differs markedly from the 2 : 3 : 5 : 6 tetramethyl gluconolactone given above. Accordingly, this can only be the 1 : 5 lactone:



2 : 3 : 4 : 6 Tetramethyl gluconolactone

From this it has been concluded that the normal tetramethyl glucose (and hence, glucose itself) is an amylene oxide.[26a]



[26a] When this book was about to be published an important paper by C. S. Hudson appeared in which is expressed considerable doubt of the validity of Haworth's assumption that rings never shift in the methylation of glucosides and glucoside-like derivatives (J. Am. Chem. Soc., **52**, 1680 [1930]).

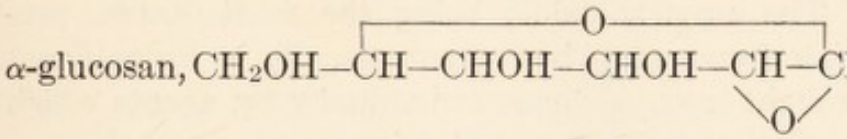
(2) Crystalline tetramethyl glucose is oxidized by nitric acid to xylo-trimethoxy-glutaric acid, as shown at the bottom of p. 50.

(3) Tetramethyl galactonolactone, derived from tetramethyl galactose is known to have an amylene-oxide configuration. Trimethyl arabinose and trimethyl xylose are also known to give 1 : 5 lactones. These three lactones have properties which are almost identical with those of the lactone obtained from tetramethyl glucose. By analogy, it has therefore been inferred that the tetramethyl glucose is also an amylene-oxide.

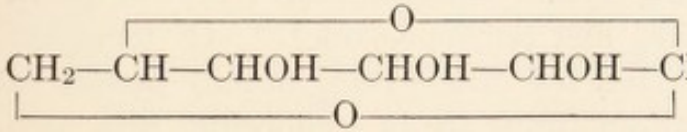
Space Relations of the H and OH Attached to Carbon Atom 1.—The positions of the H and OH, attached to carbon atom 1, in the formulas of α- and β-glucose are not arbitrarily chosen, but are based partly on the following observations:

(1) The conductivity of α-glucose in boric acid solution diminishes during mutarotation as it is converted to β-glucose, whereas the conductivity of β-glucose is increased under similar conditions as α-glucose is formed. The change in conductivity takes place with the same velocity as the mutarotation, showing that the two phenomena are related. It has been shown that an alcohol increases the conductivity of a boric acid solution if it has two hydroxyl groups attached to two neighboring carbon atoms and situated in the same plane on the same side of the carbon chain. Accordingly, it is concluded that the OH groups attached to carbon atoms 1 and 2 are on the same side in α-glucose and on opposite sides in β-glucose.

(2) On heating α-glucose (150–155° C. under a pressure of 15 mm.),



α-glucosan, $CH_2OH—CH—CHOH—CHOH—CH—CH$, is obtained. This has an ethylene-oxide structure and is formed by the loss of HOH from the two hydroxyl groups attached to carbon atoms 1 and 2. On the assumption that the hydroxyls are only able to react with one another when they are on the same side of the carbon chain, it is inferred that the OH group attached to carbon atom 1, in α-glucose, occupies the position as represented by the formula on p. 46. β-glucose yields β-glucosan,[27]



$CH_2—CH—CHOH—CHOH—CHOH—CH.$

[27] For a more complete and more critical discussion of this question, see Armstrong's " The Carbohydrates and Glucosides," p. 44. See also, P. A. Levene and H. Sobotka, J. Biol. Chem., **67**, 759 (1926). Reference may also be made to the important papers of J. Boeseken and associates, Ber., **46**, 2612 (1913); Proc. Roy. Acad. Amsterdam, **18**, 1654 (1916); Rec. trav. chim. Pays-Bas, **40**, 354 (1921); and to the paper of M. Levy and E. A. Doisy, J. Biol. Chem., **84**, 749 (1929).

Cyclic Forms of Glucose, other than the Amylene-oxide. "Active Glucose."—The stable form of glucose, as well as of mannose, galactose, fructose, xylose arabinose and probably other sugars, in aqueous solution, is the amylene-oxide form, but it is unlikely that this is the only cyclic structure which glucose (and the other sugars) may possess. Indeed, a considerable amount of evidence points to the existence of a so-called " active " (or reactive) glucose which is not an amylene-oxide compound. We cannot deal here with this question in detail, but some of the observations which have led to this belief may be pointed out.[28]

(1) Different sugars ferment at different velocities; yet the intermediate and final products are identical. This is also true of the biological oxidation of various sugars. The assumption is that the different sugars are changed to a form common to all, which then undergoes decomposition, and that this form is the so-called " active " glucose.

(2) Glycogen is more rapidly converted to lactic acid than free glucose. The conclusion drawn is that glycogen glucose is in a more labile state than are the common forms of glucose.

(3) Various derivatives of glucose have been shown to exist as several ring isomers which differ from each other in their stability.

As to the nature of the " active " glucose, there is much to indicate that it is the γ-, or butylene-oxide form, but Levene is of the opinion that in aqueous sugar solutions, all theoretically possible cyclic structures coexist in equilibrium with each other (i.e., the α- and β- modifications of the ethylene oxide, the α- and β- modifications of the propylene-oxide, as well as the two isomers of the butylene-oxide and amylene-oxide forms of glucose). The amylene-oxide, being the most stable, predominates. The other forms may be present, usually in insignificant amounts, but are brought into existence individually by agents which act specifically or preferentially on one lactal form and none other.

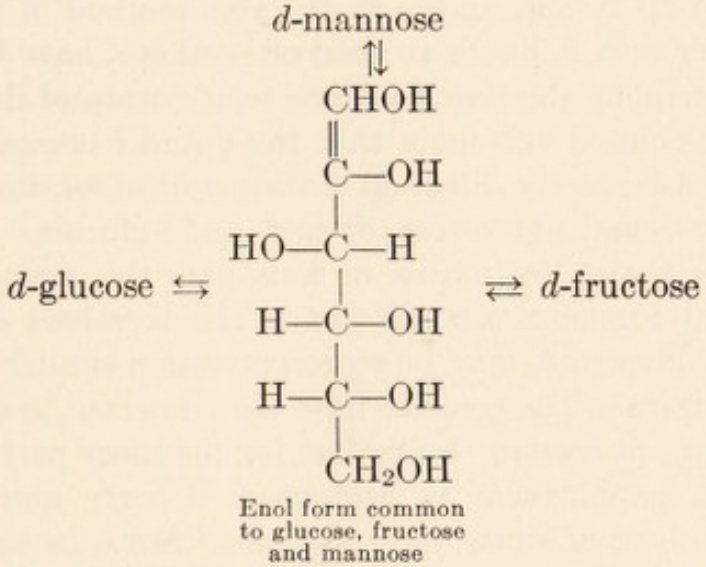
The main conclusions to be derived from the discussions in the preceding paragraphs is that glucose in solution does not behave as though it were a compound having a fixed molecular structure. On the contrary, it is to be regarded as existing in at least five, and possibly in nine different forms, one of which is represented as the open-chain aldehyde, and the other eight possessing cyclic configurations (ethylene-oxide, propylene-oxide, butylene-oxide, and amylene-oxide).[29] Of these the amylene oxide form predominates, the others being usually present in very small amounts. The various forms are in equilibrium with each

[28] P. A. Levene, Chem. Reviews, **5**, 1 (1928).

[29] If the α- and β- isomers of the hexylene-oxide, ϵ or $<1:6>$ form, the existence of which is questioned, were included, the total number of glucose isomerides would be eleven.

other, and under appropriate conditions, are convertible one into another. Nor does this concept apply only to glucose. It is very likely that what has been said with regard to this sugar applies with equal force to the other simple carbohydrates.

The Action of Alkali on Glucose and other Monosaccharides.—If *d*-glucose is treated with a dilute basic solution, such as 0.05 N $Ca(OH)_2$ it changes its optical rotation, which ultimately reaches an equilibrium. The equilibrium mixture contains *d*-glucose, *d*-fructose and *d*-mannose, as well as small amounts of *d*-glutose and *d*-pseudofructose.[30] This conversion, first studied by Lobry de Bruyn,[31] also occurs if *d*-fructose or *d*-mannose is treated with alkali, the final products being the same as with glucose. It seems that in these interconversions, the α- and β-carbon atoms are involved. It is believed that glucose, fructose and mannose are capable of forming an enol which is common to all, and that from this enol the three sugars are regenerated. The relationship may be represented as follows:



In more concentrated alkaline solutions, the sugars are oxidized spontaneously in the presence of air, with the formation of a large number of simpler compounds. Nef [32] has shown that from glucose at least 93 substances are formed in this way, and possibly as many as 116 if those compounds are included which result from the resynthesis of the initial fragments of glucose disintegration.

The Structural Relationship of the Monosaccharides.—Two methods have been especially valuable in studying the structural relationship of

[30] Glutose, when represented in the noncyclic form, is $CH_2OH—CHOH—CO—CHOH—CHOH—CH_2OH$. Pseudofructose differs from fructose (p. 57) in the β-carbon atom configuration.

[31] Rec. trav. Chim., **14**, 156, 203 (1895).

[32] Ann. d. Chem., **357**, 214 (1907); **403**, 204 (1913).

the simple sugars. The first method is the so-called cyanhydrin synthesis of Kiliani, which is based on the reaction of aldehydes and ketones with HCN. If a sugar, such as *d*-glucose, is treated with HCN, the corresponding nitrile is formed, which on hydrolysis yields an acid containing one carbon atom more than the original sugar; in this case a seven-carbon sugar acid (glucoheptonic acid). On reduction, the acid is converted to the aldehyde, glucoheptose.[33] By this method it is possible to produce from *d*-glycerose the two four-carbon monosaccharides, *d*-erythrose and *d*-threose. From these the four *d*-pentoses may be synthesized and, in turn, the eight *d*-hexoses. Continuing the cyanhydrin synthesis, it is possible to prepare heptoses, octoses and even higher monosaccharides.

The second method is that of Wohl, which depends on the reaction of aldehydes and ketones with hydroxylamine with the formation of oximes. From glucose, glucose-oxime is formed. When this is treated with concentrated NaOH, it yields the nitrile of gluconic acid, which, on heating, decomposes to HCN and arabinose.[34] This method of degradation of sugars, together with Kiliani's method of synthesis, have been especially useful in determining the stereoisomeric relationship of the sugars.

It may be pointed out again that the *d* and *l* nomenclature of the sugars is not based on the direction of their optical rotation, but on their relationship to *d*- and *l*-glycerose, or to *d*- and *l*-glucose. On p. 55, this relationship is shown by means of formulas, the arrangement being based on Emil Fischer's classification. The members of the *l*-series, derived from *l*-glycerose, may be represented in a similar way.

Pentose Sugars.—The pentoses have been detected, in small amounts, in the free form, in certain plants, but for the most part they occur in polysaccharide combination as pentosans. Cherry gum yields *l*-arabinose. Hydrolysis of straw, hay, oat hulls, corn cobs and most woods yields *d*-xylose. *d*-Ribose is a normal constituent of the mononucleotides, guanylic and inosinic acids, as well as of plant nucleic acid. Until

[33] $\underset{\text{Glucose}}{C_6H_{12}O_6} + HCN = \underset{\text{Nitrile of glucoheptonic acid}}{C_6H_{13}O_6 \cdot CN};$

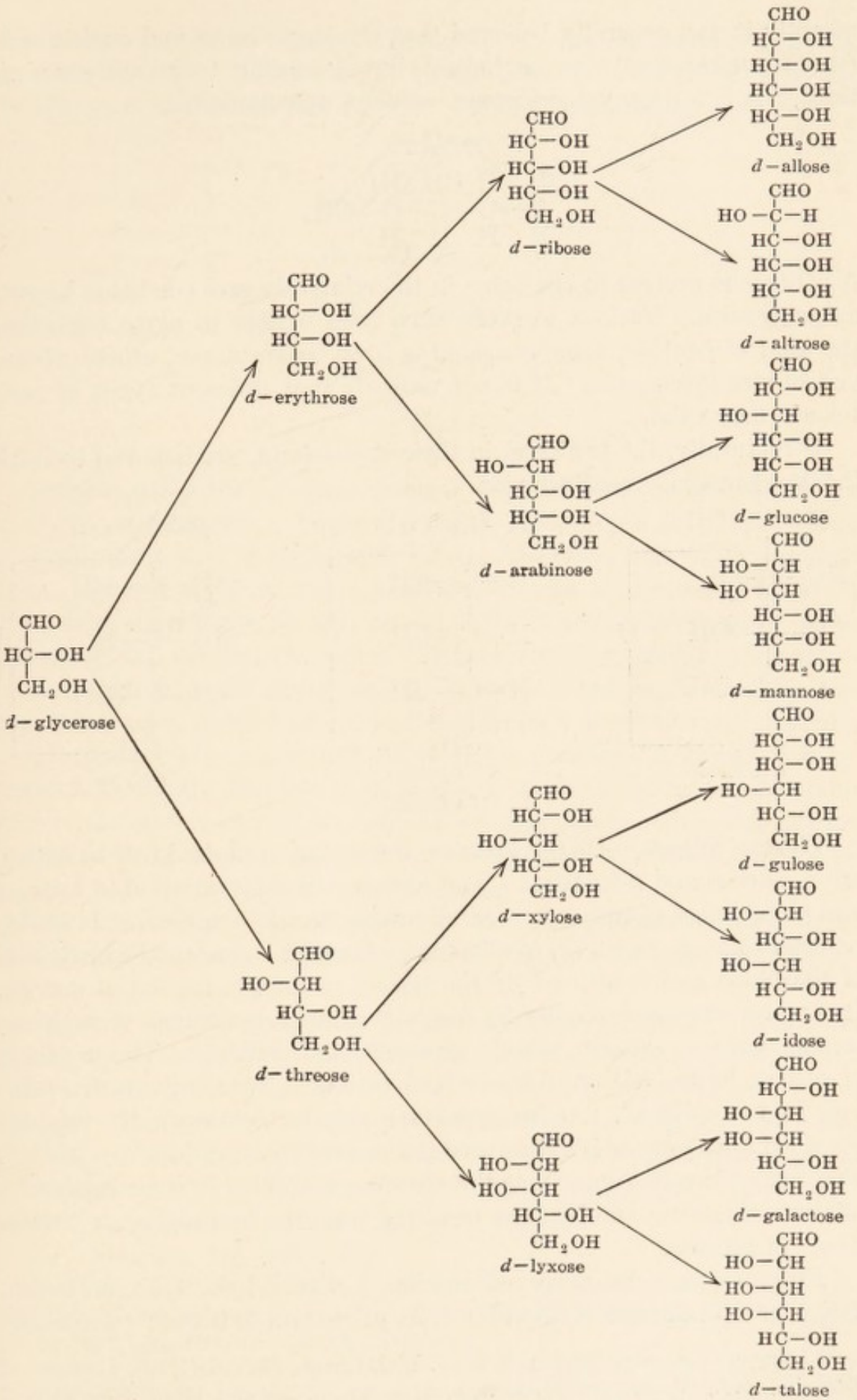
$C_6H_{13}O_6 \cdot CN + 2H_2O = \underset{\text{Glucoheptonic acid}}{C_6H_{13}O_6 \cdot COOH} + NH_3;$

$C_6H_{13}O_6 \cdot COOH \xrightarrow{-O} \underset{\text{Glucoheptose}}{C_7H_{14}O_7}.$

[34] $\underset{\text{Glucose}}{C_5H_{11}O_5 \cdot CHO} + \underset{\text{Hydroxylamine}}{NH_2OH} = \underset{\text{Glucose-oxime}}{C_5H_{11}O_5 \cdot CH : NOH} + H_2O;$

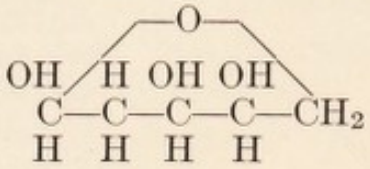
$C_5H_{11}O_5 \cdot CH : NOH + NaOH = \underset{\text{Nitrile of gluconic acid}}{C_5H_{11}O_5 \cdot CN} + NaOH + H_2O;$

$C_5H_{11}O_5 \cdot CN \xrightarrow{\text{heat}} \underset{\text{Arabinose}}{C_5H_{10}O_5} + HCN.$



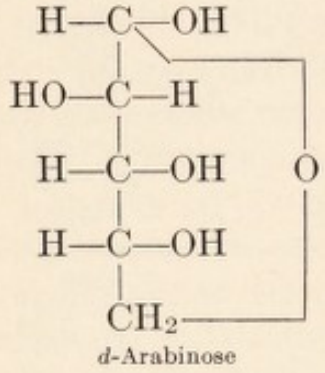
Tetrose, Pentose and Hexose Sugars Derived from *d*-Glycerose

recently it was generally believed that the sugar in animal nucleic acid (thymonucleic acid) was an hexose, but according to recent observations,[35] it is a desoxyaldopentose, namely, *d*-2-ribodesose.

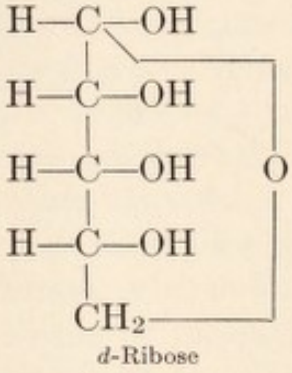


A pentose is present in the urine in the relatively rare condition known as pentosuria. Various workers have been unable to agree regarding the kind of pentose, some considering it to be arabinose, others ribose, still others xylose, etc. It is not unlikely that different types of pentosuria may exist.

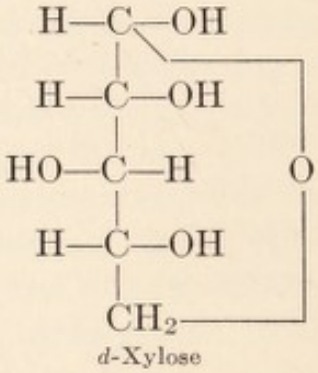
Structurally, the pentoses, in their stable form, are believed to have the amylene-oxide configuration.



d-Arabinose



d-Ribose



d-Xylose

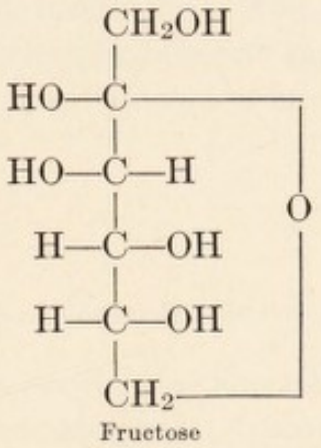
(α-forms)

Hexose Sugars.—Of the hexoses, those that are found free in nature are *d*-glucose and *d*-fructose. The former is widely distributed in fruit and plant juices (grape, sweet corn, onions, unripe potatoes). It is also referred to as grape sugar, or dextrose. Glucose is a normal constituent of the blood and is utilized by the tissues in the production of energy. It may be obtained readily by enzymic or acid hydrolysis of maltose, lactose, sucrose, dextrin, starch, glycogen, and cellulose. The products of sucrose hydrolysis are fructose and glucose. Sucrose is dextro-rotatory $[\alpha]_D = +66.5°$. As fructose is strongly levo-rotatory, the mixture obtained after sucrose is hydrolyzed is also levo-rotatory $[\alpha]_D = -19.84°$. The term " invert sugar " has therefore been applied in referring to the mixture of glucose and fructose resulting from the hydrolysis, or "inversion," of sucrose.

Fructose may be prepared readily by acid hydrolysis of inulin. Together with glucose it occurs in fruit juices and in honey. According

[35] Levene, P. A., and London, E. S., J. Biol. Chem., **83**, 793 (1929); Levene and Mori, T., *ibid.*, **83**, 803 (1929); Levene, Mikeska, L. A., and Mori, *ibid.*, **85**, 785 (1929–30).

to Haworth and Hirst,[36] normal fructose is an amylene-oxide compound, as indicated below. However, it seems that in sucrose (p. 58), the fructose part of the molecule has the γ-oxide configuration.



Fructose

d-Galactose is the constituent sugar of the polysaccharides which are classified as galactans. These are widely distributed in plants, being especially abundant in algae, and lichens, including agar-agar and Irish moss. By hydrolytic methods, galactose may be conveniently prepared from these substances, as well as from the wood of the western larch. Galactose also occurs in saponins. It is a constituent sugar of the disaccharide lactose (milk sugar) and is likewise a component of certain fat-like substances, present in brain tissue, known as cerebrosides (p. 81).

Mannose is the constituent sugar of the mannans, a group of polysaccharides widely distributed in plants, but which are especially abundant in the endosperm of the seed of the tagua palm. Recently, Levene and Mori [37] reported mannose to be a constituent of ovomucoid, a conjugated protein (p. 87) which occurs in egg white.

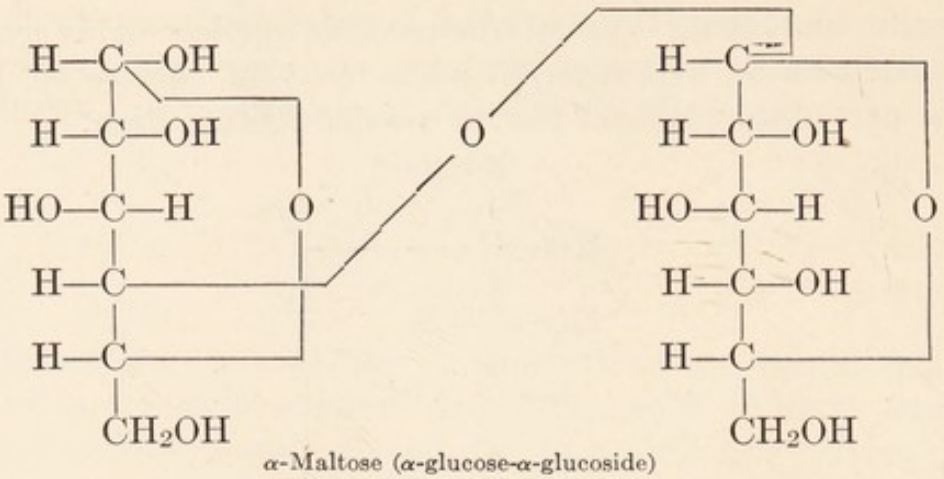
Occurrence and Constitution of the Disaccharides.—The three disaccharides that are of physiological importance are maltose, sucrose, and lactose.

Maltose (malt sugar) may be formed from starch by incomplete hydrolysis of the latter. It is a constituent of germinating cereals and malt. The sugar crystallizes in small needles, is a reducing agent, is fermented by yeasts, forms an osazone with phenylhydrazine, and exhibits mutarotation. Maltose is α- or β-glucose α-glucoside, and, on hydrolysis by acid or the enzyme maltase, yields glucose. It is represented stereochemically by the following formula:[38]

[36] J. Chem. Soc., **129**, 1858 (1926).

[37] J. Biol. Chem., **84**, 49 (1929). The occurrence of mannose in association with proteins has also been reported by Fränkel and Jellinek (Biochem. Z., **185**, 392 (1927)) and by Rimington (Biochem. J., **23**, 430 (1929)).

[38] Irvine, J. C. and Black, I. M. A., J. Chem. Soc., **129**, 862 (1926); Cooper, C. J. A., Haworth, W. N., and Peat, S., *ibid.*, **129**, 876 (1926); Haworth, W. N., and Peat, S., *ibid.*, 3094 (1926).

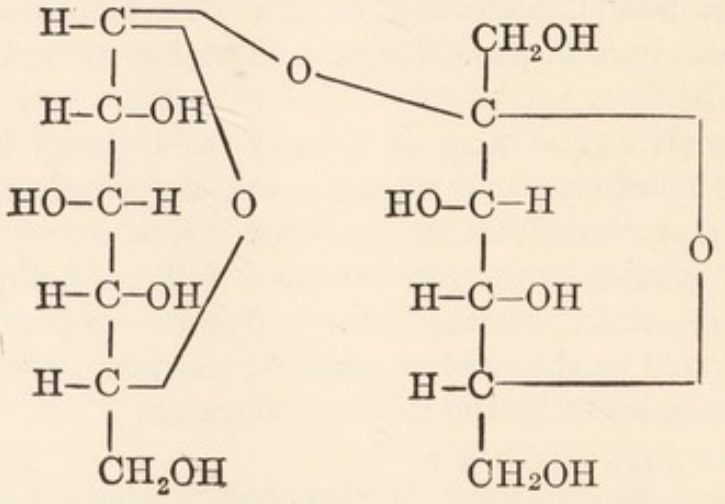


α-Maltose (α-glucose-α-glucoside)

Two isomeric modifications, α-maltose (α-glucose-α-glucoside) and β-maltose (β-glucose-α-glucoside), are possible, depending on the arrangement of the H and OH attached to carbon atom 1 of the glucose portion of the molecule.

Isomaltose is glucose-β-glucoside. It was first prepared by Emil Fischer by the condensation of glucose.

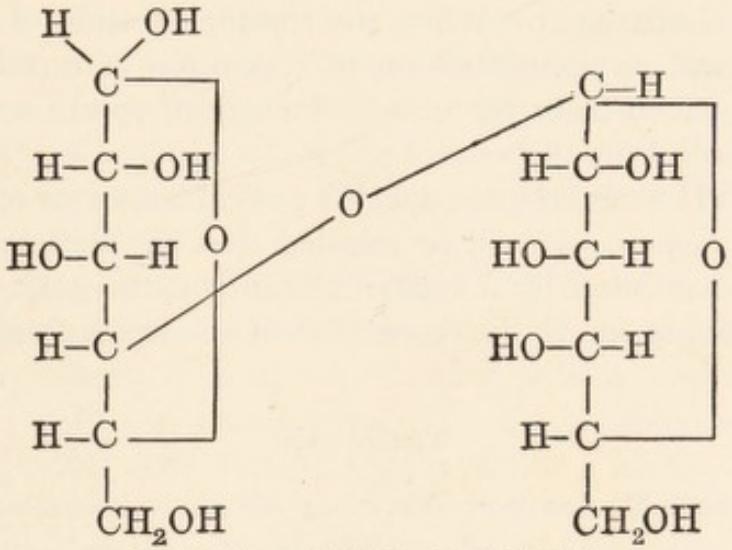
Sucrose (saccharose, cane sugar) occurs in the sugar beet, sorghum cane, sugar maple, pineapple, in the roots of carrots, and in many other plants. It is fermented by yeast, the first step being the inversion of the sucrose by the enzyme invertase which is present in the yeast. Sucrose has no reactive aldehyde group, is therefore non-reducing, does not form an osazone, and does not exhibit mutarotation. On hydrolysis it yields fructose and glucose. As has been pointed out previously, the fructose in the sucrose molecule is believed to have the γ-oxide ring. Sucrose may be represented by the following structural formula:[39]



Sucrose

[39] W. N. Haworth and E. L. Hirst, J. Chem. Soc., **129**, 1858 (1926); G. McOwan, *ibid.*, **129**, 1737 (1926). Compare this formula for sucrose with the one just published by Hudson, J. Am. Chem. Soc., **52**, 1707 (1930).

Lactose (milk sugar) occurs in the milk of mammals. It is hydrolyzed to glucose and galactose either by acid or by the enzyme lactase. Lactose is a reducing sugar, forms an osazone when treated with phenylhydrazine, and exhibits mutarotation. Accordingly, it must have a free or a potentially free aldehyde group and should be present in at least two isomeric forms. Lactose is believed to have the following molecular configuration:



Lactose (α- or β-glucose–β-galactoside)

A number of disaccharides have been obtained from naturally occurring trisaccharides. From gentianose, a trisaccharide (glucose-glucose-fructose) present in the roots of the gentian, gentibiose (glucose-β-glucoside) has been obtained. Melibiose (glucose-β-galactoside) is formed from raffinose (fructose-glucose-galactose) by incomplete hydrolysis. Turanose (fructose + glucose) is obtained by hydrolyzing the trisaccharide melicitose (glucose-glucose-fructose).

The Specific Optical Rotation of Various Sugars.—Owing to the presence of asymmetric carbon atoms, the sugars have the property of rotating the plane of polarized light. The specific rotatory power, or specific rotation, determined at 20° C. in sodium light (*D* line) may be computed from the formula:

$$(\alpha)_{D}^{20^\circ} = \frac{a \times 100}{lc},$$

in which a = observed rotation, l = length of the polariscope tube in decimeters and c = number of grams of optically active substance. The specific rotation (α) of a given compound is defined as the angle of

rotation through which a plane of polarized light (the source of illumination is sodium light) is turned in passing through a tube 1 decimeter in length, filled with a solution containing 1 gram of the substance to 1 cc. Both the temperature, 20° C., and the source of illumination, sodium light (*D* line), are stated in the formula.

Determinations have been made of the specific rotation of a large variety of substances. For sucrose, the value is +66.5°. Special types of the polariscope have been designed for use in sugar analyses. These are called saccharimeters. When the specific rotation of a given compound is known, its concentration in a solution of unknown strength may be determined from the observed angle of rotation which is produced by the solution.

In Table VII are given the specific optical rotations of a number of mutarotating sugars. It will be recalled that the shift in optical rotation which is exhibited by a sugar such as glucose, galactose, maltose, etc., in solution, is due to the conversion of one isomeric modification to another.

TABLE IX

SPECIFIC ROTATION IN WATER OF MUTAROTATING SUGARS
(After Abderhalden)*

Sugar	α Form	Equilibrium	β Form
d-Glucose	+113.4°	+52.2°	+19°
d-Galactose	+144	+80.5	+52
d-Mannose	+34	+14.6	−17
d-Fructose	−21	−92	−133.5
d-Xylose	+92	+19	−20
d-Arabinose	−54	−105	−175
Lactose	+90	+55.3	+35
Maltose	+168	+136	+118

* Taken from data in Biochemisches Handlexikon, vol. 10, p. 366.

Polysaccharides.—When present in foods, fructose, glucose, maltose, and lactose are readily utilized. The bulk of the carbohydrate foods, however, is ingested in the form of polysaccharides. Of these, starch and glycogen are of primary importance physiologically, although cellulose is also utilized to a considerable extent by herbivorous animals. Even in man, it is believed that young and tender cellulose (sometimes referred to as hemicellulose), present in cabbage and lettuce, is utilized to some degree.

Cellulose constitutes the supporting tissue of plant cells and, with the exception of the protective covering of tunicates, is found exclusively in plants. Its chemical properties, such as resistance to the action of acids, are determined by the stage of growth of the plant. A large variety of celluloses doubtless exists; but on complete hydrolysis with strong acids, cellulose, from whatever source, yields glucose as the end-product. In controlled hydrolyses, a disaccharide, cellose, or cellobiose (glucose-β-glucoside), isomeric with isomaltose, is also obtained. Usually, the chief value of cellulose in human nutrition is in providing bulk to the intestinal contents, thereby facilitating peristalsis.

Sponsler and Dore [40] have studied the constitution of ramie cellulose by means of the X-ray and are of the opinion that cellulose is essentially crystalline in structure and that the glucose units of which it is composed exist in the δ-oxide form.

Starch is the principal form in which carbohydrate is stored in the plant and is especially abundant in seeds, bulbs, and tubers. In some plants (apple, banana), during the ripening process, starch is converted into glucose; in others (corn, peas, etc.), the reverse occurs, namely, conversion of sugar into starch, a process which seems to require the presence of potassium. In the plant, the starch occurs in granules which have concentric stratifications. These are characteristic for any given species of plant.

In the raw state, starch is not soluble in cold water, nor is it readily digested by starch-splitting, or amylolytic, enzymes. This is due to the resistance of the outer layer of the starch granule. Starch is a hydrophilic colloid and may take up a considerable amount of water. When it is heated in water, the granules swell but do not necessarily rupture. Prolonged heating or fine grinding may cause the disintegration of starch granules. After raw starch has been subjected to grinding in a ball mill it is found that a portion will go into solution in cold water.

When treated with iodine, most starches give an indigo-blue color. Among the first products which starch yields on hydrolysis are the dextrins. The more complex dextrins, when treated with iodine, give colors varying from purple in the case of amylodextrin to a reddish-brown color given by erythrodextrin. The simpler dextrins (achroodextrin) yield no color when treated with iodine. The final product of the enzymic hydrolysis of starch by amylase is maltose. Starch is

[40] Colloid Symposium Monograph, Chemical Catalog Co., New York, Vol. 4, 174 (1926).

one of the most important constituents of the human diet, making up 50 to 70 per cent of the solid matter of most cereal grains and about 80 per cent of the solids of the potato. It is of interest to note that a small amount of phosphorus exists in combination in starch; the content in potato starch has been found to be about 0.06 per cent.[41]

Glycogen is the reserve carbohydrate of the animal body and is stored principally in the liver and muscles. It is widely distributed in the animal kingdom and is especially abundant in molluscs, echinoderms, and other invertebrates. Certain fungi and yeasts likewise contain glycogen. In these plants, chlorophyll is lacking, a fact which may point to a different mechanism of carbohydrate synthesis than in most plants. When purified, glycogen is a white amorphous powder, odorless and tasteless, having many properties in common with starch. Dissolved in water, it gives an opalescent colloidal solution. Iodine colors glycogen red-brown or deep red. Glycogen is apparently acted upon by starch-splitting enzymes.

Glycogenesis, or the synthesis of glycogen, takes place very rapidly in the animal body. The reverse process, glycogenolysis, is likewise a rapid one, especially when the tissues require carbohydrate for combustion. These transformations will be considered in greater detail elsewhere. Acid or enzyme hydrolysis of glycogen yields glucose as the final product, the intermediate products formed resembling the dextrins obtained from starch.

Not all polysaccharides are equally useful from the standpoint of nutrition. This question has been studied by Swartz and others. No enzymes capable of hydrolyzing the mannans, galactans, and levulans have been found in the digestive tract of man. Swartz,[42] nevertheless, observed that appreciable amounts of certain mannans and galactans disappeared from the alimentary tract. A similar utilization of inulin has been observed in the white rat by Bodey, Lewis and Huber.[43] These polysaccharides may have become available for purposes of nutrition as a result of bacterial changes rather than because of enzymes

[41] For a discussion of the chemical structure of starch and glycogen the student is referred to a review by J. C. Irvine, Chem. Rev., **4**, 225 (1926), as well as to the monograph by W. N. Haworth, "The Constitution of Sugars," Chapter X, London (1929).

The microscopy of starches is comprehensively described in E. T. Reichert's "The Differentiation and Specificity of Starches in Relation to Genera, Species, etc. Stereochemistry Applied to Protoplasmic Processes and Products, and as a Strictly Scientific Basis for the Classification of Plants and Animals." Parts I and II, Carnegie Institution of Washington, Pub. 173, Washington, D. C. (1913).

[42] Trans. Conn. Acad. Sci., **16**, 247 (1909).

[43] J. Biol. Chem., **75**, 715 (1927).

secreted in the alimentary tract. On the whole, however, it seems quite certain that the polysaccharides other than starch, glycogen, and to a less extent cellulose, are of little value in human and animal nutrition.

Immunologically Specific Polysaccharides.—Soluble polysaccharides have been isolated from certain bacterial cultures which react with the antisera of the respective bacteria to form precipitates. For example, the polysaccharides obtained from fluid cultures of the various types of pneumococci, in dilutions as high as 1 : 60,000, have been found to be specific precipitants for the antisera of the corresponding organisms. Heidelberger and Goebel [44] hydrolyzed the polysaccharide from type III pneumococcus and obtained aldobionic acid, $C_{11}H_9O_{10}\cdot COOH$, which on further hydrolysis yielded glucose and glucuronic acid. A polysaccharide, to which the empirical formula $(C_{30}H_{44}O_{26})_x$ has been assigned by Goebel, is said to occur in type A Friedlander's bacillus. On hydrolysis, this polysaccharide yields aldobionic acid, glucose, and a disaccharide of unknown constitution.

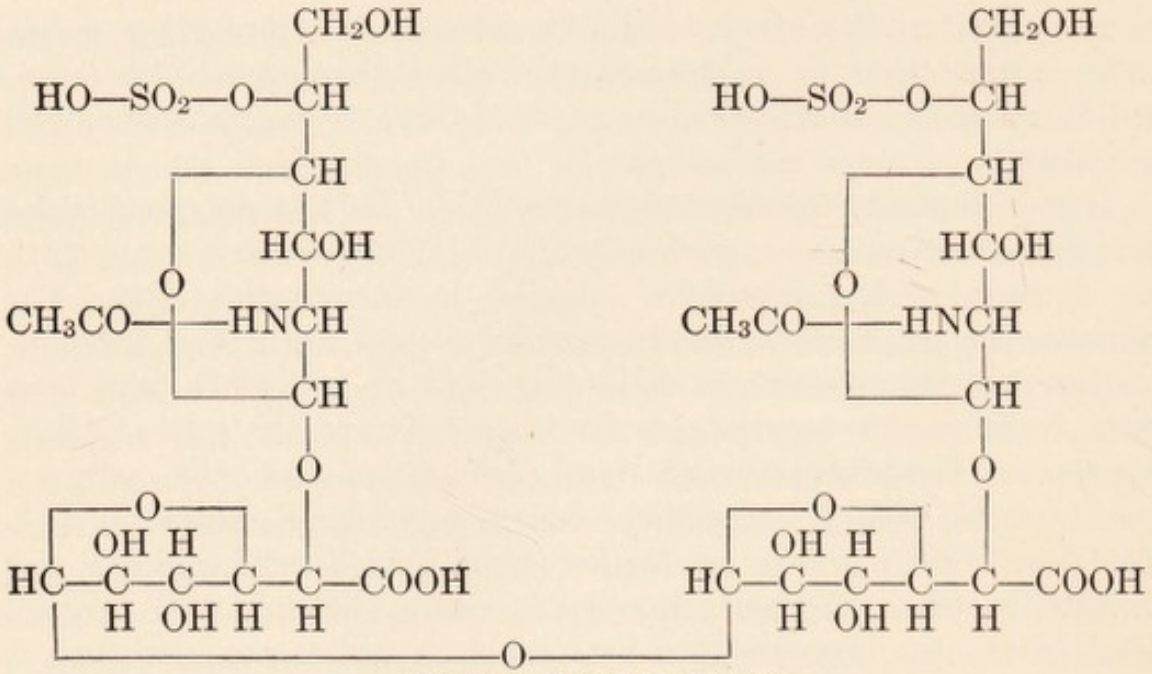
Hexosamines.—Chitin, the outer covering of insects and crustacea, on hydrolysis yields an amino-hexose, called chitosamine or glucosamine $[CHO\cdot CHNH_2\cdot (CHOH)_3\cdot CH_2OH]$. Glucosamine is also a constituent of certain mucoproteins or mucins, proteins present in mucous secretions, and it probably exists widely distributed in other combinations.[45]

The mucoproteins are classified as conjugated proteins (p. 87) and are characterized by having in combination a carbohydrate group. In certain of these, the carbohydrate group is mucoitin-sulfuric acid. This compound has been isolated from the mucin of the gastric mucosa, serum mucoid, ovomucoid, funis (umbilical cord) mucin, vitreous humor, and cornea. Mucoitin-sulfuric acid is represented by the following formula (Levene):[46]

[44] Heidelberger, M., Chem. Rev., **3**, 403 (1927); Heidelberger and Goebel, W. F. J. Biol. Chem., **70**, 613 (1926); *ibid.*, **74**, 613 (1927). Goebel, J. Biol. Chem., **74**, 619 (1927).

[45] S. Fränkel and C. Jellinek (Biochem. Z., **185**, 392 (1927)), have reported the isolation from coagulated egg white and yolk proteins of a polysaccharide composed of glucosamine and mannose. C. Rimington (Biochem. J., **23**, 430 (1929)), has reported the isolation of a carbohydrate derivative from the purified proteins of horse-serum. This is said to be a disaccharide composed of glucosamine and mannose. P. A. Levene and T. Mori (J. Biol. Chem., **84**, 49 (1929)), and P. A. Levene and A. Rothen (*ibid.*, **85**, 63 (1929)) state that the carbohydrate groups of egg proteins are probably trisaccharides, composed of 1 molecule of glucosamine and 2 molecules of mannose.

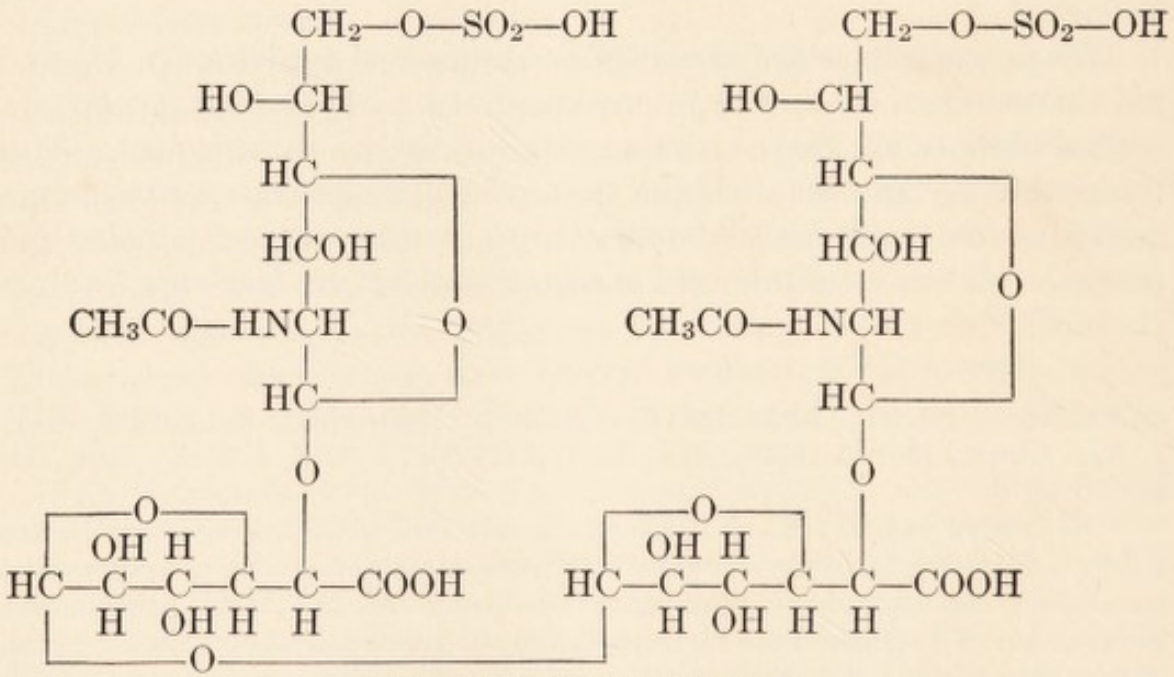
[46] P. A. Levene, Hexosamines and Mucoproteins, Longmans, Green & Co., New York, 1925, p. 84; the formula for chondroitin-sulfuric acid is on p. 82.



Mucoitin-sulfuric acid (Levene)

The components of mucoitin-sulfuric acid are: sulfuric acid, acetic acid, a hexosamine which is probably glucosamine[47] and glucuronic acid.

Chondroproteins are mucoproteins which occur in connective tissue. The carbohydrate group in these is chondroitin-sulfuric acid.



Chondroitin-sulfuric acid (Levene)

[47] Formerly, chitosamine was thought to be either glucosamine or mannosamine. The evidence now seems to be that chitosamine is derived from glucose, being 2-amino glucose. Epichitosamine, 2-amino-mannose, has been prepared synthetically. Chondrosamine is 2-amino-galactose.

On hydrolysis, chondroitin-sulfuric acid yields sulfuric acid, acetic acid, glucuronic acid and a hexosamine which is probably galactosamine, also called chondrosamine.

The Glucosides.—The glucosides are substances that on hydrolysis yield a sugar, usually glucose, and one or more additional products. The glucosides are very numerous and are widely distributed in plants. Familiar examples are *phlorhizin*, also spelled *phloridzin* (glucose + phloretin), which occurs in the bark of *Rosaceae*; *coniferin* (glucose + coniferyl alcohol), present in the bark of the fir tree; *salicin* (glucose + saligenin) in the bark of the willow tree; *amygdalin* (2 glucose + mandelonitrile), in the seeds of the bitter almond; *quercitrin* (rhamnose + quercetin), in the bark of the oak; *sinigrin* (glucose + allyl thiocyanate + $KHSO_4$), in black mustard seeds; in the leaves of the foxglove occur: *digitalin* (glucose + digitalose [$C_7H_{14}O_5$] + digitaligenin), *digitonin* (2 glucose + galactose + digitogenin); *digitoxin* (2 digitoxose [$C_6H_{12}O_4$] + digitoxigenin).

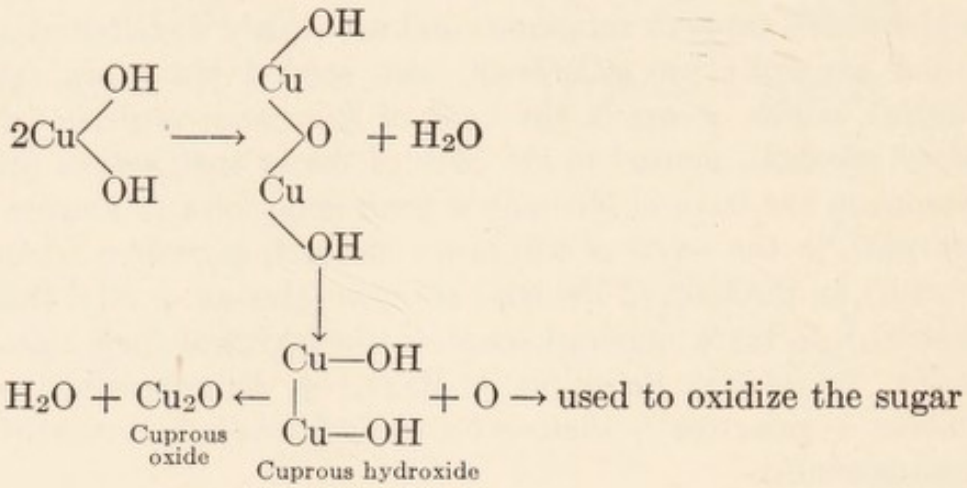
In the animal body analogous compounds are found. The cerebrosides are usually classified with the lipids but may also be considered as galactosides. The two important cerebrosides are phrenosin and kerasin. When hydrolyzed, these yield fatty acids, galactose, and sphingosine, a nitrogenous base. Then there are the nucleosides which give on hydrolysis a sugar, *d*-ribose, and either a purine or pyrimidine.

Pectins.—Pectins are carbohydrates of high molecular weight and colloidal properties which occur most abundantly in the parenchymatous tissues of fruits and vegetables, such as apples, oranges, grape fruit (in the last two, especially in the inner white rind), turnips and beets. On hydrolysis, the pectins yield galacturonic acid, arabinose and galactose. In the presence of suitable concentrations of acid and sucrose, the pectins form the familiar fruit jellies and jams.

Reactions of the Sugars.—Sugars that have an aldehyde or ketone group are easily oxidized, thereby acting as reducing agents. Of the many tests that are known, a few will be described here. For detailed directions, the student is referred to laboratory manuals on biochemistry.

Fehling's Test.—Two solutions are used: one contains copper sulfate, the other Rochelle salt and sodium or potassium hydroxide. Two or three cubic centimeters of each solution are mixed and heated. At this stage, heating should produce no change. Upon the addition of several drops of sugar solution and further heating, the solution at first becomes turbid and greenish in color. Later a yellow precipitate of cuprous hydroxide or a red precipitate of cuprous oxide separates. The cupric

hydroxide which forms when the two solutions are mixed is held in solution by the tartrate (Rochelle salt is sodium-potassium tartrate). The reduction of the copper hydroxide by the sugar may be considered to take place as follows:



Fehling's method may be applied to the quantitative analysis of sugars.

Benedict's Test.—This involves the use of only one solution and is more sensitive than Fehling's test. Benedict's solution contains sodium carbonate, sodium citrate, and copper sulfate. After preliminary heating of the reagent, several drops of sugar solution are added. A greenish yellow or red precipitate is produced on heating a second time. Benedict's test has been modified for use in the quantitative estimation of sugar. Here, however, the end-point is indicated by the disappearance of the blue color, due to the formation of white cuprous thiocyanate (CuCNS).

Nylander-Almen Test.—In this test, bismuth subnitrate is reduced by the sugar to metallic bismuth according to the following equation:

$$2Bi(OH)_2NO_3 + 2KOH \rightarrow 2Bi(OH)_3 + 2KNO_3$$

$$2Bi(OH)_3 \rightarrow Bi_2 + 3H_2O + 3O$$

Barfoed's Test.—The addition of sugar solution to Barfoed's reagent (containing copper acetate in dilute acetic acid), with heating, results in the reduction of the cupric acetate to cuprous oxide. The test is given by the monosaccharides but not readily by the disaccharides; hence it is employed in distinguishing the two groups.

Reduction of Picric Acid.—In alkaline solution, sugar readily reduces

picric acid and its salts, supposedly to picramic acid. The reaction is only partially represented by the equation:

$$C_6H_2(NO_2)_3OH \rightarrow C_6H_2NH_2(NO_2)_2OH + O$$

Upon this reaction is based the Lewis-Benedict method for the determination of sugar in small amounts of blood.

Molisch Test.—This is a general test for all carbohydrates as well as for other compounds containing a carbohydrate residue in their molecules. Several drops of an alcoholic solution of α-naphthol are added to the sugar solution. This is then stratified above a layer of concentrated sulfuric acid. At the zone of contact, a violet ring develops. The reaction depends on the condensation of furfural or its derivatives with the α-naphthol.

Seliwanoff Reaction.—This test is specific for keto-sugars. Seliwanoff's reagent is a solution of resorcinol in hydrochloric acid. On heating, the acid converts fructose, for example, into levulinic acid and methyl-hydroxy-furfural. The latter compound condenses with the resorcinol to form a red compound.

Moore's Test.—Sugars are very unstable in alkaline solution. When heated with sodium hydroxide to boiling, sugar solutions develop a brown color and an odor of caramel.

Phenylhydrazine Reaction.—The addition of phenylhydrazine to a solution of certain sugars (those having a free aldehyde or ketone group), with heating, results in the formation of yellow crystalline osazones, specific as to crystal form, melting point, etc. By this method it is possible to distinguish between glucose, lactose, maltose, and other sugars. Glucose, fructose, and mannose yield the same osazone. Sucrose does not form an osazone.

Mucic Acid Test.—Oxidation of galactose with hot nitric acid yields an insoluble dicarboxylic acid, mucic acid [COOH—$(CHOH)_4$—COOH]. The corresponding dicarboxylic acid formed from glucose, namely saccharic acid, is soluble. Lactose likewise yields mucic acid, since on hydrolysis it forms both glucose and galactose.

Fermentation.—Yeast ferments glucose, fructose, maltose, sucrose, and other sugars. Ordinary brewer's yeast (*Saccharomyces cerevisiæ*) does not ferment either galactose or lactose. The disaccharides are first inverted by enzymes present in yeast. Alcohol and carbon dioxide are the products usually obtained.

Orcinol-Hydrochloric Acid Test.—The addition of this reagent to a solution of a pentose, with heating, results in the production of a succession of colors—violet, blue, red, and green. If the sugar solution is sufficiently concentrated, a bluish-green precipitate separates.

Phloroglucinol-Hydrochloric Acid Test.—The addition of phloroglucinol and hydrochloric acid to a pentose solution, with heating, results in the development of a cherry-red color. Galactose, likewise, responds to this test.

Iodine Test.—Iodine yields with starch a blue or purple-blue color. With glycogen and the higher dextrins a wine-red color is produced.

CHAPTER III

THE LIPIDS

FATS are the triglyceride esters of fatty acids and constitute an important class of foodstuffs. They are closely associated in nature with the phosphatides, cerebrosides, sterols, and other substances. As regards the nomenclature applied to the fats and these related compounds, there has been little uniformity. The name " lipoids " is often employed as an inclusive term, but it is also used in the more restricted sense of applying only to phosphatides and cerebrosides. Recently the term " lipides " or " lipids " has found a certain amount of usage as a general group name for the fats and fat-like substances.

The following classification has been suggested by Bloor: [1]

Lipids.—Substances having the following characteristics:

a. Insolubility in water and solubility in the fat solvents, such as ether, chloroform, benzene.[2]

b. Relationship to the fatty acids as esters, either actual or potential.[3]

c. Utilization by living organisms.

Simple Lipids.—Esters of the fatty acids with various alcohols.

1. Fats—esters of the fatty acids with glycerol.
2. Waxes—esters of the fatty acids with alcohols other than glycerol.

[1] Bloor, W. R., Biochemistry of Fats, Chemical Reviews, **2**, 243 (1925–6).

[2] This property, namely solubility in fat solvents and insolubility in water, sets off the fats from the carbohydrates and proteins. Nevertheless, this property is not an absolute one; the lecithins are somewhat soluble in water and insoluble in acetone which is otherwise a good solvent for fats. The cephalins are mainly insoluble in alcohol, while sphingomyelin and the cerebrosides are difficultly soluble in ether.

[3] Bloor has wisely included this property, as well as the next one (*c*), in order to exclude organic compounds which have no biochemical relationship to the fats or fatty acids, but which from their solubilities alone would be included in the group. According to (*b*) and (*c*), the substances classified as lipids must be either ester-like combinations of the fatty acids or capable of forming such combinations, and they must be capable of performing some useful functions in living organisms.

Compound Lipids.—Esters of the fatty acids containing groups in addition to an alcohol and fatty acid.

1. Phospholipids—substituted fats containing phosphoric acid and nitrogen: lecithin, cephalin, sphingomyelin.

2. Glycolipids—compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid: phrenosin, kerasin. These are also called cerebrosides.

3. Aminolipids, sulfolipids, etc.—groups which are at present not sufficiently well characterized for classification.

Derived Lipids.—Substances derived from the above groups by hydrolysis.

1. Fatty acids of various series.

2. Sterols—mostly large molecular alcohols, found in nature combined with the fatty acids and which are soluble in the fat solvents: cholesterol ($C_{27}H_{45}OH$), myricyl alcohol ($C_{30}H_{61}OH$), cetyl alcohol ($C_{16}H_{33}OH$), etc.

Fat Synthesis.—In both plants and animals there is a close relationship between fats and carbohydrates, pointing to the origin of the former from the latter. For example, in the maturation of seeds, the increase in fat content is concurrent with a decrease in the amount of carbohydrate. This is shown in the following table, representing the results of analyses of almonds by Le Clerc du Sablon.[4]

TABLE X

CONVERSION OF CARBOHYDRATE INTO FAT DURING THE MATURATION OF THE ALMOND

Date of Gathering	Per Cent Fat	Per Cent Glucose	Per Cent Sucrose	Per Cent Starch and Dextrins
June 9.....	2	6	6.7	21.6
July 4.....	10	4.2	4.9	14.1
August 1.....	37	0	2.8	6.2
September 1.....	44	0	2.6	5.4
October 4.....	46	0	2.5	5.3

The reverse occurs during germination; during this process there is a decrease in the fat content of the seedlings, accompanied by an increase

[4] Cited by J. B. Leathes and H. S. Raper, The Fats, New York and London, 2d edition, 1925, p. 103.

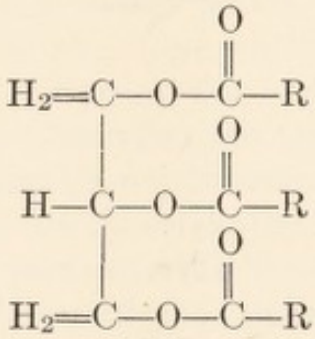
in the content of cellulose and other insoluble carbohydrates. This is well brought out in the following data obtained by Maquenne [5] in a study of the chemical changes occurring during the germination of Arachis seedlings.

TABLE XI

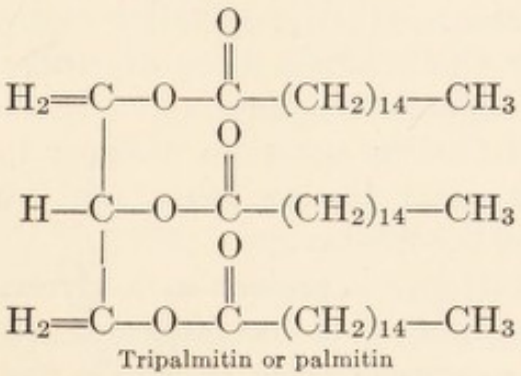
DISAPPEARANCE OF FAT DURING GERMINATION OF ARACHIS SEEDLINGS

Age in Days	Per Cent Fat	Per Cent Carbohydrate Other than Cellulose	Cellulose and Other Insoluble Carbohydrates
0	51.39	11.55	2.51
6	49.81	8.35	3.46
10	36.19	11.09	5.01
12	29.00	12.52	5.22
18	20.45	12.34	7.29
28	12.16	9.46	9.48

Constitution of the Fat Molecule.—The molecular structure of a fat may be represented by the formula:

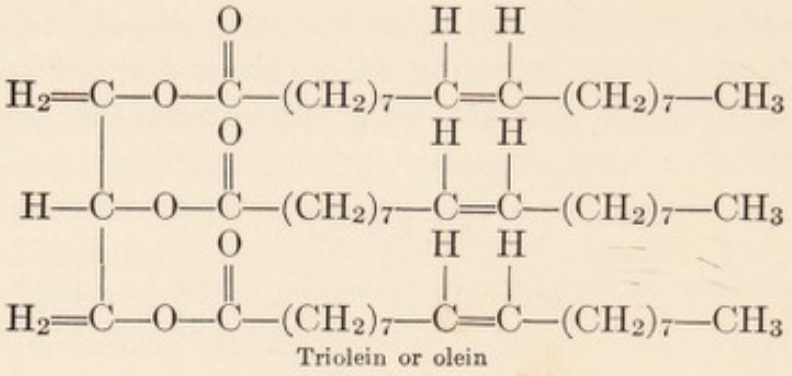


in which R represents a fatty-acid radical. The following are the formulas for tripalmitin (the tripalmitic acid ester of glycerol) and triolein (the trioleic acid ester of glycerol):



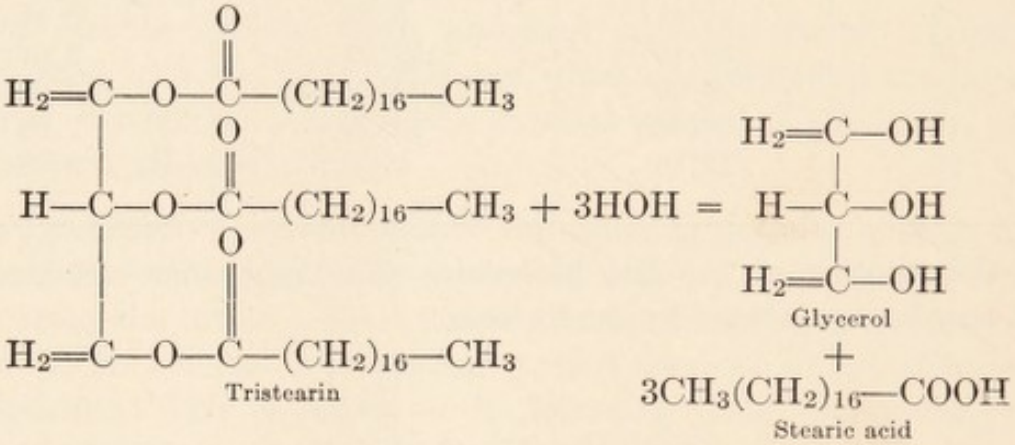
Tripalmitin or palmitin

[5] Compt. rend., **127**, 625 (1898).



Triolein or olein

Fats are hydrolyzed by the action of acids, alkalies, fat-splitting enzymes (lipases), and superheated steam. Three molecules of fatty acid and one of glycerol are formed as a result.



Tristearin

Glycerol

Stearic acid

In the presence of alkali, the fatty acid reacts to form soap. The action of alkali on fat is therefore termed saponification. Saponification, in a broader sense, is the hydrolysis of any ester with or without alkali.

Saturated Fatty Acids.—Fatty acids may be divided into two general groups, the saturated and the unsaturated. With few exceptions, the fatty acids that occur in nature contain an even number of carbon atoms. However, in the blubber of porpoises is found isovaleric acid ($C_5H_{10}O_2$), and in croton oil, tiglic acid ($C_5H_8O_2$) which is an unsaturated fatty acid. The saturated fatty acids are homologs of formic acid and have the general formula $C_nH_{2n}O_2$, ($C_nH_{2n+1}COOH$).

Formic acid (HCOOH) occurs in sweat, urine, meat juice, and the bodies of ants (especially the red ant).

Acetic acid (CH_3COOH) occurs in vinegar; in smaller amounts in sweat, muscle and other tissues, feces, and urine. It occurs as a glyceride in the oil of the spindle tree.

Butyric acid ($C_4H_8O_2$) is present as a glyceride in butter, to the extent of about 6 per cent. The free fatty acid occurs in sweat.

n-Caproic and *n*-caprylic acids ($C_6H_{12}O_2$ and $C_8H_{16}O_2$) occur as glycerides in butter, cocoanut oil, and palm-nut oil.

Capric acid ($C_{10}H_{20}O_2$) is present, in combination with glycerol, in the milk of cows and goats, as well as in cocoanut oil and palm-nut oil.

Lauric acid ($C_{12}H_{24}O_2$) occurs as glyceride in milk, more abundantly in spermaceti, laurel oil, cocoanut oil, palm-kernel oil, etc.

Myristic acid ($C_{14}H_{28}O_2$) is a constituent of nutmeg oil and also occurs as glyceride in milk and vegetable fats. In small amounts, it has been found in lard and cod-liver oil.

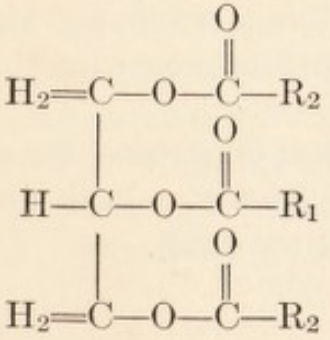
Palmitic acid ($C_{16}H_{32}O_2$) is widely distributed as glyceride in animal and vegetable fats. It occurs in cow's milk, myrtle wax, Japan wax, and palm oil. Bayberry tallow is almost pure tripalmitin. In spermaceti, a wax found in the skulls of whales and dolphins, it is present in combination as the ester of cetyl alcohol ($C_{16}H_{33}OH$); in beeswax as the ester of myricyl alcohol ($C_{30}H_{61}OH$ or $C_{31}H_{63}OH$); and in opium wax as the ester of ceryl alcohol ($C_{26}H_{53}OH$).

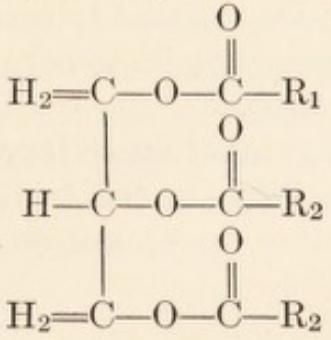
Stearic acid ($C_{18}H_{36}O_2$) is contained as a glyceride in most vegetable and animal fats.

Arachidic acid ($C_{20}H_{40}O_2$) occurs in peanut and other vegetable oils. It is also said to be present in cow's milk, and in the fat of tissues and of dermoid cysts.

There are a number of fatty acids of even greater complexity. Behenic acid ($C_{22}H_{44}O_2$) is found in the oil of ben obtained from the seeds of *Moringa pterygosperma*. Lignoceric acid ($C_{24}H_{48}O_2$) is a component of the phosphatide, sphingomyelin, and occurs also in beechwood and lignite tar. It is present as the glyceride in arachis oil. Cerotic acid ($C_{26}H_{52}O_2$) has been isolated from a variety of waxes (beeswax, carnauba wax, Chinese wax, opium wax, and wool fat). Melissic acid ($C_{30}H_{60}O_2$) occurs free in beeswax. For further details as to the distribution of the fatty acids in nature, the student is referred to Chapter I of the monograph by Leathes and Raper.

Mixed Triglycerides.—The three fatty-acid radicals in a fat molecule may be all the same, as in palmitin, stearin, and olein; but it is also possible for them to differ. Thus, if the three fatty acids are all different, three combinations are possible, each representing a different mixed triglyceride. If two radicals are alike and one is different, two combinations of these radicals are possible, namely:





Other combinations may exist. Taking into consideration the number of fatty acids occurring naturally, it is theoretically possible to have innumerable mixed triglycerides. A number of these have been isolated. Palmito-distearin occurs in lard and in beef tallow. Stearo-dipalmitin has been prepared from mutton tallow. Stearo-diolein is said to be present in the fat of the human body. Myristo-palmito-olein occurs in cacao-butter.

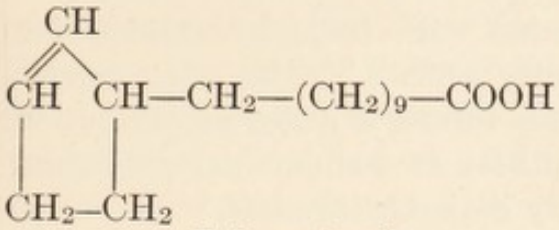
Unsaturated Fatty Acids.—The unsaturated fatty acids contain one or more pairs of carbon atoms united by a double bond. In the oleic series of fatty acids ($C_nH_{2n-2}O_2$ or $C_nH_{2n-1}COOH$) there is one such pair. Several isomers of the formula $C_{16}H_{30}O_2$ have been described. Hypogeic acid occurs in peanut and maize oils. Palmitoleic acid is said to be present in cod-liver oil. Physetoleic acid occurs in sperm and seal oils. The most important member of the series is oleic acid ($C_{18}H_{34}O_2$), a constituent of most fats and oils, where it is present in combination with glycerol. A number of fatty acids isomeric with oleic acid have been prepared in the laboratory (elaidic and iso-oleic acids). Another isomer, rapic acid, is present as glyceride in rape or colza oil. Gadoleic acid ($C_{20}H_{38}O_2$) occurs in herring, sperm, and cod-liver oils. Erucic acid ($C_{22}H_{42}O_2$) has been found in rape-seed, mustard-seed, and cod-liver oils.

Belonging to the linoleic acid series ($C_nH_{2n-4}O_2$) and the linolenic acid series ($C_nH_{2n-6}O_2$) are fatty acids of a greater degree of unsaturation than oleic acid. Linoleic acid ($C_{18}H_{32}O_2$) is an important constituent of cottonseed oil, and linolenic acid ($C_{18}H_{30}O_2$) of linseed oil. When exposed to the air, the highly unsaturated triglycerides of linolenic acid combine readily with oxygen to form solid compounds. To this are due the useful properties of linseed and other drying oils. Clupanodonic acid ($C_{22}H_{34}O_2$) has been prepared from cod-liver and sunfish-liver oils and from herring, sardine, and whale oils. Castor oil contains a monohydroxy fatty acid, ricinoleic acid ($C_{18}H_{34}O_3$), and a dihydroxy acid, dihydroxystearic acid ($C_{18}H_{36}O_4$). Arachidonic acid ($C_{20}H_{32}O_2$) has been isolated from liver tissue of pigs and is reported to be the only highly unsaturated fatty acid occurring in thyroid, suprarenal and spleen. Arachidonic acid, and possibly tetracosapentenoic acid, $C_{24}H_{38}O_2$, are said to occur in the brain, where the highly unsaturated fatty acids seem to be present in greater proportion than in other tissues (Brown).[6]

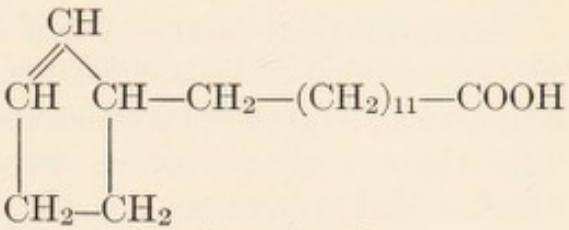
Two cyclic unsaturated fatty acids are of importance because of their therapeutic value in the treatment of leprosy. Both are found in

[6] J. Biol. Chem., **83**, 777, 783 (1929).

chaulmoogra oil. They are hydnocarpic acid ($C_{16}H_{28}O_2$) and chaulmoogric acid ($C_{18}H_{32}O_2$).



Hydnocarpic acid



Chaulmoogric acid

Properties of Fats and Fatty Acids: *Solubility.*—Mention has been made of the solubility of fats in the so-called fat solvents, ether, chloroform and benzene, and their insolubility in water. This applies more especially to the glycerides of the higher fatty acids, for those of the lower fatty acids, such as tributyrin and tricaproin are somewhat soluble in water. In ethyl and methyl alcohol and in acetone, the fats dissolve readily in the hot, but only slightly in the cold.

All fatty acids are soluble in ether, chloroform, benzene and hot alcohol. The fatty acids, lower than palmitic acid, are also soluble in cold alcohol. In water only the lowest members are readily soluble.

Hydroxy-fatty acids, as well as their glycerides, are insoluble in petroleum ether, a property which distinguishes them from other fatty acids and fats.

The fats themselves are very good solvents for other fats and fatty acids.

Consistency.—The temperature at which a fat melts is higher than the temperature at which it solidifies. Thus, the melting point of tristearin is 71.5° C., whereas the solidifying point is 52.5° C. This peculiarity of having widely differing solidifying and melting points is not exhibited by the fatty acids. The melting point of a fat depends upon the component fatty acids. The glycerides of the higher saturated fatty acids have higher melting points than the glycerides of the lower fatty acids. The glycerides of the unsaturated fatty acids have even lower melting points. Glycerides which are fluid at ordinary temperatures are often called oils, whereas those which are solid are called fats. This distinction, which is essentially one of convenience in industrial and culinary uses, is not ordinarily adhered to in chemical discussions, the term fat being used indiscriminately for a liquid as well as a solid fat.

Specific Gravity.—The specific gravity of most solid fats (mutton tallow, lard, beef tallow, cocoanut oil, etc.) is very uniform, being approximately 0.86. Somewhat greater variation is found with different

liquid fats, as is shown by the following data: olive oil, 0.915–0.918; peanut oil, 0.917–0.926; cottonseed oil, 0.921–0.926; maize oil, 0.921–0.927; linseed oil, 0.931–0.941.

Saponification Value.—When treated with basic hydroxides, fats yield glycerol and the basic salts of fatty acids. The latter are termed soaps, and the process by which they are formed is called saponification. The soaps of commerce are usually those of sodium and potassium. Calcium and magnesium soaps are very insoluble in water.

$$\underset{\text{Stearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3KOH = \underset{\text{Glycerol}}{C_3H_5(OH)_3} + 3\underset{\text{Potassium stearate (a soap)}}{CH_3(CH_2)_{16}COOK}$$

Applying the law of chemical combination to the above equation, we see that the molecular weight of fat is to three times the molecular weight of alkali as the actual weight of fat is to the actual weight of alkali. If the last three terms are known, the first can be determined. The amount of potassium or sodium hydroxide that will react with a given amount of fat in the process of saponification will depend on the average length of the constituent fatty-acid chains, for the smaller the fatty-acid molecules, the greater would be their number in a given amount of fat. Upon this principle is based a method for determining the character of different fats. The determination is made by heating a definite amount of fat (usually 1–2 grams) with a known volume of a standardized alcoholic solution of potassium hydroxide (usually 25 cc. 0.5 normal alkali) until saponification is complete. The unused alkali is determined by titration with standard acid. From the data obtained may be calculated the amount of alkali that was used in the saponification of the fat, and in turn its saponification value. *The saponification value is defined as the number of milligrams of potassium hydroxide neutralized by the saponification of one gram of fat.* Accordingly, it serves as a measure of the mean molecular weight of the fatty acids that are present in the fat. A large proportion of butter consists of the lower fatty acids; hence, butter has a relatively high saponification value (about 220–230). Similarly, coconut oil contains such large amounts of caproic, caprylic, capric, and lauric acids that it has an even higher saponification number (about 250). On the contrary, lard, mutton tallow, and cod-liver oil are composed of the higher fatty acids to a greater extent. Consequently, these have relatively low saponification values. Another factor that may influence this constant is the presence in the fat of unsaponifiable constituents. Fats containing appreciable

amounts of such substances may have low saponification values for this reason.

Hydrogenation.—The unsaturated fats, such as those contained in vegetable oils, may be saturated by hydrogen, a reaction which is catalyzed by certain finely divided metals, including nickel. By the introduction of two hydrogen atoms at the unsaturated bond in oleic acid, stearic acid is formed, and similarly linoleic acid may be converted by hydrogenation, first to oleic and finally to stearic acid. This process is of great commercial and economic importance, as it makes possible the production of valuable articles of diet from comparatively inedible oils, such as cottonseed oil. The process of hydrogenation is not carried to completion, however, as this would produce a brittle form of tallow. The various lard substitutes of commerce are the products of partial hydrogen absorption and contain approximately 20 to 25 per cent of saturated fats (stearin), 65 to 75 per cent of olein, and 5 to 10 per cent of linolein. The hydrogenated fats are as well utilized by the animal body as the natural fats.

Halogen Absorption; Iodine Number.—The unsaturated fatty acids react readily with the halogens, particularly with iodine, forming saturated halogen absorption derivatives. The more unsaturated the fatty acid, the more iodine is taken up at the double bonds, the reaction being quantitative under certain conditions. Upon this principle is based a method for determining the degree of unsaturation of fats and fatty acids. In determining the iodine number, a weighed amount of fat is treated with a known volume (usually 25 cc.) of a solution containing iodine (Wijs or Hanus solution) and allowed to stand in the dark for one to two hours. The unabsorbed iodine is then determined by titration with standard sodium thiosulfate. From the data thus obtained, the amount of iodine taken up by the fat may be calculated, and in turn the iodine number may be computed. *The iodine number is the number of grams of iodine that is absorbed by* 100 *grams of fat.* No iodine is taken up by saturated fatty acids or their corresponding glycerides. Oleic acid has one double bond and an iodine value of 90.1. Clupanodonic acid has five double bonds and an iodine number of 384. The iodine number therefore serves as an index of the degree of unsaturation of fats.

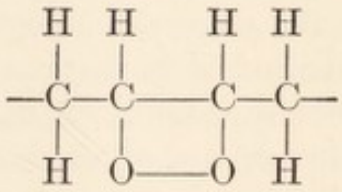
In the following table are given the melting points,[7] iodine numbers, and saponification values of certain fats:

[7] These data are taken from a table in Robertson's Principles of Biochemistry, Philadelphia and New York, 1924, p. 123.

TABLE XII

Fat	Melting-point	Iodine Number	Saponification Value
Butter fat	28°–33° C.	26–38	220–230
Pork fat	36°–46° C.	50–70	195–197
Beef fat	40°–48° C.	36–48	193–200
Human fat	17.5° C.	57–66	193–199
Cod-liver oil	0°–10° C.	144–168	175–193
Cottonseed oil	3°– 4° C.	105–117	191–196
Olive oil	2°–10° C.	78–91	185–194
Linseed oil	−27° C.	173–202	190–195

Rancidity and Oxidation of Fats.—Fats are relatively unstable substances and are susceptible to deterioration, especially when exposed to light, heat and moisture. They thus acquire characteristically disagreeable odors and flavors. At least two changes occur, one consisting in hydrolysis, with the liberation of free fatty acids. Rancid butter owes its peculiar odor partly to the formation of free butyric acid. The second change is one of oxidation and affects principally the unsaturated fatty acids, resulting in the fixing of the oxygen in the peroxide form



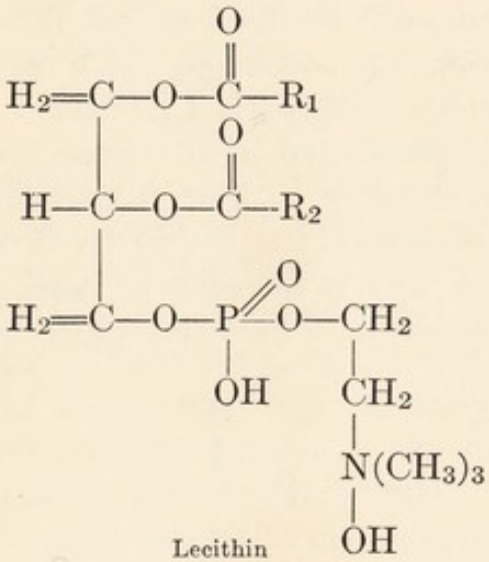
as well as in the formation of aldehydes, ketones and acids of lower molecular weight. The two processes occur simultaneously, the presence of free acid apparently increasing greatly the susceptibility of fats to oxidation. The iodine number falls as rancidity progresses.

The capacity to take up oxygen is especially marked in the highly unsaturated fats and is a property exhibited by the so-called drying oils. When thin layers of these oils are exposed to air, they absorb oxygen and are converted into tough, elastic and waterproof substances which adhere tightly to the painted surface and protect it from the weather. Linseed oil and tung, or Chinawood oil are the two principal drying oils used in the manufacture of paints, varnishes, artificial rubber, linoleum, and other coverings.[8]

[8] For a general view of the economic importance of fats, the student is referred to the interesting and brief monograph by C. L. Alsberg and A. E. Taylor, The Fats and Oils, Stanford University Press, 1928.

Phospholipids.—The phospholipids (also called phospholipins and phosphatides) are present in every animal and vegetable cell and are especially abundant in the brain, heart, muscles, liver, and eggs. On hydrolysis these substances yield fatty acids, a nitrogenous base, phosphoric acid, and usually glycerol. Of the phosphatides or phospholipids that have been described, only three type substances have been studied sufficiently to establish their chemical individuality. These are the monoamino-monophosphatides, lecithin and cephalin; and the diamino-monophosphatide, sphingomyelin.

The following formula, in which R represents a fatty acid radical, suggests that more than one lecithin is possible.



Lecithin

Great difficulty has been encountered by those who have attempted to determine the precise character of the fatty acids present in the lecithin molecule. For some time the prevailing opinion has been that lecithin contains one saturated fatty acid, either palmitic or stearic, and one unsaturated fatty acid. The lecithin of egg yolk is said to contain oleic acid. Levene [9] and his co-workers have determined the presence of a fatty acid belonging to the linoleic acid series in liver lecithin. By brominating lecithin obtained from liver, Levene and Simms [10] prepared octabrom-arachidic acid.

According to the newer concepts of fat metabolism, the formation of lecithin represents an intermediate stage in the oxidation of the fatty acids. The neutral fat is believed to be converted into lecithin, and it is in this form of combination that the constituent fatty acids undergo a successive number of dehydrogenations, being thus rendered more

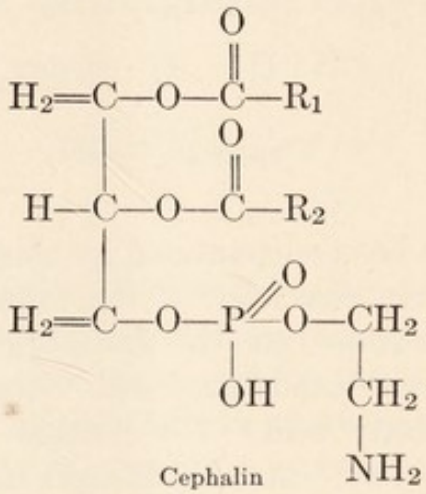
[9] Physiol. Reviews, **1**, 327 (1921).

[10] J. Biol. Chem., **51**, 285 (1922).

and more unsaturated. The lecithin content of cellular tissues remains unchanged even in extreme emaciation and it is therefore very likely that lecithin is essential to the life of the cell. Preparations of lecithin obtained from tissues do not ordinarily represent a single substance, but are actually mixtures of lecithins, containing as impurities other phosphatides, as well as cerebrosides.

Choline [$(CH_3)_3{\equiv}N(OH)—CH_2CH_2OH$], trimethyl-oxyethyl-ammonium-hydroxide, is the base obtained on hydrolysis of lecithin. The nitrogen content of lecithins from various sources is usually higher than can be accounted for on the assumption that choline is the only nitrogenous base present in the molecule. The choline content is less than the theoretical amount. This is explained by the fact that the lecithin preparations usually obtained are not pure but contain admixtures of a second phospholipid, cephalin, which on hydrolysis yields the base, amino-ethyl alcohol ($NH_2 \cdot CH_2 \cdot CH_2OH$).

Cephalin is widely distributed in all animal tissues. Like lecithin, it is a type compound and may, at least theoretically, hold in combination a variety of fatty acids. On hydrolysis it yields glycerol, fatty acids, phosphoric acid, and a nitrogenous base, amino-ethyl alcohol. The available evidence points to the following formula for cephalin:

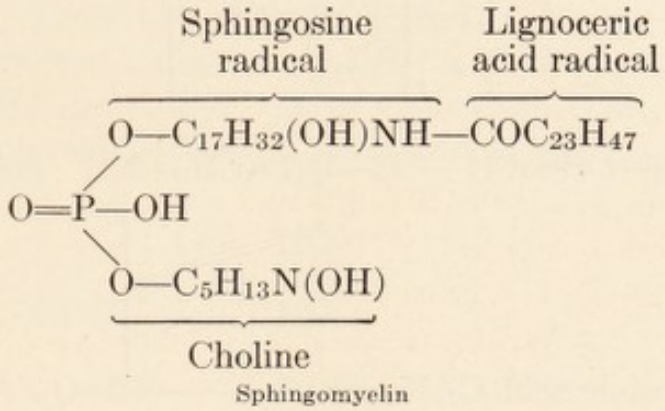


Cephalin

Properties of Cephalin and Lecithin.—Lecithin and cephalin are miscible in water, from which they may be precipitated by acetone. Lecithin is soluble in alcohol, whereas cephalin is relatively insoluble. Both are soluble in ether, chloroform, benzene and the other common fat solvents, with the exception of acetone in which they are insoluble. They oxidize readily in air, turning brown and acquiring a disagreeable odor.

Sphingomyelin is a phospholipid which yields on hydrolysis lignoceric acid ($C_{23}H_{47}COOH$), phosphoric acid, and two nitrogenous bases,

choline and sphingosine ($C_{17}H_{32}(OH)_2NH_2$). A second fatty acid, the true nature of which has not been well established, seems to be a constituent of sphingomyelin. Analyses indicate that this fatty acid is possibly an hydroxy-stearic acid. Sphingomyelin occurs in brain, kidney, liver, egg yolk, and in small amounts in blood and muscle. The following formula has been suggested by Levene:[11]



Sphingomyelin

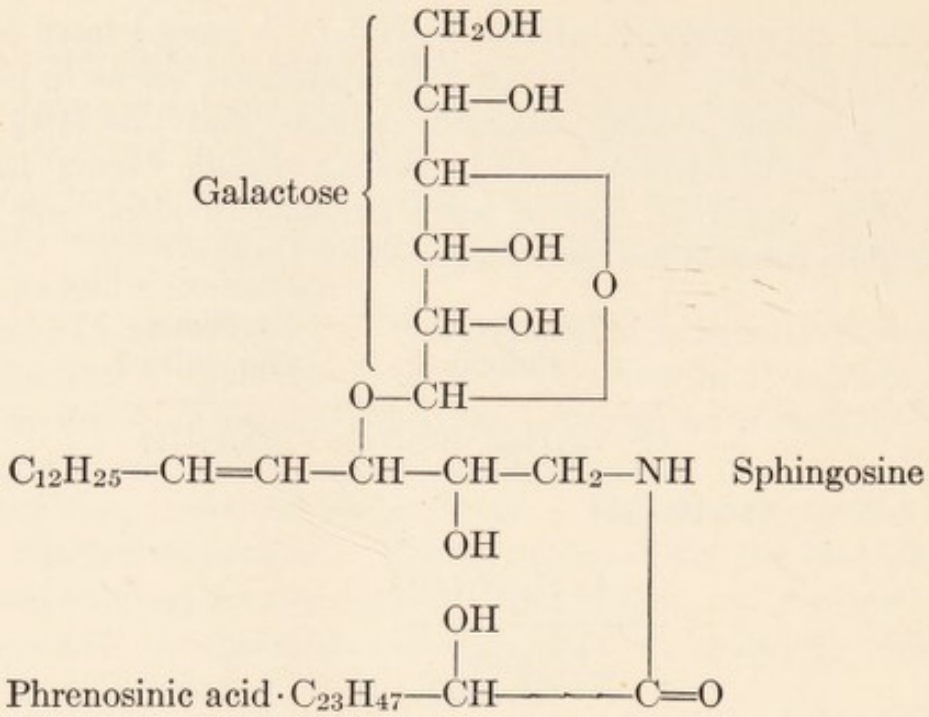
Properties.—Sphingomyelin is soluble in cold and hot chloroform, benzene, pyridine, glacial acetic acid, and hot alcohol, from which it separates on cooling in crystalline form. It is relatively insoluble in hot and cold ether. It is somewhat soluble in hot acetone, but not in cold acetone, which may be used in precipitating it from water in which it forms an opalescent suspension. Sphingomyelin is dextrorotatory. As compared with lecithin and cephalin, it is a relatively stable compound, undergoing no change on exposure to air or light.

The Cerebrosides.—Associated with the phosphatides in the tissues, particularly in the brain, are the cerebrosides which on hydrolysis yield among other products the sugar galactose. The cerebrosides are glycolipids and because galactose is the constituent sugar, they are also called galactolipids (or galactolipines). These compounds are essentially analogous to the glucosides found in plants. Two cerebrosides are known, phrenosin and kerasin, but judging from the recent observations of Taylor and Levene,[11a] it is possible that there are other compounds of this type. Phrenosin on hydrolysis yields a fatty acid, phrenosinic (cerebronic) acid ($C_{25}H_{50}O_3$), galactose, and the base sphingosine. Kerasin differs from phrenosin in that lignoceric acid is the fatty-acid constituent of the molecule. Rosenheim [12] has suggested the following constitutional formula for phrenosin:

[11] J. Biol. Chem., **24**, 69 (1916).

[11a] *Ibid.*, **84**, 23 (1929). In this paper it is also shown by Taylor and Levene that the cerebronic acid fraction obtained from phrenosin may be fractionated into several acids of higher and lower molecular weight than cerebronic acid itself.

[12] Biochem. J., **10**, 142 (1916).



Crystalline Phrenosin

Phrenosin is crystalline, but on losing one molecule of water with the formation of an anhydride, it becomes amorphous (amorphous phrenosin). The cerebrosides are soluble in hot alcohol, acetone, or benzene, and in pyridine. Like sphingomyelin, they are almost insoluble in hot and cold ether.

Owing to the difficulty involved in isolating and purifying the various phosphatides and cerebrosides, mixtures of these substances have frequently been mistaken for individual compounds. The opinion once held concerning the chemical individuality of protagon, a " compound " obtained from brain tissue, is now known to be incorrect. Protagon is really a mixture of cerebrosides and sphingomyelin with traces of other substances. Another example is that of jecorin, found in the livers of many animals. Jecorin is now known to be a variable mixture containing, among other substances, cephalin, cerebrosides, and carbohydrates. Until recently, cuorin was thought to be a phosphatide. However, Levene and Komatsu [13] have shown that it is actually a mixture of decomposition products of cephalin and lecithin.

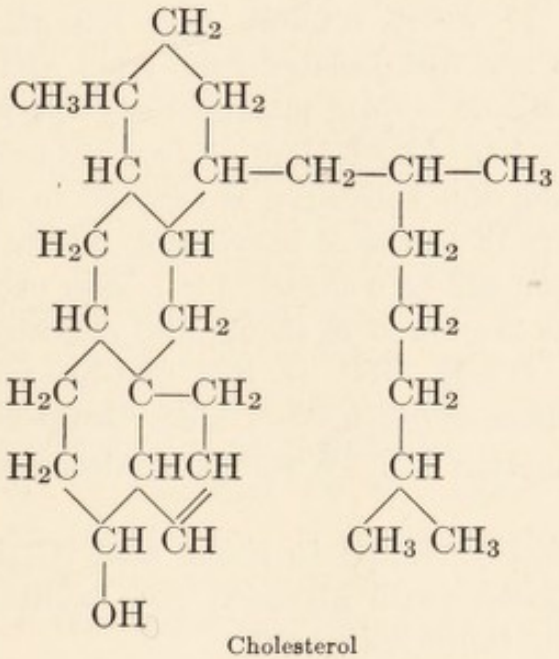
Waxes.—The waxes are fatty-acid esters of the higher monoatomic alcohols. Spermaceti, found in the skull of certain species of whales and dolphins, contains as its chief constituent the palmitic acid ester of cetyl alcohol ($C_{16}H_{33}OH$). Among the other constituents of spermaceti are esters of lauric, myristic, and stearic acids with the following alcohols: lethal ($C_{12}H_{25}OH$), methal ($C_{14}H_{29}OH$), and stethal ($C_{18}H_{37}OH$).

[13] J. Biol. Chem., **39**, 91 (1919).

The principal constituents of beeswax are the palmitic acid ester of myricyl alcohol ($C_{30}H_{61}OH$), cerotic acid ($C_{26}H_{52}O_2$), either in the free form or as an ether, and cerolein, which is probably a mixture of several substances.

Waxes are found in wool, in sperm oil, in the secretions of many animals, particularly insects, and in many forms of plant life.

The Sterols.—These are alcohols of high molecular weight closely related to the terpenes. Physiologically, the chief sterol is cholesterol. It was first isolated from gall-stones by Conradi in 1775. According to Windaus,[10] cholesterol ($C_{27}H_{45}OH$) has the following structural formula:



Cholesterol

Cholesterol was first isolated from gall-stones and derives its name from the fact that it is a constituent of bile (Greek χόλη = bile). In the tissues, cholesterol exists both free and in combination with fatty acids as esters. Cholesterol is very abundant in brain and nerve tissue as well as in certain tumors. It is said to accumulate in the arteries in arteriosclerosis. Purified lanolin, the fat of sheep's wool, is a mixture of cholesterol oleate, palmitate, and stearate. The feathers of birds are said to contain a silicic acid ester of cholesterol. Cholesterol is believed to be an essential constituent of the living cell. Since it is an alcohol and not an ester it is not hydrolyzed by alkalies, and hence is a constituent of the " unsaponifiable fat residue." Fish and liver oils are especially rich in unsaponifiable matter, containing in some cases as much as 20 per cent. Wheat oil contains about 2.5 per cent; cottonseed oil, corn oil and olive oil about 1 per cent, or somewhat less.

Cholesterol has several asymmetric carbon atoms which accounts

for the existence of a number of isomers. One of these, isocholesterol, is present in wool fat. Cholesterol is optically active, $[\alpha]_D = -31.6°$.

Cholesterol is soluble in all the fat solvents, including hot alcohol. It is also soluble in fluid fats, fatty acids (oleic), and bile. Cholesterol crystallizes from alcohol as white, glistening, rhombic plates with one irregular or broken corner. The melting point is 148° C. Owing to the presence of an unsaturated bond, cholesterol combines with iodine.

Coprosterol ($C_{27}H_{47}OH$), a constituent of feces, is formed by the reducing action of bacteria in the lower intestine. It has been prepared in the laboratory by the catalytic hydrogenation of cholesterol, using nickel, in an atmosphere of hydrogen.

Another sterol of great interest and importance is ergosterol ($C_{27}H_{41}OH$), which was first isolated from ergot and later found to be present also in yeast and certain mushrooms. Ergosterol seems to be widely distributed in fats of both plant and animal origin. Recent work has shown that when this substance is exposed to ultraviolet light, it acquires the property of curing or preventing rickets. The rôle of this substance in nutrition will be considered in a later chapter.

The phytosterols are found in plants and resemble cholesterol very closely. Sitosterol ($C_{27}H_{45}OH$) is the best-known example. Corn oil contains several isomers of sitosterol, a dihydrositosterol ($C_{27}H_{47}OH$), and stigmasterol ($C_{30}H_{47}OH$). Wheat oil contains sitosterol and dihydrosterol.

Cholesterol is closely related to cholic acid ($C_{24}H_{40}O_5$), the latter occurring in conjugation with glycocoll and taurine in the two bile acids, glycocholic and taurocholic acids.

Physiological Importance.—In connection with topics to be discussed in later chapters, the physiological importance of the fats will be considered from various angles. It is sufficient to say at this point that a part of the fat stored in the body may serve as a source of energy in periods of food deprivation. The fats have a higher caloric value than either protein or carbohydrate. The combustion of one gram of fat in the body yields 9.3 calories as compared with about 4 calories provided by a similar amount of either protein or sugar. The amount of stored or reserve fat varies in different individuals, depending on the amount of food consumed and other factors. In addition to this, fat and fat-like substances are present in every cell and form as integral and essential a part of protoplasm as protein itself. This fat is not depleted even during extreme inanition.

CHAPTER IV

THE PROTEINS

THE resemblance between plant and animal proteins was clearly stated in 1839 by the Dutch chemist, Mulder,[1] who pointed out the importance of these substances in the constitution of cell protoplasm. The protein foodstuffs not only play an extremely important part in the regeneration of worn-out tissue and in the building of new tissue, but are also used by the body in the production of energy. Most proteins contain at least the elements, carbon, hydrogen, oxygen, nitrogen and sulfur, and, as far as can be determined by chemical analysis, seem to be closely related. However, there is nothing more characteristic of the proteins as a group than their physiological specificity. No two proteins seem to be exactly alike as far as their physiological behavior is concerned. The proteins of one animal differ from those of all other animals and the same is true of plant proteins.

Proteins cannot be identified by the usual chemical methods. Accordingly, the classifications of these substances are based largely on physical properties such as solubility. The classification most frequently employed is that recommended jointly by the American Physiological Society and the American Society of Biological Chemists.[2] This classification is given below: [3]

THE PROTEINS

I. Simple Proteins.—Protein substances that yield, on hydrolysis, only amino acids or their derivatives.

The various simple proteins may be designated as follows:

a. Albumins.—Simple proteins soluble in pure water and coagulable by heat. (Examples: egg-albumin, serum-albumin, legumelin of the pea, and leucosin of wheat.)

[1] Mulder, G. J., J. prakt. Chem., **16**, 129 (1839).

[2] J. Biol. Chem., **4**, p. xlviii (1908).

[3] In the light of recent studies, this classification is not strictly correct. For example, egg albumin, serum albumin and globulin are said to contain carbohydrate groups. By definition, these should be classified as glycoproteins, a subgroup of the conjugated proteins, and not as simple proteins.

b. Globulins.—Simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases with strong acids. (Examples: serum globulin, fibrinogen, myosinogen of muscle, edestin of hemp seed, legumin of peas, excelsin in Brazil nuts, concanavalin in the jack bean.)

c. Glutelins.—Simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies. (Examples: glutenin of wheat, oryzenin in rice.)

d. Alcohol-soluble proteins—Prolamins or Gliadins.—Simple proteins soluble in relatively strong alcohol (70 to 80 per cent), but insoluble in water, absolute alcohol, and other neutral solvents. (Examples: gliadin from wheat or rye, hordein from barley, zein from maize or wheat.)

e. Albuminoids.—Simple proteins that possess essentially the same chemical structure as the other proteins, but are characterized by great insolubility in all neutral solvents. These substances form the principal organic constituents of the skeletal structure of animals and also of their external covering and its appendages. The albuminoids are also called scleroproteins. (Examples: keratin from hair, horns, hoofs, nails, etc.; elastin in elastic tissue, ligaments, and the walls of arteries; collagen in bones and cartilage; spongin found in the skeletal structure of the sponge; reticulin present in lung, kidney, spleen, liver, and lymphatic gland tissue; fibroin and sericin from silk.)

f. Histones.—Soluble in water and insoluble in very dilute ammonia; in the absence of ammonium salts, insoluble even in an excess of ammonia. They yield precipitates with solutions of other proteins, and, on heating, a coagulum which is easily soluble in very dilute acids. On hydrolysis they yield a number of amino acids among which the basic ones predominate. (The histones are found in the red corpuscles of the blood and in spermatozoa. Examples: scombron in mackerel spermatozoa, gadus histone from the codfish, globin from hemoglobin.)

g. Protamins.—Simpler polypeptides than the proteins included in the preceding groups. They are soluble in water, uncoagulable by heat, have the property of precipitating aqueous solutions of other proteins, possess strong basic properties, and form stable salts with strong mineral acids. They yield comparatively few amino acids, among which the basic amino acids greatly predominate. (Like the histones, the protamins occur in combination with nucleic acids in spermatozoa. Examples: salmine from salmon, sturine from sturgeon, scombrine from mackerel, cyprinine from carp, clupeine from herring.)

II. Conjugated Proteins.—Substances that contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

a. Nucleoproteins.—Compounds of one or more protein molecules with nucleic acid. (Present in the germ of grain and in glandular tissue.)

b. Glycoproteins.—Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. (Example: mucin.)

c. Phosphoproteins.—Compounds of the protein molecule with some as yet undefined phosphorus-containing substance other than a nucleic acid or lecithin. (Examples: caseinogen of milk, vitellin of egg yolk.)

d. Hemoglobins.—Compounds of the protein molecule with hematin or some similar substance. (Example: Hemoglobin.) (These substances are also classified as chromoproteins and include such substances as the hemocyanins.)

e. Lecithoproteins.—Compounds of the protein molecule with lecithin.

III. Derived Proteins.

1. *Primary Protein Derivatives.*—Derivatives of the protein molecule, apparently formed through hydrolytic changes which involve only slight alterations of the protein molecule.

a. Proteans.—Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes.

b. Metaproteins.—Products of the further action of acids and alkalies, whereby the molecule is so far altered as to form products soluble in very weak acids and alkalies but insoluble in neutral fluids. (Examples: Acid metaprotein, alkali metaprotein.)

c. Coagulated Proteins.—Insoluble products which result from (1) the action of heat on their solutions, or (2) the action of alcohols on the protein.

2. *Secondary Protein Derivatives.*—Products of the further hydrolytic cleavage of the protein molecule.

a. Proteoses.—Soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium sulfate or zinc sulfate.

b. Peptones.—Soluble in water, uncoagulated by heat, but not precipitated by saturating their solutions with ammonium sulfate.

c. Peptides.—Definitely characterized combinations of two or more

amino acids, the carboxyl group of one being united with the amino group of the other, with the elimination of a molecule of water.

In the classification adopted by British biochemists, the protamins, histones, albumins, globulins, glutelins, gliadins, scleroproteins, and phosphoproteins are grouped as the simple proteins. The conjugated proteins are the glucoproteins, nucleoproteins and chromoproteins. The metaproteins or infraproteins, proteoses, and polypeptides are grouped as products as protein hydrolysis.

Amino Acids.—By the action of strong acids or protein-splitting enzymes, the proteins may be broken down into simpler and simpler compounds, the end products being the amino acids. The amino acids are the units or fragments from which the protein molecule is built up. At least nineteen amino acids have been definitely determined among the cleavage products of various proteins. These are as follows:

CLASSIFICATION OF AMINO ACIDS

I. Aliphatic Amino Acids.

A. Monoamino-monocarboxylic Acids.

1. Glycine, $C_2H_5NO_2$, or amino-acetic acid. (1820, Braconnot)[4]

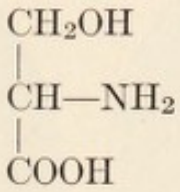
$$\begin{array}{l} CH_2—NH_2 \\ | \\ COOH \end{array}$$

2. *d*-Alanine, $C_3H_7NO_2$, or α-amino-propionic acid. (1875, Schützenberger and Bourgeois)

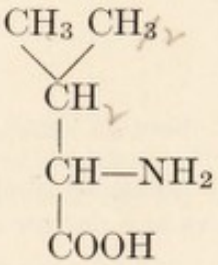
$$\begin{array}{l} CH_3 \\ | \\ CH—NH_2 \\ | \\ COOH \end{array}$$

[4] The dates of discovery and the names of the discoverers are those given by H. H. Mitchell and T. S. Hamilton in their monograph "The Biochemistry of Amino Acids." Wherever possible, these have been confirmed by reference to original sources. It is to be pointed out that not in all cases were the constitutions of the amino acids known to their discoverers, nor are the names now in use necessarily those originally given. To cite a recent example as an illustration, methionine was discovered by Mueller, but its constitution was determined by Barger and Coyne (Biochem. J., **22**, 1417 (1928)), who suggested the name. Then, also, in at least one case, alanine, the amino acid was synthesized (Strecker, 1850) long before it was obtained as a decomposition product of protein.

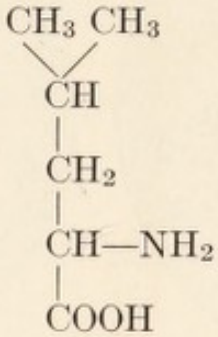
3. *l*-Serine, $C_3H_7NO_3$, or β-hydroxy-α-amino-propionic acid. (1865, Cramer)



4. *d*-Valine, $C_5H_{11}NO_2$, or α-amino-isovalerianic acid. (1856. v. Gorup-Besanez)



5. *l*-Leucine, $C_6H_{13}NO_2$, or α-amino-isocaproic acid. (1818. Proust)



6. *d*-Isoleucine, $C_6H_{13}NO_2$, or α-amino-β-methyl-β-ethyl-propionic acid. (1903, F. Ehrlich)

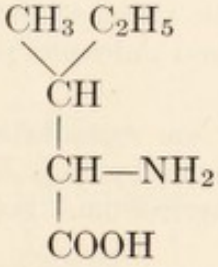


FIG. 7.

FIG. 8.

FIG. 7.—Alanine. Magnification about 45 times. Crystal habit,—rods and needles.

FIG. 8.—Leucine. Magnification about 45 times. Crystal habit,—colorless, thin, six-sided plates and narrow rod-like plates.

FIG. 9.

FIG. 10.

FIG. 9.—Tyrosine. Magnification about 54 times. Crystal habit,—thin needles and rods, aggregate into tufts and sheaves.

FIG. 10.—Cystine. Magnification about 45 times. Crystal habit,—colorless hexagonal plates and prisms.

FIGURES 7, 8, 9, 10, 11, and 12 are reproductions from micro-photographs which were kindly furnished to the author by G. L. Keenan of the Bureau of Chemistry, United States Department of Agriculture. See J. Biol. Chem., **62**, 163 (1924).

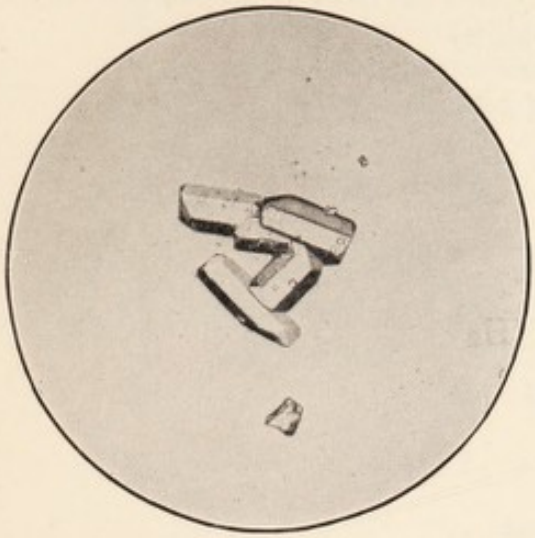


FIG. 11.

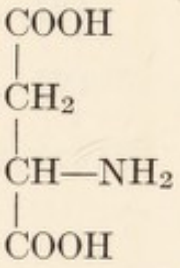
FIG. 12.

FIG. 11.—Aspartic acid. Magnification about 36 times. Ordinarily obtained as irregular fragments of crystals, having high refractive indices.

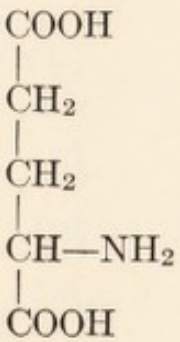
FIG. 12.—Tryptophane. Magnification about 45 times. Crystal habit,—very thin plates, rhombs and irregularly six-sided crystals.

B. Monoamino-dicarboxylic Acids.

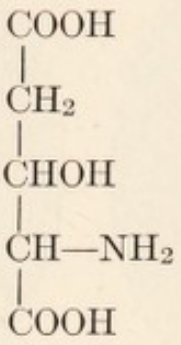
7. *l*-Aspartic acid, $C_4H_7NO_4$, or amino-succinic acid. (1827, Plisson)



8. *d*-Glutamic acid, $C_5H_9NO_4$, or α-amino-glutaric acid. (1868, Ritthausen)

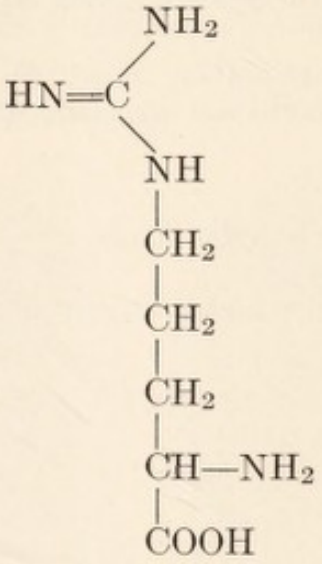


9. *d*-Hydroxyglutamic acid, $C_5H_9O_5N$, or α-amino-β-hydroxy-glutaric acid. (1918, Dakin)

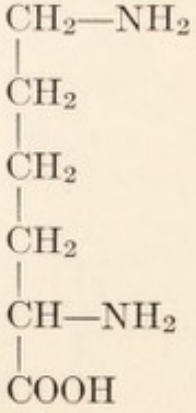


C. Diamino-monocarboxylic Acids.

10. *d*-Arginine, $C_6H_{14}N_4O_2$, or α-amino-δ-guanidine-valeric acid. (1866, Schulze and Steiger)

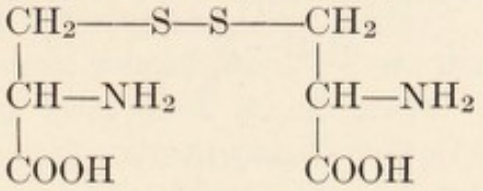


11. *d*-Lysine, $C_6H_{14}N_2O_2$, or α-ϵ-diamino-caproic acid. (1889, Drechsel)

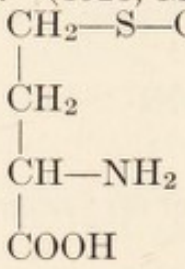


D. Sulfur-containing Amino Acids.

12. *l*-Cystine, $C_6H_{12}N_2O_4S_2$, or dicysteine, or di-(β-thio-α-amino-propionic acid). (1810, Wollaston)

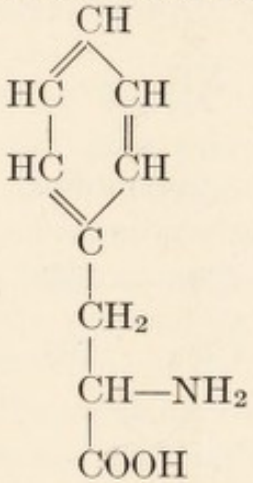


13. *l*-Methionine, $C_5H_{11}SNO_2$, or α-amino-γ-methylthiol-n-butyric acid. (1923, Mueller)

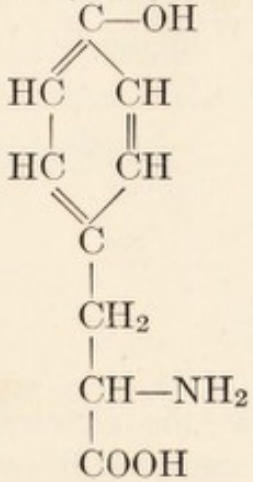


II. Aromatic Amino Acids.

14. *l*-Phenylalanine, $C_9H_{11}NO_2$, or α-amino-β-phenyl-propionic acid. (1881, Schulze and Barbieri)

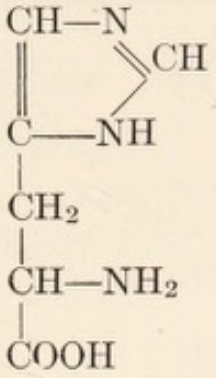


15. *l*-Tyrosine, $C_9H_{11}NO_3$, or β-parahydroxy-phenyl-α-amino-propionic acid. (1846, Liebig)

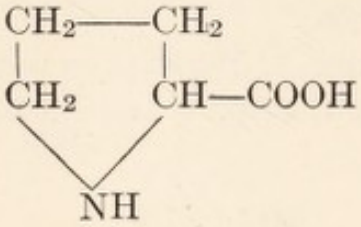


III. Heterocyclic Amino Acids.

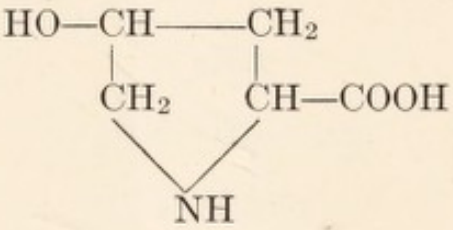
16. *l*-Histidine, $C_6H_9N_3O_2$, or β-imidazole-α-amino-propionic acid. (1896, Kossel)



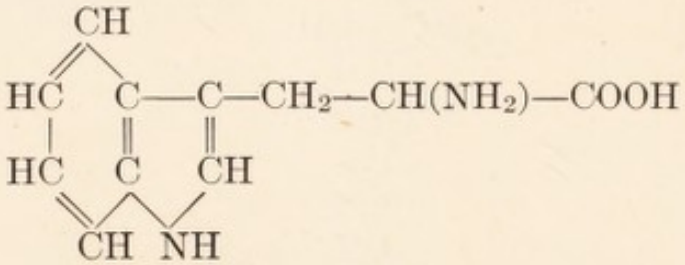
17. *l*-Proline, $C_5H_9NO_2$, or α-pyrrolidine-carboxylic acid. (1901, Fischer)



18. *l*-Hydroxyproline (oxyproline), $C_5H_9NO_3$, or γ-hydroxy-α-pyrrolidine-carboxylic acid. (1902, Fischer)



19. *l*-Tryptophane, $C_{11}H_{12}N_2O_2$, or β-indole-α-amino-propionic acid. (1901, Hopkins and Cole)



The foregoing list comprises the more important cleavage products of the protein molecule. The amino acids are present in varying propor-

tions in different proteins, and while most proteins contain all of the amino acids named there are some proteins that are lacking in one or more of these. Gelatin is made up of only fourteen or fifteen amino acids and is lacking in tyrosine and tryptophane. Glycine, tryptophane and lysine are lacking in zein of corn. Then there are the protamins, which are made up of even fewer amino acids. For example, salmine yields on hydrolysis only valine, serine, proline and arginine; sturine yields arginine, lysine and histidine. The animal body, at least in the vertebrates, is unable to synthesize certain amino acids, such as tyrosine, tryptophane, lysine, cystine, and histidine, and probably arginine and glutamic acid. These are required, however, in the formation of tissue and hence are essential for proper nutrition. The rôle of amino acids in nutrition will be considered in greater detail in a later chapter.

In addition to the nineteen amino acids that have been listed, other amino acids have been reported as products of protein hydrolysis. Among these are norleucine, or α-amino-*n*-caproic acid (Abderhalden and Weil), α-amino-*n*-butyric acid (Foreman), a dihydroxyphenylalanine (Guggenheim) and a hydroxy-valine (Schryver and Buston). It has also been claimed that cysteine ($CH_2SH—CHNH_2—COOH$) may exist as such in the protein molecule.

The method introduced by Fischer[5] for the separation of amino acids consists in converting the amino acids into esters and subsequently separating these by fractional distillation.

The products of acid hydrolysis of proteins may also be separated by extraction with various solvents, such as butyl and ethyl alcohols. This is the basis of a method suggested by Dakin.[6]

In determining the proportions of mono- and diamino acids. The protein is hydrolyzed by boiling with hydrochloric acid. The amide and ammonia nitrogen is determined by distillation with magnesium oxide *in vacuo* at 40° C. The diamino acids are precipitated with phosphotungstic acid and the amount of nitrogen in the precipitate as well as in the filtrate is determined by Kjeldahl's method. The nitrogen content of the filtrate represents the monoamino acids.

The Synthesis of Polypeptides.—Glycine, as the ethyl ester, in an aqueous solution forms an anhydride:

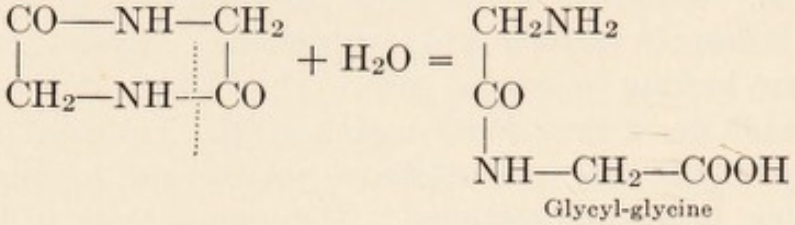
$$\begin{array}{l} COOH \\ | \\ CH_2—NH_2 \end{array} \quad \begin{array}{l} NH_2—CH_2 \\ | \\ COOH \end{array} \quad \rightleftarrows \quad \begin{array}{l} CO——NH—CH_2 \\ | \qquad\qquad | \\ CH_2—NH—CO \end{array} \quad + 2H_2O$$

2 molecules of glycine — Diketopiperazine (glycine anhydride)

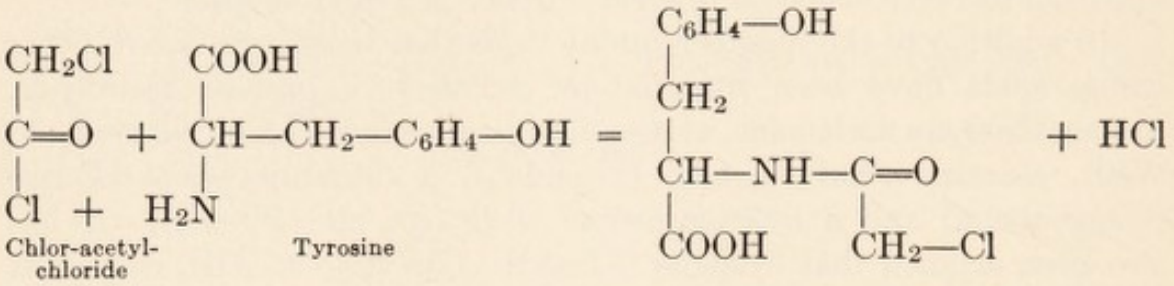
[5] Fischer, E., Untersuchungen über Aminosäuren, Polypeptide und Proteine (1899–1906), Berlin, 1906.

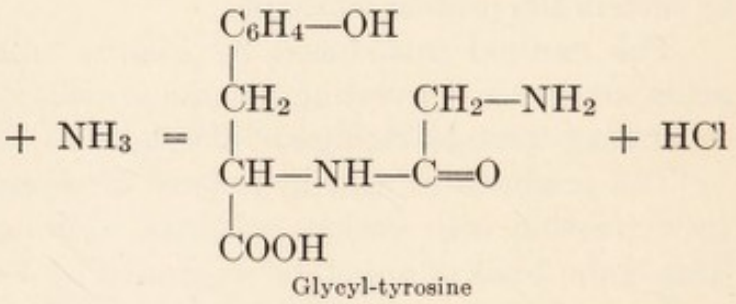
[6] Biochem. J., **12**, 290 (1918); J. Biol. Chem., **44**, 499 (1920).

On boiling the anhydride with concentrated hydrochloric acid, Fischer obtained the dipeptide glycyl-glycine:

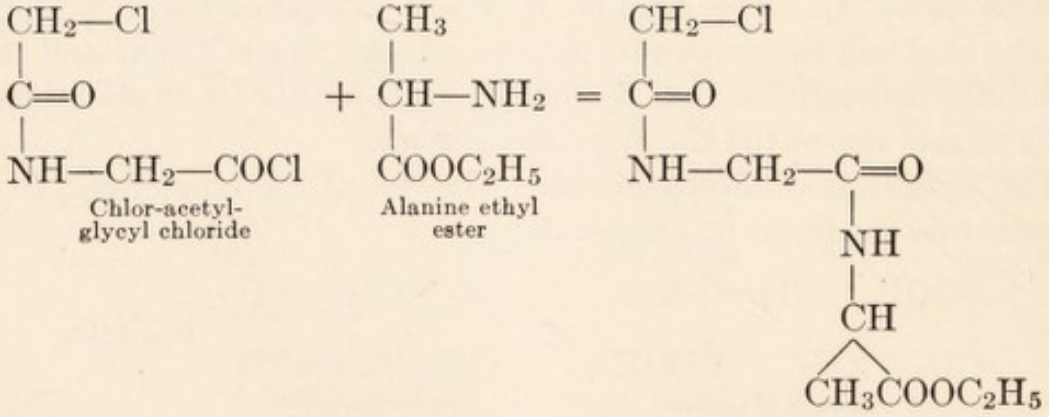


Another method employed by Fischer for the synthesis of a dipeptide consists in treating an amino acid with an α-halogen acyl radical. When the resulting compound is treated with ammonia a dipeptide is formed as represented by the following equations:





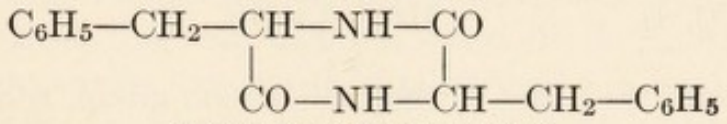
The acid chloride of the halogen acyl derivative of an amino acid reacts with amino-acid esters as follows:



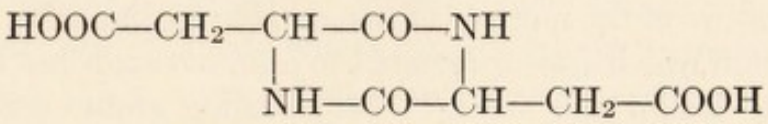
The ester group of the resulting compound may be hydrolyzed, and on subsequent treatment with ammonia, a tripeptide, diglycyl-alanine is formed. By these and similar methods, Fischer and other workers

have synthesized a variety of polypeptides from amino acids. Fischer prepared a chain compound of as many as eighteen amino acids, namely *—l-leucyl-triglycyl-leucyl-triglycyl-leucyl-octoglycyl-glycine.* Later, Abderhalden synthesized a polypeptide chain composed of nineteen amino acids. The more complex synthetic polypeptides have many points of resemblance to the proteins. They are non-diffusible through a parchment membrane, give the color reactions characteristic of proteins, and are precipitated from solution by tannic acid, phospho-tungstic acid, and other protein precipitants.

In the condensation of amino acids, compounds with cyclic structures have been formed. Glycocoll anhydride, or diketopiperazine, is a simple example. Substituted diketopiperazines may be obtained by the condensation of phenylalanine, aspartic acid, etc.

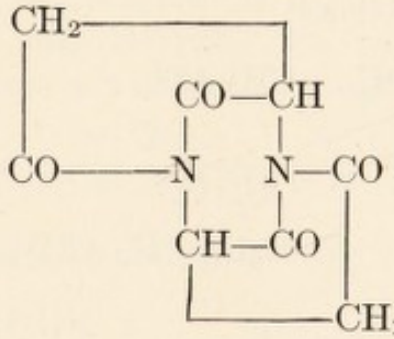


Phenylalanine diketopiperazine



Aspartic acid diketopiperazine

The dipeptide of aspartic acid (and glutamic acid) yields yet another type of cyclic compound (Blanchetier),[7] consisting of three condensed rings, as follows:



These, and a variety of other cyclic compounds, obtained by the condensation of two or more amino acids, and which some workers claim to have also isolated among the products of protein hydrolysis, have acquired considerable interest in recent years in connection with various theories of protein structure which have been proposed.

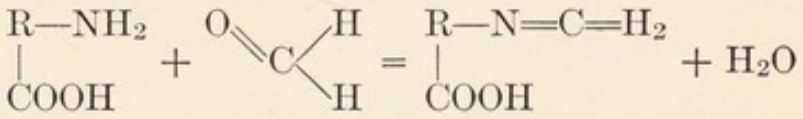
Reactions of Amino Compounds.—Nitrous acid reacts with amino compounds as represented by the equation:

$$R{-}NH_2 + HNO_2 = ROH + H_2O + N_2$$

[7] Bull. Soc. chim. biol., **6**, 854 (1924).

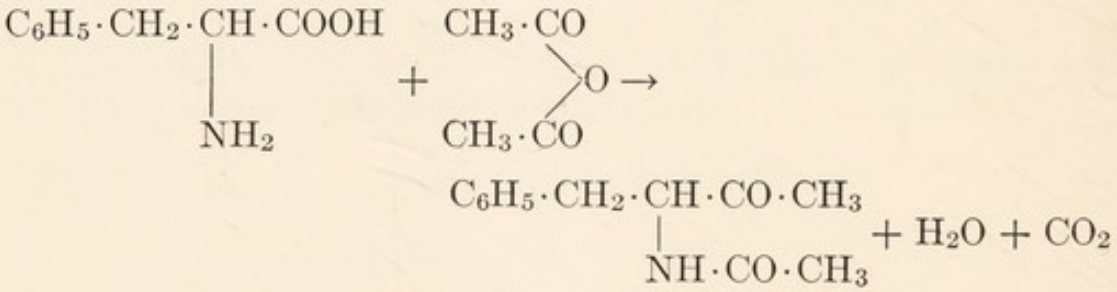
This reaction is the basis of the Van Slyke method [8] for determining the free amino groups in protein. Since hydrolysis of the protein molecule results in the presence of a larger number of free amino (NH_2) groups, the method may be employed in following the progress of protein digestion.

The reaction between amino acids and formaldehyde is the basis of Sørensen's formol-titration method [9] which may be employed in determining the number of free carboxyl groups. The reaction is adjusted to a definite alkalinity. Formaldehyde is then added to combine with the amino group, forming a methylene derivative in accordance with the equation:



Having removed the effect of the basic amino group, subsequent titration with standardized alkali to the original reaction of the solution gives a measure of the number of free carboxyl groups.[10]

A reaction which appears general to α-amino acids has been described recently by Dakin and West.[11] On warming amino acids with acetic anhydride and pyridine, carbon dioxide is evolved and two acetyl groups are introduced, one attached to nitrogen and one to carbon. The compounds have the general formula $R \cdot CH \cdot (NH \cdot COCH_3) \cdot COCH_3$ and are derivatives of acetylaminoacetone. The reaction with phenylalanine may be represented as follows:



The function of the pyridine appears to be catalytic. Proline and alkylamino acids do not react analogously, but undergo simple acetylation.

[8] J. Biol. Chem., **12**, 275 (1912).

[9] Z. physiol. Chem., **64**, 120 (1909).

[10] According to L. J. Harris, [Proc. Roy. Soc. (London), B, **95**, 500 (1923–24)] the action of formaldehyde is to increase the acid ionization constant of the amino acids and is not due to reaction with the amino group.

[11] J. Biol. Chem., **78**, 91, 745 (1928); see also Levene, P. A., and Steiger, R. E., *ibid.*, **79**, 95 (1928).

The Synthesis of Protein in Nature.—Our knowledge concerning the synthesis of proteins in nature is very limited. Evidence has been adduced, however, to show that the animal cell is capable of synthesizing certain amino acids, such as glycocoll, alanine, and serine. It seems that in the lower organisms, such as the yeasts and bacteria, the synthesis of the aromatic and heterocyclic amino acids may be accomplished, and hence protein synthesis in these organisms occurs by the utilization of carbohydrates and simple sources of nitrogen. In the higher organisms, however, the amino-acid supply is largely exogenous in origin. Animal life depends on the plants for its nitrogen supply. The dependence may be a direct one as in herbivorous animals, or it may be somewhat more remote as in the carnivorous animals.

For the most part, protein synthesis takes place in the leaves of plants from nitrogen supplied to the plant in the form of simple nitrogen-containing salts, the most important of which are the nitrates. A great many factors determine the growth of plants and their capacity to form proteins and other foodstuffs. It is obvious that the supply of nitrogen to the plant is an essential factor. Much of the nitrogen is absorbed by the plant in the form of nitrate and is reduced to nitrite under the influence of sunlight. The same effect may be achieved by exposure of potassium nitrate to the rays from a quartz mercury-vapor lamp. Further reduction of the nitrite doubtless occurs. Baudisch[12] has shown that the exposure of mixtures of potassium nitrite and methyl alcohol in aqueous solution to diffused daylight and ultraviolet light results in the reduction of the nitrite to hyponitrite and the oxidation of the methyl alcohol to formaldehyde. The two products thus formed react to give the potassium salt of formhydroxamic acid:

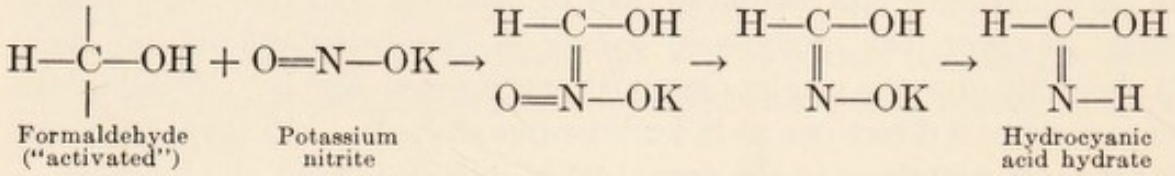
$$KNO_2 + CH_3OH = KNO + HCHO + H_2O$$

$$KNO + HCHO = H—C(=N—OK)—OH$$

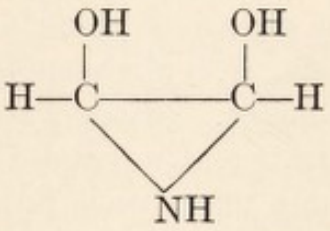
Another important factor which determines the synthesis of proteins is the available supply of carbohydrate. The synthesis of proteins in the plant can take place in the dark, provided there is an adequate supply of carbohydrate and potassium. It can be seen therefore that radiant energy may have only an indirect effect on protein formation, for it will be recalled that the formation of carbohydrates is the result of photosynthetic reactions.

[12] Ber. d. d. chem. Gesellsch., **44**, 1009 (1911); **49**, 1176 (1916); **51**, 793 (1918)

According to Treub,[13] hydrocyanic acid is the first recognizable product of nitrogen assimilation in the plant. More recently, Baly, Heilbron and Hudson [14] have reported that formaldehyde may react with potassium nitrate or nitrite to yield potassium formhydroxamate, a compound which on hydrolysis and subsequent reduction yields a hydrate of hydrocyanic acid. These changes may be represented as follows:



Formhydroxamic acid is said to condense with activated formaldehyde to yield an unstable ring compound:



which by molecular rearrangement may conceivably yield glycocoll. In this connection, Baly has reported the synthesis of histidine *in vitro*. It is emphasized by this investigator that the synthesis of nitrogen compounds by the plant is not photosynthetic except in so far as the production of formaldehyde is concerned. If the view of Baly is accepted, formhydroxamic acid may be regarded as perhaps the first product of nitrogen assimilation in the plant. It also follows that the formation of this compound precedes not only the synthesis of amino acids and protein, but that of alkaloids and other nitrogen bases as well.

The Structure of Proteins and the Types of Linkage in the Protein Molecule.[15]—Early in the study of protein structure, Fischer arrived at the conclusion that amino acids are linked together through the amino group of one amino acid and the carboxyl group of another, forming long chains of amino acids. The union between the two constituent amino

[13] Ann. du Jardin Botanique de Buitenzorg, **13**, 1 (1896).

[14] J. Chem. Soc., **121**, 1078 (1922).

[15] Two excellent reviews of the subject have appeared recently, which are especially recommended to the student. One is by E. Klarmann, Chem. Reviews, **4**, 51 (1927), and the other by H. B. Vickery and T. B. Osborne, Physiol. Reviews, **8**, 393 (1928). These reviews contain abundant references to the work of Fischer, Kossel, Hofmeister, Abderhalden, Bergmann, Karrer, and others who have attempted to solve the perplexing problem of the constitution of the protein molecule.

acids in the dipeptide, alanyl-glycine, illustrates this form of combination.

$$\underset{\text{Alanine}}{CH_3 \cdot CHNH_2 \cdot CO}OH + H\underset{\text{Glycine}}{NH \cdot CH_2 \cdot COOH} =$$

$$\underset{\text{Alanyl-glycine}}{CH_3 \cdot CHNH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH} + H_2O.$$

This type of linkage (O=C—NH—) is called the peptide binding.
|

The view that it is the principal linkage existing between amino acids in the protein molecule is based on the following considerations as enumerated by Vickery and Osborne:

1. Native protein itself contains very little amino nitrogen, but the end products of protein hydrolysis contain larger amounts. The peptide bond type of union readily accounts for this.

2. The biuret reaction (p. 114) is given by many substances which contain this group and this reaction is characteristic of proteins and their decomposition products, the proteoses. It disappears on complete hydrolysis. This strongly suggests the presence of the peptide bond in proteins and their partial hydrolysis products.

3. A number of condensation products of amino acids have been prepared which contain this group. Many of these give the biuret reaction.

4. The peptide union is also encountered in other naturally occurring substances as, for example, in hippuric acid.

5. The synthetic polypeptides obtained by Fischer from the natural isomers of optically active amino acids are hydrolyzed by the enzymes of the digestive tract.

6. Polypeptides have frequently been found among the products of incomplete hydrolysis of proteins.

7. During the hydrolysis of proteins, whether by acids or enzymes, amino groups and carboxyl groups are progressively liberated at an approximately equal rate.

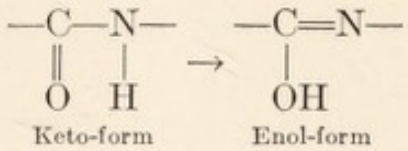
8. Hydrolysis of proteins occurs without material change in the hydrogen ion concentration of the solution. This is consistent with the view that equivalent amounts of amino and carboxyl groups are thereby produced.

9. Pepsin alone liberates as a rule about 20 per cent of the total amount of amino nitrogen which can be obtained by the complete hydrolysis of a protein. Erepsin acting on a peptic digest can liberate as much as 70 per cent more. Since there is every reason to believe that the latter enzyme acts only upon peptide bonds, it is obvious that by far the greater part of the total possible amino nitrogen of a protein has its origin in such bonds.

However, certain facts seem to point to the possibility that the protein molecule is not merely a single large polypeptide. This type of structure is believed to be inconsistent with the changes which protein undergoes in the process of denaturation by alcohol or heat. Nor is it possible to explain the insolubility in water of many proteins on the basis of a polypeptide structure. An even greater obstacle in accepting the peptide bond as being the sole link between amino acids is the behavior of pepsin toward polypeptides. Pepsin does not act on polypeptides, nor for that matter on any synthetic products formed from amino acids. In fact, it is not known which bonds in the protein molecule are attacked by this enzyme.

To explain various peculiarities in the behavior of proteins, a number of theories regarding their structure have been suggested. Of these, only two will be mentioned here. According to *Abderhalden's diketopiperazine hypothesis*,[16] the protein molecule is built up of a number of diketopiperazine containing complexes which are associated or held together by forces of secondary, or latent, valence.[16a] A theory similar to Abderhalden's has been proposed by Bergmann,[17] who believes that protein is composed of a variety of cyclic derivatives of piperazine held together by means of secondary valences.

There is little evidence for the presence of ester, ether, or imide linkages between amino acids in the protein molecule. However, in the individual amino acids, we have the guanidine binding ($NH—CH_2$) in arginine, and the disulfide linkage (S—S) in cystine. It is likely that the peptide group may be capable of rearrangement from the keto- to the enol-form, as follows:



Molecular Weights of Proteins.—The physical and chemical properties of the proteins have stimulated much interest in the question of their molecular weights. Various methods have been employed in an attempt to determine the relative size of the molecules of various proteins. The minimal weight of a protein molecule may be calculated from

[16] Naturwissenschaften, **12**, 716 (1924); Z. physiol. Chem., **128**, 119 (1923); *ibid.*, **139**, 169, 181 (1924).

[16a] According to Werner's conception there are two kinds of valence, one which he termed "primary" valence, and the residual attraction left over after the primary valence is saturated, which Werner called "secondary" valence. This idea of auxiliary or potential valence forces may be harmonized, with certain modifications, with the more modern views of atomic structure.

[17] Naturwissenschaften, **12**, 1155 (1924); *ibid.*, **13**, 1045 (1925).

analytical data by assuming the presence, in the molecule of protein, of but one molecule of a given amino acid. The molecular weight of hemoglobin has been calculated thus from the amino acid content, and also from data obtained of its iron content and of the oxygen content in oxyhemoglobin. It has also been shown that 16,721 grams of hemoglobin combine with one mol of carbon monoxide. Consequently this value was formerly taken for the minimal molecular weight of hemoglobin.

Determination of the equivalent combining weights of various proteins with acids and bases may yield useful information, especially when compared with calculations of their molecular weights based on the analyses of such elementary constituents as iron (in the case of hemoglobin), copper (in hemocyanin), sulfur, and phosphorus.

The direct determination of the molecular weights of proteins by osmotic-pressure methods has yielded results approximating those obtained by analytical methods. For the determination of the relative size of protein molecules, methods of ultrafiltration and dialysis have been found very useful. By a combination of these methods, Cohn, Hendry and Prentiss [18] have obtained the following values for the minimal molecular weights of a variety of proteins:

TABLE XIII

(After Cohn, Hendry and Prentiss)

Protein	Minimal Molecular Weight
Gelatin	10,300
Zein	19,400
Gliadin	20,700
Hemocyanin, *Limulus*	22,700
Bence-Jones protein	24,500
Edestin	29,000
Hemocyanin, *Octopus*	33,500
Egg albumin	33,800
Glutenin	36,300
Fibrin	42,000
Serum albumin	45,000
Hemoglobin (dog)	50,000
Serum globulin	81,000
Casein	192,000

[18] J. Biol. Chem., **63**, 721 (1925); see also Cohn, Physiol. Reviews, **5**, 349 (1925); Svedberg and associates have determined the molecular weights of proteins by measuring their sedimentation velocities in an ultra-centrifuge; see Svedberg and Rinde, J. Am. Chem. Soc., **46**, 2677 (1924); Svedberg and Fahraeus, *ibid.*, **48**, 430 (1926); Svedberg, Carpenter and Carpenter, *ibid.*, **52**, 241, 701 (1930).

Behavior of Proteins as Electrolytes. — According to Graham's classification, colloidal substances are distinguished from crystalloids in that the former are not capable of diffusing through parchment, collodion, or animal membranes. This, however, is not the only distinguishing property. There are in addition certain differences in the capacity of colloids and crystalloids to dissolve, to crystallize, and to react with other substances. It has been assumed that substances in the colloidal state do not react according to the law of combining weights or in stoichiometric proportions.

The proteins have long been regarded as colloids. Proteins dissolved in water give an opalescent solution. If this solution is placed in a bag of parchment paper, the protein does not diffuse through. While a number of proteins, such as hemoglobin, have definite crystal structure, most of them are amorphous and do not crystallize readily.

In 1900 Hardy [19] demonstrated that particles of coagulated egg albumin were differently influenced by an electric current, depending on whether the reaction of the solution was acid or alkaline. In a slightly alkaline solution the protein moved from the cathode to the anode, whereas in the presence of acid the protein acquired a positive charge and migrated in the direction of the cathode. According to Hardy, the H^+ or OH^- ions become entangled within the colloid particle of protein, which thereby acquires a positive charge if there is an excess of H^+ ions (acid solution) or a negative charge if the OH^- ions are in excess (alkaline solution).

An explanation offered by Loeb [20] in 1904 was to the effect that proteins behaved like amphoteric electrolytes, reacting as bases in the presence of acids and as acids in the presence of bases. Owing to the presence of the amino group in the amino-acid molecule, this reacts with acids as though it were a basic substance. When placed in an alkaline solution, amino acids behave as though they were acids, because of the carboxyl group. Since the protein molecule likewise has at least one free amino group and one free carboxyl group, it will yield a protein cation in the presence of acids and will form protein chlorides, sulfates, etc. In the presence of bases it will yield a protein anion to form such compounds as sodium, potassium, or calcium proteinates. The protein molecule is obviously capable of electrolytic dissociation. The degree of dissociation of a protein and its capacity to combine with anions or cations is conditioned by the hydrogen-ion concentration, and for each protein the degree of dissociation is negligible at a certain critical hydrogen-ion concentration, the *isoelectric point*. Michaelis has defined

[19] Proc. Roy. Soc., **66**, 110 (1900).

[20] Univ. of Cal. Publications, Physiology, **1**, 149 (1904).

the isoelectric point, as applied to amphoteric electrolytes, as that point at which the dissociation of the ampholyte is at a minimum.

For certain of the amino acids the isoelectric points have been determined by a number of investigators. The following data are taken from a table compiled by Michaelis:[21]

THE ISOELECTRIC POINTS OF AMINO ACIDS CALCULATED FROM THEIR DISSOCIATION CONSTANTS DETERMINED AT 25° C.

Amino Acid	Isoelectric Point, C_H
Arginine	3.10^{-11}
Lysine	3.10^{-10}
Leucine	$8.8.10^{-7}$
Glycocoll	$8.2.10^{-7}$
Alanine	$6.1.10^{-7}$
Histidine	$6.2.10^{-8}$
Phenylalanine	$4.4.10^{-6}$
Tyrosine	$3.9.10^{-6}$

The following are the isoelectric points of a number of proteins:

	C_H
Serum albumin (genuine)	2.10^{-5}
Serum albumin (denatured)	about 4.10^{-6}
Serum globulin	about 4.10^{-6}
Egg albumin	$1.6.10^{-5}$
Edestin	$1.3.10^{-7}$
Casein	2.10^{-5}
Gelatin	2.10^{-5}
Oxyhemoglobin	$1.8.10^{-7}$

Chemical Reactions of Proteins with Anions and Cations.—Assuming that proteins are amphoteric electrolytes, they should combine with anions only on the acid side of the isoelectric point, and with cations only on the alkaline side. At its isoelectric point, the protein should combine with but negligible amounts of either acid or base. The correctness of this assumption has been proved by Jacques Loeb [22] in a very ingenious manner:

Doses of 1 gram of finely powdered commercial gelatin (going through sieve 60 but not through 80), which happened to have a *p*H of 7.0 were brought to different hydrogen-ion concentrations by putting

[21] Die Wasserstoffionenkonzentration, Berlin, 1922, 60

[22] Jacques Loeb, Proteins and the Theory of Colloidal Behavior, New York (1924), p. 34; see also J. Gen. Physiol., **1**, 449 (1920).

them for 1 hour at about 15° C. into 100 cc. of HNO_3 solutions varying in concentration from M/8,192 to M/8. Owing to the Donnan equilibrium the hydrogen-ion concentration inside a gelatin granule is lower than that outside. After this, each dose of 1 gram of gelatin was put on a filter, the acid being allowed to drain off, and each dose was washed once or twice with 25 cc. of cold water (at 5° C. or less) to remove the greater part of the acid between the granules of the powdered gelatin. These different doses of originally 1 gram of gelatin, each of which now possessed a different *p*H, were put for 1 hour each into a separate beaker containing the same concentration, e.g., M/64, of silver nitrate at a temperature of 15° C. Each dose of powdered gelatin was then put on a filter and washed with stirring six or eight times each with 25 cc. of ice-cold water. This washing serves the purpose of removing the $AgNO_3$ held in solution between the granules, thus allowing us to ascertain where the Ag is in combination with gelatin and where it is not in combination, since the Ag not in combination with gelatin can be removed by the

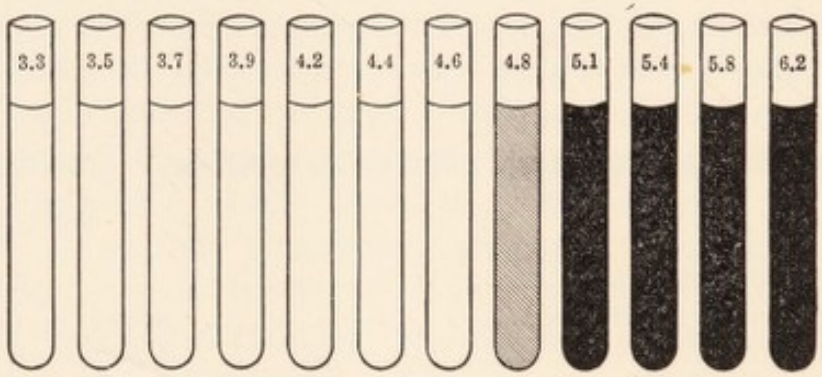


FIG. 13.—Proof that cations combine with proteins only on the alkaline side of the isoelectric point. Powdered gelatin brought to different *p*H was treated in a dark room with M/64 $AgNO_3$ and then washed with cold water to remove the silver not in combination with gelatin. The gelatin was liquefied, brought to a 1 per cent solution, and the *p*H was determined. The solutions were then poured into test-tubes and exposed to light. In about half an hour the gelatin of *p*H > 4.7 was dark while the gelatin of *p*H 4.7 or less remained permanently clear though exposed to light for over a year. The *p*H of each gelatin solution is marked at the head of each test-tube. (After J. Loeb, Proteins and the Theory of Colloidal Behavior, McGraw Hill, 1924 edition, p. 34.)

washing while the former cannot, or at least only extremely slowly (by altering the *p*H). After having removed the $AgNO_3$ not in combination with gelatin by washing with cold water, the gelatin is melted by heating to 40° C., enough distilled water is added to bring the volume of each gelatin solution to 100 cc., the *p*H of a sample of each solution is determined potentiometrically, and the solutions are exposed in test-tubes to light, the previous manipulations having been carried out in a dark room (with the exception of the determination of *p*H, for which only part of the gelatin solution was used). In 20 minutes all the gelatin solutions with a *p*H > 4.7, i.e., from *p*H 4.8 and above, upon

exposure to strong light become opaque and then brown or black, while all the solutions of pH < 4.7, i.e., from pH 4.6 and below, remain transparent even when exposed to light for months or years (Fig. 12). The solutions of pH $= 4.7$ become opaque, but remain white, no matter how long they may have been exposed to light. At this pH—the isoelectric point—gelatin is not in combination with Ag, but it is sparingly soluble. Hence, the cation Ag is only in chemical combination with gelatin when the pH is > 4.7. At pH 4.7 or below gelatin is not able to combine with Ag ionogenically.

Loeb has made similar tests with other cations, such as nickel and copper, and with basic dyes. Basic fuchsin and neutral red, after sufficient washing with cold water, stain only those gelatin solutions that have a pH above 4.7.

In order to bring out more fully the significance of the preceding observations, let the gelatin molecule be represented by the formula:

$$\left[R\begin{matrix} NH_2 \\ COOH \end{matrix} \right]$$

in which the brackets are used to indicate the inability of isoelectric gelatin to combine with either anions or cations. On the alkaline side of the isoelectric point, only the COOH reacts, in accordance with the following equation:

$$\left[R\begin{matrix} \overline{NH_2} \\ COOH \end{matrix} \right] + NaOH = \left[R\begin{matrix} \overline{NH_2} \\ COONa \end{matrix} \right] + H_2O$$

The sodium proteinate that is formed dissociates into a protein anion and a Na^+ cation:

$$\left[R\begin{matrix} \overline{NH_2} \\ COONa \end{matrix} \right] \rightleftharpoons \left[R\begin{matrix} \overline{NH_2} \\ COO^- \end{matrix} \right] + Na^+$$

When other electrolytes are present, as $AgNO_3$, an interchange of cations takes place with the formation in this case of silver proteinate.

When protein on the acid side of the isoelectric point is treated with a salt, it combines with the anion of the salt. Loeb demonstrated this by using potassium ferrocyanide and other salts. Gelatin was treated with M/128 $K_4Fe(CN)_6$ and subsequently washed. From the gelatin samples 1 per cent solutions were prepared and these allowed to stand

for several days. It was found that the gelatin solutions with a pH < 4.7 turned blue (owing to the formation of ferric ferrocyanide) whereas the gelatin samples with a pH > 4.7 remained perfectly clear (Fig. 13). This is taken as evidence that the gelatin molecule enters into chemical combination with the anion $Fe(CN)_6$ only when the pH is less than 4.7.

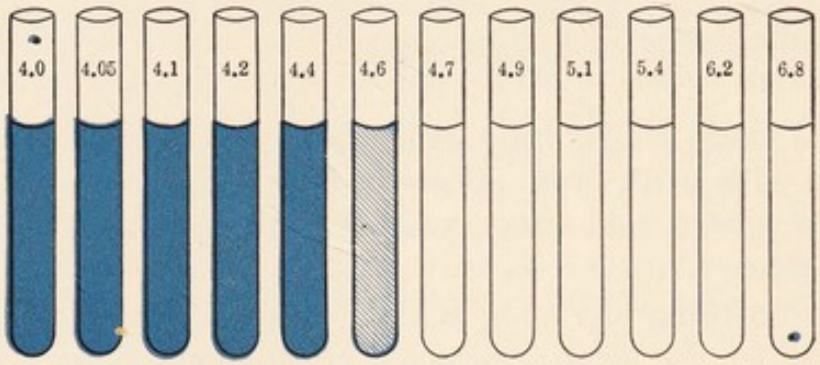


FIG. 14.—Proof that anions combine with proteins only on the acid side of the isoelectric point. Doses of powdered gelatin solutions of different pH were treated with M/128 $K_4Fe(CN)_6$ and then washed with cold water. All the samples of gelatin solution of pH < 4.7 turned blue (through the formation of some ferric salt), while all the gelatin solutions of pH 4.7 or above remained colorless.

On the acid side of the isoelectric point, the amino group of the protein molecule behaves like ammonia in its ability to add an acid. This may be represented by the equation:

$$\left[R\begin{matrix}NH_2\\COOH\end{matrix}\right] + HCl = \left[R\begin{matrix}NH_2\cdot HCl\\COOH\end{matrix}\right]$$

The hydrochloride dissociates into a protein cation and an anion (Cl^-).

$$\left[R\begin{matrix}NH_3Cl\\COOH\end{matrix}\right] \rightleftarrows \left[R\begin{matrix}NH_3^+\\COOH\end{matrix}\right] + Cl^-$$

It is to be appreciated, of course, that the protein molecule very probably contains more than one reactive carboxyl and amino group.

Reaction of Proteins with Acid.—The formerly accepted view that the addition of acids and alkalies to proteins results in the formation of adsorption compounds must give way to the chemical viewpoint postulated by Loeb and supported by his quantitative proof that proteins combine with acids and bases in stoichiometric proportions.[23]

[23] Loeb, Proteins and the Theory of Colloidal Behavior (1924), Chapter IV.

It has been pointed out that proteins combine with acids at a *p*H below that of the isoelectric point. It also happens that most weak dibasic and tribasic acids dissociate as monobasic acids in solutions more acid than that equivalent to a *p*H of 4.7. For example, H_3PO_4 dissociates into H^+ and $H_2PO_4^-$ ions. On the other hand, H_2SO_4, being a strong acid, splits off both hydrogen ions in dilute solutions. Oxalic acid behaves as a monobasic acid below *p*H 3.0 but above this value the second hydrogen atom begins to split off.

If acids combined stoichiometrically with isoelectric protein, it would require exactly three times as many cubic centimeters of 0.1N H_3PO_4 to bring a 1.0 per cent solution of isoelectric gelatin, egg albumin, casein, or any other protein to a given hydrogen-ion concentration as it does of 0.1N HCl, HNO_3 or H_2SO_4. The correctness of this assumption was demonstrated in a series of experiments in which varying amounts of acid were added to equal concentrations of isoelectric protein and the *p*H of the resulting solutions measured with the hydrogen electrode. The titration curves in Fig. 15 were obtained by plotting the concentration of acid against *p*H. From the curves it can be seen that it required three times as many cubic centimeters of 0.1N H_3PO_4 as of 0.1N HCl or H_2SO_4 to bring the albumin solution to the same *p*H. To bring the protein to *p*H 3.4 it required 4 cc. of 0.1N HCl or H_2SO_4 and 12 cc. of 0.1N H_3PO_4. As indicated by the titration curves, twice as much 0.1N

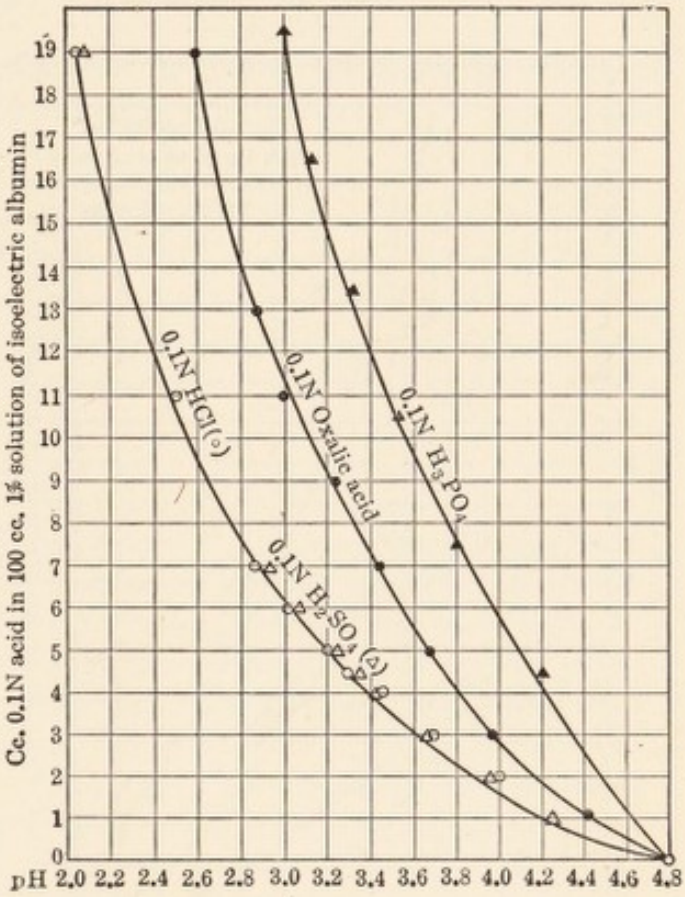


FIG. 15.—The ordinates represent the number of cubic centimeters of 0.1N HCl, H_2SO_4, oxalic and phosphoric acids required to bring 1 gram of isoelectric crystalline egg albumin to the *p*H indicated on the axis of the abscissæ. Enough H_2O was added to bring the albumin and acid to a volume of 100 cc. For the same *p*H, the ordinates for HCl, H_2SO_4, and phosphoric acid are approximately 1 : 1 : 3. The ratio of HCl to oxalic acid is a little less than 1 : 2 when *p*H is >3.0. (After Loeb, Proteins and the Theory of Colloidal Behavior, 1924 edition, p. 53.)

oxalic acid as 0.1N hydrochloric acid was required to bring the albumin solution to the same *p*H below *p*H 3.0. Above *p*H 3.0 it required less than twice as many cubic centimeters of oxalic acid as of hydrochloric or sulfuric acid.

From the titration curves, the amount of acid actually in combination with the protein may be calculated. The curves in Fig. 16 represent the actual amounts of each of the four acids in combination with one gram of originally isoelectric egg albumin in 100 cc. of solution. The values for HCl and H_2SO_4 are practically identical. For H_3PO_4 the values are always about three times as large as those for HCl, any slight deviations being due to the limitations in the accuracy of the method.

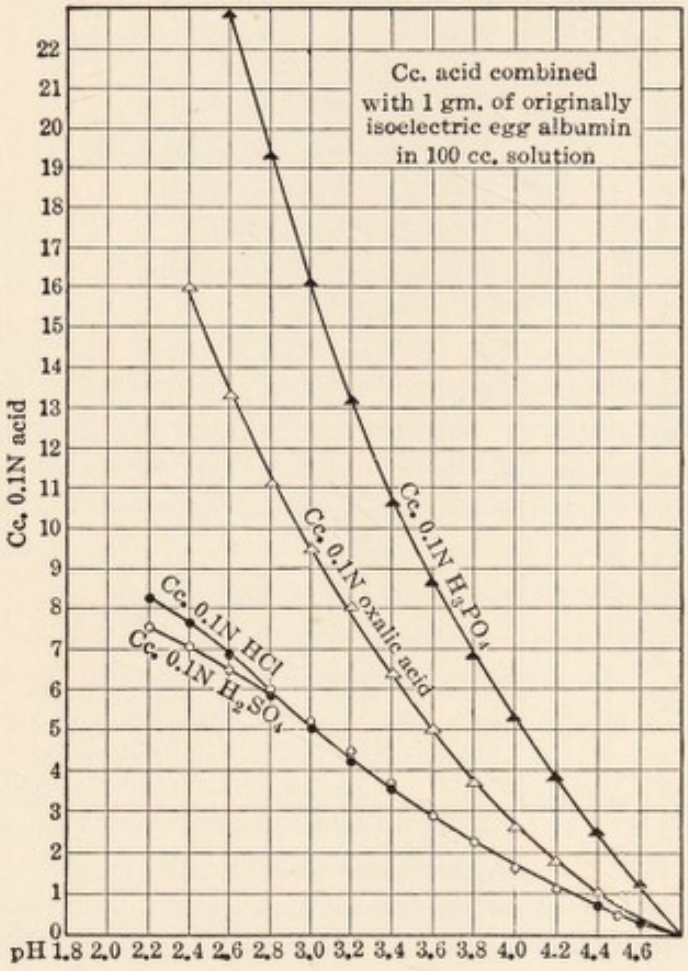


FIG. 16.—Proof of the stoichiometrical character of the combination of acids with isoelectric albumin. The same mass of albumin combines with three times as many cubic centimeters of 0.1N H_3PO_4 as with HCl or H_2SO_4; and with twice as many cubic centimeters of 0.1N oxalic acid below *p*H 3.0. (After Loeb, p. 56.)

Reaction of Proteins with Base.—The combination of isoelectric protein with bases likewise occurs in stoichiometric proportions. Loeb has determined the number of cubic centimeters of 0.1N KOH, NaOH, $Ca(OH)_2$, and $Ba(OH)_2$, respectively, that must be contained in 100 cc. of a 1 per cent solution of crystalline egg albumin, isoelectric albumin, casein, or gelatin to bring the solution to a given *p*H. He found that the numbers of cubic centimeters is the same in each case and that the values for the four bases lie on the same curve. This is illustrated in Fig. 17a for casein and in Fig. 17b for gelatin.

Rawlins and Schmidt[24] have titrated casein, fibrin, gelatin and edestin with certain basic dyes, methylene blue, safranine Y, and induline scarlet

[24] J. Biol. Chem., **82**, 709 (1929).

and found that the union between protein and basic dye occurred in stoichiometric proportions.

Loeb has pointed out that the failure of the earlier investigators to recognize the stoichiometric character of the reactions of proteins with acids and bases was due to the fact that these workers were unable to measure the hydrogen-ion concentration of a protein solution and did not appreciate its significance.

Solubility of Proteins.—Solutions in which the ultimate units are molecules or ions have been defined as true solutions, as disinguished from colloidal solutions in which the ultimate units are aggregates of molecules. Molecular aggregation or association is not limited, however, to colloidal particles. Many substances in the liquid state (water, acetic acid, methyl and ethyl alcohol) are not made up of discrete molecules, as can be demonstrated by comparing the molecular weights determined in the liquid state with those obtained in the vapor state. It has been generally assumed that proteins do not form true aqueous solutions but that the relatively large molecules form, by coalescence or aggregation, still larger particles, which remain in suspension.

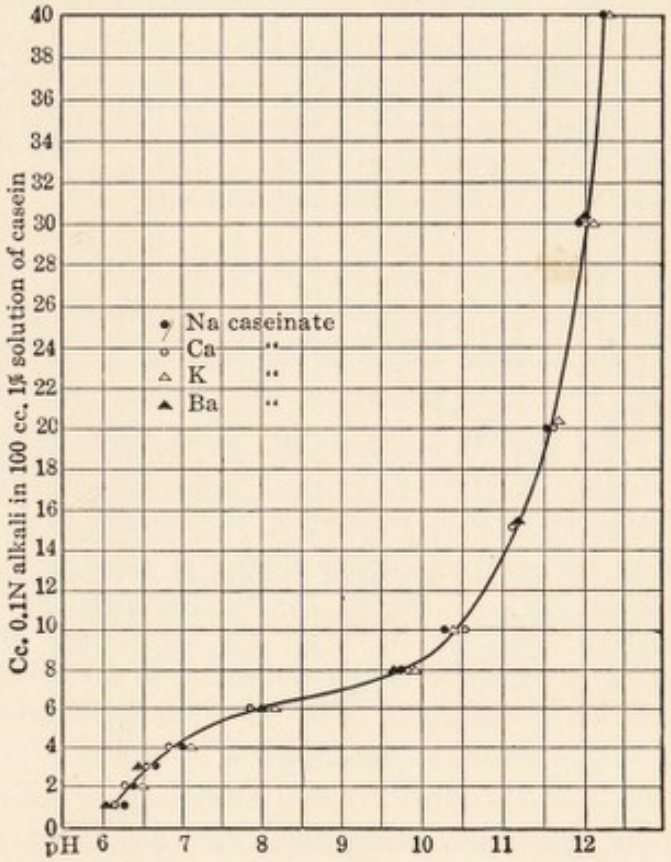


FIG. 17a.—Ordinates are the cubic centimeters of 0.1N NaOH, KOH, $Ca(OH)_2$, and $Ba(OH)_2$ in 100 cc. of 1 per cent solution of casein. Abscissæ are the *p*H of the solution. The curves for the four alkalies are identical, proving that Ba and Ca combine with casein in equivalent proportion. (After Loeb, p. 69.)

The stability of colloidal particles suspended in water depends on the weak forces of repulsion due to the electrical double layer, of measurable potential difference, between each colloidal particle and the water. Diminution of the potential difference of the double layer below a certain critical value results in a coalescence of the particles into larger and larger aggregates, which settle out. The neutralization of the electrical charge of the colloidal particles may be accomplished even by

relatively low concentrations of neutral salts, the effect being greater in the case of polyvalent ions having a charge opposite to that of the particles in suspension.

On the other hand, the stability of individual molecules and ions in true solution in water is due to the strong forces of attraction between the molecules or ions of the solute and of the solvent. Substances in true solution are not easily precipitated, therefore, by chemically non-reacting substances. Large concentrations of salts are required to precipitate substances from true solution and emulsoids. It is not a question of the neutralization of an electric charge, for the precipitation may be accomplished by an ion having a charge similar to that of the ion or molecule that is being removed from the solution.

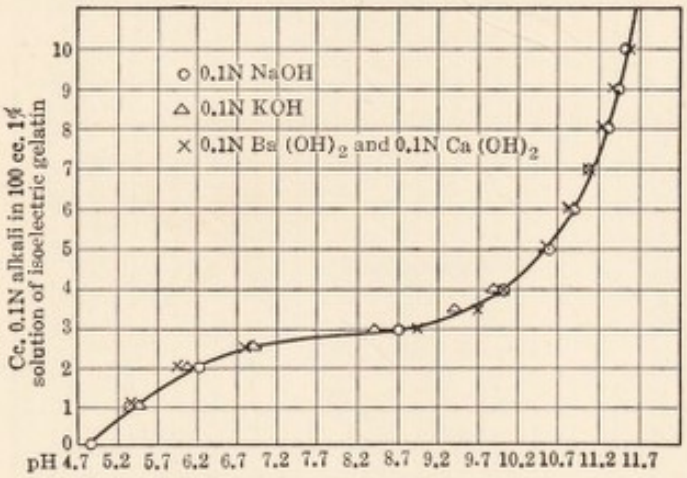


FIG. 17b.—Curves for the number of cubic centimeters of 0.1N NaOH, KOH, $Ba(OH)_2$, and $Ca(OH)_2$ required to bring the same mass of about 0.8 gram of isoelectric gelatin in a 100 cc. solution to different *p*H. All four curves are identical. (After Loeb, p. 70.)

We may now consider whether proteins in solution behave as crystalloids or as colloids. If, for precipitation, proteins required low concentrations of salts, this would be evidence for the suspensoid nature of their solutions. The fact that they require very large concentrations has been used in arguing for the emulsoid character of protein solutions. Some workers, however, maintain that this may also be used as an argument for the non-colloidal nature of protein solutions. In the precipitation of proteins, the active ion need not necessarily have a charge opposite to that of the protein.

Proteins are least soluble at the isoelectric point, a property which is also characteristic of other amphoteric electrolytes. It has been shown that true crystalloids, such as the amino acids, have minimum solubility at their isoelectric points. In other words, amphoteric electrolytes and ionized protein salts are more soluble than the un-ionized molecules. Quantitative evidence that proteins may form true solutions has been furnished by Cohn and Hendry.[25]

The Colloidal Behavior of Proteins.—It is beyond the scope of this work to enter into a detailed discussion of Loeb's work on the behavior

[25] J. Gen. Physiol., **5**, 521 (1922–23).

of proteins. He has postulated that, as far as the proteins are concerned, it is incorrect to distinguish between colloids and crystalloids. With regard to their chemical reactions and solubility, the proteins behave like crystalloids. These constitute therefore the crystalloidal properties of proteins. On the other hand, the protein ion, on account of its large size, does not diffuse through membranes or gels which are permeable to smaller crystalloidal ions. This constitutes the colloidal property of the protein ion. Evidence has been adduced to show that the behavior of proteins can be explained on the basis of Donnan's theory of membrane equilibria, which applies to the equilibria established between ions on the two sides of a membrane impermeable to one of the ions. The effect of electrolytes on the swelling of proteins, osmotic pressure, membrane potentials, and the viscosity of protein solutions may be accounted for, according to the Loeb school, by application of Donnan's postulates.[26]

Hoffman and Gortner,[27] in an exhaustive study of the physicochemical behavior of the prolamins reach the conclusion that a chemical type of combination between proteins and acids or bases occurs only between hydrogen-ion concentrations corresponding to *p*H 2.5 to 10.5. Working with a large variety of proteins belonging to this group, these authors found that the amount of acid or alkali bound at any hydrogen-ion concentration is dependent on the chemical composition of the protein. This may be taken as evidence of a chemical type of combination within this range of *p*H values. However, at hydrogen-ion concentrations higher than that represented by *p*H 2.5 or lower than that represented by *p*H 10.5, all the proteins, regardless of their chemical composition, combine with the same amount of acid or alkali, as the case may be. Moreover, with increases in hydrogen-ion concentration, protein salts, such as the chloride, increase in ionization, so that at a *p*H of 2.5, the protein chloride is highly ionized. But, when the hydrogen-ion concentration is increased above *p*H 2.5, there is no further increase in the ionization of the protein salt. These and similar observations, according to Hoffman and Gortner, argue for the adsorption type of combination between proteins and bases or acids outside the *p*H range of 2.5 to 10.5.

Bancroft and Barnett [27a] have recently studied the reactions of proteins with gaseous ammonia and hydrogen chloride. They found

[26] See David I. Hitchcock's Review of Proteins and the Donnan Equilibrium, Physiol. Reviews, **4**, 505 (1924).

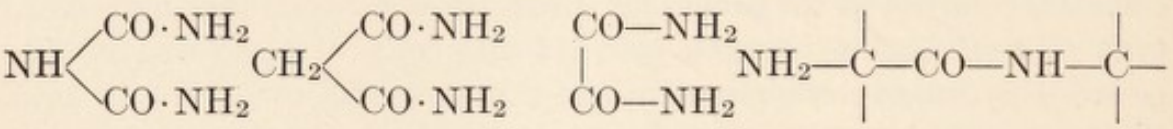
[27] W. F. Hoffman and R. A. Gortner, Colloid Symposium Monograph, vol. 2, p. 209, etc., The Chemical Catalogue Company, New York, 1925.

[27a] J. Phys. Chem., **34**, 449 (1930).

that casein, zein, arachin, fibrin and gliadin adsorbed ammonia readily, but could obtain no evidence for the formation of definite chemical compounds. On the contrary, hydrogen chloride reacted in stoichiometric proportions and formed definite compounds with casein, arachin, fibrin, gliadin and edestin, but not with zein.

Color Reactions of the Proteins.—Most proteins exhibit characteristic color reactions when treated with certain reagents. The colors are due to specific linkages or to amino acids, and some reactions are specific for a particular amino acid. Hence these reactions may be employed in the qualitative characterization of proteins. Among the more familiar tests are the following:

The biuret reaction.—This is obtained by treating a protein solution first with strong alkali and then with a very dilute copper sulfate solution. A reddish-violet to violet-blue color is produced. The reaction depends on the presence of the peptide linkage in the protein molecule. According to Schiff, the following groups are responsible for the reaction:



Millon's reaction.—The addition of Millon's reagent, a solution containing mercuric nitrate and nitrite in a mixture of nitrous and concentrated nitric acids, to protein solution, with heating, results in the formation of a brick-red precipitate. This reaction is due to the presence of the tyrosine group and is exhibited, as far as the red color is concerned, by substances, other than proteins, that contain the hydroxyphenyl group.

The xanthoproteic reaction.—Nitric acid added to proteins produces a yellow color which deepens to an orange-yellow on the addition of alkali. The yellow color is due to the formation of nitrated benzene derivatives. The reaction depends on the presence of tyrosine, phenylalanine and tryptophane. Some authors deny that phenylalanine gives the xanthoproteic reaction.

Hopkins-Cole reaction.—The addition of a small amount of glyoxylic acid to a protein solution stratified above concentrated sulfuric acid results in the formation of a reddish-violet ring, provided the protein contains the amino-acid tryptophane.

Precipitation and Coagulation Reactions.—The proteins are precipitated from solution by a large variety of substances. Among these are the neutral salts, such as sodium sulfate, magnesium sulfate and ammonium sulfate. Large amounts of these are required for the separation of the proteins. The process is frequently spoken of as *"salting out."*

The *salts of heavy metals*, such as those of copper, mercury, and lead, are good precipitants. Precipitation of proteins is also brought about by strong mineral acids. On the addition of nitric acid to a protein solution, a ring of protein is formed at the junction of the acid and the solution (*Heller's test*).

The so-called *alkaloidal reagents* precipitate proteins more or less completely from slightly acid solution. Among these reagents are phosphotungstic acid, phosphomolybdic acid, tannic acid, picric acid, potassium mercuric iodide, and potassium bismuth iodide.

Ferrocyanic acid, trichloracetic acid, sulfonyl-salicylic acid, and dinitrosalicylic acid are likewise efficient protein precipitants.

Coagulation is probably associated with dehydration of the protein molecule and the formation of anhydrides. This may be accomplished by the application of heat to solutions of protein acidified with acetic acid, or by the addition of alcohol to neutral or acid solutions.

The Proteins as Foodstuffs.—The proteins of vegetable origin play a very important part in animal nutrition. They have been very carefully and thoroughly studied by Osborne and his co-workers.[28] The proteins found in a variety of cereals enter into the human dietary. Wheat contains gliadin, glutenin, and the albumin, leucosin, in addition to a proteose. The mixture of gliadin and glutenin, when moistened, absorbs water to a greater degree than other proteins of the cereals. Rye contains a gliadin, differing from that of wheat, and a glutelin. The swelling property of rye proteins is less than that of the proteins of wheat. Rye flour therefore yields a dough which is less elastic and less capable of becoming porous than wheat dough.[29]

The prolamin of barley is hordein. It differs markedly from the gliadin of wheat or rye. The remaining proteins of barley resemble those of wheat. The prolamin of corn is zein.

The biological value of proteins is largely determined by the proportions of their constituent amino acids. Deficiency in even one of the amino acids that are essential for tissue construction limits the value of a protein to the animal body. Osborne and Mendel [30] and other workers have studied this problem very exhaustively by feeding experiments performed on animals. It has been shown, for example, that maintenance and growth are difficult on a diet in which casein is the sole protein, because casein is deficient in cystine. Supplementing this diet with small amounts of cystine results in normal growth. The relation between

[28] T. B. Osborne, The Vegetable Proteins, Longmans, Green & Co., 1924.

[29] See chapter by Carl L. Alsberg on The Colloid Chemistry of the Cereals in R. H. Bogue's The Theory and Application of Colloidal Behavior, New York (1924).

[30] A long series of papers in J. Biol. Chem., 1910. See also Chap. XVII.

the chemical composition of proteins and their value in nutrition will be considered in another connection. It is desirable at this point, however, to bring out the variations in the amino-acid composition of a variety of proteins.

TABLE XIV

QUANTITATIVE COMPARISON OF AMINO ACIDS OBTAINED BY HYDROLYSIS FROM PROTEINS *

	Casein	Gelatin	Gliadin	Zein	Lactal-bumin	Edestin	Salmine (Rhine salmon)	Silk-Fibroin (Italian)
Glycocoll	0.45	25.5	0.00	0.00	0.37	3.80	.....	36.0
Alanine	1.85	8.7	2.00	9.79	2.41	3.60	+	21.0
Valine	7.93	0.0	3.34	1.88	3.30	6.20	4.3	0.0
Leucine	9.70	7.1	6.62	19.55	14.03	14.50	+	1.5
Proline	7.63	9.5	13.22	9.04	3.76	4.10	11.0	0.3
Oxyproline	0.23	14.1	?	?	?	?	.....	.....
Phenylalanine	3.88	1.4	2.35	6.55	1.25	3.09	.....	1.5
Glutaminic acid	21.77	5.8	43.66	26.17	12.89	18.74	.....	0.0
Oxyglutaminic acid	10.50	0.0	2.4	?	10.00	?	.....	.....
Aspartic acid	4.1	3.5	0.58	1.71	9.30	4.50	.....	.....
Serine	0.5	0.4	0.13	1.02	1.76	0.33	.....	.....
Tyrosine	4.5	0.01	1.61	3.55	1.95	2.13	.....	10.5
Cystine	?	?	0.45	?	1.73	1.00	.....	.....
Histidine	2.5	0.9	1.49	0.82	2.61	2.19	0.0	+
Arginine	3.81	8.2	2.91	1.55	3.47	14.17	87.4	1.0
Lysine	7.62	5.9	0.63	0.00	9.87	1.65	0.0	+
Tryptophane	1.50	0.00	1.0	0.00	2.40	1.50	.....	.....
Ammonia	1.61	0.40	5.22	3.64	1.31	2.28	.....	.....
Total	90.17	91.31	87.61	85.27	83.41	83.78	110.5	73.4

* The data in this table are taken largely from a compilation by H. B. Vickery. The analyses are combinations of what appear to be the best determinations by various chemists. *Cf.* L. B. Mendel, Nutrition—The Chemistry of Life—New Haven, 1923, p. 115. See also Plimmer, The Chemical Constitution of the Proteins, Part I, p. 111, etc.

CHAPTER V

SOURCES AND COMPOSITION OF FOODSTUFFS

It seems appropriate at this stage to consider briefly the sources of the protein, carbohydrate and fat of our diet and also the composition of some of the more important articles of food.

The extensive statistical studies of Raymond Pearl [1] have provided us with very valuable information regarding the food consumption in the United States of America for the period 1911–1918. He has shown that despite significant fluctuations in food production and food exports and imports, the total annual consumption of food shows remarkable uniformity from year to year. The following summary is based on data contained in his book, " The Nation's Food."

Of the protein consumed in the United States, 47 per cent comes from primary food sources, i.e., food directly gathered or harvested, such as, for example, potatoes, fish, oysters; or food derived by process of manufacture from a raw plant product, such as, for example, wheat flour or cottonseed oil. The remaining 53 per cent is obtained from so-called secondary sources, i.e., edible products of animals obtained either directly (without involving the death of the producing animal), such as honey, eggs, milk, or derivatively (involving the death of the animal), such as meats.

Primary food sources provide only 18 per cent of the fats and secondary sources 82 per cent. Most of the fat of the American's diet is therefore derived from animal sources.

The condition is reversed in the case of carbohydrates, 95 per cent being furnished from primary and 5 per cent from secondary food sources.

Approximately 36 per cent of the total protein consumed comes from grain, 26 per cent from meat, and 20 per cent from dairy products. All but 18 per cent of the nation's food protein is therefore supplied by these three great commodity groups.

Meats furnish 51 per cent of the fat; dairy products, 27 per cent; vegetable oils and nuts about 12 per cent; and grains about 4 per cent.

[1] R. Pearl, The Nation's Food, Philadelphia, 1920; see also, R. Pearl, Studies in Human Biology, Baltimore, 1924, Chap. XIV.

Grains provide 56 per cent of the carbohydrate; sugars, 26 per cent; vegetables about 9 per cent; dairy products, 5 per cent; and fruits, 4 per cent.

Thirty-five per cent of the energy representing the total food consumption is derived from grains; 22 per cent from meats; 15 per cent from dairy products; 13 per cent from sugars; about 5 per cent from vegetables; 5 per cent from vegetable oils and nuts; 2 per cent from poultry; and about 2 per cent from fruits.

Of the grains, wheat is by far the most important as a source of protein and carbohydrate, representing 29 per cent of the total protein and 42 per cent of the total carbohydrate consumption. Wheat provides the nation with 26 per cent of its food calories. However, it contributes only 1.8 per cent to the total fat consumption.

Corn ranks second among the grains, furnishing 5.55 per cent (6.4 per cent during the World War) of the protein, 1.9 per cent of the fat, 11 per cent of the carbohydrate and 7 per cent of the calories.

Rye, which is an important food in Russia and elsewhere in Europe, is little used in America. It supplies only 0.31 per cent of the protein, 0.03 per cent of the fat and about 0.8 per cent of the carbohydrate.

Dairy products furnish 20 per cent of the protein, 27.5 per cent of the fat, 5.5 per cent of the carbohydrate, and 15.26 per cent of the energy of the total food consumed.

Of the meats, beef is the most important from the standpoint of protein, representing 14.47 per cent of the total protein consumption. It contributes about 10 per cent to the fat consumption and provides 5.3 per cent of the total calories. Pork ranks first among all foods from the standpoint of fat; it provides 39.57 per cent of the total fat consumed in this country. It ranks fourth from the standpoint of protein, supplying 10.74 per cent of the total. The energy value represented by the pork consumption is 15.74 per cent, second only to wheat.

Of the vegetables, the potatoes are of greatest importance, representing 3.14 per cent of the total protein, 5.7 per cent of the total carbohydrate and 3.36 per cent of the total energy consumption.

As compared with other foods, fish occupy a relatively unimportant position in this country. Only 2.32 per cent of the total protein is derived from this source.

These figures represent gross consumption, being based on averages for the six years, 1911–12 to 1916–17, and do not take into account losses through wastage. Pearl has estimated the probable loss of edible food through wastage to be: for protein, about 5 per cent; fat, at least 25 per cent; carbohydrate, 20 per cent.

Composition of Some Foods.—With the relative importance of various foods in mind, we may now present the results of analyses of the protein, fat and carbohydrate contents of some of the more common foods.[2] Unless stated otherwise, the data are based on the edible portion only. The inorganic constituents and vitamins will be considered elsewhere.

TABLE XV

Composition of Edible Portion of Various Meats

	Per Cent Protein (N × 6.25)*	Per Cent Fat	Portion in Grams Equivalent to 100 Calories
Beef, chuck, thin	19.2	9	63
chuck, medium	18.6	16	45
chuck, fat	17.6	22	37
loin, thin	18.6	16	46
rib, medium	17.4	23	36
liver	20.4	4.5	78
sirloin steak	18.9	18.5	41
Porterhouse steak	21.9	20.4	37
Pork, chops	16.6	30.1	30
sausage	13.0	44.2	22
Bacon, smoked	10.5	64.8	16
Ham, fresh, lean	25.0	14.4	44
Lamb, chops, broiled	21.7	29.9	28
leg, roast	19.7	12.7	52
Mutton, leg	19.8	12.4	52
Veal, breast	20.3	11.0	56
cutlet	20.3	7.7	66

* The nitrogen content of most proteins is approximately 16 per cent, whence the factor 6.25.

[2] The sources of the data contained in these tables are: Bulletin 28 (Atwater and Bryant), of the Office of Experiment Stations, U. S. Department of Agriculture, Washington; H. C. Sherman, Chemistry of Food and Nutrition, Macmillan, New York, 1928, Appendix B; U. S. Department of Agriculture Circulars No. 50 and 389.

TABLE XVI

Composition of Fish and Oysters

	Per Cent Protein (N × 6.25)	Per cent Fat	Portion in Grams Equivalent to 100 Calories
Flounder	14.2	.6	161
Haddock	17.2	.3	140
Cod, salt	25.4	.3	96
Halibut steaks	18.6	5.2	83
Mackerel	18.7	7.1	72
salt	21.1	22.6	35
Shad, whole	18.8	9.5	61
Salmon, whole	22.0	12.8	49
Whitefish	22.9	6.5	67
Oysters *	6.2	1.2	199

* Oysters contain about 3.7 per cent carbohydrate.

TABLE XVII

Composition of Poultry and Eggs

	Per Cent Protein (N × 6.25)	Per Cent Fat	Portion in Grams Equivalent to 100 Calories
Chicken, broilers	21.5	2.5	92
Fowls	19.3	16.3	45
Turkey	21.1	22.9	34
Eggs, uncooked	13.4	10.5	68

TABLE XVIII

COMPOSITION OF SOME DAIRY PRODUCTS

	Per Cent Protein (N × 6.25)	Per Cent Fat	Per Cent Carbohydrate	Portion in Grams Equivalent to 100 Calories
Milk, whole	3.3	4.0	5.0	145
condensed, sweetened	8.8	8.3	54.1	31
evaporated	6.7	8.1	10.3	71
Cream	2.5	18.5	4.5	50
Butter	1.0	85.0	....	13
Cheese, American pale	28.8	35.9	.3	23
cottage	20.9	1.0	4.3	91
full cream	25.9	33.7	2.4	24
Swiss	27.6	34.9	1.3	23

TABLE XIX

COMPOSITION OF SOME FRUITS, BERRIES AND NUTS

	Per Cent Protein (N × 6.25)	Per Cent Fat	Per Cent Carbohydrate	Portion in Grams Equivalent to 100 Calories
Apples	.4	.5	14.2	159
Bananas	1.3	.6	22.0	101
Grapes	1.3	1.6	19.2	104
Grapefruit	.6	.1	12.2	193
Cherries, fresh	1.0	.8	16.7	128
Figs, fresh	1.4	.4	19.6	115
dried	4.3	.3	74.2	32
Oranges	.8	.2	11.6	195
Olives, green	1.1	27.6	11.6	33
ripe	1.7	25.0	4.3	40
Peaches, fresh	.7	.1	9.4	242
Pears, fresh	.6	.5	14.1	158
Strawberries	1.0	.6	7.4	269
Blackberries	1.2	1.1	7.8	160
Blueberries	.6	.6	13.9	146
Raspberries, red	1.0	.....	12.6	184
Almonds	21.0	54.9	17.8	15
Brazil nuts	17.0	66.8	7.0	14
Peanuts	25.8	38.6	24.4	18
Walnuts (California or English)	18.4	64.4	13.0	14

TABLE XX

Composition of Grain Products and Cereals

	Per Cent Protein (N × 6.25)	Per Cent Fat	Per Cent Carbohydrate	Portion in Grams Equivalent to 100 Calories
Flour, wheat, patent baker's grade	13.3	1.5	72.7	28
rye	6.8	.9	78.7	29
Corn meal	9.2	1.9	75.4	28
Bread, average white	9.2	1.3	53.1	38
whole wheat	9.7	.9	49.7	41
Macaroni	13.4	.9	74.1	28
Oatmeal	16.1	7.2	67.5	25
Barley	8.5	1.1	77.8	28
Rice	8.0	.3	79.0	29

TABLE XXI

Composition of Some Vegetables and Legumes

	Per Cent Protein (N × 6.25)	Per Cent Fat	Per Cent Carbohydrate	Portion in Grams Equivalent to 100 Calories
Asparagus, cooked	2.1	3.3	2.2	213
Beets, cooked	2.3	.1	7.4	252
Cabbage	1.6	.3	5.6	317
Celery	1.1	.1	3.3	542
Lettuce	1.2	.3	2.9	525
Onions, fresh	1.6	.3	9.9	206
Potatoes, white, raw	2.2	.1	18.4	120
sweet	1.8	.7	27.4	81
Spinach, fresh	2.1	.3	3.2	417
Squash	1.4	.5	9.0	217
Tomatoes, fresh	.9	.4	3.9	438
Beans, string, fresh	2.3	.3	7.4	241
baked, canned	6.9	2.5	19.6	78
Peas, canned	3.6	.2	9.8	180
dried	24.6	1.0	62.0	28
green	7.0	.5	16.9	100

CHAPTER VI

DIGESTION AND THE CHEMISTRY OF ENZYME ACTION

THE breakdown of the three great classes of foodstuffs, after they enter the body, into substances capable of absorption from the intestinal tract and utilizable for the production of energy and living tissue is fundamentally due to the action of a group of substances known as *enzymes*.[1] So apparent is the relation between enzyme action and the processes of living organisms that at various times it has been believed that the former was dependent upon the unit structure of the latter, the cell. Thus, though Kirchoff [2] in 1814 had discovered the catalytic action of the glutinous component of wheat meal capable of converting starch to sugar and dextrin; though Payen and Persoz [3] (1833) had separated a similar substance from malt extract, and Liebig and Wöhler [4] (1837) noted the cleavage of amygdalin by the emulsin of bitter almonds, yet Pasteur [5] many years later (1878) ascribed the processes of fermentation to the metabolism of micro-organisms. It was Buchner,[6] in 1897, who established firmly the concept that the action of enzymes was independent of the cell structure. To-day we broadly think of enzymes as material substances, formed by living cells, but the action of which is independent of their presence.

The definition given by Waldschmidt-Leitz[7] is: "*Enzymes are definite material catalyzers of organic nature with specific powers of reaction, formed indeed by living cells, but independent of the presence of the latter in their operation.*"

Gortner [8] supplements this definition by appending the phrase, "*and when in the moist state, readily destroyed by heat.*"

[1] The term, coined by Kühne, is derived from the Greek words, ἐν ζύμῃ, meaning *in yeast.*

[2] Schweigger's J. für Chemie u. Physik, **14**, 389 (1815).

[3] Ann. Chim. Phys., **53**, 73 (1833); **56**, 337 (1837).

[4] Annalen d. Chem., **22**, 1 (1837); Pogg. Ann., **41**, 345 (1837).

[5] Die Alkoholgärung, 2. Aufl., Stuttgart, 1878.

[6] Ber., **30**, 117 (1897).

[7] E. Waldschmidt-Leitz, Enzyme Actions and Properties, translated and extended by R. P. Walton, John Wiley & Sons, Inc., New York (1929), p. 3.

[8] R. A. Gortner, Outlines of Biochemistry, John Wiley & Sons, Inc., New York (1929), p. 713.

Preparation and Purification of Enzymes.—The general methods used in the preparation and purification of enzymes differ considerably, depending on the kind of enzyme, whether it is being separated from a secretion, such as the saliva or gastric juice, or from a tissue, depending also on its solubilities and other properties. Some of the information which we have about certain enzymes, pepsin, for example, was obtained directly by working with the gastric juice, but in most cases the enzymes, if they are to be made available for study and other uses, must be liberated from tissues. This may be accomplished by macerating the cells, as was done by Buchner in his classical experiments with yeast. Or the tissues may be allowed to undergo autolysis, a process of cellular dissolution which occurs after tissues die. In either case the juice which may be separated from the mass of disintegrated cells contains the enzyme, at least a portion.

Another procedure is to dry the tissue at low temperature, or with some non-injurious chemical agent and subsequently grinding and extracting the preparation with a suitable solvent. Weak acids and bases, glycerol and alcohol are commonly used for this purpose.

Enzyme preparations obtained by any of these methods contain large amounts of extraneous material and purification methods have been developed for separating these. For the removal of inorganic impurities, dialysis is used. For the separation of enzymes from their solutions, various methods of precipitation have been employed, but these have many limitations, the most serious of which is that a large proportion of the extraneous material, such as protein, is precipitated along with the enzyme. Nevertheless partial success has attended such methods, particularly in the case of pepsin. The isoelectric point of this enzyme is said to be at *p*H 2.5 (Forbes,[9] Fenger and Andrew[10]). Recently Fenger, Andrew and Ralston[11] by isoelectric precipitation at *p*H 2.5 obtained an exceedingly active product, possessing a proteolytic potency as high as 1 : 65,000.[12]

The use of suitable adsorbents, such as colloidal aluminum hydroxide and kaolin for separating enzymes from associated substances has been adopted by many modern workers and has led to important advances in our knowledge of enzymes. Using alumina gel as an adsorbing agent, a preparation of saccharase has been obtained, 12,000 times more active

[9] J. Biol. Chem., **71**, 559 (1927).

[10] *Ibid.*, **73**, 371 (1927).

[11] *Ibid.*, **80**, 187 (1928).

[12] This means that one part of the enzyme preparation digested 65,000 times its own weight of coagulated egg albumin, the method of assay being that of the United States Pharmacopœia (X edition, Philadelphia, 1926, p. 280).

than living yeast. These methods have been further developed to the point of making possible the separation of enzymes from each other. Thus, in the case of the pancreas enzymes (p. 163) Willstätter and Waldschmidt-Leitz [13] found that of the three enzymes, lipase, amylase and trypsin, the first is most readily adsorbed both by alumina and kaolin and may thus be separated from the last two. Amylase, if relatively free from concomitant foreign substances, is indifferent both to kaolin and alumina, whereas trypsin is readily adsorbed by kaolin. Repeated treatment with kaolin in an acid solution removes the trypsin, leaving the amylase behind. Finally, the trypsin may be freed from kaolin by elution with dilute alkali.[14]

The Chemical Nature of Enzymes.—Our first interest in enzymes as material substances is their chemical nature. Investigation in this direction has consisted in analyzing highly active enzyme preparations with the ultimate aim of isolating and analyzing the pure principle involved. Among the earlier investigations in this field were Pekelharing's[15] (1902) and Dezani's [16] (1911) studies of pepsin. Their preparations gave the color reactions characteristic of proteins. Dezani obtained on hydrolysis the following amino acids: leucine, tyrosine, arginine, histidine and lysine. Sherman [17] in the case of amylase from the saliva and from pancreatic juice and Osborne [18] in the case of malt amylase also obtained the color reactions and composition analyses of proteins. Euler and Josephson [19] found positive biuret, xanthoproteic and ninhydrin reactions in very active preparations of invertase. Willstätter and Kuhn,[20] however, have described a preparation of invertase entirely free from protein, carbohydrate and phosphorus.

On the whole, investigation in this direction has been very indecisive. With the exception of two cases reported recently, it has not been possible to assign an enzyme to a definite group of chemical substances. The view of Willstätter's school, the most energetic and intensive group of investigators in this field, is that enzymes are composed of a specific active group and a colloidal bearer or carrier and that the specific group binds the enzyme to the substrate.

[13] Z. Physiol. Chem., **125**, 132, 142 (1922–23).

[14] As it is impossible to consider here the details of the methods developed by Willstätter and his school, the student is referred to Chap. VII, of Waldschmidt-Leitz's book, previously cited.

[15] Z. Physiol. Chem., **35**, 8 (1902).

[16] Arch. ital. Biol., **54**, 15 (1911).

[17] J. Am. Chem. Soc., **33**, 1195 (1911); **34**, 1104 (1912); **35**, 1790 (1913).

[18] *Ibid.*, **17**, 587 (1895); **18**, 536 (1896).

[19] Ber. **56** B, 1097 (1923); **57** B, 299 (1924); Z. Physiol. Chem., **133**, 279 (1924).

[20] Z. Physiol. Chem., **125**, 28 (1923).

One of the two exceptions referred to is the enzyme urease, the isolation of which as a crystalline globulin (colorless octahedra) has been reported by Sumner.[21] These crystals have an activity of 129,000 units, i.e., 1 gram will produce 129,000 mgs. of ammonia nitrogen from a urea phosphate solution in five minutes, at 20° C., or it will decompose its own weight of urea in less than 1.4 seconds.

The second enzyme, the isolation of which in pure form has been claimed recently, is pepsin (Northrop).[22] It is reported to be a protein-like substance crystallizing in small hexagonal prisms and having a peptic activity as high as 1 : 20,000 U. S. P.

The Mode of Enzyme Action.—Our second and most immediate interest in enzymes is their mode of action. Enzymes are part of a larger group of substances known as catalysts. These are defined as bodies which alter the rate of an existing reaction without themselves becoming permanently changed (Ostwald).

While in most cases catalysis is regarded as causing an increased reaction velocity (positive catalysis), it is theoretically possible for a catalytic agent to decrease the velocity of a reaction (negative catalysis). A frequently cited example of negative catalysis is the behavior of a trace of ether vapor in depressing the oxidation of phosphorus.

J. J. Thompson attributes to catalysts the property of initiating a reaction, a view that is becoming more generally accepted. Bayliss[23] has pointed out that this idea is not necessarily in disagreement with Ostwald's definition. It may be conceived that the catalyst overcomes an influence resisting the initiation of a reaction. From this standpoint, Bayliss defines a catalyst as a substance that changes the rate of a reaction which is actually in progress or which is capable of proceeding without any supply of energy from without, if certain resisting influences are removed. The resisting influences, according to Bayliss, may be conceived of as being somewhat analogous to the force of friction which would hold back a weight from sliding down an inclined plane.

While the degree of acceleration of a reaction is proportional to the concentration of the catalyst present, the final amount of products formed is independent of the amount of catalyst used. The time factor alone is affected by the catalyst.

Attempts to correlate our knowledge of enzyme action have resulted in the formulation of two main rival theories. Bayliss has advanced a colloid chemical point of view. According to this, the substances which react are first adsorbed on the surface of the enzyme particles. The

[21] J. Biol. Chem., **69**, 435 (1926); **70**, 97 (1926); **76**, 149 (1928).

[22] Science, **69**, 580 (1929).

[23] W. M. Bayliss, The Nature of Enzyme Action (1925).

chemical reaction then takes place at the interface. Though this chemical reaction may be subject to the law of mass action,—namely, that the rate of reaction at any moment is proportional to the concentration at the moment of the reacting substances, still it is the adsorbed portion of the substances which is the controlling factor.

The Michaelis school, on the other hand, has assumed that enzyme and substrate unite chemically, as ions would, to form an intermediate substance and that the rate of reaction is proportional to the concentration of this intermediate enzyme-substrate compound. These assumptions imply that the substances involved act as if they were in homogeneous solution. A good exposition of Michaelis' theory and the work upon which it is based is given by Waldschmidt-Leitz.

Reversible Reactions.—A characteristic feature of certain reactions is that they never reach completion. For example, acetic acid and ethyl alcohol react according to the following equation:

$$CH_3COOH + C_2H_5OH \rightleftharpoons CH_3COOC_2H_5 + HOH.$$

A point of equilibrium is reached when the velocity of the reaction in one direction is equivalent to that in the opposite direction. Starting with molar concentrations of ethyl alcohol and acetic acid, equilibrium occurs when $\frac{2}{3}$ mol. of ethyl alcohol and $\frac{2}{3}$ mol of acetic acid have been transformed to ethyl acetate. Increasing the concentration of either constituent on the left-hand side of the equation produces a shift to the right, until a new equilibrium is reached, whereas adding ethyl acetate to the reaction mixture results in its hydrolysis. This type of reaction is said to be *reversible*. The addition of a small amount of hydrochloric acid increases the velocity of the reaction in either direction. It is with reactions of this general type, namely those which occur relatively slowly and are reversible, that we are ordinarily concerned with in the study of catalysis, whether by inorganic catalysts or enzymes.

The Specificity of Enzyme Action.—A distinction is frequently made between the enzymes and the inorganic catalysts on the basis of their relative specificity. Colloidal platinum catalyzes a variety of reactions, such as the decomposition of hydrogen peroxide, the hydrolysis of esters of the simple alcohols, and the formation of nitric acid and sulfuric acid. In other words, platinum as a catalyst is not limited to a single reaction. Similarly unspecific in their action are the inorganic acids and bases. Hydrogen and hydroxyl ions catalyze the hydrolysis of proteins, fats and carbohydrates indiscriminately. On the other hand, a given enzyme which digests protein is never known to act on fat or carbohydrate. Catalase decomposes hydrogen peroxide, as does platinum, but it differs from platinum in having no effect on any other reac-

tion. Invertase acts on sucrose, but not on maltose, which is hydrolyzed by maltase, or on lactose, which is specifically acted on by the enzyme lactase.

In enzymic reactions, specificity may be either with respect to the substance attacked as indicated above or the type of products formed. Thus both yeast saccharase and emulsin hydrolyze the trisaccharide, raffinose. But the first forms fructose and melibiose; the latter forms sucrose and galactose. Glucose may undergo several types of fermentations, each being caused by a specific enzyme. The substrate is the same, but the products, lactic acid, alcohol, etc., are different. On the other hand, the reaction products may be identical but the reaction path different. Both the saccharase of yeast and that of *aspergillus oryzae* invert cane sugar to glucose and fructose. But according to Kuhn,[24] the former does so by attaching itself to the fructose component of cane sugar, whereas the enzyme of the mold fungus is supposed to attach itself to the glucose part of the molecule.

However, it should be emphasized that these distinctions in specificity are not so sharply drawn as it may appear. Inorganic catalysts also show some degree of specificity. Tungstic acid aids in the oxidation of hydriodic acid by hydrogen peroxide, but does not accelerate the oxidation of hydriodic acid by a persulfate. Iron salts catalyze the oxidation of potassium iodide by a persulfate, whereas no effect is produced by the iron in the oxidation of sulfurous acid by a persulfate. Even platinum black exhibits certain peculiarities in behavior toward esters. Hydrolysis of the esters of simple alcohols is accelerated, whereas the effect produced on the glycerol esters is hardly appreciable. Taylor [25] points out that acids, though very active in many hydrolytic reactions, have no effect on the conversion of adenine and guanine into xanthine and hypoxanthine.

Trypsin exhibits a marked degree of specificity, its action being limited to the proteins themselves and their higher degradation products, whereas substances of the simple peptide type are attacked by erepsin, which recent work indicates is not a single enzyme, but is composed of two enzymes, a dipeptidase and a polypeptidase.

In the case of some enzymes, the specificity seems to be relative, rather than absolute. Thus, the lipases act on a variety of ester linkages, and maltase acts not only on maltose but also on other α-glucosides, whereas the enzyme emulsin behaves similarly on a variety of β-glucosides. In general, the action of enzymes is limited not so much to certain

[24] Z. Physiol. Chem., **129**, 57 (1923).

[25] A. E. Taylor, Digestion and Metabolism, Lea & Febiger, Philadelphia, 1912, p. 104.

substances as to certain atomic groups or linkages within the molecule. It is more a question of specificity for a certain group in the molecule than of substance specificity.

Owing to their specificity, the enzymes are more limited in their action than inorganic catalysts, but, on the other hand, their efficiency is much greater. Catalase prepared from red corpuscles, for example, is about 20 times more effective in decomposing hydrogen peroxide than an equivalent amount of colloidal platinum.[26]

Classification and Nomenclature.—The classification and nomenclature of the enzymes are based on the type of reactions which they catslyze or on the substrate on which they act. The hydrolytic enzymes are most numerous and take part in reactions of hydrolysis. To this group belong the following:

I. The Proteases, proteolytic, or protein-splitting enzymes.

(*a*) *Pepsin.*—Found in gastric juice. Pepsin acts on native proteins, usually digesting them to proteoses and peptones.

(*b*) *Trypsin.*—Present in pancreatic juice. It digests proteins, proteoses and peptones to polypeptides and possibly to dipeptides.

(*c*) *"Erepsin."*—Recent work shows that the erepsin of the pancreas and intestinal mucosa is not a single enzyme, but that it is probably a mixture of two enzymes, a polypeptidase and a dipeptidase, the actions of which are to convert polypeptides and dipeptides to amino acids.

(*d*) *Rennin.*—Pancreatic rennin is found in the pancreatic juice and gastric rennin in the gastric juice. Rennin converts casein into paracasein.

(*e*) In addition to these are the plant proteases, the best known of which are *papain*, present in the melon-tree (papaw, *carica papaya*), and *bromelin*, occurring in the pineapple.

II. The Fat and Ester-splitting Enzymes, Lipases and Esterases.—These are sometimes classed together under the general head of *esterases.* The best known is the *lipase, steapsin* of the pancreatic juice. Other lipases are especially abundant in oil-containing seeds, such as the castor bean. The liver is said to contain an esterase which acts on simple esters. Among the other known esterases are: (1) *chlorophylase*, which occurs in green leaves and hydrolyzes chlorophyl *a* to chlorophylide *a* and phytol, (2) *pectase*, which is widely distributed in plants and molds and hydrolyzes pectin to pectic acid and methyl alcohol, (3) *tannase*, found in certain molds, which converts tannin into glucose and gallic

[26] Waldschmidt-Leitz, Enzyme Actions and Properties, tr. by R. P. Walton, p. 213.

acid, (4) *sulfatase*, present in *aspergillus oryzae*, which hydrolyzes sulfuric acid esters, (5) *phosphatase*, present in yeast, which hydrolyzes (and may perhaps synthesize) phosphoric acid esters.

III. Carbohydrases.—To this group belong the polysaccharide digesting enzymes and those concerned with the hydrolysis of sugars, glucosides, etc.

(*a*) *Amylases.*—These are starch-splitting enzymes. The best-known members of this group are (1) *ptyalin* of the saliva and (2) *amylopsin* of the pancreatic juice. Another is *malt amylase*. The action of amylase is the conversion of starch to maltose.

(*b*) *Sucrase, saccharase, or invertase* present in the intestinal mucosa and intestinal juice, as well as in yeast. It converts sucrose into fructose and glucose. It also hydrolyzes raffinose and other trisaccharides, the former to fructose and melibiose.

(*c*) *Maltase*, present in the saliva, pancreatic and intestinal secretions, yeast, etc. It is actually a member of the sub-group, the *α-glucosidases*. Maltase hydrolyzes maltose to glucose and probably acts on other α-glucosides.

(*d*) *Emulsin*, found chiefly in bitter almonds, is a member of the sub-group of *β-glucosidases*. It hydrolyzes amygdalin to glucose and mandelonitrile and may act similarly on other β-glucosides.

(*e*) *Lactase*, present in intestinal mucosa and intestinal juice. It hydrolyzes lactose to glucose and galactose and may have a similar effect on other β-galactosides.

(*f*) Among the other carbohydrases of importance may be mentioned the *cellulases*, concerned with the hydrolysis of cellulose, and *inulinase*, which hydrolyzes the polysaccharide inulin. These enzymes do not occur in the digestive secretions of man.

Other hydrolytic enzymes are *urease*, found in the soy bean and jack bean, which converts urea into carbon dioxide and ammonia. It is grouped with the *deaminases*, or *desamidases*, enzymes which hydrolyze amino acids and other nitrogen compounds into hydroxy acids and ammonia. In the latter group are included *asparaginase*, which hydrolyzes asparagine to aspartic acid and ammonia, *arginase*, which hydrolyzes arginine to urea and ornithine, and also *guanosine desamidase*, which hydrolyzes guanosine, and *adenosine desamidase*, which acts on adenosine. To this group also belongs guanase, which converts guanine into xanthine and ammonia, and *adenase*, which acts on adenine with the formation of hypoxanthine and ammonia.

Intestinal juice contains an enzyme, *nucleinase*, which hydrolyzes tetranucleotides into mononucleotides. The purine nucleotides are

hydrolyzed into nucleosides and phosphoric acid by another group of enzymes, the *nucleotidases*, which may be classified as phosphatases. The intestinal mucosa contains *nucleinase*, *nucleotidases* and, in addition, a third group of enzymes, the *nucleosidases*, capable of hydrolyzing the purine nucleosides into sugar and purine bases. Less is known of the enzymes capable of acting on the pyrimidine nucleosides.[27, 28]

Another important group of enzymes are the oxidizing enzymes, or oxidases. Of the enzymes that have been described and placed in this category there are some that are capable of converting phenols to quinones, tyrosine to black pigments, aldehydes to acids, and ethyl alcohol to acetic acid. The peroxidases reduce a variety of organic peroxides with the liberation of oxygen. The catalases behave similarly, though their action is limited to the disintegration of hydrogen peroxide. A number of enzymes concerned with the reduction of perhydrides have been described; to these the terms reductase, reducase, and hydrogenase have been applied. It is to be pointed out that the action of the oxidases and peroxidases results in the oxidation of one compound and the reduction of another. Much confusion exists in the literature with regard to the physiological significance of the oxidases, peroxidases, catalases, and reductases. Further reference to this subject will be necessary in the chapter concerned with oxidations and reductions in animal tissues.

Still another group of enzymes are the zymases. These act on the simpler sugars to form a variety of products, such as lactic acid (lactic-acid fermentation), carbon dioxide, and alcohol (alcoholic fermentation).

Long usage has retained for the more familiar enzymes their former nomenclature. This is true in the case of ptyalin, pepsin, rennin, trypsin, etc. In accordance with a suggestion made by Duclaux, most of the hydrolytic enzymes are now designated by the suffix *ase* added, usually, to a portion of the name of the substrate or substance on which the enzyme acts. Thus malt*ase* is the enzyme that hydrolyzes maltose; lact*ase* acts on lactose; the prote*ases* digest proteins; whereas the fat-splitting enzymes are called lip*ases*. The protein-splitting enzymes are also frequently referred to as proteo*lytic* enzymes; the enzymes that attack starch are amylo*lytic*, and those that act on fats, lipo*lytic*.

Factors Influencing Enzyme Action.—In trying to understand the nature of enzymes, not by means of their chemical composition but through a study of the actions which they exert, we are aided by a great

[27] Levene and Medigreceanu, J. Biol. Chem., **9**, 65, 375, 389 (1911); Levene and La Forge, *Ibid.*, **13**, 507 (1913).

[28] W. C. Rose, Physiol. Reviews, **3**, 544 (1923).

amount of data which has been collected either empirically or in line with various theories of enzyme action. Thus for a great many enzymes we know just how the rate at which the substrate changes, in the presence of the enzyme, varies with the following factors:

(1) Concentration of the substrate,
(2) Concentration of the enzyme,
(3) Temperature,
(4) Reaction or hydrogen-ion concentration of the medium,
(5) Light and other radiations,
(6) Electrolytes,
(7) Inhibiting agents or poisons,
(8) Products of the reaction.

Concentration of the Substrate.—In the case of most enzymic reactions it has been found that, starting with zero concentration of substrate, the velocity of the reaction increases with increasing concentration of substrate, then remains constant for a considerable variation in concentration, and finally decreases in very concentrated solutions of substrate.

In Fig. 18 are represented the results of one of Northrop's [29] experiments, showing the effect of substrate concentration on the rate of digestion of protein. Protein solutions containing 8, 4, 2, 1, and 0.5 per cent egg albumin were hydrolyzed at 25° C. in the presence of 1 cc. of 2 per cent pepsin, 25 cc. of the solution being used in each case. All solutions were brought to a *p*H of 1.8 with hydrochloric acid. Since the products of protein digestion conduct the electric current better than the original protein, the specific conductivity of the digests, determined at various intervals, was used as the measure of the amount of digestion. For purposes of comparison, the rate of digestion of the 8 per cent solution is designated in the figure as 100, and the rates of digestion of the 4, 2, 1, and 0.5 per cent egg albumin are represented on this basis. The curve (Fig. 18) shows that in low concentration the increase in rate is

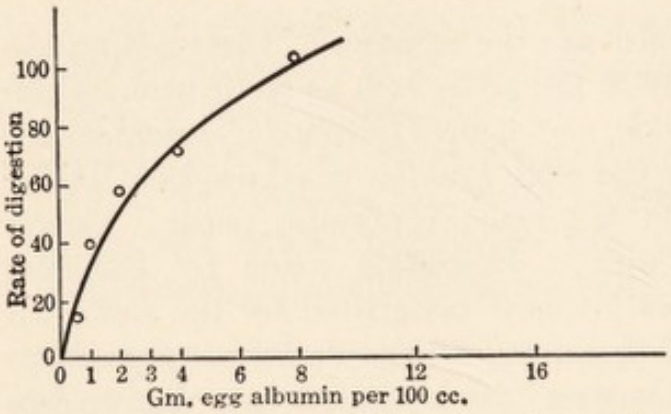


FIG. 18.—Relative rate of digestion of egg albumin solutions of different concentration when digested with the same concentration of pepsin solution.

[29] J. Gen. Physiol., **2**, 595 (1920).

nearly proportional to the increase in substrate concentration, but that in high concentrations the rate increases more slowly.

Concentration of the Enzyme.—The final equilibrium of an enzymic reaction does not depend on the quantity of enzyme. The velocity of the reaction does depend, however, on this factor. In the case of pepsin, for example, a relationship exists between the mass of protein transformed, x; the time, t; the initial concentration of the substrate, a; and the concentration of the enzyme, E. The relationship, known as the Schütz-Borissov rule, may be expressed by the formula:

$$x = k\sqrt{aEt}.$$

Northrop [30] has studied the relation between the concentration of pepsin and the rate of digestion. The latter was determined by measuring the time required to produce a given change in the conductivity of an egg-albumin solution to which pepsin had been added. The reciprocal of this time, $\left(\frac{1}{T \text{ hours}}\right)$, being proportional to the mean rate of digestion, was taken to represent the amount of "active" pepsin. All factors, such as concentration of substrate, hydrogen-ion concentration, etc., were kept constant, the amount of pepsin alone being altered. The observations recorded by Northrop are outlined in Table XXII. In the

TABLE XXII

ENZYME CONCENTRATION AND RATE OF DIGESTION (after Northrop)

Pepsin solution. 10 per cent solution of Grübler's pepsin in HCl, pH 2.0.

$$K = 7.2 \qquad d = \frac{30}{v} *$$

V = volume containing 1 cc. of original pepsin solution	E = total pepsin per cc.	$Q = \frac{1}{T}$ = active pepsin per cc.					ET
		Observed				Calculated	
		1	2	3	Average		
1	26.9	9.1	9.7	10.0	9.6	9.7	269
2	13.44	6.25	6.30	6.67	6.39	6.40	206
4	6.72	4.17	3.70	3.57	3.81	4.05	175
8	3.36	2.38	2.50	2.17	2.35	2.42	145
16	1.68	1.39	1.43	1.35	1.39	1.38	120
32	0.84	0.83	0.80	0.78	0.80	0.77	106
64	0.42	0.41	0.40	0.39	0.40	0.40	100
128	[0.21]	0.22	0.20	0.20	0.20	0.20	100

* K = equilibrium constant (see equation below);
d = concentration of peptone present at the beginning of the reaction.

[30] J. Gen. Physiol., **2**, 113, 471 (1919–20).

last column, ET represents the product of the total concentration of pepsin and the time necessary to cause 10 per cent of the total change in the conductivity of the substrate. If the rate of digestion were directly proportional to the enzyme concentration, the value for ET would remain constant. From the data presented it will be seen that ET is constant only for low concentrations of pepsin. This is clearly indicated also by the curve in Fig. 19, based on the data in Table XXII.

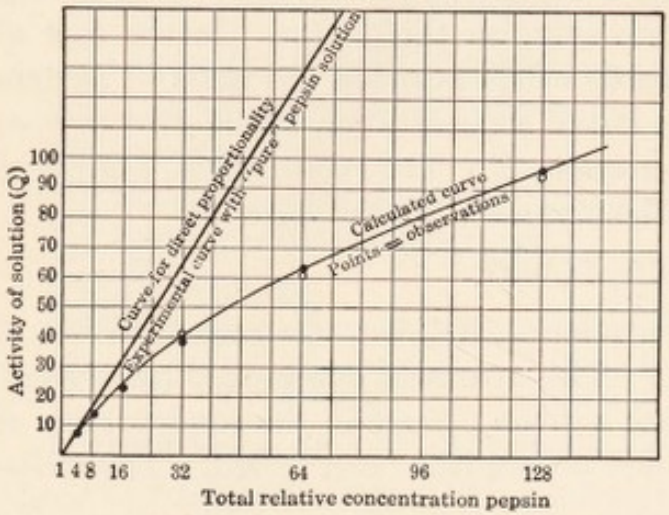


FIG. 19.—Curves showing pepsin concentration and rate of digestion (*cf.* Table XXII)

The divergence from direct proportionality is attributed to the fact that the enzyme in solution is in equilibrium with another substance, possibly peptone, and that the uncombined pepsin alone affects the hydrolysis of the protein. This equilibrium may be expressed quantitatively, according to the law of mass action, by the equation:

$$K = \frac{\text{Concentration of pepsin} \times \text{concentration of peptone}}{\text{Concentration of pepsin-peptone}}.$$

Northrop has tested the Schütz-Borissov equation in the case of pepsin and finds that it holds under certain conditions only, namely, when the concentration of peptone is large with respect to pepsin, and the concentration of substrate relatively small. The causes of the divergence from the Schütz-Borissov equation are indicated in the following chart (Fig. 20). For further discussion of this work, the reader is referred to Northrop's papers.

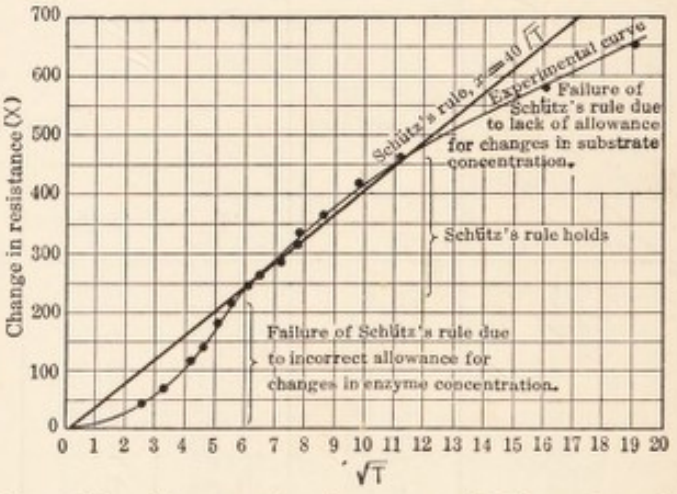


FIG. 20.—Curves showing rate of digestion of egg albumin and deviations from Schütz's rule.

Where the substrate is in true solution, as in the action of invertase on sucrose and urease on urea, the velocity of the reaction is directly proportional to the concentration of enzyme. As an illustra-

tion of this we may consider an experiment of Hudson [31] in which was determined the relation of the time required for transforming a definite amount of sucrose to the amount of invertase used. In the table given below are the data obtained with a 4.55 per cent solution of sucrose, the time being that required in each case to bring about approximately 73 per cent hydrolysis of the sucrose.

TABLE XXIII

Relative concentration of the enzyme, invertase	Time in minutes required to hydrolyze approximately 73 per cent of the sucrose (initial concentration 4.55 per cent)
.25	120
.50	60
1.00	30
1.50	20
2.00	15

Effect of Temperature.—A rise in temperature increases the velocity of a chemical reaction. Van't Hoff has formulated a rule according to which an increase in temperature of 10° C., within certain limits, about doubles or trebles the velocity of a reaction. The temperature coefficient for any given temperature range can be calculated from the formula:

$$\text{Temperature coefficient} = \frac{\text{Velocity at } (T° + 10° C.)}{\text{Velocity at } T°}.$$

The equilibrium of an enzymic reaction is only slightly dependent on the temperature when the heat change accompanying the reaction is small. Up to a certain point, increase in temperature results in an increased velocity. Opposing this effect, is the influence of heat in inactivating and destroying the enzyme. Bayliss and others relate this property to the colloidal nature of the system of which the enzyme forms a part.

An experiment of Chodat [32] on the tyrosine-tyrosinase reaction may be taken as illustrating the influence of temperature elevation on enzyme action. The time required to produce sufficient change to give a certain

[31] J. Am. Chem. Soc., **30,** 1564 (1908).

[32] Cited by Euler in Euler-Pope's "General Chemistry of the Enzymes," John Wiley & Sons, 1912 edition, p. 240.

degree of color intensity was taken as the criterion for comparing the activity of the enzyme at various temperatures.

Temperature	0°	10°	20°	30°	45°	50° C.
Time (minutes)	180	100	60	40	20	10

The following temperature coefficients have been recorded for a number of enzymes:

TABLE XXIV

Substrate and Enzyme	Temperature Interval	Temperature Coefficient $\frac{k_{t+10}}{k_t}$
Sucrose-sucrase	0–20°	2.0
Sucrose-sucrase	20–30	1.4
Sucrose-sucrase	30–40	1.5
Sucrose-sucrase	40–50	1.4
Hydrogen peroxide-catalase	0–10	1.5
Starch-amylase	20–30	2.0
Milk-rennin	20–30	2.1
Milk-rennin	30–40	3.2
Casein-trypsin	20.7–30.7	5.3

k = velocity constant.

The *optimum temperature* is the temperature at which the activity of an enzyme is at a maximum. Most enzymes exhibit maximum activity between 37° and 53° C. The optimum temperature may be influenced by various factors, such as the reaction of the medium, the concentration of the enzyme, and the nature and concentration of the substrate.

Certain enzymes from vegetable sources are known to exhibit higher temperature optima than those from animal tissues. Papain and bromelin, which are proteolytic enzymes found in plants, have an optimum temperature of about 60° C. Rennin, from animal sources, has an optimum temperature of about 45° C., but the milk-coagulating enzymes obtained from plants have much higher optima, approaching 80–85° C.

Exposure of enzymes to temperatures above 60° C. usually results in their inactivation. Here again, various factors may exert modifying influences. Invertase is more readily destroyed in the absence of sucrose (about 50° C.) than when sucrose is present (only partly inactivated at 70° C.). Trypsin is very readily destroyed at 100° C. in an alkaline solution, but not quite as readily in an acid solution. It has

been observed, in experiments with invertase, trypsin, and taka-diastase, that these enzymes inactivated by heating may regain a certain amount of their activity when allowed to stand in aqueous solution.

TABLE XXV *

THE EFFECT OF TEMPERATURE ON THE COAGULATION OF MILK BY RENNIN (ANIMAL)

Temperature 0° C.....	25°	30°	33°	36°	39°	42°	45°
Time required for coagulation, in minutes and seconds.....	1–40	1–00	0–40	0–30	0–25	0–35	0–45

Observed optimum at 39° C.

* From data obtained by Gerber and cited by Euler, p. 242.

TABLE XXVI *

THE EFFECT OF TEMPERATURE ON THE COAGULATION OF MILK BY RENNIN (PLANT)

Temperature, ° C.	Time, sec.	Temperature, ° C.	Time, sec.
72°	74	79°	56
73°	73	82°	53
74.5°	68	83°	52
75°	67	85°	57
78°	57	87°	78

Observed optimum at 83° C.

* From data of A. Bodansky obtained with the milk-coagulating enzyme present in *Solanum eleagnifolium*, J. Biol. Chem., **61**, 365 (1924).

Effect of the Reaction or Hydrogen-ion Concentration.—The activity of enzymes is markedly influenced by acids and alkalies and is usually limited to a definite range of acid or alkali concentration. Pepsin acts only in an acid medium and loses its activity in an alkaline solution, whereas trypsin digests proteins in either a neutral or an alkaline solution but not in the presence of free acid.

Sørensen [33] first pointed out that the velocity of enzymic reactions varied with the hydrogen-ion concentration and he as well as numerous other workers have studied this relation for various enzymes.

[33] Biochem. Zeit., **21**, 131, 201 (1909); **22**, 352 (1909).

As an illustration, one of Northrop's [34] experiments may be cited. Peptic digestion of purified egg albumin was studied at varying H-ion concentrations. At the end of a four-hour interval of digestion, the amount of proteolysis was determined by analyzing the digests for the increase in amino nitrogen. The results obtained are represented in Fig. 21. The curve shows that the optimum acidity for digestion, as determined by the increase in amino nitrogen, corresponds to about *p*H 1.0. This is somewhat lower than the values obtained by other workers (see Table XXVII) and is probably representative not only of the optimum for the digestion of the protein in question, but also of some of the intermediate products of the proteolysis.

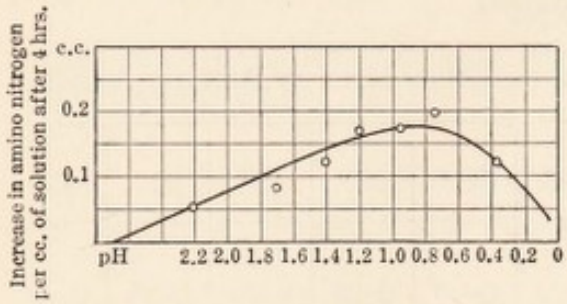


FIG. 21.—Influence of *p*H on the rate of digestion of egg albumin.

Northrop believes that the determining factor in the digestion of proteins by pepsin is the amount of ionized protein in solution. The degree of ionization is very slight in the case of most proteins at a *p*H of 4.5. Now it is of interest to note that pepsin becomes practically inactive at this *p*H, showing that it is the protein ion which is acted on by the enzyme. The optimum hydrogen-ion concentration for the activity of pepsin coincides with the hydrogen-ion concentration at which the protein solution contains the greatest number of protein ions.

The nature of the acid is not an important factor in enzymic activity. Northrop [35] has shown that at equivalent hydrogen-ion concentrations the rate of pepsin digestion of gelatin, egg albumin, casein, and edestin is the same in solutions of hydrochloric, nitric, sulfuric, oxalic, citric, and phosphoric acids. Acetic acid diminishes the rate of digestion of all proteins except gelatin. The peculiarity of acetic acid in this regard is probably due to some effect on the protein rather than on the enzyme.

Another illustration of the dependence of enzymes on hydrogen-ion concentration is the Activity-*p*H curve in Fig. 22 for the enzyme invertase from yeast, based on the experimental data of Michaelis and Davidsohn.[36] It is to be noted that this enzyme exhibits a broad optimal zone of action, this being between *p*H 3.5 and 5.5.

In the following table are given a number of values which have been

[34] J. Gen. Physiol., **3**, 211 (1920).
[35] J. Gen. Physiol., **1**, 607 (1919).
[36] Biochem. Z., **35**, 386 (1911).

obtained for the optimum hydrogen-ion concentrations of various enzymes.

TABLE XXVII *

Enzyme	Optimum *p*H	Authority
Sucrase, yeast	4.4–4.6	Sørensen; Fales and Nelson
Sucrase, intestinal	6.8	Euler and Svanberg
Amylase, saliva	6.0	Norris
Amylase, pancreatic	7.0	Sherman, Thomas, and Baldwin
Pepsin (edestin, casein)	1.4	Michaelis and Mendelssohn
Pepsin (egg albumin, ½–1 hour)	1.6	Sørensen
Pepsin (egg albumin, 12 hours)	1.2	Sørensen
Pepsin (gelatin)	3.0–3.5	Dernby
Trypsin, pancreatic	8.0	Lundén
Erepsin, intestinal	7.7	Rona and Arnheim
Catalase	7.0	Sørensen

* Compare with table in Falk's The Chemistry of Enzyme Actions, New York, 1924 edition, p. 97.

These values have been obtained with the enzymes in a relatively crude state and it seems probable that totally different results might be obtained with highly purified preparations. In the case of at least one enzyme has this been shown to be true. Lipase extracted from the gastric mucosa was found by Willstätter and his pupils [37] to exhibit optimum activity at *p*H 5.5 to 6.3 and a minimum (the point at which the enzyme was completely inhibited) at *p*H 8.6. However, after repeated purification with kaolin, the optimum *p*H was found to be 7.1–7.9 (identical with pancreatic lipase) and the minimum *p*H, 4.7. Presumably the enzyme as secreted in the stomach is associated with a substance which inhibits its action in an alkaline medium.

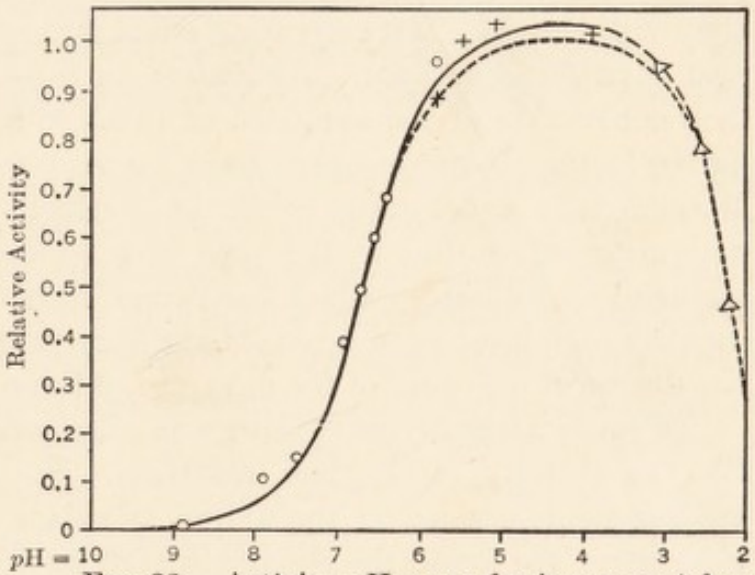


FIG. 22.—Activity-*p*H curve for invertase (after Michaelis and Davidsohn).

[37] Willstätter, Waldschmidt-Leitz and Memmen, Z. physiol. Chem., **125**, 93 (1922–23); Willstätter, Haurowitz and Memmen, *ibid.*, **140**, 203 (1924).

The dependence of enzymes on definite concentrations of hydrogen or hydroxyl ions has been ascribed by Kuhn [38] to the influence of the latter on the decomposition velocity of the enzyme-substrate combinations. Abderhalden's view [39] of the mechanism of enzyme action is likewise suggestive of a marked hydrolytic effect on certain enzyme-substrate complexes which may be exerted by relatively low concentrations of acids and bases. Formerly this dependence was associated with the amphoteric nature of enzymes, which, as the work of several investigators has shown, behave as weak electrolytes. Reference has already been made to a possible direct effect on the substrate, as in the case of peptic digestion of protein, which occurs best at the hydrogen-ion concentration at which there is maximum dissociation of the protein. These views suggest the possibility that the effect of hydrogen or hydroxyl ions may be a multiple one.

Light and Other Radiations.—Most enzymes are not very sensitive to visible light rays. Maltase and rennin, however, are partly inactivated when exposed to light. The same is true of certain other enzymes. Catalase is very sensitive to light when oxygen is present. On the other hand, the reduction of methylene blue by xanthine oxydase is markedly accelerated by light, provided that only traces of oxygen are present.[40] Ultraviolet rays exert a more pronounced inhibiting effect than visible light. An activating effect due to ultraviolet radiations has also been demonstrated. Activation in these cases is presumably due to a conversion of the zymogen or inactive form of the enzyme to the active form. Roentgen rays have no very marked effect on enzymes. Radium rays and radium emanations usually exhibit a stimulating or activating effect, but in the case of a number of enzymes (invertase, emulsin) an inhibitory effect has been observed. The conflicting statements which have been reported are very probably due to variations in the degree of exposure of the enzymes to these radiations.[41]

Effect of Electrolytes.—Inorganic salts may either increase or diminish the velocity of enzymic reactions. Falk [42] studied the effect of various neutral salts on the action of castor bean lipase toward ethyl butyrate. He found the change in activity to be a continuous function of the concentration of the salt added. Monovalent salts, the chlorides and nitrates of barium and calcium (except for very dilute solutions), magnesium chloride and nitrate, and dilute solutions of sodium sulfate

[38] Naturwissenschaft, **11**, 713 (1923).

[39] Proc. XIII International Physiol. Congress, Am. J. Physiol., **90**, 258 (1929).

[40] Bernheim, F., and Dixon, M., Biochem. J., **22**, 113 (1928).

[41] For recent work on this problem the student is referred to the studies of R. G. Hussey, J. Gen. Physiol., **9**, 211, 217, 309 (1925–1926).

[42] J. Am. Chem. Soc., **35**, 601 (1913).

were found to exert a depressing effect. Dilute solutions of $BaCl_2$, $CaCl_2$, $MgSO_4$, $MnCl_2$, and $MnSO_4$ produced increased activity. Lithium salts had a more depressing effect than the salts of sodium or potassium. In the case of the sodium and potassium halides, fluorides had the maximum inhibiting effect, iodides next, bromides next, and chlorides last. Bromides have a very marked effect in accelerating amylolytic activity.

Calcium and magnesium salts are said to have both an activating and an accelerating effect on trypsin. Amylase requires neutral salts, particularly chlorides or phosphates. The latter also seem to play an essential part in enzymic reactions involving the disintegration of glucose. This is believed to be due to the action of the enzyme, not on glucose, but on glucose phosphate.

The term *co-enzyme* is applied to substances, including both electrolytes and non-electrolytes, that are specifically required by a particular enzyme as a condition of its activity. Phosphates are required in the case of ptyalin, and the bile salts in the action of lipase. The co-enzymes are to be distinguished from the so-called *activators* which are supposed to convert the inactive form of certain enzymes to their active form. The examples most frequently cited are the conversion of pepsinogen to pepsin by hydrochloric acid and the action of enterokinase or calcium salts in the transformation of trypsinogen to trypsin. The specificity and action of these activators will be considered in other connections.

Much of the information at present available on the influence of electrolytes in enzymic reactions will require careful revision in view of the failure of the earlier investigators to control adequately the various factors affecting enzyme action, notably the hydrogen-ion concentration.

Inhibiting and Inactivating Agents, or Poisons.—Enzymes are very sensitive to a large variety of organic and inorganic substances by which they are inactivated. While in most cases the degree of inhibition depends on the concentration of the inactivating substances, many of these are effective even in very great dilution. The salts of the heavy metals, mercury, silver, lead, arsenic, and copper, are very injurious to enzymes. Mercury and silver salts are less injurious to invertase in the presence of sucrose than in its absence. Urease is especially sensitive to mercury and its salts.

Fluorides have a pronounced inhibiting action on animal lipase and this together with certain other compounds, frequently employed as antiseptics, are injurious to enzymes. Chloroform injures maltase, amylase, ptylain, rennin, and urease. Toluene, on the other hand, is practically harmless. Glycerol inhibits rennin; thymol is particularly

destructive to the oxidases. Hydrocyanic acid has a strong inhibiting effect on catalase, as well as on oxidases (Warburg[43]), has no effect on rennin or pepsin and activates papain.

A certain amount of specificity is exhibited with regard to the inhibition of enzymes by poisons. Rona and his co-workers[44] have shown that pancreatic lipase is inhibited by quinine but not by atoxyl, whereas liver lipase is sensitive to atoxyl. Kidney lipase, on the contrary, is not affected by quinine but is injured by atoxyl.

Enzyme inhibition was formerly believed to be due to adsorption phenomena. While these are probably of importance, there is some reason for believing that the chief cause is the chemical combination of the inhibiting agent with the enzyme. Euler and Myrbäck[45] have found that the inhibiting action of certain amines corresponds to their affinity for formaldehyde, paraphenylene-diamine being very injurious to enzymes. This points to a chemical combination between the enzyme and the inactivating agent, with the result that the enzyme is no longer available to act on its substrate.

It may also be mentioned that the behavior of the so-called anti-enzymes is perhaps similar. The failure of enzymes to digest the tissues in the living organism with which they are in contact—as, for example, in the case of pepsin and the gastric mucosa, trypsin and the intestinal mucosa—has been attributed to the presence in these tissues of *anti-enzymes*, substances that inhibit enzymes, the nature of which is unknown. Hussey and Northrop[46] have show that serum albumin exerts an anti-tryptic effect which they attribute to a combination of the enzyme with the serum albumin, the effect of this combination being to prevent the trypsin from combining with the substrate.

Effect of Products of Digestion, Enzymic Syntheses.—The accumulation of the products of an enzymic reaction is accompanied by a reduction of its velocity. This may be due, in part, to the direct effect of the combination of the enzyme with the products of digestion. It has been shown[47] that invertase enters into combination with either fructose and glucose. Amylase is said to combine with maltose[48] and pepsin with peptone.[49]

[43] Ber., **58**, 1001 (1925).

[44] Biochem. Z., **134**, 108, 118 (1922); **141**, 222 (1923).

[45] Z. physiol. Chem., **121**, 177 (1922).

[46] J. Gen. Physiol., **5**, 335 (1922–23).

[47] L. Michaelis and M. L. Menten, Biochem. Zeit., **49**, 333 (1913); R. Kuhn, Z. physiol. Chem, **79**, 57 (1923); J. M. Nelson and R. S. Anderson, J. Biol. Chem., **69**, 443 (1926).

[48] G. McGuire and K. G. Falk, J. Gen. Physiol., **2**, 224 (1920).

[49] J. H. Northrop, J. Gen. Physiol., **2**, 471 (1920).

Retardation in the rate of reaction is also brought about by the approach or attainment of equilibrium, since the tendency to reversion is determined by the concentration of the products of a reaction. Removal of the products of the reaction produces a continuous shift in the equilibrium, with the result that the reaction progresses more and more toward completion.

In this connection the synthetic action of the hydrolyzing enzymes may be considered briefly. Croft-Hill,[50] in 1898, treated a very concentrated solution of glucose with yeast maltase and obtained a disaccharide, first thought to be maltose, but later shown to be isomaltose.

The equilibrium position of a reversible hydrolytic reaction is determined by the ratio $\frac{\text{velocity of hydrolysis}}{\text{velocity of synthesis}}$. Attainment of equilibrium in the case of a 0.5N solution of sucrose, when subjected to the action of invertase, requires about six days; at the end of this time 99 per cent of the sucrose has been inverted. On this basis, it has been calculated, it would require ten months to attain equilibrium by the synthetic action of invertase on glucose and fructose.

Taylor [51] accomplished the synthesis of salmine by the action of trypsin prepared from the liver of a mollusc (*Schizotherus nutalli*) on the products of digestion originally obtained from salmine. In this experiment, the yield was about 0.5 per cent of the original protein.

As early as 1896, the synthesis of protein from the products of peptic proteolysis was reported by Danilewski,[52] but his proof did not seem to be complete at the time. Since then, however, his work has been confirmed by Robertson and others [53] and in a remarkable series of investigations by Wasteneys and Borsook,[54] whose work moreover has other elements of interest and importance. Wasteneys and Borsook found that peptic synthesis of protein may be accelerated if carried out in emulsions formed by certain substances, such as benzaldehyde, benzoic acid, benzene, toluene and chloroform. Emulsions formed with oleic acid or olive oil had no accelerating effect. It appears that those emulsions which accelerate the synthesis are capable of producing some protein from suitable digests even in the absence of pepsin.

The synthetic proteins were found to vary depending on the method

[50] J. Chem. Soc., **73**, 634 (1898).

[51] J. Biol. Chem., **3**, 87 (1907).

[52] Danilewski, quoted from Wasteneys and Borsook, Colloid Symposium Monograph, **6**, 155 (1928).

[53] J. Biol. Chem., **3**, 95 (1907); **5**, 493 (1908–1909); also Henriques and Gjaldbäk, Z. physiol. Chem., **71**, 485 (1911); **81**, 439 (1912).

[54] J. Biol. Chem., **62**, 15, 633, 675 (1924–25); **63**, 563 (1925); Colloid Symposium Monograph, New York, **6**, 155 (1928).

employed. Thus, protein synthesized by benzene alone had the highest base-combining capacity, and that synthesized by benzaldehyde alone had a somewhat lower base-combining capacity. On the other hand, the proteins formed by pepsin alone, or by pepsin and benzene combined with much less base. The proteins also differed in solubility, in the proportion of free amino and free carboxyl groups and apparently also in their isoelectric points, for they precipitated at different hydrogen-ion concentrations.

Wasteneys and Borsook attribute the effect of the emulsions essentially to adsorption or surface phenomena. They also advance the interesting speculation with regard to the proteins synthesized that the " variation in physical and chemical properties with the emulsifying agent employed suggests a possible mechanism by which the many proteins of the organism may be synthesized, as they are, *in vivo*, from a common substrate."

The enzymic synthesis of an ester by an esterase was first reported by Kastle and Loevenhart,[55] and of fat (triolein) from glycerol and fatty acids by Hamsick.[56] Similarly, claims have been made for the synthesis of starch- and glycogen-like substances by the action of amylase on simple sugars. While it is true that the amount of synthesis in most of these cases has been very small, it is nevertheless a fact of great physiological importance that the enzymes capable of hydrolyzing certain substances are likewise capable of synthesizing them. In the plant and animal organism, products thus formed by the synthetic action of enzymes are for the most part insoluble and are removed from the sphere of the reaction by being laid down and stored in the tissues. Removal of the synthetic products results in a continuous shift of the equilibrium, and hence in continued synthesis.

However, in most cases, an unequivocal relationship between the enzyme content of tissues and their assumed synthetic function has not been established. The logical supposition may seem to be that tissues rich in fat would contain more lipase than tissues poor in fat, but that this is not the case has been stated by Bradley.[57] Not only is there no broad correlation between the fat and lipase content of tissues, but some fat-producing organs are relatively poorer in lipase than many organs which normally never contain or produce more than a small amount of fat. Likewise, the enzymic synthesis of lactose by the mammary gland has been questioned by Bradley,[58] since he was unable to find any lac-

[55] Am. Chem. J., **24**, 491 (1900).
[56] Z. physiol. Chem., **59**, 1 (1909); **65**, 232 (1910).
[57] J. Biol. Chem., **13**, 407 (1913).
[58] *Ibid.*, **13**, 431 (1913).

tase in this tissue. On the other hand, the starch-storing tissues of plants are found to contain amylase and there usually seems to be a correlation between the content of starch and enzyme, which has led Bradley and Kellersberger [59] to conclude that in the plant the synthesis of polysaccharides from the sugars of the sap is enzymic in character.

Autolysis.—In 1889, Salkowski [60] described the non-bacterial, chemical liquefaction or " self-digestion " of tissues which occurs *post mortem*, and pointed out its similarity to digestion in the alimentary tract. Since then this process, termed " autolysis " by Hofmeister,[61] has been studied by numerous investigators and certain broad generalizations have been made.[62]

Glandular tissues, such as kidney, pancreas, spleen, liver, and thyroid autolyze more rapidly than other tissues, such as muscle. In the process, the tissue becomes acid through the formation of lactic acid and the cleavage of fatty acids from fats. Inorganic colloids (colloidal Ag, Pt, Cu, Au, Fe, MnO_2, etc.), iodides and bromides and certain other inorganic salts, particularly those of arsenic, accelerate autolysis, whereas alkaline substances, such as carbonates, bicarbonates, or the oxides of the alkaline earths, inhibit this process.

The enzymes engaged in autolysis are not limited in their action to the particular tissue undergoing dissolution. When to an autolyzing mixture are added gelatin, casein, or various protein cleavage products, these are digested. On the other hand, egg albumin is said to be unaffected and is even reported to exert an inhibiting effect.

Apparently autolysis may occur within a wide range of hydrogen-ion concentration and it is therefore assumed that there is in the tissues at least one enzyme capable of digesting protein in an acid medium and another which is active in an alkaline medium. Amino acids are found among the end products of tissue autolysis.

Atrophy, which may be defined as a loss of tissue, is essentially similar to autolysis, being likewise associated with enzymic activity. It occurs physiologically, as in the mammary gland after lactation, or pathologically, as in acute yellow atrophy of the liver. The process may be very rapid, as in the disease just mentioned, or it may be relatively slow, as in old age, starvation, in an immobilized limb, etc.

Owing to its exceptional blood supply and partly to its low protein content, the brain is normally relatively resistant to autolysis, but any

[59] *Ibid.*, **13**, 425 (1913).

[60] Z. physiol. Chem., **13**, 506 (1889).

[61] Die Chemische Organisation der Zelle, Braunschweig, 1901.

[62] For reviews of the subject, consult Bradley, H. C., Physiol. Rev., **2**, 415 (1922); Wells, H. G., Chemical Pathology, Philadelphia, 5th edition, 1925; Levene, P. A., Harvey Lectures (1905–06), p. 73.

process which produces asphyxia, such as pressure, trauma, thrombosis, carbon-monoxide poisoning, etc., may be accompanied by autolysis, or " softening."

DIGESTION

The carbohydrates, fats, and proteins, together with water, oxygen, certain inorganic salts, and a number of substances known as accessory food factors or vitamins and not yet completely defined, constitute the essential ingredients of the diet of man and animals. Of these, as has been pointed out, the fats, proteins, and carbohydrates are ingested in forms not readily absorbed into the circulation and for this reason are not available to the organism for purposes of nutrition until they have become converted into small particles which can diffuse through the intestinal epithelium. The carbohydrates are converted into monosaccharides, the fats into glycerol and fatty acids, and the proteins into amino acids. To some extent, hydrolytic changes occur during the process of cooking, but the major part of the disintegration of the foodstuffs takes place in various portions of the alimentary tract. The chemical transformations by which foods are converted into small diffusible particles constitute the process of digestion. The enzymes which take part in this process are secreted in various glands and pass into certain compartments of the digestive tract, the mouth, stomach, and small intestine. In the following paragraphs the fate of the foodstuffs in digestion will be traced.

Salivary Digestion.—The saliva is a mixed secretion produced partly by three pairs of glands, the submaxillary, sublingual, and parotid, and partly by the mucous membrane and the buccal glands of the mouth, throat, and esophagus. The saliva is a viscous, frothy, slightly opalescent fluid, containing many constituents, including the glycoprotein mucin. The salivary glands possess two kinds of cells, the serous or albuminous, which secrete a fluid containing protein and enzyme, and the mucous cells which secrete a ropy fluid containing mucin. A mixed secretion is obtained from the submaxillary gland which has both serous and mucous cells. The sublingual glands are chiefly mucous; and the parotid, chiefly serous.

Salivary secretion is under the control of the nervous system, the glands being supplied by two sets of nerves, the cranial, or cerebral, and the sympathetic.

Salivary flow is normally caused by a variety of stimuli. Psychic secretion is brought about by a reflex stimulation. The excitation,

which may be caused by the sight or smell of food or by the hearing of sounds associated with the giving of food, travels along afferent paths, the stimuli being transmitted along efferent pathways to the salivary glands. Mechanically, salivary flow may be induced by the presence in the mouth of solid particles, such as food, sand, or paraffin. Dry food calls forth a greater amount of saliva than moist food. Many chemical agents, such as acids, salts, and flavored substances, stimulate the secretion of saliva. During vomiting, the abdominal fibers of the vagus nerve are stimulated and cause an increased flow as a result of reflex stimulation of the salivary centers.

The total amount of saliva secreted in twenty-four hours by a normal man has been calculated to be about 1500 cc. Among the many factors that influence the daily volume of saliva are (*a*) the amount of water consumed, (*b*) the amount of food intake and the degree of its mastication, and (*c*) the character of the food. Chewing and smoking usually increase the flow of saliva.

The quantitative composition of mixed saliva varies considerably in different individuals, and in the same individual at different times during the day. A number of analytical results which have been obtained for mixed human saliva are tabulated below. The results are in parts per 1000.

TABLE XXVIII *

	Berzelius	Jacubowitsch	Frerichs	Herter	Hammerbacher
Water	992.9	995.16	994.1	994.7	994.2
Solids	7.1	4.84	5.9	5.3	5.8
Mucus and epithelium	1.4	1.62	2.13	.....	2.2
Soluble organic substances (ptyalin of early investigators)	3.8	1.34	1.42	3.27	1.4
Sulfocyanides	.....	0.06	0.10	.....	0.04
Salts	1.9	1.82	2.19	1.30	2.2

Hammerbacher found in 1000 parts of the ash from human saliva: potash, 457.2; soda, 95.9; iron oxide, 50.11; magnesia, 1.55; sulfuric anhydride (SO_3), 63.8; phosphoric anhydride (P_2O_5), 188.48; and chlorine 183.52.

* Taken from data in a table in Hammarsten-Mandel's, "A Text Book of Physiological Chemistry," John Wiley & Sons, New York, 1915 edition, p. 458.

Reaction of the Saliva.—Many of the early investigators ascribed to saliva a slightly alkaline reaction equivalent to about 0.08 per cent

sodium carbonate. Starr [63] analyzed 610 specimens of human saliva, obtained from 228 healthy, normal subjects, and found the reaction to vary from *p*H 5.75 to 7.05. In 86 per cent of the analyses, the variations were within a narrower range, namely, *p*H 6.35–6.85. That the reaction of the saliva is usually slightly acid has also been reported by Henderson and Millet,[64] who observed, moreover, that the salivary *p*H falls just before meals and remains low just after meals. Between meals, the reaction of the saliva approaches neutrality. The belief that an acid reaction of the saliva is harmful and that it is desirable to change it from acid to alkaline is probably without scientific basis. In fact, the saliva is a well-buffered mixture and it is practically impossible to change its reaction for periods longer than a few minutes by the addition of even moderate amounts of either acid or base.[65]

Mechanically, owing to the water and mucin, the saliva aids in the mastication of foods and serves as a solvent for some of the constituents. Chemically, the saliva takes part in the digestion of carbohydrates. The enzyme ptyalin in the saliva is capable of hydrolyzing starch into a variety of dextrins and ultimately into maltose which may be further digested to glucose by maltase, a second enzyme found in the saliva in small amounts. However, the digestion of starch is far from being completed in the mouth. There are no enzymes in the saliva which cause the digestion of either fat or protein.

The conversion of starch into simpler products by the saliva may be demonstrated *in vitro*. This process can be followed by means of the well-known starch-iodine test. As the starch is hydrolyzed, on testing with iodine, the original blue color exhibited by starch gives way to a reddish color due to the so-called erythrodextrins. As the process continues, the digest yields paler tints, and finally it fails to yield any color whatever with iodine. Concurrently, the content of reducing sugars increases. The methods employed in studying starch digestion may be found in laboratory manuals of physiological chemistry.

Salivary digestion, therefore, consists in the transformation, with the aid of ptyalin, of a portion of the starch of the diet into simpler polysaccharides and maltose. As the food remains in the mouth for a relatively short period, very little digestion occurs even in the case of the carbohydrates. Carbohydrate digestion by the salivary enzymes may

[63] J. Biol. Chem., **54**, 55 (1922).

[64] J. Biol. Chem., **75**, 559 (1927).

[65] Bloomfield, A. L., and Huck, J. G., Bull. Johns Hopkins Hosp., **31**, 118 (1920); Carlson, V. R., and McKinstry, Dental Cosmos, **66**, 840 (1927); Clark, G. W., and Carter, K. L., J. Biol. Chem., **73**, 391 (1927); Editorial, J. Am. Med. Assoc., **92**, 899 (1929).

continue for some time in the stomach or until the food comes in contact with the hydrochloric acid of the gastric juice which inactivates the ptyalin.

Gastric Digestion.—Gastric digestion is concerned primarily with the partial disintegration of the protein of the diet. This is accomplished by the enzyme pepsin in the presence of hydrochloric acid.

The food, after being swallowed, remains in the stomach for a variable period, usually between one and five hours. In this way the stomach functions as a food reservoir.

Among the more important of the earlier contributions to our knowledge of gastric digestion are the observations of Réaumur (1752) and of Spallanzani (1783). These investigators studied gastric secretion in birds, fishes, and mammals, and demonstrated that gastric juice is acid, that it prevents putrefaction, that the juice has digestive properties *in vitro*, and that the process of digestion is essentially a chemical one.

In 1825, William Beaumont,[66] a young American surgeon, began a classical investigation of digestion, which lasted until 1833, on a patient with clinical gastrostomy. The patient's name was Alexis St. Martin, and he was first observed by Beaumont in 1822. Little was known at that time concerning the mechanism of gastric secretion. Beaumont was a very careful worker and painstakingly studied the factors that influence the flow of gastric juice. He found that the presence of food in the stomach stimulates gastric secretion, and that irritating condiments, alcohol, anger, fear, and fever diminish it. He failed to observe, however, that gastric secretion may occur in the absence of food.

Beaumont recorded observations showing that gastric flow may be induced by mechanical stimulation of the gastric mucosa, a view which was later regarded as erroneous largely as a result of the work of Pavlov. More recently, however, Ivy [67] and his co-workers were able to demonstrate mechanical stimulation of gastric secretion. In their experiments this was brought about by distending the stomach with a rubber balloon. These results corroborate the early observations of William Beaumont.

Some time before Beaumont began his experiments, Tiedemann and Gmelin and later Prout reported that the acid in the stomach was hydrochloric acid and, this was apparently known to Beaumont. One of the more important conclusions deduced by this brilliant investigator was that gastric juice contains a substance, other than hydrochloric acid, which has a solvent action on food material. He thus

[66] Experiments and Observations on the Gastric Juice, Plattsburg, 1833.

[67] Ivy, Lim, McCarthy, and Farrell, Am. J. Physiol., **72**, 203, 232 (1925).

anticipated by about six years the actual discovery of pepsin by Wassman.

Following the work of Beaumont, experimental methods were introduced for the study of gastric secretion. The removal of juice by means of a stomach tube is a method still employed clinically. Juice may also be collected readily from an artificial fistula. A gastric fistula is made by cutting an opening into the stomach and sewing the cut portions to the abdominal wall. The collection of pure gastric juice, uncontaminated with food, was first made possible by Heidenhain. His method consisted in cutting through the walls of the stomach, sewing the flaps into a pouch which was then sewed to the abdominal wound. By this operation, however, most of the extrinsic and intrinsic nerve connections were severed. Hence, there remained the possibility that the gastric secretion formed in the pouch was not normal. This difficulty was overcome by Pavlov, who devised an improved technique for making an isolated gastric pouch. The following is Pavlov's description of the method:[68]

The first incision, which begins in the fundus of the stomach, 2 cm. from its junction with the pyloric end, is carried in the longitudinal direction for 10 to 12 cm., and divides both the anterior and posterior walls. A triangular flap is thus formed, the apex of which lies in the long axis of the stomach. A second incision is made exactly at the base of this flap, but only through the mucous membrane, the muscular and peritoneal coats remaining intact. The margins of the mucous membrane all around these incisions are separated for a little way from the submucous tissue: on the side of the stomach for a width of 1 to $1\frac{1}{2}$ cm.; on the side of the flap for 2 to $2\frac{1}{2}$ cm. The raised edges of mucous membrane belonging to the large stomach are applied to each other for half their width and sewn together. Out of the piece which belongs to the flap a cupola is formed. Both the stomach and the margins of the flap are then closed by sutures along the edges of the first incision. A septum is thus made between their respective cavities, consisting of two layers of mucous membrane; one, that of the cupola, being intact, the other stitched along the middle.

By means of the Pavlov pouch, it is possible to obtain gastric juice which is similar in character to that secreted in the main stomach and which is not contaminated with saliva or food material. In his numerous experiments, Pavlov employed dogs with gastric pouches and dogs with gastric and esophageal fistulas. If the esophagus is divided and the two ends sutured to the skin, an opening is formed. Food swallowed by dogs with esophageal fistulas does not reach the stomach but falls through the upper end of the fistula; hence, this is known as sham feeding.

[68] Pavlov, The Work of the Digestive Glands, translated by Thompson, London, 1902 edition, p. 11.

The following diagrams illustrate the operation:

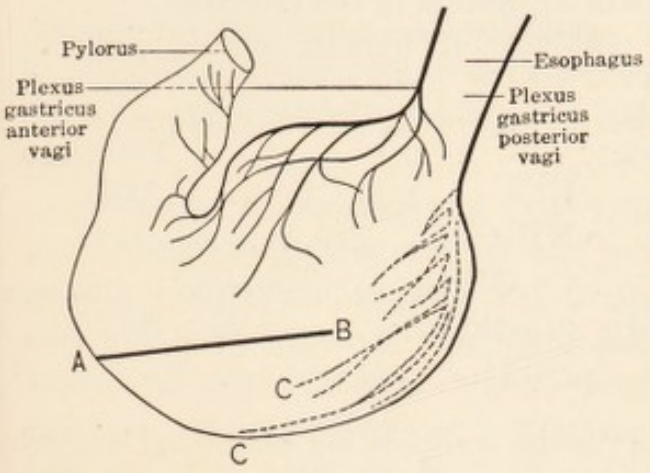


FIG. 23*a*.

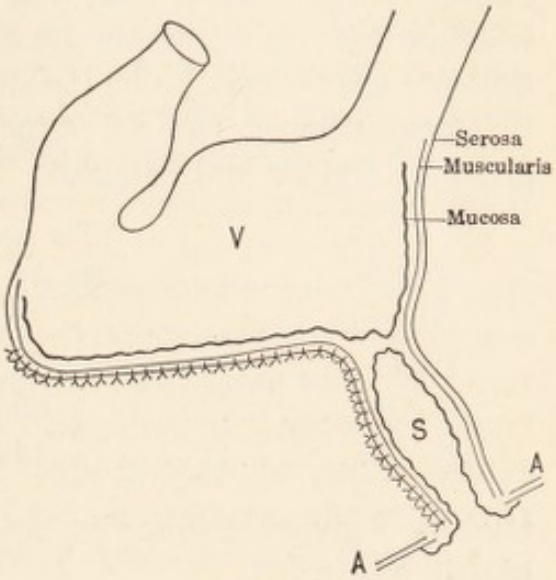


FIG. 23*b*.

FIG. 23*a*.—*A*–*B*, line of incision; *C*, flap for forming stomach pouch of Pavlov.
FIG. 23*b*.—*V*, cavity of the stomach; *S*, Pavlov's pouch. *S* is separated from *V* by a double layer of mucous membrane. *A*, abdominal wall. (After Pavlov.)

The essential results of Pavlov's work on gastric digestion may be summarized as follows:

1. The amount of juice secreted is normally proportional to the amount of food and depends to a considerable extent on the character of the food to be digested.

2. In sham feeding there is no chemical or mechanical stimulation. The stimulation to gastric flow is psychic. Psychic secretion of gastric juice may be caused by the sight or smell of food or by established conditioned reflexes. If at the time of feeding, a bell is rung, a conditioned reflex is established in the course of time, so that eventually the mere ringing of the bell, without the presentation of food, causes gastric secretion.

3. Mechanical stimulation, according to Pavlov, produces no secretion. This is not in accord with Beaumont or with the recent work of Ivy.

4. Many substances, especially those having a flavor, produce secretion.

The Gastric Mucosa.—The mucous membrane of the stomach is lined with columnar epithelial cells. At the pyloric end these are finely granulated and are known as chief cells. In the fundic portion of the stomach, there are two kinds found, the chief or peptic and the parietal or border cells. Though the hydrochloric acid is formed largely in

the fundus, and the evidence is in favor of the view that it is produced by the border cells, yet there is still some uncertainty as to whether it is formed within the cell or outside. The chief (central, peptic) cells are believed to be the site of formation of the enzymes.

Composition.—The following analysis of human gastric juice is given by Bidder and Schmidt:[69]

	Per cent		Per cent
Water	99.44	KCl	.055
Organic	.32	NH_4Cl	.....
HCl	.20	$Ca_3(PO_4)_2$	.0125
$CaCl_2$	.0061	$Mg_3(PO_4)_2$	
NaCl	.146	$FePO_4$	

Carlson[70] gives the following data for the composition of normal human gastric juice:

Acidity { Free HCl = 0.40–0.50 per cent.
Total acidity = 0.45–.60 per cent.

Solids { Organic = 0.42–0.46 per cent.
Inorganic * = 0.13–0.14 per cent.

Specific gravity = 1.006–1.009.

Osmotic concentration = − 0.55° to − 0.52° C.

Total nitrogen = 0.051–0.075 per cent.

Amino-acid nitrogen = 3–10 mgm. per 100 cc.

Ammonia = 2–8 mgm. per 100 cc.

Chlorides = 0.50 − 0.58 per cent.

* This refers to inorganic constituents other than hydrochloric acid.

The amount of gastric juice secreted daily by the normal individual, on an ordinary diet, probably varies between 2 and 3 liters. Many factors influence the quantity and composition of the secreted juice.

Effect of Diet.—There seems to be a qualitative and quantitative adaptation of gastric secretion to food. In the first place, the quantity of juice may be affected by psychological factors. Food that is not relished will not call forth as copious a secretion of "*appetite juice*" as will food that by experience or association is likely to prove appetizing. On a bread diet, Pavlov found that the stomach secreted more than double the quantity of pepsin that was secreted on a diet of an

[69] Cited by A. P. Mathews, Physiological Chemistry, Fourth Edition, p. 357.
[70] Physiol. Reviews, **3**, 1 (1923).

equal amount of meat or milk protein. On the other hand, the acidity on a bread diet was found to be lower than on a meat diet. These differences, according to Carlson, may be only apparent and not due to the specific effects of the various foodstuffs as secretagogues, that is, substances that stimulate secretion. Differences in the motility of the stomach and in the rate of secretion of the gastric juice may be responsible for the qualitative difference between " bread juice " and " meat juice."

Contrary to the views of Beaumont and Pavlov, it has been found more recently that in the fasting stomach there occurs a continuous secretion of gastric juice, having an acidity which varies with the secretion rate and which is somewhat less than that of " appetite juice." Luckhardt and Johnston [71] have demonstrated that the gastric glands respond more promptly to a test meal that is ingested when the subject is under hypnosis than to one taken when he is in the waking state.

Products of protein hydrolysis stimulate gastric secretion. This has been accepted as a fact for a long time, but only recently has it been demonstrated satisfactorily by Ivy and Javois.[72] These workers have studied the effect of protein-split products, including amino acids, on gastric secretion. β-alanine is a powerful excitant whereas α-alanine acts but feebly. Histamine, ethylamine, and methylamine hydrochlorides and other amines are very active in stimulating gastric secretion.

Gastrin.—Many workers have shown that the injection of extracts prepared from the pyloric mucous membrane causes increased secretion of gastric juice. This effect is attributed to a specific secretagogue, hormone-like in character, called gastric secretin, or *gastrin*. Other workers, however, have found that a gastric secretagogue can be secured not only from the pyloric mucosa but also from the mucosa of the entire alimentary tract and from the liver, thyroid, plant tissues, etc. Luckhardt has recently found that gastrin is not specific in its action since it also stimulates pancreatic secretion. Carlson states that it is probable that the gastrins are artefacts formed in the decomposition of the foods or in the extraction of the mucosa and do not represent a physiological mechanism. From the work of Koch, Luckhardt and Keeton,[73] it appears that gastrin may be an imidazole derivative related to histamine and pilocarpine.

[71] Am. J. Physiol., **70**, 174 (1924); also Johnston and Washeim, *ibid.*, **70**, 247 (1924).

[72] Am. J. Physiol., **68**, 132 (1924).

[73] Am. J. Physiol., **52**, 508 (1920).

In their contribution to the subject, Lim, Ivy, and McCarthy [74] have analyzed the factors concerned in the excitation of gastric secretion as follows: (1) The cephalic phase, heretofore referred to as the "psychic secretion" demonstrated by Pavlov, which is excited chiefly by the taste, smell, and mastication of palatable food, and by sight, thought or hypnotic suggestion of palatable food. The term " psychic secretion " is rejected because it is not necessarily psychic, having been shown to occur in the absence of the cerebral cortex. (2) The gastric phase, in which mechanical and chemical stimuli are effective. (3) The intestinal phase, in which the stimuli are certain chemical substances acting in the intestine. Ivy, Lim, and McCarthy [75] have demonstrated that the intestinal phase of gastric secretion is due to the action of the products (e.g., peptone, amino acids, and amines) of digested complex food substances and apparently not to the food in its raw state (meat, carbohydrates, and neutral fat).

The Origin of Hydrochloric Acid.—No adequate explanation of the formation of hydrochloric acid by the gastric mucosa has as yet been offered. In the concentration in which it is formed, the acid might be expected to be injurious to the cell protoplasm. This fact, together with more direct evidence, has contributed to the view that the acid is not actually formed within the border cells but rather in the fovea of the glands, presumably from the chlorides of weak bases secreted by the cells.

It has been suggested that hydrochloric acid is set free from ammonium chloride by hydrolysis:

$$NH_4Cl + H_2O = NH_4OH + HCl$$

According to this view, the ammonium hydroxide is absorbed, leaving the hydrochloric acid behind. The mold *Penicillium glaucum* is capable of effecting this reaction. In further support of this view, evidence has been offered to show that the gastric mucosa contains a somewhat greater amount of ammonia than other tissues. The evidence in favor of this hypothesis is altogether too indirect and insufficient, and while a small amount of hydrochloric acid might conceivably be formed in this way, the theory, on the whole, does not appear plausible.

When a solution of phosphates is enclosed within a parchment membrane, there results a diffusion of acid with a consequent increase in the alkalinity of the fluid within the dialyzer due to the more rapid diffusion velocity of acids than of alkaline salts. A similar mechanism has been suggested for the formation of hydrochloric acid by the gastric

[74] Quart. J. Exp. Physiol., **15**, 13 (1925).

[75] *Ibid.*, **15**, 55 (1925).

mucosa from the alkaline phosphates of the blood. An objection to this view has been raised by Robertson [76] who states that this theory proves too much, for, by parity of reasoning, all the secretions of the tissues should be acid in reaction, whereas, actually, the majority of the secretions are alkaline.

The formation of hydrochloric acid may possibly be accounted for by the following equations representing reactions which may occur, perhaps, between three of the blood constituents:

$$H^+HCO_3^- + Na^+Cl^- = H^+Cl^- + Na^+HCO_3^-,$$

$$Na^+H_2PO_4^- + Na^+Cl^- = H^+Cl^- + Na_2^{++}HPO_4^{--}$$

If this view is correct, the secretion of large amounts of hydrochloric acid in the stomach should be followed by an accumulation in the blood of basic radicals. This actually occurs, frequently to such an extent that despite the loss of alkali in the pancreatic and intestinal secretions, sufficient base remains to give the urine an alkaline reaction. The change in the reaction of the urine, following food intake, from the normal acid reaction to one that is alkaline, is referred to as the *alkaline tide.*

A very interesting experiment and one that may have some bearing on the problem of acid formation is that of T. B. Osborne.[77] He dissolved edestin in sodium chloride solution and later precipitated it with a stream of carbon dioxide. The precipitate contained edestin in combination with hydrochloric acid, whereas the solution contained $NaHCO_3$. Obviously, in the presence of the protein edestin the reaction represented by the equation $NaCl + HHCO_3 = NaHCO_3 + HCl$ was facilitated.

Behavior similar to that exhibited by edestin can be demonstrated with red blood corpuscles. If these are washed with isotonic solution of sodium chloride until the washings are neutral, then suspended in neutral sodium chloride solution and treated with a stream of carbon dioxide, it is found that the solution becomes alkaline and the corpuscles richer in chlorine. From these observations, Robertson infers that the secretion of an acid juice depends upon the existence in the secreting cells of a protein that is capable of decomposing sodium chloride in the presence of carbon dioxide, the appearance of the free hydrochloric acid in the secretion being attributable to the colloidal, indiffusible character of the protein base. The validity of this suggestion remains to be determined by further study.

[76] Robertson, Principles of Biochemistry (1924), p. 366.

[77] Am. J. Physiol., **5**, 180 (1901).

If it is assumed that in the acid-forming cells, the hydrochloric acid is combined with protein or some other cell constituent to which the cell membrane is impermeable, an explanation for the secretion of free hydrochloric acid may be based on Donnan's theory of membrane equilibria. The original state may be represented by the following diagram:

$$\begin{array}{c|c|c} R^+ & \| & H^+ \\ Cl^- & \| & OH^- \end{array}$$

R represents the positively charged protein ion (or other cell constituent). The membrane being permeable to the Cl^-, H^+, and OH^- ions, an interchange of ions will occur, so that at equilibrium the situation will be as follows:

$$\begin{array}{c|c|c} R^+ & \| & H^+ \\ Cl^- & \| & Cl^- \\ OH^- & \| & \end{array}$$

The soundness of this theory has been confirmed experimentally by Donnan [78] who has shown that under such conditions, hydrochloric acid may actually be "secreted" across a membrane. Donnan is of the opinion that "the proper ampholyte can easily give rise by this mechanism alone to a concentration of hydrogen ions in the external liquid as great as that found in the gastric juice."

The formation of acid in animal organisms is not limited to the gastric mucosa. The salivary glands of the mollusc *Dolium galea* produce a secretion containing 4–5 per cent of sulfuric acid. Certain related species of molluscs produce aspartic acid in large concentration.

Hunger Contractions.—The sensation of hunger is due in large part to the contraction of the walls of the stomach. Contractions usually begin about the time of the accustomed meal or soon thereafter. Bulatao and Carlson [79] have suggested recently that in the empty stomach, under ordinary conditions, the increase in gastric tonus and contraction is parallel with the reduction of the tissue glycogen and with the enforced energy metabolism of lipids on the part of the motor tissues of the stomach. Normal gastric hunger contractions may be inhibited in conditions of experimental hyperglycemia produced by intravenous injection of glucose. Similar injection of lactose or sodium chloride does not produce this effect, showing that the hypertonicity of the

[78] J. Chem. Soc., **99**, 1554 (1911); **105**, 1941 (1914); **115**, 1313 (1919); cited by Gortner, Outlines of Biochemistry (1929), p. 285.

[79] Am. J. Physiol., **69**, 107 (1924); A. J. Carlson, The Control of Hunger in Health and Disease, Chicago, 1916.

injected solution is not the cause of the inhibition. Insulin (p. 294) stimulates motility of the stomach as well as of other parts of the gastrointestinal tract (Quigley).[80]

The Acidity of the Gastric Juice.—The concentration of free hydrochloric acid in the stomach is subject to considerable variation, but this is not due to differences in the strength of acid secreted, as this appears to be strikingly uniform in normal individuals, approaching a concentration of 0.5 per cent. A part of this acid is neutralized by the regurgitated alkaline intestinal contents as shown by the work of Boldyreff.[81] During digestion another considerable portion combines with the protein of the food. McCann [82] has recently made the important observation that these are by no means the only factors which regulate the acidity of the gastric contents. He has pointed out that after the acid has stopped combining with the food, there is a gradual reduction in the rate of hydrochloric acid secretion. A portion of this changing volume of acid combines with mucus, secreted by the mucus-forming cells, especially in the fundus. In the resting stomach, the acid secretion may be so slow that all the acid produced enters into combination with the mucus.

Pepsin.—In the stomach, by virtue of the vigorous muscular movements, the food is subjected to a churning process and becomes intimately mixed with the gastric secretion which contains, among other substances, the enzymes pepsin and rennin. The hydrochloric acid of the gastric juice transforms the protein of the diet to acid metaprotein, converts the inactive pepsinogen of the mucosa into the active enzyme, pepsin, and furnishes a favorable hydrogen-ion concentration for its action. The evidence for the existence of pepsinogen is that neutral extracts of gastric mucosa, which have not been previously treated with acid, are more resistant to the action of alkali than pepsin, which is quickly decomposed in an alkaline medium. The existence of the inactive pre-stage of the enzyme is not as generally accepted now as formerly. According to Waldschmidt-Leitz,[83] the differences in the behavior between pepsin and its zymogen, pepsinogen, are to be attributed to C_H effects. The difference in stability to alkali, he believes, might be attributed to different amounts of associated substances in the enzyme solutions compared. Whether the associated substances exert a protective effect, or the opposite, is not clear. The pepsin digests the metaprotein, as well as native protein, to proteoses

[80] Am. J. Physiol., **90**, 89 (1929); **91**, 488 (1929–30).

[81] Ergeb. d. Physiol., **11**, 156 (1911).

[82] Am. J. Physiol., **89**, 483 (1929).

[83] E. Waldschmidt-Leitz, Enzyme Actions and Properties, Trans. by R. P. Walton, John Wiley & Sons, Inc., New York, p. 129.

and peptones. Usually, the food does not remain sufficiently long in the stomach for any further transformation.

Rennin.—Rennin is secreted by the gastric mucosa and is said to be especially abundant in young animals. The essential feature of the process of milk clotting, with which rennin is concerned, is the hydrolysis of the casein molecule. Calcium is essential for rennitic action. If the calcium is removed by treating the milk with an oxalate, clotting does not occur on the addition of rennin. On the addition of calcium to milk so treated, clotting occurs. If milk, to which rennin has been added after the removal of the calcium, is allowed to stand for some time and then boiled to destroy the enzyme, and calcium is finally added, clotting occurs. This shows that the rennin must have acted on the casein in some way in the absence of calcium. Many theories have been advanced for the clotting process of milk, but tentatively, the changes may be represented by the following equations:

$$\text{Casein} + \text{Rennin} = \text{Paracasein (soluble)}.$$

$$\text{Paracasein} + \text{Ca} = \text{Ca-paracasein (insoluble)}.$$

The action of rennin may also be represented by the equation:

$$\text{Ca-casein} + \text{Rennin} = \text{Ca-paracasein}.$$

The curdling of milk may be brought about likewise by the addition of acid. In the precipitation of calcium caseinate by hydrochloric acid, isoelectric casein, which is insoluble, is formed:

$$\text{Ca-casein} + \text{HCl} = \text{Casein} + \text{CaCl}_2$$

The Identity of Pepsin and Rennin.—The identity of rennin and pepsin has been the subject of much discussion in the literature. According to Pavlov, protein digestion and rennitic action are due to the same enzyme. This view is based on the wide distribution and coexistence of rennin and pepsin both in plants and in animals. Contrary to this view is the opinion of Hammarsten and others that pepsin and rennin are distinct enzymes. This is more in accord with many experimental facts. Pepsin preparations without rennitic action and rennin preparations without peptic action have been prepared. However, there are those who deny the validity of this kind of evidence and who hold that proof of the non-identity of rennin and pepsin can only be obtained by the complete separation of the two enzymes.

Gastric Lipase.—The gastric secretion of the human organism contains a lipolytic enzyme. However, as the hydrogen-ion concentration

of the stomach is unfavorable both for the emulsification of fat and for the action of lipase, the amount of fat digestion in the stomach is very slight, being limited to the partial disintegration of the highly emulsified fat such as is found in egg yolk, butter, and milk.

Discharge of Food from the Stomach.—Constriction waves propel the semi-liquefied food, or chyme, toward the pyloric sphincter, which is apparently able to resist the passage of large or solid food fragments, but which opens from time to time to permit the passage of small amounts of chyme. Formerly, the stimulus for opening of the pyloric sphincter was attributed to the accumulation of acid on the stomach side, whereas the stimulus for closure was thought to be the presence of acid on the duodenal side. During the period of relaxation, there probably is not only the ejection of material from the stomach, but the reverse passage of intestinal contents into the stomach. The view that the acid is the essential factor for the alternate opening and closure of the sphincter is no longer generally accepted, for it appears that contraction of the sphincter may be produced not only by acid, but by mechanical irritation, and even by alkali. The stomach, moreover, can also empty itself of an alkaline contents. Carlson and Litt [84] believe that ordinary sensory stimuli may induce contraction of the pylorus.

This view finds some support in the recent work of McCann.[85] In animals in which the pyloric sphincter is resected, there is only partial reduction in the efficiency of food retention in the stomach. This observation, adequately controlled by fluoroscopic examinations and fractional analyses of the gastric contents, has led McCann to conclude that the emptying of the stomach is not controlled by the sphincter alone, but that the whole pyloric antrum is actively engaged in the process. The vigorous tonic and peristaltic contractions of the pylorus observed early in digestion, McCann believes to be due to its irritability. As digestion progresses and the food is reduced to a semi-fluid consistency, this stimulus gradually diminishes, giving way to a progressive relaxation of the pars pylorica, including the sphincter, and results in the more rapid emptying of the stomach. Neither the free hydrochloric acid or the products of digestion seem to be the specific influence for the relaxation.

The type and amount of food determine the rate of evacuation of the stomach. When fed separately, carbohydrate food remains in the stomach for a shorter period than protein food. Fat remains in the stomach for a longer period than proteins. The digestion of protein food is markedly delayed on a high-fat diet. In part, the differences

[84] Arch. Int. Med., **33**, 281 (1924).

[85] Am. J. Physiol., **89**, 497 (1929).

in the rate of emptying of the stomach are due to the variations in the time required for mechanical disintegration and enzyme action.[85a]

Germicidal Properties of Gastric Juice.—The hydrochloric acid of the stomach is said to have antiseptic properties. While this is true to some extent, as evidenced by the fact that gastric juice, when allowed to stand, does not putrefy readily, nevertheless, the antiseptic properties have been overestimated. The presence of yeasts and bacteria in normal gastric juice has frequently been demonstrated.

Anti-enzymes.—In view of the proteolytic action of pepsin, the question may be raised as to the failure of the stomach mucosa to digest itself. One explanation is that the gastric mucosa contains an anti-enzyme which inhibits the action of pepsin. According to another explanation, the blood and lymph bathing the cells of the stomach have an alkaline reaction which is unfavorable to peptic digestion. It is obvious, however, that the latter explanation cannot hold in the case of the intestinal mucosa, which is not attacked by either the intestinal or the pancreatic enzymes despite the favorable reaction. Many regard the mucins to be of much importance in preventing autodigestion of the gastric mucosa.

Gastric Absorption.—Except for the absorption of small amounts of the monosaccharides, inorganic salts, alcohol, alcoholic solutions, and some drugs, the stomach is not an absorptive organ.

Gastric Analysis.—Clinically, the composition of the gastric juice has been regarded for a long time as a very valuable aid in diagnosis. Marked variations from the normal frequently occur, both in the quantity and in the character of this secretion. Gastric hyposecretion occurs in cancer, anemia, pellagra, and fevers. Hyperacidity, though not a constant feature, occurs in gastric and duodenal ulcers. The subject of gastric analysis will be treated very briefly in this connection, as it belongs more properly in textbooks devoted to laboratory methods.

Gastric flow may be stimulated by the ingestion of a so-called test meal or by hypodermic injection of histamine. The Ewald test meal consists of a piece of toast and a cup of tea. One hour after the food has been taken on an empty stomach, the gastric contents are removed by means of a stomach tube and analyzed. Instead of a single specimen, several samples of gastric juice may be removed from the stomach at definite intervals. This is the basis of the Rehfuss fractional method of gastric analysis.

The concentration of free hydrochloric acid may vary in the normal individual from 0.2 to 0.4 per cent, although greater variations occur

[85a] A recent review on the subject of the emptying mechanism of the stomach is that of J. R. Murlin, Nutrition, **2**, 311 (1929).

frequently. Not all of the hydrochloric acid is present in the free form. Some is present in combination with protein and is termed " combined " hydrochloric acid. Titration of the gastric juice with sodium hydroxide gives values for the free hydrochloric acid (with dimethylaminoazobenzene as the indicator), for free hydrochloric acid plus organic acids (with alizarin), and for total acidity (with phenolphthalein). The combined hydrochloric acid is calculated from the difference in the titration values for the total acidity and the free acid plus organic acid.

In pathological conditions, the gastric juice may contain excessive amounts of organic acids and such abnormal constituents as blood, pus, and bile. The presence of less than the normal amount of hydrochloric acid is known as hypochlorhydria or hypoacidity, whereas the presence of excessive amounts is termed hyperchlorhydria or hyperacidity. Even in normal individuals, however, the acidity may vary within wide limits. There may also be a deficiency of enzymes in the gastric juice in abnormal conditions.

Digestion in the Intestine.—The partly digested food material, after entering the small intestine, is subjected to the action of three separate secretions, the pancreatic juice, the intestinal juice or succus entericus, and the bile. The pancreas is a long, irregularly shaped gland lying close to the duodenum. In the adult, the organ usually weighs about 70–90 grams. Two secretions are formed by the pancreas, an internal secretion which is concerned with regulating carbohydrate metabolism, and an external secretion which has digestive properties and which is conveyed to the duodenum by one or more ducts.

Mechanism of Pancreatic Secretion.—Pancreatic secretion, although, in part, under the control of the nervous system, does not seem to be influenced by psychic stimuli, as is the case with salivary and gastric secretion. It has been shown (Pavlov) that the presence of acid chyme in the intestine normally causes active secretion by the pancreas. This excitation Pavlov thought to be due to a reflex stimulation, since the pancreas is under the control of both the vagi and the splanchnic nerves. However, in 1902, Bayliss and Starling [86] were able to show that even after nervous communication with the pancreas had been destroyed, secretion could be induced by the introduction of acid into the intestine. Working on the assumption that the secretory mechanism was under chemical control, Bayliss and Starling prepared an acid extract from the intestinal mucosa, and after neutralizing it, injected it into the circulation of dogs. This resulted in copious secretion of pancreatic juice.

This chemical mechanism is believed to consist in the transformation of a substance known as prosecretin, which is present in the intestinal

[86] J. Physiol., **28**, 325 (1902).

mucosa, into secretin. Secretin is a hormone, or chemical messenger, which enters the circulation and is carried to the pancreas which it rouses to activity.[86a]

Of the many experiments which have confirmed the work of Bayliss and Starling, several reported by Ivy and Farrell [87] are especially convincing. These workers transplanted the tail of the pancreas of dogs, subcutaneously, beneath the mammary gland, and in the same animals made a Thiry fistula of the jejunum. When dilute acid was applied to the Thiry fistula, the pancreatic transplant was stimulated to secrete. As this occurred after ligation of the bile duct and injection of atropine (the latter inhibits gastric secretion), the secretion of the transplant could not have been due to bile or to gastric juice flowing into the duodenum. In another experiment, loops of the jejunum were transplanted subcutaneously in animals with a pancreatic transplant. The application of dilute acid to the intestinal transplant stimulated the pancreatic transplant.

Many other substances when introduced into the upper intestine are said to stimulate pancreatic secretion in varying degree. Among these are water, solutions of various organic and inorganic acids, tetramethylamine and other quaternary amines, fat and other foodstuffs, and bile. Pilocarpine and curarine, when injected, likewise stimulate the flow of pancreatic juice.

Composition of the Pancreatic Juice.—The pancreatic juice is a clear liquid having an alkalinity corresponding to a *p*H of approximately 8.0. The following are analyses of human pancreatic juice:[88]

	Glaessner	Wohlgemuth
Water	98.72%	98.70%
Solids	1.27	1.30
Coagulable protein	0.174	0.093
Nitrogen	0.0983	0.0813
Alcohol-soluble substances	0.508	0.523
Specific gravity	1.0075	1.00713

Ash:

K	1.10%	Cl	50.75%	P_2O_5	1.85%
Na	36.56	SO_3	2.05	SO_2	0.34
				CO_2	0.11

Traces of Ca, Mg, Fe, SiO_2.

[86a] Mellanby, J., J. Physiol., **66**, 1 (1928), and Still, E. U., Am. J. Physiol., **91**, 405 (1929–30), have recently described methods for the isolation of secretin.

[87] Am. J. Physiol., **77**, 474 (1926); **78**, 325 (1926); J. Am. Med. Assoc., **89**, 1030 (1927).

[88] Glaessner, K., Z. physiol. chem., **40**, 465 (1904); Wohlgemuth, J., Biochem. Z., **39**, 302 (1912); see also Mathews' "Physiological Chemistry," New York, 1925, edition, p. 402.

Pancreatic Enzymes.—The enzymes of the pancreatic juice are trypsin, steapsin, amylopsin, maltase, a pancreatic rennin, a lactase (especially in young animals), pancreatic erepsin, and possibly invertase. As secreted by the pancreas, trypsin is in the inactive form, trypsinogen. In the intestinal mucosa there is a substance, enterokinase, which appears to have the properties of an enzyme and which has a specific effect in activating trypsin. Since the discovery of enterokinase by Schepovalnikov,[89] a pupil of Pavlov, in 1899, it has been thought that its activating effect is due to some sort of transformation of the trypsinogen to trypsin. A more precise idea of the mode of activation has been furnished by the work of Waldschmidt-Leitz,[90] which shows that enterokinase combines slowly with the relatively inactive trypsin in definite, or stoichiometric, proportions, and that the resulting compound (trypsin-enterokinase) is the activated enzyme. After the trypsin is activated, it is possible to separate it by means of adsorption methods into its two components. The trypsin-enterokinase complex is capable of ionic dissociation.

Tryptic Digestion.—The digestion of protein by trypsin occurs most readily in a slightly alkaline solution (about *p*H 8.0). The sodium bicarbonate of the pancreatic juice transforms any unchanged protein that may reach the intestine into alkali metaprotein. In the process of digestion by trypsin, alkali metaproteins, native proteins, and the products of gastric digestion pass through the stages of proteoses, peptones, and amino acids.

Aiding trypsin in the digestion of protein is a pancreatic erepsin. Certain peptide linkages, the so-called resistant groups, are not acted upon by trypsin, and it is believed that the erepsin of the intestinal juice is concerned with their hydrolysis. The possibility that erepsin is not a single enzyme will be considered presently.

Pancreatic Lipase, Steapsin.—The fat-splitting enzyme of the pancreas, steapsin, is relatively inactive in the form in which it is secreted. However, in the presence of certain substances, such as bile, bile salts, egg albumin, calcium salts, and calcium soaps, the enzyme seems to be activated. This type of activation is obviously non-specific. Willstätter [91] and his pupils have shown that these activating agents exert their effect on pancreatic lipase by providing a specially favorable adsorption condition for the contact of the water-soluble enzyme with its insoluble substrate, the fats. This conception is a departure from the view, which until recently has been generally accepted, that the bile

[89] Maly's Jahresbericht, **29**, 378 (1899).
[90] Z. physiol. Chem., **132**, 181 (1923–24).
[91] Z. physiol. Chem., **125**, 93 (1922–23).

salts transform the inactive zymogen, steapsinogen, into steapsin, and that, in addition, the bile salts accelerate fat hydrolysis because of their co-ferment action toward the active steapsin.

Other Enzymes of the Pancreatic Juice.—Amylopsin is the starch-splitting enzyme of the pancreatic juice. It is active in a neutral or slightly alkaline solution. The starchy food reaching the small intestine is digested by pancreatic amylase through the maltose stage. The maltose is hydrolyzed to glucose by the pancreatic and intestinal maltases. Lactase is not found uniformly in pancreatic tissue of adults, but occurs more consistently in children and other young mammals. This enzyme converts lactose into glucose and galactose. For the most part, the disaccharides are acted on by the intestinal enzymes. However, invertase is occasionally found in pancreatic juice. There is, likewise, a rennitic enzyme. The pancreatic juice is without influence upon nucleic acids.

Relation of the Pancreas to Digestion.—The quantity of pancreatic juice varies with the type of food, probably because of an interrelationship with gastric secretion. The secretion of the pancreatic juice begins when the acid chyme enters the duodenum, the quantity secreted being more or less conditioned by the amount of acid admitted. It will be recalled that the acid secretion in the stomach is determined to some extent by the character of the food. Cessation of pancreatic secretion in pathological conditions, as in obstruction of the pancreatic duct by a tumor, or in experimental occlusion of the pancreatic ducts by ligation, is usually followed by a reduction in the digestion of protein and fat. It has been stated (Yesko)[92] that under such conditions there is a delayed emptying time of the stomach which permits gastric digestion to proceed further than normally. Nevertheless, large amounts of material remain undigested and are found in the feces. This occurs, likewise, in animals after pancreatectomy. Even in these animals, with special care in the selection of the diet, fair nutrition may be maintained by virtue of the digestive powers of the gastric and intestinal secretions.

The Enzymes of the Intestinal Juice.—Closely associated with the pancreatic juice in the digestive processes that occur in the intestines is the intestinal juice, or *succus entericus*. This secretion is produced most abundantly in the duodenum and is formed in progressively smaller quantities in the lower portions, the jejunum and the ileum. The juice, which is alkaline in reaction, is produced by two types of glands present in the mucous membrane of the entire small intestine, the so-called Brunner's and Lieberkühn's glands. There are apparently two distinct types of secretions, only one of which is associated with

[92] Am. J. Physiol., **86**, 483 (1928).

digestion. The other, which is periodic, occurring about every two hours even during starvation, is rich in the glycoprotein, mucin, and poor in enzymes, and contains a number of constituents which are very probably products of excretion. This periodic secretion and the bile form the major portion of the feces eliminated in starvation.

The discharge of food into the intestine results in the rapid secretion of a juice which possesses marked digestive properties. The secretion is under the control of both a nervous and a hormone mechanism. The latter is very probably the same as that concerned with the stimulation of pancreatic secretion. In addition to these, mechanical stimulation is probably a factor; it is said to cause the secretion of juice of low enzyme content.

Amylase, maltase, invertase, lactase, erepsin, lipase, rennin, the nucleinases, and nucleotidases are found in the intestinal juice and intestinal mucosa. The nucleosidases, which act on purine nucleosides, are found only in the intestinal mucosa. A peptic enzyme, active in acid solution, has been found to be produced by Brunner's glands. The function of this enzyme in intestinal digestion is probably insignificant. There are, in addition, a number of less well-defined enzymes, including emulsin which is capable of hydrolyzing β-glucoside linkages. Enterokinase, the enzyme which activates trypsin, is an important constituent of the intestinal mucosa.

The digestive properties of intestinal erepsin, discovered by Cohnheim [93] in 1901, are of especial importance. It will be recalled that trypsin fails to act on certain peptide linkages. In tryptic digestion, the unhydrolyzed portion may be equivalent to as much as 10–20 per cent of the total protein nitrogen. This fraction constitutes the so-called resistant group of polypeptides. Erepsin is capable of completing protein digestion by cleaving these resistant polypeptides. Erepsin is said to hydrolyze, in addition, peptones, casein, fibrin, the protamins, and the histones, but is not capable of acting on native proteins such as the albumins, globulins, and muscle proteins. (Abderhalden and Fischer.[94])

An interesting recent development is the report by Waldschmidt-Leitz, Balls, and Graser [95] that the so-called erepsin of the animal intestinal tract and of other tissues is not a single enzyme, but a mixture of two independently acting enzymes, a polypeptidase and a dipeptidase. The former splits tri, tetra, penta, and hexapeptides composed of leucine and glycine residues, and, according to these authors, would, in all probability, split the still higher members of these series.

[93] Z. physiol. Chem., **33**, 451 (1901).
[94] *Ibid.*, **46**, 52 (1905).
[95] Ber., **62**, 956 (1929); Am. J. Physiol., **90**, 549 (1929).

Somewhat earlier Grassmann[96] showed that the erepsin of yeast could be separated into two fractions, a dipeptidase and polypeptidase.

The Bile.—The bile is continuously formed by the liver cells and, between periods of digestion, is stored in the gall-bladder. As it reaches the intestine, the bile is composed not only of the secretions of the liver cells, but likewise of the mucosa of the gall-bladder and the biliary passages. The quantity of bile secreted is subject to great variation, and accurate determinations are not available. In man, the secretion for twenty-four hours has been estimated at between 500 and 1200 cc.

Bile is usually yellow, brownish yellow, or olive green in color. It is very bitter to the taste. In addition to its properties as a digestive fluid, the bile is an excretory channel for a variety of substances, including toxins, metals, and cholesterol. It is alkaline in reaction.

The more important constituents of the bile are the bile pigments, bile salts, and cholesterol. Among the other constituents are fats, fatty acids and lecithin. The bile contains .6 to 1.1 per cent inorganic substances. These include the chlorides of sodium and potassium; calcium, magnesium, and iron phosphates; traces of copper, and possibly zinc. Bile obtained from a biliary fistula differs in composition from that found in the gall-bladder, chiefly with regard to the solid constituents, liver bile containing a much lower per cent of total solids. While in the gall-bladder, the bile becomes concentrated by the reabsorption of a certain amount of water. Mucin and possibly other substances are added to the bile, being secreted from the wall of the gall-bladder.

The following are analyses of human gall-bladder bile obtained from normal individuals who had been either executed or accidentally killed. The data are in parts per hundred.

	I*	II	III	IV
Water	86.00	85.92	82.27	89.81
Solids	14.00	14.08	17.73	10.19
Bile salts	7.22	9.14	10.79	5.65
Mucin and pigments	2.66	2.98	2.21	1.45
Cholesterol	0.16	0.26	4.73	3.09
Fat	0.32	0.92		
Inorganic substances	0.65	0.75	1.08	0 62

* Analysis I and II are those of Frerichs; Analyses III and IV are from the work of v. Gorup-Besanez. These data are taken from Hammarsten-Mandel's "Physiological Chemistry," 1915 edition, p. 437.

[96] Z. physiol. Chem., **167**, 202 (1927).

The composition of human liver bile (according to Hammarsten) is given below.

Water	97.48	96.47	97.46
Solids	2.52	3.53	2.54
Mucin and pigments	0.53	0.43	0.52
Bile salts	0.93	1.82	0.90
Taurocholate	0.30	0.21	0.22
Glycocholate	0.63	0.16	0.15
Fatty acids from soaps	0.12	0.14	0.10
Cholesterol	0.06	0.16	0.15
Lecithin	0.02	0.06	0.07
Fat		0.10	0.06
Soluble salts	0.81	0.68	0.73
Insoluble salts	0.03	0.05	0.02

The Bile Pigments.—The color of bile is due to the presence of a variety of pigments, chief among which is bilirubin, $C_{33}H_{36}N_4O_6$, a substance closely related to porphyrin (p. 208). On oxidation, this yields a green pigment, biliverdin, $C_{33}H_{36}N_4O_8$. The latter, on oxidation forms a number of compounds, among which is the blue pigment, bilicyanin. Bilicyanin does not occur in normal bile, but is found in gallstones, together with bilirubin and biliverdin, as well as with certain other pigments, choleprasin, bilifuscin, biliprasin and bilihumin. In diarrhea, the feces may have a greenish color due to biliverdin. The brown color of normal feces is due to stercobilin, which is a reduced bilirubin. Another reduction product of bilirubin is urobilin, a pigment found in the urine.

Origin of the Bile Pigments.—Until recently, it was generally held that the bile pigments were formed in the liver from hemoglobin liberated upon the disintegration of red corpuscles. It has been shown, however, by Whipple and his co-workers,[97] that cells other than those of the liver have the capacity to change hemoglobin into bile pigment at a rapid rate. Thus, in a number of experiments where the liver was excluded from the circulation, it was found that this conversion occurred in the blood stream of the head and thorax, and in the serous cavities.

With regard to the question of normal pigment metabolism, it has been assumed that the food taken into the system, including iron, was transformed into hemoglobin by the bone marrow. Ultimately, the hemoglobin was believed to be broken down into bile pigments, which were later converted into stercobilin in the intestinal tract and partly

[97] Physiol. Reviews, **2**, 440 (1922).

eliminated in the feces, the remainder, following reabsorption, being changed into urobilin and urochrome and excreted in the urine. Whipple denies the reabsorption of stercobilin; nor does he believe that there is any evidence pointing to a transformation of stercobilin into urobilin. Moreover, Whipple and Hooper[98] have shown that bile pigment is not necessarily related directly to destruction of red cells and hemoglobin, but may have its origin directly in the food. It is to be recalled that, in common with hemoglobin, proteins and the green coloring matter of plants contain the pyrrol ring which must enter into the "pigment complex" of the bile pigment molecules. The term "pigment complex" is used here, as it is used by Whipple, to indicate a group of substances which are essential parts of the mature body pigments.

On the other hand, Rich,[99] in his review of the subject of bile pigment formation, states that while hemoglobin may be regarded as a source of bile pigment, proof is lacking that there are other sources.

Whipple's ideas concerning pigment metabolism in the animal organism are summarized in Fig. 24.[100]

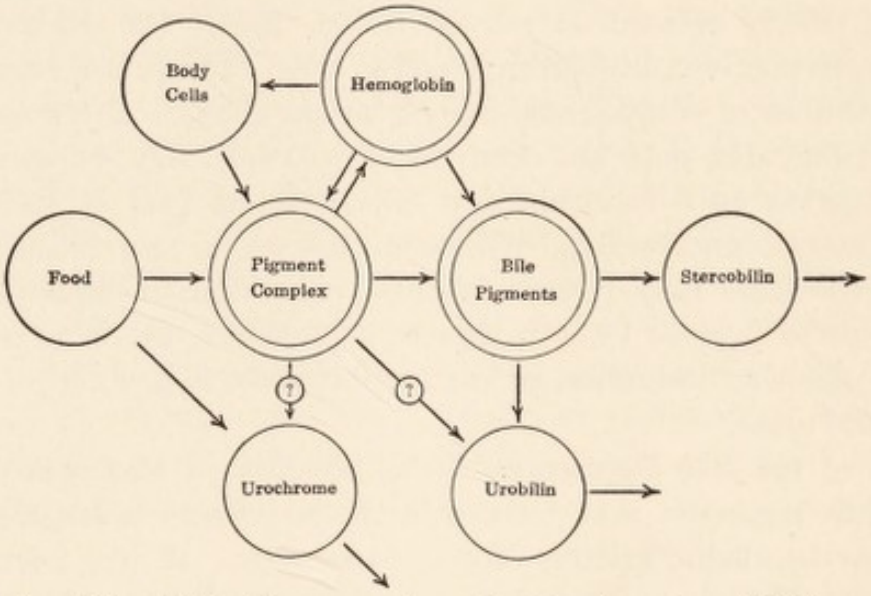


FIG. 24.—Whipple's conception of body pigment metabolism.

The Bile Salts.—Among the constituents of all bile are the salts of the bile acids. Human bile contains glycocholic acid ($C_{26}H_{43}NO_6$) and taurocholic acid ($C_{26}H_{45}NSO_7$). In addition, glycocholeic acid ($C_{26}H_{43}NO_5$ or $C_{27}H_{45}NO_5$) has been detected in human bile and, more recently several new bile acids have been described. Glycocholeic is present in considerable amount in ox bile. Taurocholeic acid

[98] Am. J. Physiol., **40**, 349 (1916).
[99] Physiol. Reviews, **5**, 182 (1925).
[100] Arch. Int. Med., **29**, 711 (1922).

($C_{26}H_{45}NSO_6$ or $C_{27}H_{47}NSO_6$) is present in dog bile and ox bile but has not been found in human bile. Hyo-glycocholic acid ($C_{27}H_{43}NO_5$) occurs in the bile of pigs and cheno-taurocholic acid ($C_{29}H_{49}NSO_6$) in the bile of geese.

On hydrolysis, glycocholic acid yields cholic acid and glycocoll, whereas taurocholic acid is converted into cholic acid and taurine (amino-ethyl-sulfonic acid).

$$\underset{\text{Glycocholic acid}}{C_{23}H_{39}O_3\cdot CO\cdot HN\cdot CH_2\cdot COOH} + H_2O = \underset{\text{Cholic acid}}{C_{23}H_{39}O_3\cdot COOH} + \underset{\text{Glycocoll}}{CH_2NH_2\cdot COOH}$$

$$\underset{\text{Taurocholic acid}}{C_{23}H_{39}O_3\cdot CO\cdot HN\cdot CH_2CH_2SO_2\cdot OH} + H_2O = \underset{\text{Cholic acid}}{C_{23}H_{39}O_3COOH} + \underset{\text{Taurine}}{H_2N\cdot CH_2\cdot CH_2\cdot SO_2OH}$$

Glycocoll and taurine are present in the animal body, the latter being derived, very probably, from cysteine. Cholic acid is structurally related to cholesterol, and it is possible that it may be derived from cholesterol in metabolism, but of this there is no clear-cut evidence.[101]

Very little is actually known concerning the source and metabolism of the bile acids, except for the fact that the bile salts are readily absorbed from the intestine and reappear in the bile. Thus, by continued circulation, the bile repeats its function many times. Variation in the daily output of bile salts seems to be largely dependent upon the diet. A greater excretion occurs on a diet of meat and meat products [102] than on a carbohydrate diet. The excretion of taurocholic acid is very uniform in fasting dogs. When these are fed sugar, the level of excretion is lowered. As will be shown in a later chapter, carbohydrates have a sparing action on tissue protein. There is thus an interesting parallelism between bile-acid excretion and urinary nitrogen excretion, pointing to a relationship between body-protein metabolism and bile-acid metabolism. Moreover, within certain limits an increased intake of food protein raises the bile-acid excretion. There is some basis, therefore, for the conclusion that both endogenous and exogenous factors influence bile-acid metabolism.

A striking effect which follows the administration of bile or bile salts is the increased secretion of bile. Together with a number of other substances, including certain drugs—salicylates, chloral hydrate, soaps, acids, albumoses—the bile salts have been classified as cholagogues,

[101] In the last few years important work has been done by Wieland, Windaus, and others in elucidating the chemical constitution of the bile acids. For a résumé of their results, the student is referred to the Annual Reports on the Progress of Chemistry, issued by the Chemical Society of London, **24**, 128 (1927); **25**, 157 (1928).

[102] Smith, H. P., Groth, A. H., and Whipple, G. H., J. Biol. Chem., **80**, 659 (1928).

i.e., substances that stimulate the secretion of bile. Whipple points out that of these the only active cholagogues are bile or bile salts. Other substances may cause slight fluctuations in the secretion of bile, but these are insignificant when compared with the prompt reaction caused by bile salts. A recent study of the cholagogue action of bile acids is that of Greene and Snell.[103]

The bile salts diminish the surface tension of the limiting membrane of red corpuscles and most other cells. In sufficient quantity they may exert a solvent effect on the cell lipids, causing the complete disintegration of the cells. To these properties of the bile salts are attributed the toxicity of bile when it leaves its normal channels, the biliary tract and alimentary canal, as in bile peritonitis and obstructive jaundice (Horrall and Carlson).[104]

Cholesterol.—The origin of bile cholesterol is not clear. Human fistula bile contains usually about 0.07 to 0.10 per cent cholesterol, larger amounts being present in gall-bladder bile. Many suggestions have been advanced concerning the source of cholesterol. Its origin has been attributed to the secretions of the epithelium of the bile tract and to degenerating liver cells. According to another view, the adrenals are regarded as controlling the metabolism of cholesterol. It has also been suggested that cholesterol in the bile results from red-cell disintegration as well as from tissue destruction in general. Feeding excessive amounts of cholesterol or administering it intravenously or otherwise has been found to have very little effect in increasing the cholesterol content of the bile in fistula dogs. On the other hand, lipid-free diets may be accompanied by low values for bile cholesterol. The formation of cholesterol in the body is indicated by the fact that the elimination of cholesterol may exceed the cholesterol intake. No definite relationship seems to exist between the cholesterol content of human bile and disease, except possibly in diabetes and pregnancy where the amounts eliminated are larger than normal. Very wide variations are observed normally.

In man, cholesterol is converted in the intestine to coprosterol, or stercorin ($C_{27}H_{47}OH$). This is a constituent of human feces from which it may be obtained by extraction with ether.

Functions of the Bile.—Normal bile flow appears to be necessary for life; yet bile-fistula animals may tolerate the exclusion of bile over considerable periods. Especially is this true when the diet is carefully selected. Whereas fistula dogs kept on a diet of kitchen scraps usually die within two months, they may live in good condition for four to ten months when fed a diet of milk, cooked potatoes, rice, and bread.

[103] J. Biol. Chem., **78**, 691 (1928).

[104] Am. J. Physiol., **85**, 591 (1928).

These animals usually develop bony abnormalities, however, the essential features of the condition being a loss of inorganic salts from the bones, which thus become thin and fragile. This condition is very likely due to the excessive loss of calcium in the bile.

Exclusion of the bile leads to serious digestive disturbances. The bile is a good emulsifying agent; it also promotes the solution of fats, fatty acids and other lipids, and, according to the older view, exerts a direct effect in activating and accelerating lipases. Because of these properties the bile plays a very important rôle in the digestion and absorption of fats. Fat digestion is intimately associated with the digestion of other foodstuffs. The formation of a fatty layer around food particles diminishes the amount of surface exposed to the action of enzymes. As a result, in the absence of bile, a relatively large amount of undigested or partially digested food finds its way into the large intestine, where it is likely to undergo putrefactive changes. The bile ordinarily diminishes putrefaction by aiding in the digestion and absorption of fats as well as by its natural laxative properties. The bile stimulates peristalsis.

In his lucid review of the extra-hepatic functions of the bile, Schmidt [105] emphasizes the importance of the bile as a reservoir for alkali. The bile, together with the pancreatic and intestinal juices, neutralizes the hydrochloric acid which enters the intestines from the stomach. Owing to its recirculation, the bile affords a method of bringing alkali to the intestinal tract.

The bile is a channel for the elimination of a variety of excretory products—cholesterol, lecithin, drugs, toxins, bile pigments, copper, iron, and other inorganic substances.

Another function which may be ascribed to the bile is the cholagogue effect of the bile acids. Bile taken internally stimulates biliary secretion.

Functions of the Gall-bladder.—The sphincter of Oddi, a muscular band surrounding the common bile duct near its duodenal end, closes when digestion ceases. The continuous secretion of bile raises the pressure in the ducts and, it is believed, after a certain pressure is reached, namely one of about 70 mm. of water, bile begins to flow into the gall-bladder. Discharge of bile into the duodenum occurs when the pressure in the ducts rises above 100 to 120 mm., which is presumably the pressure maintained during digestion. Considerable variations in pressure in the bile passages have been observed under different conditions.

Not all animals have a gall-bladder. The horse, certain deer, and the rat are among the species of animals in which it is absent.

[105] Physiol. Reviews, **7**, 129 (1927).

As to the functions of the gall-bladder, considerable differences of opinion exist, as may be judged from the excellent reviews of the subject by Mann [106] and by Halpert.[107] The oldest, and perhaps even yet the most widely accepted view, is that the gall-bladder serves as a reservoir during the intervals between digestion, when the bile is not needed. However, the gall-bladder is not a reservoir in the same sense as the urinary bladder, for the bile which it can hold is only a portion of the total which enters the intestinal tract. In man the capacity of the gall-bladder is approximately 3 per cent of the total daily bile flow. Actually, however, the importance of the gall-bladder as a reservoir may be much greater than this figure would indicate, if due account is taken of the fact that the bladder bile is much more concentrated than hepatic bile.

The observation that the gall-bladder is an organ of absorption of bile constituents has led many investigators to consider that absorption is its main function, by virtue of which certain valuable materials, secreted in the bile, are restored to the organism. In fact, it has been stated [108] that the bile which once enters the gall-bladder does not leave it, under ordinary conditions, through the cystic duct, but is resorbed by the mucous membrane of the gall-bladder, and the constituents are then returned into the general circulation and the liver.

On the other hand, various substances, such as mucus and toxic agents, are added to the bile during its stay in the gall-bladder. Accordingly, it has been suggested that the gall-bladder is secretory, and perhaps also excretory, in function.

The view has been advanced that the gall-bladder is concerned in regulating the flow of bile, making possible an intermittent, rather than a continuous flow into the intestine. Finally, there is the plausible theory that, because it is an expansible chamber, the gall-bladder regulates the pressure in the biliary passages.

Both nervous and hormone mechanisms are said to control the flow of bile. The afferent nerve endings of the mucous membrane of the intestine are thought to be excited by the acid chyme when it enters the duodenum, resulting in a reflex contraction of the gall-bladder and the flow of bile into the duodenum. Many workers have questioned the importance of this factor.[109]

The hormone secretin, which, we have seen, stimulates the pancreas

[106] Physiological Reviews, **4**, 251 (1924).

[107] Archives of Surgery, **19**, 1037 (1929).

[108] Halpert, B., Med. Klin., **20**, 408, 1830 (1924); Halpert, B., and Hanke, M. T., Am. J. Physiol., **88**, 351 (1929); Sweet, J. E., Internat. Clinics, **1**, 187 (1924).

[109] Alvarez, W. C., The Mechanics of the Digestive Tract, New York, Second Edition (1928), p. 262.

and intestinal glands, has been thought to act also on the liver cells, causing increased bile formation. Recently, Ivy and Oldberg [110] reported having prepared an extract of the upper intestinal mucosa which when injected intravenously caused the contraction and evacuation of the gall-bladder. The view has been advanced by these workers that when acid is injected into the duodenum something gets into the blood which causes the gall-bladder (in cats, dogs and guinea pigs, but not in rabbits) to contract. The active principle, presumably a hormone, has been named " cholecystokinin."

The introduction of fat into the duodenum is said to stimulate a copious flow of bile.

Gallstones.—Biliary concretions, or gallstones, are occasionally formed in the gall-bladder, usually around some foreign body, injured epithelial cells, or bacteria. Although gallstones may contain a preponderance of one constituent, as in the case of cholesterol stones, or of two constituents, as in the calcium carbonate-bile pigment stones, they all contain small, although at times only minute, amounts of other substances. Fats, soaps, fatty acids, lecithin, mucin, copper, zinc, iron and manganese are among the organic and inorganic substances which may be present. Strictly, therefore, there are no *pure* gallstones, but for purposes of classification, it is convenient to designate as such certain concretions which consist mainly of one substance. The cholesterol stones, for example, may contain as much as 98 per cent of pure cholesterol. A convenient classification of gallstones has been proposed by Halpert.[111]

One species of whale (*Physeta macrocephalus*) develops biliary concretions containing a substance, ambrine, which closely resembles cholesterol. These concretions are often found in the excreta of these animals and are known as ambergris.

Summary of Digestion.—Reactions in living tissues are catalyzed by certain substances known as enzymes, which behave, in many respects, like inorganic catalysts.

Most of the reactions of physiological importance are those of hydrolysis. To the group of hydrolytic enzymes belong the proteolytic, lipolytic, and amylolytic enzymes.

The activity of enzymes is influenced by a variety of factors, such as the concentration of the substrate, the concentration of the enzyme, the temperature, the reaction of the medium, and the presence of inhibiting or accelerating agents.

It has been shown that the very same enzymes that are capable

[110] Am. J. Physiol., **86**, 599 (1928).

[111] Archives of Pathology, **6**, 623 (1928).

of bringing about the hydrolysis of a given substance may be capable of accelerating the reaction of synthesis of the substance from the hydrolytic products. Thus, protein synthesis has been accomplished with the aid of trypsin, and ester formation with the aid of lipases, and starch-like substances have been prepared by the action of amylases.

The chemical transformations by which the foods are converted into small diffusible particles constitute the process of digestion. These changes are accomplished with the aid of enzymes distributed in the upper portion of the alimentary tract. By this means, the food becomes available for absorption and subsequently for metabolism.

The enzymes concerned in the digestion of starch are the ptyalin of the saliva and the amylopsin of the pancreatic juice. The maltase, and lactase of the pancreatic juice, and the maltase, lactase, and invertase of the intestinal juice and intestinal mucosa, are concerned with hydrolysis of the disaccharides. Thus, the carbohydrates of the diet are reduced to monosaccharides.

Protein digestion occurs in the stomach and small intestine. The pepsin of the gastric juice, the trypsin of the pancreatic juice, and the polypeptidases and dipeptidases of the intestinal secretions are primarily concerned with the hydrolysis of proteins. Rennins are found in all three of these secretions. Rennin takes part in the clotting of milk, a process associated with the conversion of casein into paracasein.

The nucleoproteins are partly digested by the proteolytic enzymes of the stomach and pancreas. The nucleic acids that are split off are hydrolyzed by an enzyme, nucleinase, of the intestinal juice. The change consists in the hydrolysis of the tetranucleotide molecule into four mononucleotide molecules. The mononucleotides are further acted upon by an enzyme or a group of enzymes, the nucleotidases. It is not certain whether the pyrimidine nucleotides are acted upon by the intestinal juice. At any rate, the purine nucleotides are broken down to phosphoric acid and nucleosides. The purine nucleosides are hydrolyzed by the nucleosidases which are present in the intestinal mucosa, yielding a sugar and a purine base. These changes will be considered in more detail in a later chapter.

Lipases are found in the secretions of the stomach, pancreas, and intestine. Fat digestion is accomplished almost entirely, however, by the enzymes poured into the intestine. The bile aids both in the digestion and in the absorption of fat.

TABLE XXIX

The Rôle of Enzymes in Digestion

Site	Secretion	Reaction	Enzyme	Substrate	Amount of Digestion	Products of Digestion
Mouth	Saliva	Neutral, acid or slightly alkaline ($pH = 5.75–7.05$)	Ptyalin	Starch	Slight	Dextrins, maltose
			Maltase	Maltose	Very slight	Glucose
Stomach	Gastric Juice	Acid	Pepsin	Protein	Incomplete	Proteose, peptones, some polypeptides
			Rennin	Casein	Usually complete	Paracasein
			Lipase	Highly emulsified fat	Very slight	Fatty acids, glycerol
Intestine	Pancreatic Juice	Alkaline	Trypsin	Protein, proteoses, peptones and polypeptides	Nearly complete	Polypeptides, amino acids
			Steapsin	Fat	Nearly complete	Fatty acids, glycerol
			Amylopsin	Starch	Nearly complete	Dextrins, maltose
			Maltase	Maltose	Fairly marked	Glucose
			Lactase	Lactose	Appreciable	Glucose and galactose
			Invertase (?)	Sucrose	(?)	Glucose and fructose
			Rennin	Casein		
			'Erepsin' (polypeptidase and dipeptidase)			
Intestine	Intestinal Juice and Intestinal Mucosa	Alkaline	'Erepsin' (polypeptidase and dipeptidase)	Certain proteins, casein, protamins, etc., polypeptides, dipeptides	Complete	Amino acids
			Amylase	Starch	Nearly complete	Maltose
			Rennin			
			Enterokinase			
			Lipase	Fat	Nearly complete	Fatty acids, glycerol
			Maltase	Maltose	Complete	Glucose
			Lactase	Lactose	Complete	Glucose galactose
			Invertase	Sucrose	Complete (usually)	Glucose, fructose
			Nucleinases	Nucleic acids	.....	Mononucleotides
			Nucleotidases	Mononucleotides	.....	Nucleosides, phosphoric acid
			Nucleosidases (in mucosa)	Nucleosides	.....	Purine bases, sugar

CHAPTER VII

ABSORPTION AND INTESTINAL PUTREFACTION

The end-products of digestion diffuse through the wall of the small intestine, pass into the small blood and lymph vessels of the intestinal wall, and are then transported by the blood and lymph to the tissues. The undigested, unabsorbed residue is propelled to the large intestine and finally excreted as feces.

So much of the general plan is known. As to the precise mechanism involved in intestinal absorption, we are very much in the dark. Essentially, the problem is but one phase of the more general problem of cell permeability. Some of the more puzzling questions, pertaining to this subject, which we are as yet unable to answer satisfactorily, are set forth in the excellent review of Jacobs,[1] here quoted:

Beginning with the alimentary system, the problem of cell permeability arises in many forms. Why, for example, does practically no absorption, even of water, occur in the stomach, while taking place with the greatest ease in the small intestine? Why, in the latter, are some substances absorbed much more rapidly than others; for example, NaCl more rapidly than Na_2SO_4, dextrose more rapidly than sucrose, etc.? Why does NaCl readily enter the blood stream from a solution introduced into the gut but pass with difficulty in the reverse direction? Does the wall of the intestine show evidence of a one-sided permeability to water? What are the means by which water is taken up, not merely from hypotonic, but from isotonic and hypertonic solutions as well? What is the mechanism of normal absorption of the different kinds of digested food materials? . . .

Factors in Absorption.—Among the more important factors influencing the amount of absorption from various parts of the alimentary canal may be mentioned the following:

1. Character of the lining epithelium;
2. Area of the absorbing surface;
3. Time during which food remains in contact with the absorbing surface in a particular region;
4. Amount of digested material present.

Mouth and Esophagus.—The epithelium of the oral cavity, pharynx, and esophagus is relatively thick. There is but a very slight amount

[1] In Cowdry's General Cytology, 1924 edition, p. 99.

of carbohydrate digestion and no protein and fat hydrolysis in the mouth. Moreover, the food remains in this region a very short time, with the result that no food is absorbed from these areas. Certain drugs, however, are absorbed, owing to their ready penetration and the vascularity of the tongue and the lining of the oral cavity.

Stomach.—There is, likewise, very little absorption in the stomach. While the gastric mucosa secretes large amounts of water, it normally absorbs but little or none. An exception to this is the absorption of water from the psalterium of ruminating animals. The gastric mucosa is permeable, however, to alcohol or alcohol solutions, as well as to small amounts of sugar, amino acids, and other organic compounds. It is stated that condiments, such as mustard, increase the permeability of the gastric mucosa.

Small Intestine.—The small intestine is best adapted for absorption, especially the jejunum and the lower part of the duodenum. Superficially, the surface of the small intestine measures about ½ sq. m. However, the mucous coat is so irregular, because of its folds (plicae circulares) and its numerous smaller projections or villi, that the actual absorbing surface is about 10 sq. meters. Moreover, the food remains in the small intestine for several hours. It usually requires four to six hours, from the time the stomach begins to discharge its contents or acid chyme, before intestinal digestion and absorption are complete. The distribution of the chyme over so large an area as is offered by the small intestine greatly facilitates the absorption of diffusible substances.

Function of the Villi.—The villi are of primary importance in absorption. They are small finger-like projections consisting largely of a framework of reticular tissue containing many leucocytes in its meshes. The lining is simple columnar epithelium, containing many goblet cells. In size, the villi may vary between 0.5 and 0.7 mm. In man, the villi number between four and five millions.

Two channels take part in the removal of material. In the center of each villus is the central lacteal which opens into a plexus of lymphatics lying in the muscularis mucosae. Fluid is forced from the lacteal toward the larger lymphatics by the contraction of muscle fibers which run lengthwise in the villus. The flow of fluid in the reverse direction is prevented by valves present in the deeper plexuses. After reaching the larger lymph vessels, the absorbed material, consisting largely of fat in emulsion, flows to the thoracic duct and enters the blood near the junction of the left subclavian with the jugular vein.

The capillary blood vessels of the villus constitute the second channel of absorption. The material diffusing into the capillaries is carried to the radicles of the portal vein and subsequently by the portal vein

to the liver. The circulation of blood in capillaries is very rapid as compared with the sluggish flow of lymph. This is, no doubt, an important factor determining the distribution of material between the lymph stream and the blood.

Carbohydrate Absorption.—Only the monosaccharides are readily absorbed. The disaccharides are not found in the blood except when excessively large amounts are fed or when they are injected directly into the circulation. Under these conditions, sucrose and lactose behave as foreign substances and are excreted as such by the kidneys. Maltose behaves somewhat similarly, although a certain amount is said to be transformed into glucose by a maltase present in the blood. Intestinal contents containing the disaccharides, but washed free from enzymes, are readily absorbed; the sugar appearing in the circulation is, however, in the form of glucose. Obviously, then, the intestinal mucosa takes part in intracellular hydrolysis of disaccharides. No absorption of starch or dextrin occurs under similar conditions. The transformation of levulose and galactose to *d*-glucose has also been affirmed but is probably not complete when large amounts of these sugars are taken. The absorbed sugar is carried by the blood of the portal vein to the liver where much of it is removed and stored as glycogen.

Other tissues, particularly muscle, likewise store carbohydrate in this form. The sugar also enters the lymph circulation, as has been shown by Hendrix and Sweet,[2] who observed that during the absorption of glucose, the sugar concentrations of the lymph and blood rise to the same level. A marked increase in the concentration of sugar in the blood occurs soon after large amounts have been fed, but in the normal individual the blood sugar soon returns to normal levels even while absorption still continues. This shows that the liver and other tissues are capable of removing the sugar at a faster rate than it is absorbed. This capacity is markedly diminished in diabetes and in conditions in which the liver is involved.

In a quantitative study of carbohydrate absorption, Cori [3] found that sugars are absorbed from the intestine at a rate which is constant for each sugar and which is independent of the initial concentration of the sugar in the intestine. The following is the order of the rates of absorption of the sugars studied by Cori: galactose > glucose > fructose > mannose > xylose > arabinose.

Fat.—The possibility of the passage of unsplit fat across the intestinal wall cannot be overlooked entirely, but, as Bloor [4] points out,

[2] J. Biol. Chem., **32**, 299 (1917).
[3] J. Biol. Chem., **66**, 691 (1925).
[4] Physiol. Reviews, **2**, 103 (1922).

there is every reason to believe that fat is completely hydrolyzed before it passes from the intestine. The sequence of events is not entirely known, but there is abundant evidence to show that the products of fat hydrolysis are taken up by the epithelial cells and there resynthesized. This occurs not only when both fatty acid and glycerol are available, but also when fatty acid alone is fed. We have not a lucid understanding of the synthetic function of the intestinal epithelium, but it is believed that the lipases perform the dual function of fat hydrolysis and synthesis. R. G. Sinclair [5] has recently suggested that absorbed fatty acids are transformed into phospholipid within the intestinal mucosa as an essential step in the resynthesis of neutral fat. It has also been suggested that the passage of fat into and out of a cell is preceded by hydrolysis. How long the fat remains in the epithelial cells depends on the amount and rate of absorption. The next step in the process, according to the view of Heidenhain, is the expulsion of the fat into the lacteal by contraction of the cell protoplasm. The more generally accepted explanation, however, is that of Schäfer, according to which the leucocytes take part in the transportation of fat from the lining epithelium to the lacteal. The presence of fat globules in the leucocytes, during absorption, can be easily demonstrated by histological methods. What remains to be learned is whether fat undergoes hydrolysis before being taken up by the leucocytes or whether neutral fat is absorbed as such, owing to its high degree of subdivision.

After entering the lacteals, the fat appears as a milk-white emulsion to which the term *chyle* has been applied. The chyle enters the larger lymphatics of the mesentery, passes to the receptaculum chyli, then by way of the left thoracic duct enters the blood at the junction of the left subclavian and jugular veins.

However, only about 60 per cent of the absorbed fat can be accounted for in the chyle. What happens to the remainder has always been somewhat of a mystery. Perhaps a small amount is directly absorbed by the blood. Recently Eckstein [6] studied fat absorption through channels other than the left thoracic duct. While his results are not altogether consistent, they show, nevertheless, that when all of the thoracic lymph is diverted from the blood stream, an appreciable, though small, augmentation of the fatty-acid content of the blood follows the absorption of neutral fat from the duodenum. It has been suggested, likewise, that a portion of the fat that is unaccounted for may be stored somewhere along the path of transport to the blood, or that it may be catabolized in the tissues before reaching the blood.

[5] J. Biol. Chem., **82**, 117 (1929).

[6] J. Biol. Chem., **62**, 737 (1925).

In the blood, the fat is transported as neutral fat, fatty acid, and lecithin, and in the form of cholesterol esters. This question will be considered again in relation to the intermediary metabolism of fat.

The paraffin hydrocarbons are not absorbed, for example, petrolatum.

Function of the Bile.—By increasing the solubility of the fatty acids and soaps in the intestine, the bile aids greatly in fat absorption. The occlusion of the bile ducts or the production of a biliary fistula is followed by a very pronounced reduction in the utilization of fat.

Absorption of Proteins.—The digestion of protein to amino acids serves many purposes. Except for minute amounts, the intestinal epithelium is normally impermeable to protein as well as to its intermediate digestion products—proteoses, peptones, and higher polypeptides.[7] For the most part, only amino acids are absorbed, although the simpler peptides are no doubt also diffusible through the intestinal epithelium. Were it nor for this exclusion of nearly everything except the amino acids, much of the protein ingested would be of little use to the animal organism. To synthesize proteins characteristic of itself, the organism must begin with the simplest building-stones possible. The building specifications, so to speak, must be observed most rigidly, a difference in even a single peptide bond being sufficient to alter the architecture and properties of the protein molecule.

The amino acids are absorbed into the blood capillaries of the villi, the rate of absorption in all probability being somewhat different for individual amino acids (Wilson and Lewis).[8] Several hours after a meal, the amino-acid content of the blood, and especially of the corpuscles, is increased considerably. This does not mean, as is commonly supposed, that the blood is the only channel of amino-acid absorption. Evidence of absorption into the lacteals has been adduced by Hendrix and Sweet,[2] who found the amino nitrogen of the chyle to increase considerably during absorption, becoming much greater in concentration than in the blood of the systemic circulation.

Not only would the absorption of proteins, proteoses and peptones as such prove useless to the animal organism, but their entrance into the blood is usually attended by a severe form of intoxication, termed "shock." Proteins differ in their toxicity and in the manner in which they act. This effect is especially pronounced in the case of the proteoses and peptones and has been attributed to a variety of constituents

[7] According to Sussman, Davidson, and Walzer (Arch. Int. Med., **42**, 409 (1928)), absorption of detectable amounts of unaltered egg protein from the digestive tract was noted in 85.3 per cent of 34 subjects tested and may, according to these authors, therefore, be considered a normal phenomenon. The absorption of fish protein was likewise noted.

[8] J. Biol. Chem., **84**, 511 (1929).

which may be supposed to arise during the hydrolysis of protein. It has been suggested that either histamine or substances related to it may be the fundamental cause of peptone shock.

Proteins, therefore, are foods when absorbed in the usual way as amino acids, and poisons when introduced directly into the blood. One of the most violent poisons known is ricin, the protein of the castor bean. The injection of a protein that is foreign to the tissues of an animal results in the excretion of most of it in the urine. If the injection is repeated a few days later, no ill effects ensue. Continued injection of small amounts of a given protein at short intervals establishes an immunity for that protein, due, it is believed, to the formation of a precipitin, in the presence of which the foreign protein is precipitated. If, however, the second injection is administered several weeks after the first, severe shock is induced. This phenomenon is termed *anaphylaxis* and has among its symptoms a marked fall in blood pressure and a reduction in the coagulability of the blood. According to some investigators anaphylactic shock and peptone shock are essentially the same, the former being due to the development in the sensitized animal of an enzyme capable of converting the foreign protein in question into proteoses and peptones. "Serum-sickness" frequently occurs in individuals sensitized against horse-serum proteins, and develops after the injection of antitoxins, such as diphtheria antitoxin. Under these conditions typical anaphylactic shock may occur and may terminate fatally.

Idiosyncrasies toward food proteins are likewise known. Certain individuals are unable to tolerate egg or milk proteins. Others, after eating strawberries or sea food, develop skin eruptions, asthma, and other anaphylactic reactions. These idiosyncrasies are attributed to the absorption of native or unchanged proteins found in these foods. Exceedingly small amounts (less than one milligram) are frequently sufficient to produce typical intoxications.

Occasionally, therefore, unchanged protein may be absorbed from the intestine. When this happens, the protein behaves as a foreign substance, or, where the individual has been previously sensitized to that protein, it behaves as a poison. Ordinarily, however, protein is absorbed almost entirely in the form of amino acids.

Mechanism of Intestinal Absorption.—In the present state of our knowledge, we cannot speak with assurance concerning the forces involved in the absorptive process. No doubt, diffusion or osmotic forces play an important part, but other factors are likewise involved. In a general way, the more diffusible substances are absorbed more readily. Thus the diffusion velocities of sugar, amino acids, and fatty acids diminish in the order given and likewise do their rates of absorp-

tion. There is, however, no quantitative relationship between the two rates as there would be if the laws of osmosis were to hold. Moreover, the osmotic pressure of intestinal contents may bear little relation to the rate of absorption. Sugar solutions of varying concentration enter the blood with approximately equal rapidity. Voit and Bauer, in a classical experiment, demonstrated the absorption of the blood serum of an animal from a loop of the intestine of the same animal.

The intestinal epithelium may be injured, as in poisoning with sodium fluoride, in which case more definite relationships between osmotic forces and absorption become manifest. Similarly, dead intestinal epithelium behaves in many respects like an artificial gelatin membrane. Neither exhibit the characteristics of selective absorption shown by the living intestinal wall.

Heidenhain [9] has shown that sodium chloride solutions of osmotic pressure greater than or equal to the osmotic pressure of the blood, when injected into an isolated loop of the intestine, are absorbed indifferently. According to the laws of osmosis, no water should pass from the intestine into the blood in the first case, and in the second, no absorption of any kind should take place.

Cohnheim [10] studied the interchange of substances between the intestine and the circulating fluid in dead animals by pumping through the blood vessels a solution of sodium chloride (0.94 per cent). A sugar solution was placed in an isolated loop of the intestine, with the result that interchange of material occurred in both directions, sugar passing into the circulating fluid and sodium chloride into the intestine. There was no diminution in the volume of the intestinal contents. These observations have led many to the conclusion that absorption is due to some specific activity of the living epithelium. This, however, does not bring us any closer to the solution of the problem. It should be borne in mind that the circulating fluid in Cohnheim's experiment could not be expected to have the properties of blood. On the basis of what has been said in regard to Donnan's theory of membrane equilibrium (page 30), the blood proteins must play an important rôle in absorption by determining the distribution of electrolytes on the two sides of the intestinal membrane. The entire problem of intestinal absorption is a difficult one. The literature pertaining to the subject has been reviewed by Goldschmidt.[11]

Formation of Feces.—The intestinal contents, upon reaching the ileocaecal valve, are not like feces in appearance and composition.

[9] Pflüger's Archives, **56**, 579 (1894).

[10] Z. f. Biol., **37**, 443 (1899).

[11] Physiol. Reviews, **1**, 421 (1921).

They are semi-fluid in consistency, and frequently acid in reaction, whereas the feces are usually alkaline. At this stage the intestinal contents consist largely of undigested food remnants, the remains of the digestive and intestinal secretions, and cellular elements, including cell débris from the alimentary tract. The transformation of this material into feces occurs in the large intestine where the food residues remain for one or more days. Here, certain substances, especially water, are partly resorbed.

The character of the feces depends only partly on the diet. Thus, on a diet consisting exclusively of rice, the feces may have the same composition as on an exclusively meat diet. The two foods, which differ in composition, are presumably almost completely digested and the feces are derived largely from the secretions of the alimentary tract. In a starving animal, the feces are diminished in amount, but the composition may be the same as in a normally fed animal. The feces are bulky when the food contains much indigestible material, like cellulose. Normally, the color is dark brown, but when much fat is present the stool acquires a characteristic lighter color. The composition of feces is about 60–70 per cent water, 5–10 per cent nitrogen, 10–20 per cent fatty material and 10–20 per cent ash. Human feces is approximately neutral in reaction. According to Robinson [12] the normal fecal reaction of healthy men lies between *p*H 7.0–7.5.

Feces may contain the following food residues: cellulose, fruit seeds and skins (also made up largely of cellulose), muscle fibers, shreds of connective tissue, starch, fat, fatty acids, and soap. Among the remains of bile and intestinal secretions are to be found bile acids, bile pigments, cholesterol, coprosterol, mucin, and a variety of inorganic constituents, especially iron. Cellular elements derived from the alimentary tract are likewise present. One-fourth or more of the feces consists of bacteria, the number excreted per day having been estimated to vary between 50 and 500 billions.[13]

The fecal excretion of fat has recently been the subject of careful study. Hill and Bloor [14] and Sperry and Bloor [15] have shown that the amount and composition of fecal fat are to a large extent independent of the fat in the diet. In an experiment in which the effect of diet was studied, the amount of fecal fat on a fat-free diet was 1.76 gm., having an

[12] J. Biol. Chem., **52**, 445 (1922).

[13] For an excellent account of the nature and composition of the feces, the student is referred to Chap. II of Lusk's "Science of Nutrition," 4th edition, Philadelphia (1928).

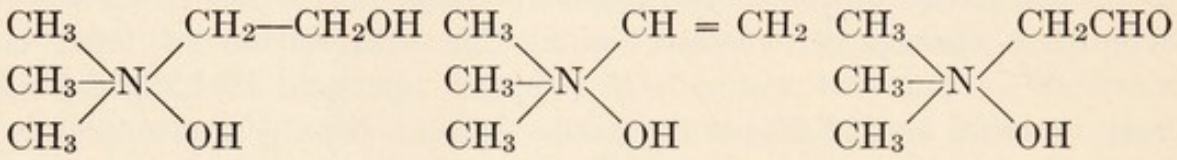
[14] J. Biol. Chem., **53**, 171 (1922).

[15] *Ibid.*, **60**, 261 (1924).

iodine number of 32.7. When this diet was supplemented with 50 grams of cocoanut oil, which has an iodine number of 8.8, the fat excretion was 2.50 grams, and its iodine number was 24.8. When 50 grams of olive oil (iodine number, 88.2) was added to the diet, the feces contained 2.24 grams of fat, having an iodine number of 44.6.

Fecal fat differs in composition from food fat, but resembles closely the lipids of the blood. Approximately one-third of the fecal lipids are unsaponifiable.[16] It has also been shown (Sperry)[17] that nearly all of the fecal lipids are contained in the bodies of bacteria, desquamated epithelial cells, and other cellular structures of the feces.

Intestinal Putrefaction and Auto-intoxication.—The contents of the large intestine undergo bacterial or putrefactive changes. Concerning the putrefaction of fat, little can be said except that it results in the formation of fatty acids and glycerol. From lecithin may be formed choline, neurine, muscarine, and related compounds.



Choline Neurine Muscarine

The carbohydrates yield a variety of substances, including oxalic acid, the lower fatty acids[18] and their derivatives—formic, acetic, propionic, lactic, butyric, oxybutyric, and succinic—acetone, and the gases, carbon dioxide, methane, and hydrogen.

The effects resulting from the intestinal absorption of acids have been recently discussed by E. B. Boldyreff.[19] He found that the introduction into the intestine of acids (hydrochloric and lactic), even in low concentration, was followed by a destruction of red corpuscles. Chronic anemia was produced by the repeated administration of acids. These results are highly suggestive in view of the possibility that a similar effect may result from the absorption of organic acids formed in fermentative and putrefactive processes in the alimentary tract.

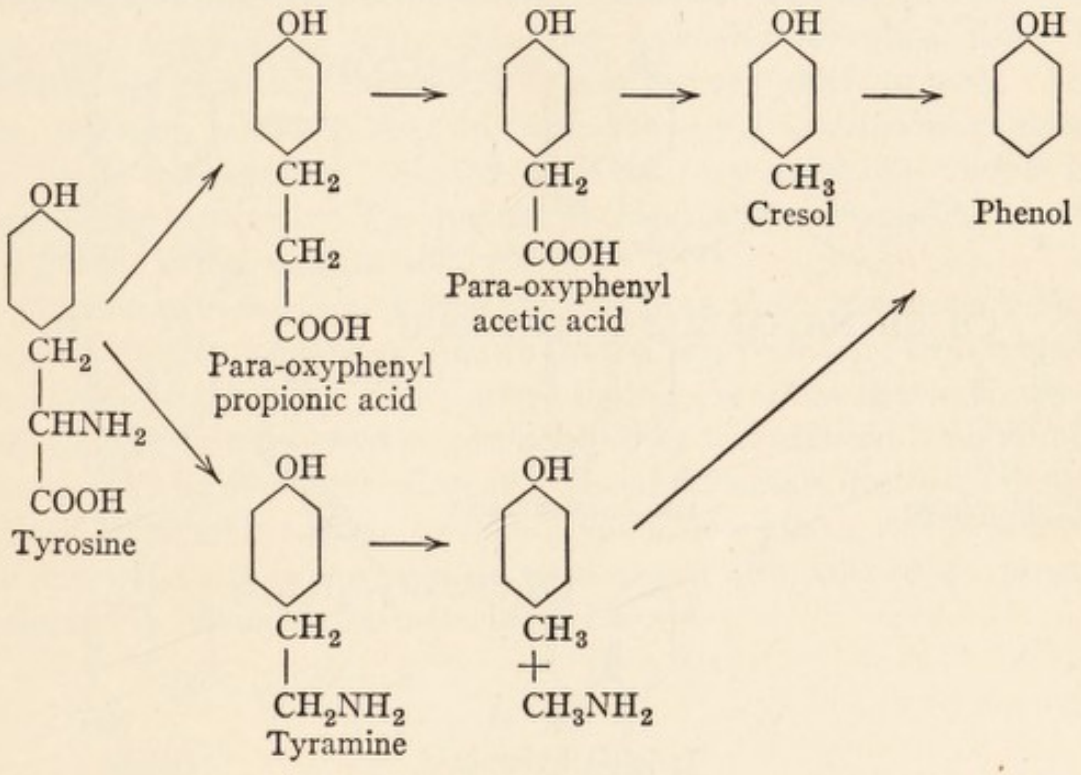
Putrefaction of proteins yields proteoses, peptones, amino acids, ammonia, and hydrogen sulfide. From the aromatic amino acids are formed indole, skatole, phenol, cresol, tyramine, and other substances.

[16] *Ibid.*, **68**, 357 (1926); **71**, 351 (1926–27).
[17] *Ibid.*, **81**, 299 (1929).
[18] Grove, Olmsted and Koenig, J. Biol. Chem., **85**, 127 (1929–30).
[19] Mich. Acad. Sci., **10**, 397 (1928).

Cadaverine, putrescine, ethylidene-diamine are among the toxic amines or ptomaines formed from amino acids in putrefaction. The fate of the fatty-acid radicals of the amino acids is similar to that of other fatty acids. Ethyl mercaptan (C_2H_5SH), methyl mercaptan (CH_3SH), and hydrogen sulfide owe their origin to the putrefaction of cystine.

With regard to the formation of these substances, much remains to be learned. Nevertheless, sufficient is known to enable us to consider briefly the chemistry of the reactions involved. Among the more important of these is one involving the removal of a carboxyl group (decarboxylation), presumably due to an enzyme, carboxylase, present in the bacteria. Another reaction consists in the splitting off of an amino group by deaminization. Reduction, possibly due to a reductase, is likewise of importance. There may also be reactions of hydrolysis as well as of oxidation.

The following substances result from bacterial action on tyrosine:



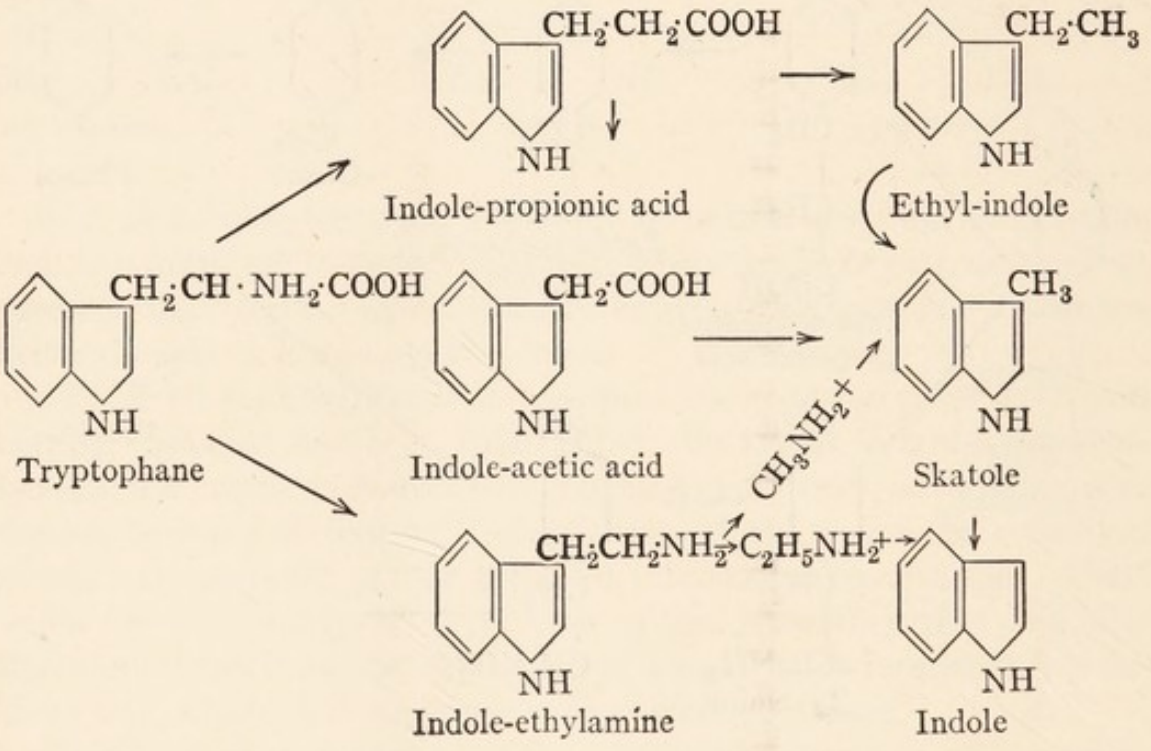
Tyramine is formed in the putrefaction of cheese and is known to be a constituent of certain cheeses—Camembert, Roquefort, Emmenthal, etc.[20] It has likewise been isolated from intestinal contents. Tyramine is a pressor base (raises blood pressure) but is weaker in its action than epinephrine, to which it is closely related chemically. The

[20] F. P. Underhill, The Physiology of the Amino Acids, 1915, New Haven, p. 42.

action of ergot on the uterus is believed to be due in part to the tyramine which it contains.

Cresol, phenol, and probably phenyl-acetic acid, after absorption, are partly conjugated with sulfuric acid and glucuronic acid (with the latter especially in herbivorous animals). Folin and Denis,[21] as well as Dubin,[22] have shown that 30–90 per cent of the phenols (this term is here applied to phenol and its derivatives) are excreted in the urine in the free form, the total amount eliminated usually varying between 200–400 mm. per day. Quantitatively, paracresol is most important. The process of conjugation with sulfuric acid, which takes place in the liver primarily but in other tissues as well, is a mechanism which the organism employs in detoxifying relatively toxic substances.[23] The fate of foreign organic compounds in the animal organism has been discussed recently by Sherwin [24] and will be referred to again.

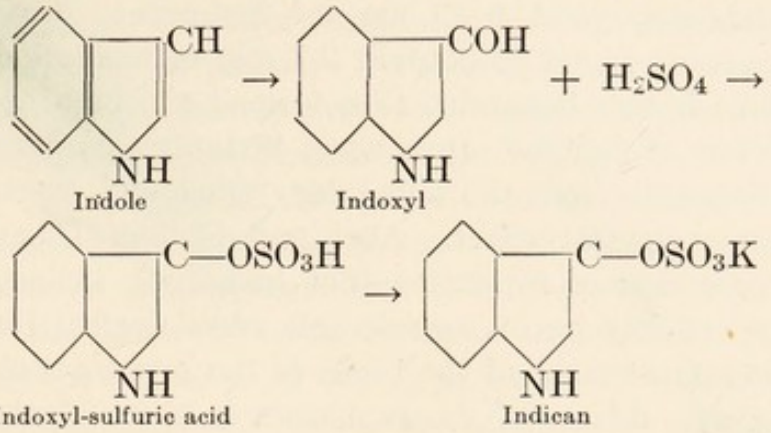
Tryptophane.—The disagreeable and characteristic odor of feces is said to be due largely to two compounds, indole and skatole. These are formed from tryptophane, as indicated by the following formulas:



A portion of the indole is oxidized either before or after absorption and is subsequently conjugated to form the potassium salt of indoxyl-

[21] J. Biol. Chem., **22**, 309 (1915).
[22] J. Biol. Chem., **26**, 69 (1916); **31**, 255 (1917).
[23] Pelkan, K. F., and Whipple, G. H., J. Biol. Chem., **50**, 513 (1922).
[24] Physiol. Reviews, **2**, 238 (1922).

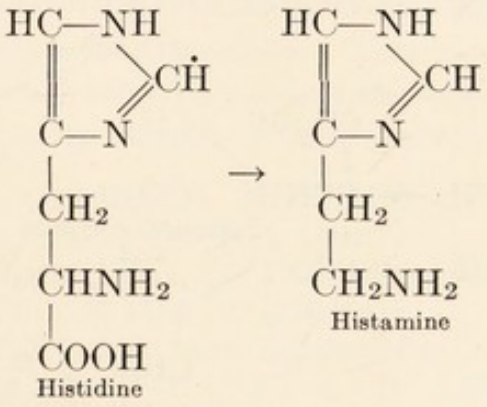
sulfuric acid (indican), in which form it is excreted by the kidneys.



The daily elimination of indican varies considerably, but is usually between 12 and 20 mg. The indole content of the feces averages about 50–60 mg. on an ordinary diet. In carcinoma of the liver, large amounts of indican appear in the urine.

As much as 1 gram of indole may be administered to a dog without producing unusual symptoms. Larger amounts (2 grams) produce diarrhea and hematuria. The quantities normally absorbed from the intestine are probably insufficient to produce any effect in man. Very large amounts, however, are said to produce torpor, feeble heart action, and lower temperature. Skatole behaves very much like indole but is somewhat less toxic. The amount of this substance normally excreted in the urine is less than 10 mg.

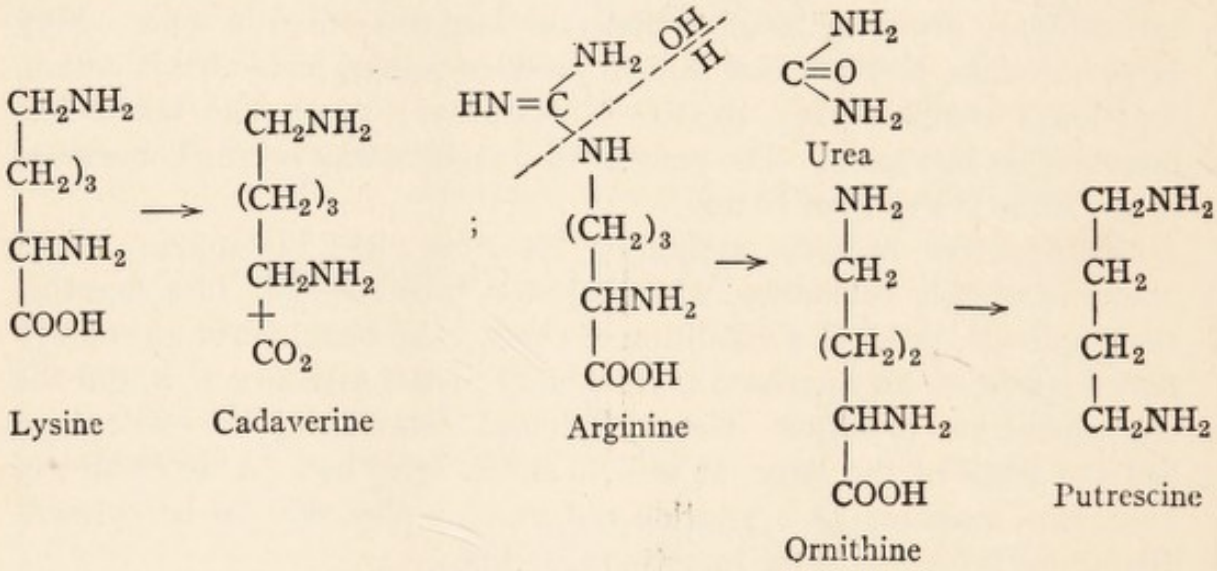
Putrefactive bacteria acting on histidine yield histamine. Small amounts of this substance, when injected intravenously into anesthetized animals, produce a condition of shock; the blood becomes concentrated owing to an increased permeability of the capillary wall and the consequent loss of water. Gerard [25] found histamine in the contents of isolated loops of the large, as well as small, intestine. A derivative of histamine, possibly of a peptide nature was also said to be present. Histamine is formed in the intestine as follows:



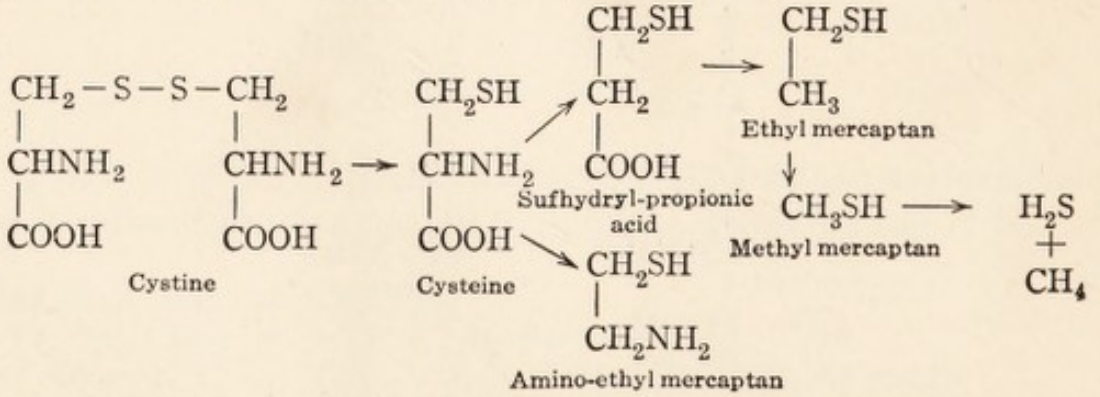
[25] J. Biol. Chem., **52**, 111 (1922).

Hanke and Koessler [26] report that human feces, 500–600 grams, from normal individuals, yield 6–20 mg. of histamine. Cæcal contents, 600–1200 cc., were found to contain 2.7 mg. of histamine. However, it is doubtful whether histamine thus formed can be a cause of intoxication, in view of the fact that when histamine is administered by mouth it disappears from the alimentary tract and exerts no demonstrable pharmacological effect. Abel and Geiling [27] have suggested that some precursor of histamine (not histidine), instead of being a poison, may actually be of considerable physiological importance by aiding in the maintenance of the tonus of the intestinal muscle as well as by acting as a dilator of the capillaries in those places where more blood is needed for digestion.

Lysine yields a diamine, cadaverine (pentamethylene-diamine), first discovered in human cadavers. A closely related substance is the compound putrescine (tetramethylene-diamine), derived from arginine. Both of these have been found in the urine in two pathological conditions, cystinuria and cholera. Their formation may be represented as follows:



Cystine undergoes the following changes when acted upon by bacteria:



[26] *Ibid.*, **59**, 879, 889 (1924).

[27] J. Pharmacology and Exp. Therapeutics, **23**, 1 (1924).

It is a popular belief that the products of intestinal putrefaction are responsible for many ills, as well as for the symptoms associated with constipation—headache, malaise, irritability, nausea, insomnia, drowsiness, etc. Actually, there is very little experimental evidence to support this view, as has been shown recently by Alvarez in reviewing the literature on the subject of intestinal auto-intoxication. Very little absorption takes place from the colon, especially when the feces are hard. If auto-intoxication were due to the absorption of toxic substances from the colon it would be more frequent in diarrhea than otherwise. Alvarez [28] ascribes the symptoms of constipation to the mere plugging of the lower end of the alimentary canal. A plug of cotton introduced into the rectum produces the same effect. He has also found that his patients with jejunal fistulas become sleepy when the intestine is made to contract on a small balloon inserted through the fistula. Muscular activity and nervous stimuli arising in the digestive tract are probably important factors in producing the symptoms ordinarily associated with constipation. Metchnikoff's well-known views concerning the relation between auto-intoxication and arteriosclerosis and senility, presented in his book, " The Prolongation of Life," have little, if any, experimental evidence to support them.

Intestinal Obstruction.—Occlusion of the intestine results in severe toxic symptoms, anorexia, weakness, profound depression, oliguria, continuous vomiting, sometimes muscular twitching and tetany, etc. The rapid onset, severity and usually fatal outcome have led to two views as to the cause of the toxemia, one being that it is due to a toxic agent formed above the point of obstruction and the other that the noxious substance has its origin in the mucosa of the intestine or stomach. However, a closer study of the problem has revealed totally different factors to which the symptoms may be attributed. In the first place, it seems that the intestinal contents in obstruction are not more toxic than normal intestinal contents.[29] Then it has been observed that in pyloric obstruction,[30] or in obstruction of the duodenum or upper ileum,[31] there are definite chemical changes in the blood. These include a striking fall in the concentration of chloride, a marked increase in the alkali reserve (carbon dioxide-combining capacity), and a terminal increase in non-

[28] Physiol. Reviews, **4**, 352 (1924); see also W. C. Alvarez, "The Mechanics of the Digestive Tract," Second Edition, New York, 1928.

[29] Wangensteen, O. H., and Chunn, S. S., Arch. Surgery, **16**, 606 (1928); Wangensteen, O. H., and Waldron, G. W., *ibid.*, **17**, 430 (1928).

[30] MacCallum, W. G., *et al.*, Bull. Johns Hopkins Hospital, **31**, 1 (1920); Hastings, A. B., Murray, C. D., and Murray, H. A., J. Biol. Chem., **46**, 223 (1921).

[31] Haden, R. L., and Orr, T. G., J. Exp. Med., **37**, 377 (1923); *ibid.*, **49**, 955 (1929); J. Am. Med. Assoc., **91**, 1529 (1928); Foster, W. C., *ibid.*, **91**, 1523 (1928); McIver M. A., and Gamble, J. L., *ibid.*, 1589 (1928).

protein nitrogen. Considerable evidence has accumulated in the last few years to indicate that the depletion of chloride and water, due to the copious vomiting, and the accompanying derangement of the acid-base balance are chiefly responsible for the symptoms in acute intestinal obstruction and possibly are also factors in other conditions where excessive loss of gastric juice occurs.

A crucial experiment demonstrating the effects of total loss of gastric juice is that of Dragstedt and Ellis.[32] These workers isolated the stomach of dogs by section at the cardia and pylorus. The duodenum was anastomosed to the lower end of the esophagus. The cardiac end of the isolated stomach was closed and the pyloric end brought to the surface as a fistula. Precautions were taken not to interfere with the vagus nerves or blood vessels supplying the isolated stomach. The total loss of the gastric secretion which was drained away through the fistula resulted in symptoms of weakness, anorexia, loss of weight (chiefly because of anhydremia), oliguria and profound depression. Death occurred in five to eight days. Accompanying these symptoms and proportionate to their intensity the following changes were noted in the blood: decrease in concentration of chloride, the values ranging between 340–108 mg. per 100 cc., an increase of the CO_2-combining capacity, reaching a value as high as 140 cc.; an increase in *p*H (7.3–7.75), and a terminal increase in non-protein nitrogen. Dragstedt was able to relieve the symptoms, restore the blood constituents toward the normal and prolong the lives of the animals (in one case to over 76 days) simply by intravenous injection of Ringer's solution. It was therefore concluded that while food deprivation was one of the main effects of this experimental procedure, the symptoms and fatal outcome could be attributed to hypochloremia, alkalosis and dehydration.

[32] Am. J. Physiol, **90**, 331 (1929).

CHAPTER VIII

THE BLOOD AND LYMPH

In the last chapter we considered the mechanism whereby the food material enters the blood and lymph. We shall now consider how the absorbed material reaches the tissues. Together with the endothelium, which lines the arteries, veins, and capillaries, the blood may be regarded as a tissue of somewhat greater fluidity than other tissues. The lymph, according to Starling, is derived from the blood by a process of passive filtration. It resembles blood plasma in chemical composition but contains in addition a large number of lymphocytes. A portion of the lymph is contained in lymph vessels or lymphatics and is thus carried about the body, occasionally passing through lymph nodes or glands which act as filters in removing and destroying foreign material, such as bacteria. Situated near the junction of the internal jugular and left subclavian veins is the thoracic duct through which the lymph gains entrance into the blood. The blood itself is usually not in direct contact with the tissue cells; on the other hand, the lymph bathes the tissues and is in immediate contact with the cells, so that, in the exchange of material between the blood and tissues, the material must pass through the lymph.

Functions of the Blood.—The functions of the blood may be summarized as follows:

1. The blood transports food material and other substances absorbed from the intestine to the tissues.
2. It is concerned with the transportation of oxygen from the lungs to the tissues.
3. The waste products formed in metabolism are carried by the blood to the organs of excretion—kidneys, lungs, intestine, and skin.
4. The blood is the channel for the exchange of products formed in one tissue or organ and used by another. The hormones are transported in the blood.
5. The white corpuscles of the blood constitute a defense mechanism against invading bacteria.
6. The blood takes part in maintaining the temperature of the

body at a fairly constant level. It also aids in the maintenance of the normal reaction of the tissues.

Volume.—The quantity of blood in the body has been estimated to be about 8.8 per cent, or between one-eleventh and one-twelfth, of the body weight. There is little variation from this value normally, but in pathological conditions changes in either direction may occur. Thus an increase in volume (plethora) occurs in polycythemia, in chlorosis, and, at times, in anemia. Concentration of the blood is especially likely to occur in infants with diarrhea when the loss of fluid is not made up. Animals deprived of water become somewhat " desiccated." In histamine shock, the blood volume is reduced owing to the passage of fluid through the walls of the capillaries, which the histamine renders more permeable. The cellular elements of the blood become more concentrated in influenza, following the inhalation of war gases,[1] following severe burns, and in intestinal obstruction. The term anhydremia has been applied to the condition in which the concentration of the blood is greater than normal. For a review of the literature on the subject, the student is referred to the article by W. McKim Marriott.[2]

Specific Gravity.—The specific gravity of whole blood usually varies between 1.054 and 1.060. In new-born infants, the values are somewhat higher, about 1.066. The plasma has a specific gravity of 1.0237–1.0276, and the corpuscles of approximately 1.088.

Formed Elements, Plasma, Serum.—The formed elements are the red corpuscles or erythrocytes, the white corpuscles or leucocytes, the blood platelets, and the so-called blood dust or hemoconein. Together these constitute 30–40 per cent by volume of the whole blood, the remainder being occupied by the plasma. In man, there are normally 5,000,000 red cells per cubic millimeter of blood; the count is somewhat lower in women. Variations occur frequently, especially after exercise or a heavy meal, or at high altitudes. Except in camels, which have elliptical corpuscles, the shape of the mammalian corpuscle is that of a circular, non-nucleated, biconcave disk. The average diameter usually given is 7.7μ, a value obtained by examining dried preparations of blood and considered by Ponder [3] to be too low. Ponder's own observations, made on red cells in the fresh state, show the human corpuscle to have an average diameter of about 8.8μ. When circulating in the blood vessels, the red cell does not maintain a fixed shape but changes its

[1] F. P. Underhill, The Lethal War Gases, Physiology and Experimental Treatment, New Haven, 1920.

[2] Physiol. Reviews, **3**, 275 (1923).

[3] The Erythrocyte and the Action of the Simple Hemolysins (1924), p. 21.

form continually, especially in the small capillaries. The red blood corpuscles are continually undergoing destruction, new corpuscles being formed to replace them.[4] The average life of red corpuscles has been estimated by various investigators to be between three and six weeks. Preceding destruction, changes in the composition of the cells are believed to occur which render them less resistant. To a large extent, the corpuscles are taken up by the phagocytic cells of the reticulo-endothelial system, such as the Kupffer cells of the liver. In the process of destruction, the lipids of the membrane are dissolved and the hemoglobin which is liberated is the most important, though probably not the only, source of bilirubin. Formerly the liver was believed to be the only site of red cell disintegration. This view is no longer generally held, for it seems that the destruction of corpuscles may occur in the blood stream, as well as in other tissues.

The leucocytes, of which there are several forms, usually number between 7000 and 15,000 per cubic millimeter. These increase in number in disease, particularly when there is bacterial infection. Considerable variation in the number of platelets occurs frequently, the usual number being about 300,000 per cubic millimeter.

If blood, after being removed from the blood vessels, is allowed to stand, it soon forms a clot in which the cellular elements of the blood are enmeshed. Clotting may be prevented by the addition of hirudin or of a soluble oxalate, citrate, or fluoride. Clot formation is due essentially to the conversion of the soluble protein, fibrinogen, into the insoluble protein, fibrin. Calcium is needed for this process. If the effect of the calcium is removed by its combination with an oxalate or citrate radical, the blood remains fluid. Blood treated in this way is said to be oxalated or citrated.

The formed elements may be separated from the plasma by centrifuging the whole blood.

When allowed to stand, a blood clot eventually shrinks; in the process of shrinking, a pale yellow liquid, the serum, is expressed. This phenomenon of shrinking is exhibited by other colloidal gels and is termed syneresis. Serum is blood from which the corpuscles and fibrinogen have been removed. Plasma is whole blood from which the corpuscles have been removed; it differs from serum in that it contains fibrinogen.

The fibrin may be removed in the form of stringy masses by rapidly stirring freshly drawn blood with a rod or some other contrivance. As a result defibrinated or whipped blood is obtained.

[4] A recent review on the subject of red cell regeneration is that of F. S. Robscheit-Robbins, Physiol. Reviews, **9**, 666 (1929).

Hemolysis.—Erythrocytes undergo hemolysis, or laking, when blood is diluted with water or treated with ether, chloroform, soap, fatty acids, ultra-violet rays, bile acids, snake venom, specific hemolysins, and other substances. Sometimes partial hemolysis is due to the disruption of erythrocytes by purely mechanical influences. When placed in a hypotonic solution, the red corpuscle swells, thus stretching the membrane. If the stretching is sufficient, the membrane bursts. There is some reason to believe that the distended membrane is more permeable than the normal membrane. Under these conditions, the hemoglobin may pass out of the corpuscle into the plasma. However, it does not seem that the red cell undergoes complete destruction at once, for there remain behind the so-called "ghosts." From their appearance, it seems possible that the "ghosts," are made up of the stroma, or network, of the original corpuscles. The disintegration of the stroma, or stromatolysis, probably follows hemolysis when red corpuscles are subjected to the action of hemolytic agents.

Composition.—Quantitatively, water is the most important constituent. The plasma of mammalian blood, including that of man, contains 90–92 per cent of water; the red corpuscles, 64–65 per cent. The framework and membrane of the red cell varies somewhat in composition in different animals; but, in all, the lipids and proteins are the most important constituents. Chief among the lipids are lecithin and cholesterol; of the proteins, the most significant are hemoglobin, a globulin, and, in certain animals (cat, rabbit), a nucleoprotein. The lipids may exist partly in combination with protein as lipoprotein. An idea of the character of the blood constituents in various domestic mammals may be gathered from the accompanying table of data (Table XXX) taken from Abderhalden. While these data are based on analytical results obtained before the advent of modern methods of blood analysis, they are essentially accurate and agree closely with more recent determinations where such have been made.

A discussion of the chemical constituents of the blood without reference to their physiological significance would have little meaning. The composition of the blood is so intimately tied up with its properties and varied functions that we are forced to approach the subject from several angles. In the first place, it would be desirable to correlate the chemical composition of the formed elements, particularly of the red corpuscles, with their structure and properties. Thus the amount of lecithin may be related to the maintenance of the normal permeability of the corpuscles; there is some evidence that an increase in the lecithin content of corpuscles increases their permeability. The influence of cholesterol can be studied in like manner. Any consideration of hemo-

globin must obviously include a discussion of its relation to the transportation of oxygen, the buffer mechanism of the blood, and the transportation of carbon dioxide. The inorganic constituents, as well as the plasma proteins, have a bearing on the buffer mechanism, osmotic equilibrium, and other properties of the blood.

We must also consider such constituents as glucose, amino acids, fats and other lipids, as well as oxygen and a certain proportion of the inorganic salts of the blood that are of nutrient value to the organism and are in transit to the tissues. In the normal individual, the amount of glucose in the blood is usually stated to be about 0.09–0.11 per cent. A man weighing 70 kg. would therefore have about 6 grams of sugar in circulation. When moderate amounts of carbohydrate are ingested, the sugar content of the blood in the portal vein increases appreciably, but in the systemic circulation little change is noted, owing to the rapid removal of excessive amounts of sugar by the liver and other tissues. However, when more than moderate amounts of sugar are taken—50 to 100 grams—the sugar in the blood may increase to as much as 0.15 per cent or even higher (see p. 269). The increase occurs during the first hour and is soon followed by a return of the blood sugar to normal levels. Sugar is an essential constituent of the blood; when it is reduced below a certain level (0.045 per cent), life processes are impaired.

The methods for determining blood sugar are based on the reducing action of glucose, under rigorously controlled conditions, on such regents as an alkaline solution of copper tartrate. Several methods in common use give somewhat different results. Actually, the results obtained by such methods are a measure, not only of the amount of glucose, but of other reducing substances as well. In order to differentiate between the so-called " true sugar " of the blood and other reducing constituents, especially non-sugars, advantage is taken of the fact that glucose is readily fermentable by yeast. Suitably prepared blood filtrates are analyzed before and after being subjected to the action of yeast, and in this way values are obtained for the total reducing substances (or apparent sugar) of the blood and for the non-sugar reducing substances. The difference between the two represents the concentration of glucose. According to Somogyi,[5] the amount of reducing non-sugars of the blood is very uniform, averaging 27 mg. per 100 cc. (expressed in terms of glucose). These reducing substances are unevenly distributed between the plasma and corpuscles, the concentration in the plasma averaging somewhat less than 10 mg., and the concentration in the corpuscles being about 40 mg. per 100 cc. The concentration of " true

[5] J. Biol. Chem., **75**, 33 (1927); **78**, 117 (1928).

TABLE XXX

I. 1000 Parts by Weight

	Cow	Bull	Sheep I	Sheep II
Water	808.9	814.8	821.7	824.6
Total solids	191.1	185.2	178.3	175.5
Hemoglobin	103.1	106.4	92.9	102.8
*Albumin	69.80	61.79	70.85	58.66
Sugar	0.7	0.68	0.732	0.708
Cholesterol	1.935	1.209	1.332	2.038
Lecithin	2.349	2.197	2.220	2.417
Fat	0.567	2.363	0.937	0.864
Fatty acids	.....	0.495	0.488	0.490
Phosphoric acid as nucleic acid	0.0267	0.0283	0.0285	0.0344
Soda	3.635	3.712	3.638	3.677
Potash	0.407	0.407	0.405	0.408
Ferric oxide	0.544	0.562	0.492	0.545
Lime	0.069	0.064	0.070	0.069
Magnesia	0.0356	0.036	0.033	0.033
Chlorine	3.079	3.081	3.080	3.091
Phosphoric acid in total ash	0.404	0.392	0.412	0.391
Inorganic phosphoric acid	0.171	0.174	0.190	0.145

II. 1000 Parts by Weight

Water	913.6	913.4	917.4	916.8
Total solids	86.36	86.62	82.56	83.19
Albumin	72.5	69.73	67.50	68.40
Sugar	1.05	1.02	1.06	1.04
Cholesterol	1.238	0.901	0.879	1.309
Lecithin	1.675	1.869	1.709	1.599
Fat	0.926	3.542	1.352	1.262
Fatty acids	.....	0.743	0.710	0.721
Phosphoric acid as nucleic acid	0.0133	0.0134	0.0106	0.0161
Soda	4.312	4.316	4.303	4.285
Potash	0.255	0.262	0.256	0.254
Ferric oxide	.....	.....	.....	.....
Lime	0.119	0.111	0.117	0.131
Magnesia	0.045	0.042	0.041	0.041
Chlorine	3.69	3.686	3.711	3.697
Phosphoric acid in ash	0.244	0.235	0.232	0.240
Inorganic phosphoric acid	0.085	0.062	0.073	0.085

III. 1000 Parts by Weight

Water	591.86	618.63	604.79	627.78
Total solids	408.14	381.39	395.23	372.24
Hemoglobin	316.74	318.27	303.29	322.05
Albumin	64.20	46.00	78.45	37.90
Sugar	.....	.....	.....	.....
Cholesterol	3.379	1.824	2.360	3.593
Lecithin	3.748	2.850	3.379	4.163
Fat	.....	.....	.....	.....
Fatty acids	.....	.....	.....	.....
Phosphoric acid as nucleic acid	0.0546	0.0580	0.069	0.0736
Soda	2.232	2.509	2.135	2.380
Potash	0.722	0.696	0.744	0.739
Ferric oxide	1.671	1.681	1.606	1.707
Lime	.....	.....	.....	.....
Magnesia	0.0172	0.026	0.016	0.0187
Chlorine	1.813	1.878	1.651	1.801
Phosphoric acid in ash	0.735	0.705	0.822	0.714
Inorganic phosphoric acid	0.350	0.397	0.455	0.275

* Albumin (Eiweiss) is used here in the sense of protein other than hemoglobin. It obviously includes not only the albumins of the blood but the globulins as well. These data are taken from

(AFTER ABDERHALDEN)

OF BLOOD CONTAIN

Goat	Horse I	Horse II	Pig	Rabbit	Dog I	Dog II	Cat
803.9	749.0	795.0	790.6	816.9	810.1	792.0	795.5
196.1	251.0	205.0	209.4	183.1	190.0	208.0	204.5
112.6	166.9	125.8	142.2	123.5	133.4	145.6	143.2
69.72	69.7	62.70	46.61	25.02	39.68	36.41	44.78
0.829	0.526	0.900	0.686	1.026	1.09	0.72	0.851
1.299	0.346	0.576	0.444	0.611	1.298	0.922	0.895
2.466	2.913	2.982	2.309	2.827	2.052	1.994	2.325
0.535	0.611	0.534	1.095	0.734	0.631	0.914	0.373
0.395	.....	0.387	0.475	0.507	0.759	0.684	0.280
0.039	0.060	0.059	0.058	0.055	0.054	0.054	0.072
3.579	2.691	2.630	2.406	2.785	3.675	3.657	3.686
0.396	2.738	1.475	2.309	2.108	0.251	0.258	0.260
0.547	0.828	0.592	0.696	0.615	0.641	0.706	0.694
0.066	0.051	0.054	0.068	0.072	0.062	0.049	0.053
0.040	0.064	0.066	0.089	0.057	0.052	0.054	0.059
2.923	2.785	2.384	2.690	2.898	2.935	2.908	2.815
0.397	1.120	1.126	1.007	0.986	0.809	0.812	0.830
0.142	0.806	0.807	0.749	0.685	0.576	0.583	0.555
OF SERUM CONTAIN							
907.7	902.1	915.1	917.6	925.6	924.0	923.0	926.9
92.31	97.95	84.94	82.39	74.40	76.02	76.98	73.07
78.07	84.24	70.82	67.74	53.57	60.14	61.12	58.60
1.26	1.176	1.49	1.21	1.65	1.83	1.32	1.52
1.070	0.298	0.521	0.409	0.547	0.709	0.658	0.600
1.727	1.720	1.746	1.426	1.760	1.699	1.755	1.716
0.624	1.300	0.834	1.956	1.193	1.051	1.642	0.788
0.611	.....	0.604	0.794	0.809	1.221	1.254	0.499
0.018	0.020	0.015	0.0218	0.025	0.016	0.017	0.016
4.326	4.434	4.358	4.251	4.442	4.263	4.293	4.439
0.246	0.263	0.254	0.270	0.259	0.226	0.259	0.262
.....	.....	.....	.....	.....	.....	.....	.....
0.121	0.111	0.111	0.122	0.116	0.113	0.111	0.110
0.041	0.045	0.046	0.041	0.046	0.040	0.046	0.043
3.691	3.726	3.655	3.627	3.883	4.023	4.138	4.170
0.237	0.240	0.242	0.197	0.242	0.242	0.250	0.236
0.070	0.071	0.076	0.052	0.064	0.080	0.082	0.071
OF BLOOD CORPUSCLES CONTAIN							
608.72	613.15	613.20	625.61	633.53	644.26	627.16	624.17
391.30	386.84	386.82	374.38	366.48	355.75	372.85	375.82
324.02	315.08	316.31	326.82	331.90	327.52	328.81	329.95
54.03	56.78	50.41	19.19	12.22	9.918	5.32	26.77
.....	.....	.....	.....	.....	.....	.....	.....
1.730	0.388	0.661	0.489	0.720	2.155	1.255	1.281
3.856	3.973	4.855	3.456	4.627	2.568	2.296	3.119
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	0.0603	0.062	.....	0.088	.....	.....
0.0806	0.095	0.125	0.1045	0.107	0.110	0.101	0.145
2.174	.....	.....	.....	.....	2.821	2.856	2.705
0.679	4.935	3.326	4.957	5.229	0.289	0.257	0.258
1.575	1.563	1.488	1.599	1.652	1.573	1.594	1.599
.....	.....	.....	.....	.....	.....	.....	.....
0.0403	0.0809	0.098	0.150	0.077	0.071	0.065	0.0806
1.480	1.949	1.460	1.475	1.236	1.352	1.361	1.048
0.699	1.901	2.466	2.058	2.244	1.635	1.519	1.605
0.279	1.458	1.916	1.653	1.733	1.298	1.214	1.186

E. Abderhalden's Physiological Chemistry, trans. by W. T. Hall and G. Defren, John Wiley and Sons, 1911 edition, pp. 554–5.

sugar" in the blood, according to the data given by Somogyi is frequently less than 90 mg. per 100 cc.

Mention should also be made of the probable occurrence of hydrolyzable sugars in the blood. The analyses of Everett and Sheppard [6] indicate the presence of about 3 mg. of such carbohydrates per 100 cc. of plasma and about 10 mg. per 100 cc. of corpuscles.

The significance of data for blood sugar should therefore be clearly understood. Glucose is not the only reducing substance present in the blood, although this sugar is undoubtedly predominant. In the present discussion the point to be especially emphasized is that the glucose of the blood is on its way to the liver, muscles and other tissues, either to be stored or oxidized. The blood going to the tissues (arterial blood) normally contains more sugar than the blood returning from the tissues (venous blood) as shown by the work of Foster [7] and others.

The amino acids of the blood are also to be regarded as nutrient material *en route* to the tissues. During digestion of a protein-rich meal, there is a very marked increase in the amino-acid nitrogen of the blood, as has been shown by György and Zunz,[8] Van Slyke and Meyer,[9] and other workers. In the work of György and Zunz, the distribution of amino acids between the plasma and the corpuscles was studied before and four hours after the ingestion of raw beef. Dogs were used as the experimental animals. The results of one of these experiments are given below and show the marked increase in the amino-acid nitrogen both in the plasma and in the corpuscles.

TABLE XXXI

AMINO-ACID N PER 100 CC. OF CAROTID BLOOD

	Whole Blood	Plasma	Corpuscles
Three to four hours after bleeding....	6.1	2.1	4.0
Four hours after bleeding followed immediately by ingestion of raw beef.	11.6	3.8	8.0

Van Slyke and Meyer [9] found the blood of fasting dogs to contain 3–5 mg. of amino-acid nitrogen in 100 cc. In one of their experiments, 12 grams of alanine were injected, the injection lasting thirteen minutes.

[6] J. Biol. Chem., **80**, 255 (1928).

[7] *Ibid.*, **55**, 303 (1923); see also Friedenson, Rosenbaum, Thalheimer and Peters, *ibid.*, **80**, 269 (1928).

[8] J. Biol. Chem., **21**, 511 (1915).

[9] *Ibid.*, **12**, 399 (1912).

Five minutes after the injection was over, the blood was analyzed. Only 1.5 grams of the alanine could be accounted for at this time. At the end of thirty-five minutes only 0.4 gram remained. During this interval 1.5 grams of the amino acid were excreted. These observations show that the tissues are capable of removing amino acids with great rapidity.

In a series of 20 analyses of the blood of normal individuals, Greene, Sandiford and Ross [10] found the amino acid nitrogen to vary between 5.2 and 7.2 mg. per 100 cc. Approximately the same results were obtained in a series of more than 400 observations on individuals suffering from various pathological conditions, the concentrations varying between 4.8 and 7.8 mg. per 100 cc. of whole blood. The concentration of amino acids is greater in the corpuscles than in the plasma.

Several hours after the intake of a meal rich in fat, the fat and fatty-acid content of the blood increases considerably, the plasma, when separated, often presenting a milky appearance due to fat globules in emulsion. The lecithin and cholesterol esters of the blood are likewise known to increase. Bloor [11] has suggested that lecithin, and possibly cholesterol, take part in the transportation of fat to the tissues, a view that is being substantiated by other workers. We see, therefore, that lecithin has at least a double significance in the blood, being connected, on the one hand, with the permeability and structure of the lipid membrane of the red corpuscles, and, on the other, with fat transport and metabolism.

The amount of phospholipid in human blood is approximately 0.3 per cent. More is present in the corpuscles than in the plasma. Cholesterol, on the other hand, is approximately evenly distributed between the plasma and corpuscles. The concentration in whole blood usually varies between 0.14 and 0.17 per cent. In the plasma it occurs chiefly in combination with fatty acids, as cholesterol esters, whereas in the corpuscles it is present almost entirely as free cholesterol. The fats are likewise about equally distributed between the plasma and corpuscles, the concentration in whole blood being usually between 0.3 and 0.45 per cent.

Many of the blood constituents may be considered as waste products of metabolism, in transit from the tissues to the organs of excretion. Blood contains approximately 45 to 65 volumes per cent of carbon dioxide. The total non-protein nitrogen of the blood, which, as the term implies, represents all the nitrogenous constituents of the blood, except the proteins, varies normally between 30 and 35 mg. per 100 cc. Of this,

[10] J. Biol. Chem., **58**, 845 (1923–24); see also Blau, *ibid.*, **56**, 861 (1923).

[11] Physiol. Reviews, **2**, 92 (1922).

about 14 to 20 mg., or roughly 50 to 60 per cent, represents urea nitrogen. The amount of urea in the blood is therefore approximately 30 to 40 mg. per 100 cc. Blood of normal individuals contains 2 to 3 mg. of uric acid, 1 to 2 mg. of creatinine, 3 to 6 mg. of creatine (which is not to be regarded as a waste product necessarily), an exceedingly small amount of ammonia (about 0.1 mg. per 100 cc.) and small amounts of other nitrogenous constituents, constituting the "undetermined nitrogen" fraction. Wu[12] has made a study of the distribution of the non-protein nitrogenous and other constituents of the blood in man. The following average results were obtained for twenty individuals:

TABLE XXXII

	Corpuscles, Mg. per 100 cc.	Plasma, Mg. per 100 cc.
Total non-protein N	49.3	28.8
Urea N	17.1	19.3
Uric acid	1.93	3.92
Creatine + creatinine	8.32	1.47
Pre-formed creatinine	2.48	1.24
Amino-acid N	9.47	5.52
Sugar	99.9	103.2
NaCl	309.7	615.2

The chemistry of the blood is of much importance in relation to intermediary metabolism and the functional activity of the organs of excretion, notably the kidneys. In diabetes, owing to a derangement in carbohydrate metabolism, the sugar in the blood may increase considerably. More than normal amounts of uric acid are formed and excreted when there is excessive metabolism of glandular material. Retention of the non-protein nitrogenous constituents of the blood occurs in many forms of nephritis, a condition in which the kidneys are damaged. The detailed study of certain of these substances is deferred for the reason that their significance will be more fully appreciated after something has been said of their origin. This will be discussed in the chapters on Intermediary Metabolism.

A newly discovered constituent of the blood is the sulfur-containing compound, ergothioneine, or betaine thiolhistidine (p. 342).

Inorganic Constituents.—This part of the discussion would not be complete without reference to the inorganic constituents of human blood.

[12] J. Biol. Chem., **51**, 21 (1922).

The values given below are taken from a recent review of Myers,[13] and give one an idea of the normal ranges as well as of pathological variations.

TABLE XXXIII

MINERAL CONSTITUENTS OF BLOOD

Constituents	Normal		Pathological Range, Mg. to 100 cc.
	Range, Mg. to 100 cc.	Average, Mg. to 100 cc.	
Sodium (serum) as Na	320–350	335	220–460
Potassium (serum) as K	18–22	20	10–35
Potassium (whole blood) as K	150–250	200	50–400
Calcium (serum) as Ca	9–11	10	2–12
Magnesium (serum) as Mg	1.5–3	2.5	1–4
Iron	.....	50	15–70
Chlorides (plasma) as Cl	340–370	360	300–510
Chlorides (plasma) as NaCl	570–620	500	500–850
Chlorides (whole blood) as NaCl	450–520	480	120–700
Phosphorus as P:			
Inorganic (serum)	2.5–5.5	3.5	1–40
Lipid (plasma)	5–12	7.5	
Organic (corpuscles)	40–75	53	
Sulfates as S	0.5–0.9	0.5	0.4–16

Many of the variations noted above are due to differences in the relative amounts of plasma and corpuscles. Certain other points of interest may be brought out in this connection. In man the blood sodium is found almost entirely in the plasma, whereas the potassium is present in the corpuscles. Kramer and Tisdall [14] analyzed the blood of thirteen adults and obtained an average value of 428 mg. of potassium per 100 cc. of corpuscles. Sodium represents about 92 per cent of the fixed base of serum, potassium practically all that of the corpuscles. This does not apply, however, to the blood of the cat and dog, in which sodium instead of potassium is present in greater abundance in the corpuscles. Calcium is contained almost entirely in the plasma and is reduced following the removal of the parathyroids in animals, the reduction being accompanied by the development of tetany. The calcium content of the blood is restored to normal levels and tetany is relieved following the administration of the parathyroid hormone to parathyroidectomized animals

[13] Physiol. Reviews, **4**, 274 (1924).

[14] J. Biol. Chem., **53**, 241 (1922).

(Collip).[15] In advanced nephritis, the amount of calcium in the blood is low (Marriott and Howland,[16] Denis and Hobson,[17] Schmitz, Rohdenburg, and Myers).[13] Calculated as NaCl, the chloride content of whole blood normally varies between 0.45 and 0.50 per cent; for the plasma the values are somewhat higher, 0.57–0.62 per cent. From 2.5 to 5.5 mg. per 100 cc. of inorganic phosphorus is normally present in the serum, the lower values in adults and the higher values in children. Of the abnormal variations noted, the most pronounced occur in severe nephritis, apparently as a result of the retention of the acid phosphates. Inorganic sulfates likewise accumulate in the blood in nephritis, as shown by Denis,[18] who found values 3000 per cent above normal in some cases.

Blood Proteins.—The total solids of the blood of various domestic animals, as indicated by Abderhalden's data (Table XXX), amount to 17.55–25.10 per cent. Values of similar magnitude, 19–23 per cent, have been obtained for human blood. Of these amounts, all but about 1.5–3.0 per cent consists of protein.

Human blood contains, per 100 cc., about 13–16 grams of hemoglobin, all of which is in the corpuscles, where usually its content is approximately 32 per cent. The most important change in composition occurs in various types of anemia, where the reduction in hemoblogin is very marked. It may or may not parallel the decrease in the number of erythrocytes. In pernicious anemia, for example, the diminution in the red cell count is usually greater than the reduction in hemoglobin, so that the hemoglobin content of the average corpuscle is somewhat greater than normal. On the other hand, in the secondary anemias, the hemoglobin may be reduced to a much greater extent than the number of corpuscles.

Besides hemoglobin, the red blood corpuscles contain small amounts (about 1 per cent or less) of other proteins. These vary considerably in amount in different animals and under different conditions. The protein content of the plasma is about 7 per cent. The plasma proteins will be considered presently.

Hemoglobin.—Hemoglobin is the red pigment of the blood and belongs to the group of conjugated proteins. One of the most characteristic properties of this compound is its power of combining with oxygen to form oxyhemoglobin, a substance which is readily dissociated when exposed to an environment of low oxygen tension. The iron content of hemoglobin is practically the same for many species of animals

[15] J. Biol. Chem., **63**, 395 (1925).
[16] Arch. Intern. Med., **18**, 708 (1916).
[17] J. Biol. Chem., **55**, 183 (1923).
[18] J. Biol. Chem., **49**, 311 (1921).

and amounts to about 0.0335 per cent. On the assumption that the hemoglobin molecule contains at least one atom of Fe, the minimum molecular weight has been calculated to be 16,669, while the empirical formula, $C_{759}H_{1208}N_{210}S_2FeO_{204}$ has been assigned to the compound. However, it is almost certain that the hemoglobin molecule contains not one but four atoms of iron. The osmotic pressure determinations of Adair [19] have led to the value of 68,000, or four times the minimal molecular weight. Confirmation of this value has been obtained recently by several investigators, employing different experimental methods.[20]

When white light is transmitted through solutions of hemoglobin, or of compounds related to it, certain wave lengths are absorbed, with the result that these solutions, when examined spectroscopically, exhibit absorption spectra. Oxyhemoglobin or diluted arterial blood shows two absorption bands between the Fraunhofer lines D and E, one narrower than the other. The center of the narrower or α band corresponds to the wave length $\lambda = 579\mu\mu$, and that of the second or β band to $\lambda = 542\mu\mu$. The β band disappears first on dilution. A third band, having its center at $\lambda = 415\mu\mu$, i.e., in the extreme violet region, may be seen in spectro-photographs of oxyhemoglobin.

Hemoglobin (reduced hemoglobin) shows a spectrum with one broad band between D and E and nearer to D. The center and darkest part of the band corresponds to the wave length $\lambda = 559\mu\mu$.

Methemoglobin is formed when blood is treated with ozone, potassium permanganate, potassium ferricyanide, chlorates, nitrites, nitrobenzene, pyrogallol, acetanilide, and many other substances. These compounds when introduced into the organism lead to the presence of methemoglobin both in the blood and urine. Methemoglobin is oxidized hemoglobin but differs from oxyhemoglobin in that the oxygen is very firmly united. In acid solution, methemoglobin shows one band, the center of which corresponds to a wavelength of about $\lambda = 634\mu\mu$.

Carbon monoxide has a greater affinity for hemoglobin than oxygen. The relationship between the proportions of hemoglobin that would unite with oxygen and carbon monoxide at varying gas pressures has been formulated by Douglas, Haldane, and Haldane,[21] and is expressed mathematically by the equation:

$$\frac{[HbCO]}{[HbO_2]} = K\frac{pCO}{pO_2}$$

[19] Proc. Roy. Soc. (London), **109A**, 292 (1925).

[20] Svedberg and Fahraeus, J. Am. Chem. Soc., **48**, 430 (1926); Svedberg and Nichols, J. Am. Chem. Soc., **49**, 2920 (1927); Vickery and Leavenworth, J. Biol. Chem., **79**, 377 (1928); Northrop and Anson, J. Gen. Physiol., **12**, 543 (1929).

[21] Douglas, C. D., Haldane, J. B. S., and Haldane, J. S., J. Physiol., **44**, 275 (1913).

The brackets indicate the concentrations of hemoglobin combined as carbon-monoxide-hemoglobin and as oxyhemoglobin; pCO and pO_2 the gas tensions, and K the relative affinity constant for hemoglobin for the two gases.

A recently estimated value of K shows that the tendency of hemoglobin (of hemolyzed human blood) to form carbon monoxide-hemoglobin is approximately 210 times greater than the tendency to form oxyhemoglobin.[22] When carbon monoxide is breathed, a large proportion of the hemoglobin combines with it. If thereby the amount left to combine with oxygen is sufficiently diminished, the tissues do not obtain sufficient oxygen to maintain life and death from asphyxiation results. In non-fatal cases of severe carbon monoxide poisoning, permanent injury to the central nervous system may occur, often consisting of a softening of the lenticular nuclei, with a resulting syndrome of paralysis agitans.

The carbon monoxide-hemoglobin spectrum shows two bands; the middle of the first corresponds to wave length $\lambda = 570\mu\mu$, and that of the second to $\lambda = 542\mu\mu$. Carbon-monoxide hemoglobin absorption spectra can be distinguished from oxyhemoglobin spectra by the fact that reducing substances, such as ammoniacal ferrous tartrate (Stokes' reagent), have a less marked effect on the absorption bands of carbon monoxide hemoglobin than on those of oxyhemoglobin.

There are many other hemoglobin derivatives. Sulfur methemoglobin is formed by the action of hydrogen sulfide on oxyhemoglobin. Cyanhemoglobin is formed by the action of cyanides. Among others that might be mentioned are nitric oxide-hemoglobin and an acetylene hemoglobin compound. The absorption spectra of a number of the more common hemoglobin compounds are represented on page 205.

When hemoglobin is treated, under appropriate conditions, with glacial acetic acid, in the presence of sodium chloride, and the mixture warmed gently, a substance is obtained which crystallizes readily as brown crystals (Fig. 26). This substance is hemin, $C_{34}H_{32}N_4O_4FeCl$ (Küster). Hemin has been recently synthesized by H. Fischer.[23] It contains four methyl pyrrol radicals and an atom of iron and according to Küster,[24] may be represented by the structural formula shown at the top of p. 206.

In passing it should be mentioned that several other structural formulas have been suggested for this compound and that various authorities differ somewhat as regards the empirical formula. Thus,

[22] Sendroy, J., Liu, S. H., and Van Slyke, D. D., Am. J. Physiol., **90**, 511 (1929).
[23] Annalen, **468**, 98 (1929).
[24] Z. Physiol. Chem., **163**, 282 (1927).

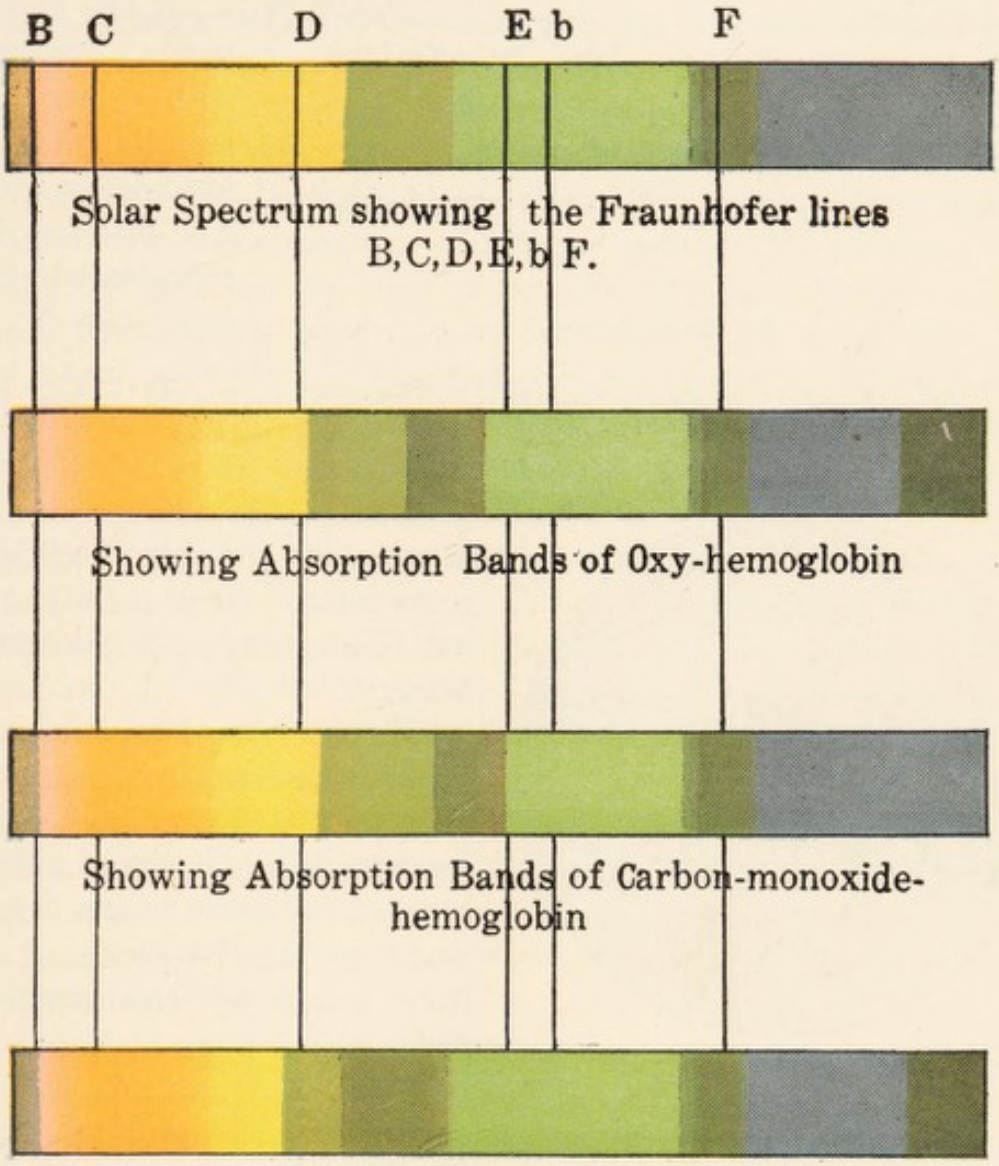
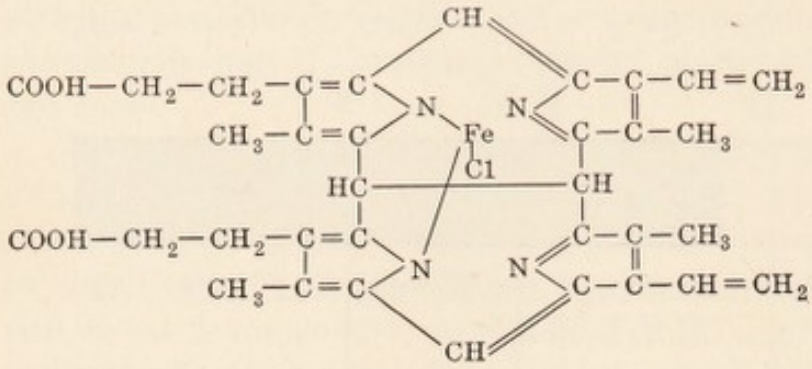


FIG. 25.—Absorption Spectra.

according to Willstätter, the formula for hemin is $C_{33}H_{32}O_4N_4FeCl$, and Barcroft [25] writes it as $C_{34}H_{30}N_4O_4FeCl$.



Hemin (Küster).

If the crystals of hemin are treated with sodium hydroxide, the corresponding base is liberated. The reaction may be represented by the equation:

$$\underset{\text{Hemin}}{C_{34}H_{32}N_4O_4FeCl} + NaOH = \underset{\text{Hematin or Heme}}{C_{34}H_{32}N_4O_4FeOH} + NaCl$$

Fig. 26.—Hemin crystals. (After Nencki and Zaleski, Z. physiol. Chem., **30**, 423 (1900).)

This substance was formerly called *hematin* and the term is still adhered to by some. *Heme* is the name that has been given to it by Anson and Mirsky.[26]

Hematin (or heme) may be reduced to a substance which some call *reduced hematin* or *reduced alkali hematin*, but which Anson and Mirsky have called *reduced heme*. Reduced heme may also be produced directly from hemin by treatment of the latter with sodium hydroxide, in the presence of a reducing agent, such as sodium hydrosulfite, $Na_2S_2O_4$. The formation of reduced heme may be indicated as follows:

$$\underset{\text{Heme}}{C_{34}H_{32}N_4O_4FeOH} \rightarrow \underset{\text{Reduced Heme}}{C_{34}H_{32}N_4O_4Fe}$$

What is the relation of these compounds to hemoglobin? It has been taught that hemoglobin is made up of a protein, globin, in conjugation with a non-protein substance, hematin, to which Hoppe-Seyler assigned the formula $C_{34}H_{34}N_4O_5Fe$. Notwithstanding the slight difference in the formula, this is the substance which has been referred to above as hematin, or as heme, the latter being the terminology of Anson and Mirsky.

It has also been taught that when hematin is reduced in an alkaline

[25] Respiratory Function of the Blood, Part II, Cambridge, 1928, pp. 8, 18, 19, etc.

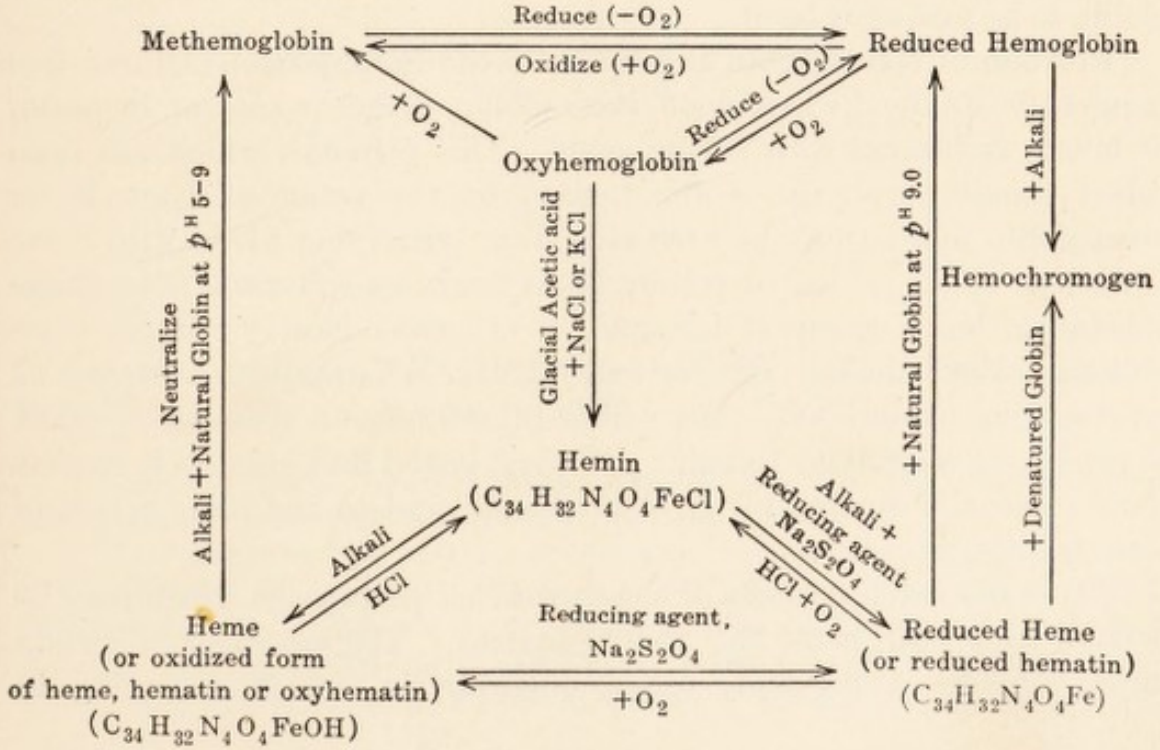
[26] J. Physiol., **60**, 50, 161, 221 (1925); Anson, Barcroft, Mirsky and Oinuma, Proc. Roy. Soc., B, **97**, 61 (1925); J. Gen. Physiol., **12**, 273 (1928–29); *ibid.*, **12**, 581 (1929).

solution, or when hemoglobin is treated with alkali and a reducing agent, a substance called hemochromogen is formed, to which the formula $C_{34}H_{35}N_4O_4Fe$ was given by Hoppe-Seyler. This compound was thought to be responsible for the characteristic spectrum obtained when hemoglobin was treated with alkali and a reducing agent. This view was apparently incorrect.

To Anson and Mirsky we owe a more correct understanding of the relationship between hemoglobin and hemochromogen. They showed that when hemin was reduced in an alkaline solution, the resulting compound did not give the spectrum of hemochromogen. However, when they added globin, the spectrum of hemochromogen appeared. The work of Anson and Mirsky, the details of which cannot be considered here, established the fact that the substance which gave what until then had been called the hemochromogen spectrum was a conjugated protein, consisting of globin and the base $C_{34}H_{32}N_4O_4Fe$.

Hill and Holden [27] have described a method for preparing undenatured globin from hemoglobin. This globin combines with heme (or hematin) over the range *p*H 5 to 10 to yield methemoglobin. Methemoglobin may be reduced to reduced hemoglobin. Subsequent oxidation is said to yield oxyhemoglobin. Or, reduced heme may be treated with globin with which it combines at *p*H 9.0 to yield reduced hemoglobin directly. This remarkable accomplishment has also been reported by Anson and Mirsky.[28] The evidence for these transformations, it should be pointed out, is entirely spectroscopic.

The relations of the compounds which we have just considered may be summarized as follows:



[27] Biochem., J., **20**, 1326 (1926); **21**, 625 (1927).

[28] J. Gen. Physiol., **12**, 273, and particularly 285 (1928)

In hemoglobin, heme is attached to natural, i.e., undenatured globin. In hemochromogen, the combination is with denatured globin. It is also stated that hemochromogen is molecularly less complex than hemoglobin. Heme can be combined with a large variety of nitrogenous substances, the resulting compounds being called hemochromogens. Anson and Mirsky [26] have prepared not only globin-hemochromogen, but also compounds of heme with albumin, pyridine, nicotine, piperidine, hydrazine, cyanide, ammonia, glycocoll and other amino acids. These nitrogenous substances do not combine with heme with equal ease. Thus, the affinity of ammonia for heme is very much less than that of globin. Indeed only a few of these, namely, pyridine and nicotine, have an affinity which in any way approaches that possessed by globin. The relation of heme to hemochromogen, both natural and synthetic, may be represented as follows:

$$\text{Heme} + \text{Nitrogenous substance} \rightleftarrows \text{Hemochromogen.}$$

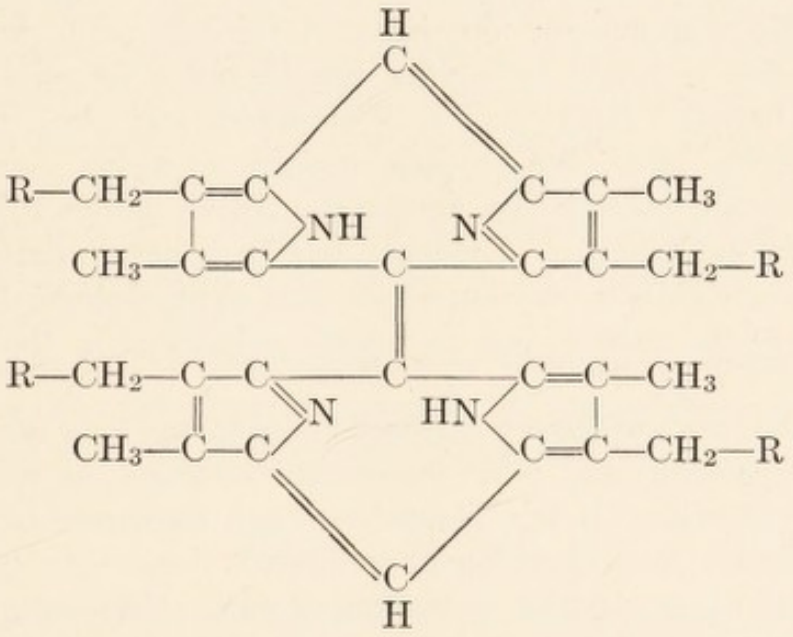
The various synthetic hemochromogens do not give identical absorption spectra, but they are sufficiently close to explain why the older workers, using less accurate spectroscopes, did not recognize the true nature of hemochromogen. In liberating the base from hemin, ammonia was often used. On subsequent reduction, instead of obtaining the reduced base, as was thought, a complex of this substance with ammonia, or as it would now be called, ammonia hemochromogen, was formed. This substance gives an absorption spectrum which sufficiently resembles that given by the hemochromogen obtained directly from hemoglobin to be mistaken for it.

Relation of Hemoglobin and Heme to the Porphyrins.—An iron-free pigment is obtained when blood, hemoglobin, hemochromogen, hematin, or hemin is treated with strong acids. This pigment, which has been called hematoporphyrin, is also formed by the action of bacteria on hemoglobin and it may be extracted from putrefying blood with ether and acetic acid. It has been stated that hematoporphyrin is not a single substance, but a group of isomeric, or otherwise closely related, compounds. Nevertheless, the formula $C_{34}H_{34}O_4N_4$ may be accepted as representing hematoporphyrin. This pigment has a remarkable effect in producing sensitivity to light. When injected into animals it renders them especially susceptible to light stimuli and to the toxic action of ultraviolet rays.

There is a large number of closely related porphyrins which may be derived from both plant and animal sources. The egg-shells of certain birds contains a pigment, called oöporphyrin, which according to

Fischer [29] is identical with hematoporphyrin. He has therefore suggested the term protoporphyrin for this substance to distinguish it from other porphyrins.

When hematoporphyrin is heated with soda lime, it yields a porphyrin having the formula, $C_{32}H_{36 \text{ or } 38}N_4$ (Fischer). This is identical with ætioporphyrin derived from chlorophyll. Thus we see the chemical relationship of chlorophyll and hemoglobin. A derivative of ætioporphyrin has been described, containing four carboxyl groups. This is coproporphyrin, $C_{36}H_{36 \text{ or } 38}O_8N_4$. It may be a product of intestinal putrefaction, for it is a constituent of feces. It has also been detected in the serum of various animals, including man. Uroporphyrin, $C_{40}H_{36 \text{ or } 38}O_{16}N_4$, is excreted in the urine in congenital porphyrinuria. The copper salt of this compound is the pigment turacin,[30] present in the feathers of the turaco, a South African bird. These are but a few of the many examples which may be cited to illustrate the widespread distribution and chemical relationships of the porphyrin derivatives in nature. The following formula of ætioporphyrin will serve to bring out the structural relationship of the porphyrins to heme.



In ætioporphyrin, R represents CH_3. In coproporphyrin, R = CH_2COOH; in uroporphyrin, R is $CH(COOH)_2$.

[29] Z. physiol. Chem., **135**, 253 (1924); Annalen, **448**, 178 (1926), and numerous other papers by Fischer and his associates in these journals.

[30] This pigment and the related metaloporphyrins have been studied by Laidlaw, P. P., J. Physiol., **31**, 464 (1904); Milroy, J. A., *ibid.*, **38**, 384 (1909); Keilin, D., Proc. Roy. Soc., London, B., **100**, 129 (1926). It was first described by Church, A. H., Proc. Roy. Soc., London, **51**, 399 (1892); Phil. Trans., B, **183**, 511 (1892). The relationship to uroporphyrin is discussed by Fischer and Hilger, Z. Physiol. Chem., **138**, 49 (1924).

The Biological Significance of Heme Compounds; Cytochrome.—If the occurrence of heme were limited only to hemoglobin, it would still be one of the most widely distributed and most important substances in nature. However, hemoglobin is not the only heme compound and there is at least one which is much more widely distributed. There exists an intracellular pigment in aerobic bacteria, yeast, higher plants and animals. It was first observed in muscle and other tissues by MacMunn,[31] in 1886, but it is to the more recent work of Keilin [32] that we owe most of our knowledge of the subject. This pigment, named cytochrome, is capable of existing in an oxidized and in a reduced form. In the latter condition, it exhibits an absorption spectrum of four bands. Whatever the source of the cytochrome, these bands occupy approximately the same positions, namely, $a = 6046$; $b = 5665$; $c = 5502$; $d = 5210$, expressed in Ångstrom units.

According to Keilin, cytochrome is not one chemically defined substance, but a mixture of three independent hemochromogen-like compounds, a', b', c', capable of being oxidized and reduced independently from each other. In addition to these hemochromogens, or even in their absence, all cells of aerobic organisms contain a free unbound hematin (heme), which is apparently identical with the heme of hemoglobin.

Cytochrome is found in highest concentration in cells capable of active metabolism. Heart muscle of mammals and birds, the pectoral muscle of flying birds, the thoracic muscles of flying insects, baker's yeast and certain bacteria are among the active tissues that are especially rich in cytochrome. Evidence has been accumulating recently that cytochrome plays a very important part in physiological oxidations. This phase of the subject will be briefly considered in the succeeding chapter.

Helicorubin is a respiratory pigment found in the liver and gut of the snail (*Helix pomatia*) and other pulmonate molluscs, as well as in the liver of the crayfish. It is a hemochromogen composed of globin and heme. Artificial hemochromogens prepared from this pigment are identical with those derived from hemoglobin. Helicorubin combines with oxygen, forming a compound capable of dissociation, thus resembling hemoglobin. However, it differs from hemoglobin in that its affinity for oxygen is greatest in a slightly acid medium.

Actiniohematin is a respiratory pigment, resembling helicorubin, which occurs in certain actinia. *Chlorocruorin* occurs in marine worms of the polychæte family. In concentrated solution it is reddish,

[31] Phil. Trans. Roy. Soc., **177**, 267 (1886).

[32] Proc. Roy. Soc., B, **98**, 312 (1925); **100**, 129 (1926); **104**, 206 (1928–29); Nature, **119**, 670 (1927).

whereas in dilute solution it has a green color. It may be oxidized and reduced like hemoglobin, which it resembles in other ways. Chlorocruorin yields derivatives corresponding to methemoglobin, hemochromogen, hematin and hematoporphyrin. The artificial hemochromogens prepared from chlorocruorin, however, yield an absorption spectrum differing from the hemochromogen derived from hemoglobin and it is therefore concluded that the porphyrin of chlorocruorin is different from protoporphyrin (hematoporphyrin).

Crystallization of Hemoglobin.—Although hemoglobin is a protein, it may be crystallized with relative ease from the blood of certain animals, such as the horse, dog and guinea pig. The hemoglobins of the ox and rat crystallize with difficulty, whereas other hemoglobins, such as that of the frog, have not been obtained in crystalline form. The most important study of the crystalline structure of the hemoglobins is that of Reichert and Brown,[33] who showed that oxyhemoglobin crystals differ not only with the species but also with the genus of the animals from which they are obtained, no two hemoglobins forming identical crystals. There is, however, some similarity in crystal structure of the hemoglobins of genetically related animals. Since heme, the non-protein part of all hemoglobins, is the same, the specificity and possible differences in the constitution of different hemoglobins presumably reside in the globin. When Reichert and Brown were engaged in their monumental work, the importance of the reaction and salt concentration were not yet fully appreciated. As has been pointed out by Hastings,[34] it would be of considerable importance if a crystallographic study comparable to that of Reichert and Brown were made to-day on crystals from isoelectric, salt-free solutions of hemoglobin.

Mechanism for the Transportation of Oxygen.—The transportation of oxygen by the blood from the lungs to the tissues depends on a reversible chemical reaction between hemoglobin and oxygen, as represented by the equation:

$$Hb + O_2 \rightleftharpoons HbO_2,$$

where Hb stands for hemoglobin.[34a] Reduced hemoglobin is readily

[33] The Crystallography of Hemoglobins, Carnegie Institution of Washington (1909).

[34] Colloid Symposium Monograph **6**, 140 (1928).

[34a] Since it has been shown that the molecular weight of hemoglobin is four times what it was formerly thought to be, oxyhemoglobin is more accurately represented by the formula Hb_4O_8, where Hb_4 denotes a molecule of reduced hemoglobin having a molecular weight of 66,800. The question has been raised whether this is the only form of combination of hemoglobin with oxygen. Indeed, Adair [J. Biol. Chem., **63**, 529 (1925)] has attempted to explain the equilibrium between oxygen and hemo-

oxidized to oxyhemoglobin when exposed to oxygen of such concentration as exists in the lungs; oxyhemoglobin is, in turn, dissociated at low oxygen tensions, such as obtain in the tissues. These reactions occur with extreme rapidity, requiring but a fraction of a second, as has been shown by Hartridge and Roughton.[35] It should also be mentioned at the outset that not all of the hemoglobin is oxidized in the lungs, nor is all of the oxygen given up in the tissues.

In a mixture of gases, each gas exerts its own partial pressure. The oxygen content of the air at sea level is about 21 per cent. From this it follows that the partial pressure of the oxygen in the air is about 160 mm. of mercury, when the atmospheric pressure is 760 mm. In the alveoli of the lungs the oxygen content is only about 13 per cent; this is equivalent to approximately 100 mm. of mercury. Ordinarily, this is the maximum oxygen tension to which the hemoglobin of the blood is exposed in the course of its circulation.

One liter of plasma saturated with alveolar air takes up about 3 cc. of oxygen. The oxygen capacity of the blood is about 1 liter, this amount of oxygen being ordinarily sufficient for tissue needs. If we were dependent, therefore, upon the solubility of oxygen in the blood alone, our circulatory system would have to contain about 300 liters of fluid. Our blood, however, is less than one-tenth, rather than four times, our body weight. That it nevertheless can handle the enormous quantity of oxygen which we need is due to the presence of hemoglobin. As stated by Barcroft, " the warm-blooded creation owes its existence, or at all events its activity, to hemoglobin."

In man, and perhaps in other animals, the quantity of hemoglobin appears to be regulated by the demand of oxygen and its supply. Thus, at high altitudes, where the amount of oxygen is reduced the quantity of hemoglobin in the blood is increased. A striking illustration of this is to be found in the observations of Barcroft [36] and his associates who studied

globin on the assumption that the latter combines with oxygen in steps to form Hb_4O_2, Hb_4O_4, Hb_4O_6 and Hb_4O_8. The equilibrium relations between oxygen and hemoglobin have also been studied recently by Ferry and Green [J. Biol. Chem., **81**, 175 (1929)] and by Conant and McGrew [*ibid.*, **85**, 421 (1929–30)]. The latter workers found that if solutions of oxyhemoglobin are deoxygenated, the Hb_4O_8 persists and does not disappear as would happen if it were converted into the intermediate products (Hb_4O_6, Hb_4O_4, Hb_4O_2),which have much higher solubilities than the fully oxidized hemoglobin. Conant and McGrew have suggested that if intermediate oxidation products of hemoglobin are formed, they are present in very small quantities.

[35] Proc. Roy. Soc. London, **104** A, 395 (1923).

[36] Phil. Trans. Royal Soc., Ser. B, **211**, 351 (1922–23). A fascinating account of the expedition to Cerro de Pasco is given in J. Barcroft's " The Respiratory Function of the Blood," Part I, Lessons from High Altitudes, 2d Edition, Cambridge, 1925.

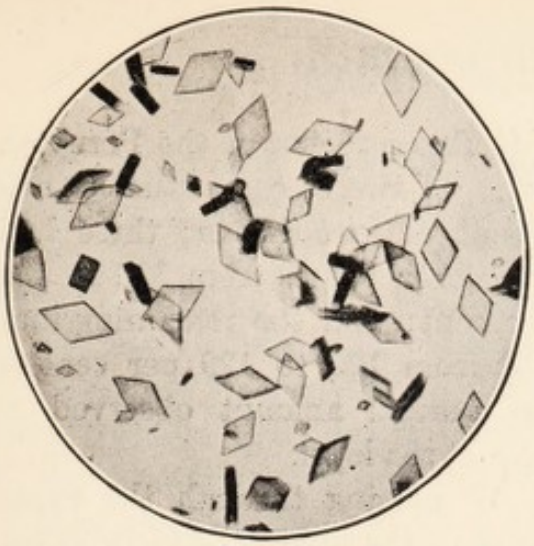


FIG. 27

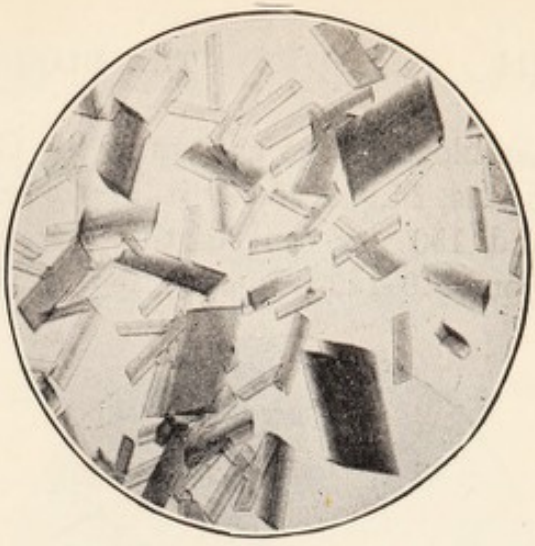


FIG. 28.

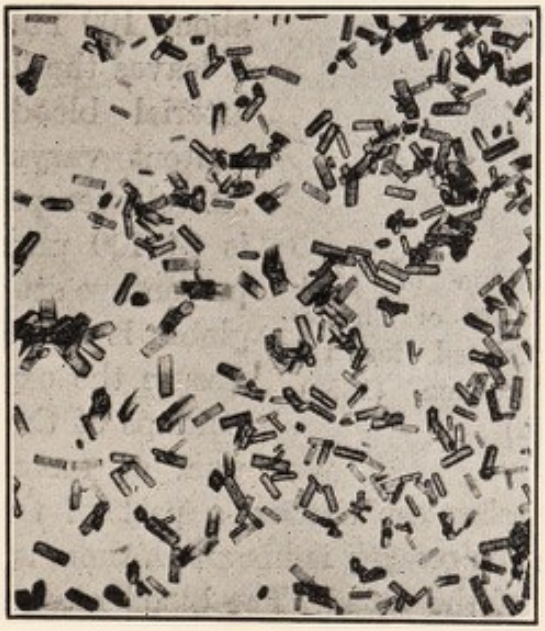


FIG. 29.

FIG. 30.

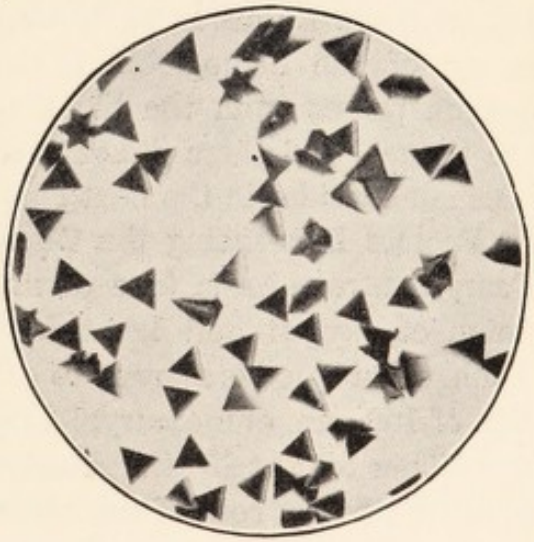


FIG. 31.

FIG. 27.—Oxyhemoglobin of the Guinea-fowl. FIG. 28.—Oxyhemoglobin of the Tasmanian Devil. FIG. 29.—Reduced Hemoglobin of the Tiger. FIG. 30.—Oxyhemoglobin of the White Rat. FIG. 31.—Oxyhemoglobin of the Guinea-pig.

(FIGURES 27, 28, 29, 30 and 31, after Reichert and Brown, The Crystallography of Hemoglobins, Carnegie Institution of Washington Publications, No. 116.)

the blood of the natives in the Cerro de Pasco region of the Peruvian Andes, 14,000–15,000 ft. above sea level. Of twelve cases studied, one had 150 per cent of the normal amount of hemoglobin; three had from 140–149 per cent; four gave hemoglobin values from 130 to 139 per cent; and the remaining four had from 120 to 129 per cent of the normal amount observed in man at sea level.

Fig. 32. — Oxyhemoglobin of Man. (After Otto Funke, Atlas of Physiological Chemistry. Printed for the Cavendish Society, London (1853), Plate X.)

In passing through the capillaries of the lungs, the blood is exposed to an oxygen tension of about 100 mm. of mercury. As it leaves the lungs, the blood (i.e., arterial blood) has an oxygen content varying between 17 and 22 volumes per cent; the tension is 84–100 mm. of mercury; the percentage saturation of the hemoglobin is 93–98 per cent. In the tissues the oxygen tension is low, perhaps of the magnitude of 0–10 mm. of mercury. As a result, when the blood reaches the tissues, a part of the oxyhemoglobin is dissociated, and, as the oxygen is liberated, there is a progressive fall in the oxygen tension of the blood. The blood returning from the tissues to the lungs, i.e., venous blood, has an oxygen content of 11–16 volumes per cent and a tension of 30–60 mm. of mercury; the percentage saturation of the hemoglobin is usually 62–85 per cent. The liberated oxygen passes from the blood to the lymph and from the lymph to the tissues, and there takes part in the processes of oxidation, which we shall consider in the next chapter.

Factors Influencing the Combination of Hemoglobin and Oxygen; Temperature.—As is true of many other chemical combinations, the union of oxygen and hemoglobin is less the higher the temperature. Thus, when blood is allowed to come to equilibrium with oxygen at a tension of 100 mm. of mercury, 93 per cent will become saturated at 38° C., and 98 per cent at 25° C. Under a pressure of 10 mm., at a temperature of 38° C., 56 per cent of the hemoglobin will still be in combination with oxygen, whereas at 25° C., the amount in combination will be 88 per cent. This means that, with a drop in oxygen tension commensurate with the difference between the pressure in the lungs and in the tissues, the amount of oxygen that becomes available for purposes of metabolism is greater

at the higher than at the lower temperature. From this point of view, the advantage of being a warm-blooded animal is fairly obvious. These relationships are more completely illustrated in the accompanying diagram (Fig. 33) in which are presented a number of oxyhemoglobin-dissociation curves obtained at different temperatures.

Effect of Electrolytes.—At low oxygen tensions, oxyhemoglobin is more readily dissociated in the presence of salts than in pure solution. If the temperature is maintained constant at 38° C., the saturation of hemoglobin in the presence of electrolytes may be reduced to less than one-half of what it is in pure solution, at an oxygen tension of 10 mm. of mercury. That this effect is not operative at higher pressures is shown by the curves in Fig. 34, where an increase in the combining capacity of the hemoglobin is actually indicated at 100 mm.

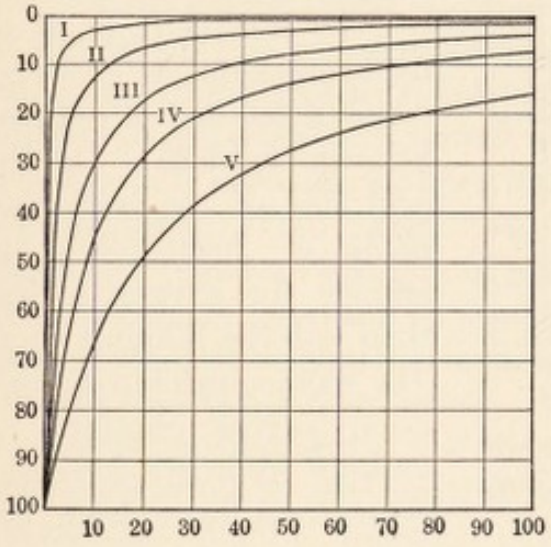


FIG. 33.

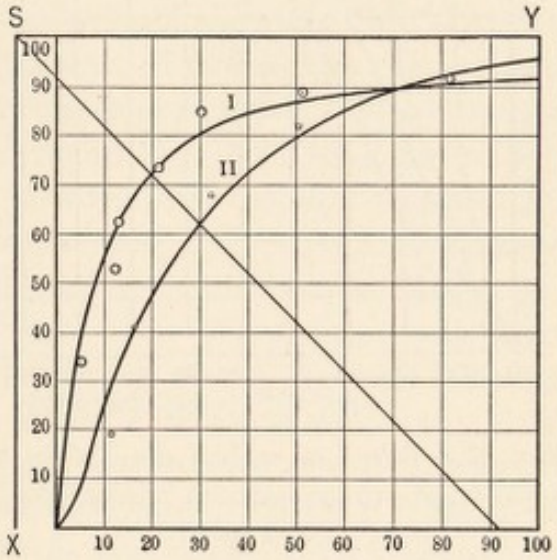


FIG. 34.

FIG. 33.—Dissociation curves of oxyhemoglobin at different temperatures. Curves I, II, III, IV, and V correspond to 16°, 25°, 32°, 38°, and 49° C., respectively. Ordinates = percentage of reduced hemoglobin; abscissæ = tension of oxygen in millimeters of mercury. (After Barcroft and Hill, J. Physiol., **39**, 422 (1909–10).)

FIG. 34.—Effect of electrolytes on the dissociation curve of oxyhemoglobin. Ordinates = percentage saturation of hemoglobin with oxygen; abscissæ = tension of oxygen in millimeters of mercury. ° Points determined from dialyzed solution. □ Points determined from undialyzed solution. Curve I (electrolytes absent) = rectangular hyperbola; $xy = 800$. Curve II (electrolytes present in low concentration) = Bohr's dissociation curve for hemoglobin (see Zentrbl. f. Physiol. **17**, 682,688 (1903–04)). (After Barcroft and Roberts, J. Physiol., **39**, 146 (1909–10).)

Effect of Carbon Dioxide.—A third factor influencing the efficiency of hemoglobin as a carrier of oxygen is carbon dioxide. In view of the acidity of carbonic acid, the effect of carbon dioxide may be referred to the hydrogen-ion concentration. This relationship has been studied

by Barcroft and Poulton [37] and others. More recently, Bock, Field, and Adair [38] obtained oxygen-dissociation curves for normal blood at carbon-dioxide tensions of 3, 20, 40, and 80 mm. of mercury. From their results, some of which are reproduced below (Fig. 35), it may be seen that carbon dioxide, while not hindering, to any appreciable extent, the formation of oxyhemoglobin in the lungs, greatly facilitates its dissociation in the tissues. In the removal of carbon dioxide from the tissues, which is intimately associated with its rôle in the transportation of oxygen hemoglobin plays an important part. This function will be discussed presently.

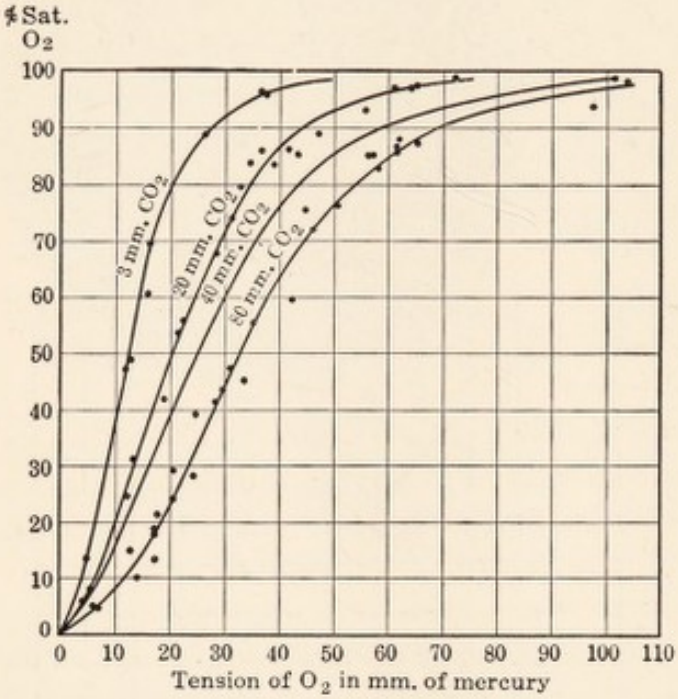


FIG. 35.—Effect of carbon dioxide on the dissociation of oxyhemoglobin. (After Bock, Field and Adair.)

The isoelectric point of oxyhemoglobin as given by Adair is 6.6 and of reduced hemoglobin, 6.81. On the acid side of the isoelectric point of hemoglobin its affinity for oxygen is less than on the alkaline side. The oxyhemoglobin dissociation curves obtained at various hydrogen-ion concentrations have been described by Adair.[39]

Plasma Proteins.—The protein content of the plasma amounts to about 7 per cent in man. The proteins may be separated into several fractions, the method most commonly employed consisting in salting out the fractions with varying concentrations of certain inorganic salts. From the work of Howe [40] and others, it appears that the precipitation of the protein fractions occurs with some regularity. Thus, in a 0.75 molar solution of sodium sulfate, fibrinogen separates out. Euglobulin is salted out in a 1.00 molar solution. In a solution that is 1.25 molar, fibrinogen, euglobulin, and the so-called pseudoglobulin I are precipitated. All the globulins are separated from the albumins in a 1.50 molar solution, the last of the globulin fractions being pseudo-

[37] J. Physiol., **46**, iv (1913).
[38] J. Biol. Chem., **59**, 353 (1924).
[39] J. Biol. Chem., **63**, 529 (1925).
[40] Physiol. Reviews, **5**, 439 (1925).

globulin II. Similarly, various albumin fractions are precipitated in concentrations above 1.50 molar. While Howe recognizes the probability that the precipitates of protein may not be chemical identities, he has nevertheless suggested the grouping of the albumins into five fractions: V, VI, VII, VIII, and IX, which are precipitated in 1.75, 2.00, 2.25, 2.50, and 2.75 molar solutions of sodium sulfate, respectively.

In the study of special problems, Howe's method of protein fractionation is very useful, but ordinarily in the analysis of plasma or serum the procedure is to determine all the globulins together as the globulin fraction and all the albumins as the albumin fraction.

For normal human serum, Linder, Lundsgaard and Van Slyke [41] obtained an average value of 6.73 per cent for the total protein, of which 2.61 per cent was globulin and 4.12 per cent albumin. The highest normal value obtained was 7.45 per cent; the lowest 6.22 per cent. Wu [42] analyzed the blood serum of five normal individuals and obtained an average of 6.94 per cent for total protein, of which 2.09 per cent was globulin. Pathologically much lower values are known. Wu gives one analysis of the blood of a patient with nephritis, whose total serum protein was 3.07 per cent, 2.54 per cent of which was globulin and 0.53 per cent albumin. This patient was edematous. The relation between plasma proteins and edema, i.e., accumulation of fluid in the tissues, has attracted much attention in recent years. One of the important functions of the plasma proteins is to maintain the normal osmotic relations between the blood and tissues. It is to be remembered that at the hydrogen-ion concentration of the blood, the proteins are to a considerable proportion in combination with base and accordingly exert an appreciable osmotic pressure. There is somewhat better agreement now than formerly as to the approximate value of this pressure. Krogh[43] states that in his laboratory determinations of the osmotic pressure of the serum proteins in twelve normal human cases gave an average result of 380 mm. of water pressure. Of the serum proteins the albumins are osmotically more active than the globulins on account of their relatively smaller molecular size. Serum albumin, according to Adair,[44] has a molecular weight of 62,000, as compared with 130,000 to 150,000 for pseudoglobulin and 174,000 for euglobulin. If there is any pathology in the kidney which makes the glomeruli permeable to protein, the fractions that are lost to the blood in greater proportion are the proteins of

[41] Proc. Soc. Exp. Biol. Med., **20**, 320 (1922–23).

[42] J. Biol. Chem., **51**, 33 (1922).

[43] The Anatomy and Physiology of Capillaries, Yale University Press, New Haven, 1929, pp. 286–290.

[44] Cited by Krogh, p. 284; also Proc. Physiol. Congress, Stockholm.

smaller molecular weight, or the osmotically more active proteins, which are the albumins. This is well brought out in a group of cases of various renal diseases described by Iversen and Nakazawa,[45] whose work also indicates that when the effective osmotic pressure of the serum (pressure due to the proteins) is below 250 mm., edema develops.

The production of experimental edema has been accomplished by Leiter.[46] He bled dogs, removing considerable amounts of the blood at a time, separated the plasma from the cells, suspended the latter in saline and reinjected them. In this way he was able to reduce the serum proteins sufficiently to produce marked edema.

A similar experiment was performed by Whipple [47] with a different object in view. The plasma proteins are primarily responsible for the viscosity of the blood and if reduced sufficiently, which Whipple did by replacing whole blood by the red corpuscles suspended in Locke's solution, a condition of shock is produced. This may prove fatal if the serum protein depletion is sufficient.

Extensive hemorrhage is usually followed by a marked increase of the lipid content of the blood, which, according to Fishberg [48] may be interpreted as an effort of the organism to compensate for the decrease in osmotic pressure produced by the loss of serum proteins.

Clinically, low concentrations of serum proteins are not necessarily always due to extensive hemorrhage or kidney injury, nor is edema only an accompaniment of nephritis. Severe and chronic malnutrition is an important cause. During and after the World War, a nutritional edema was commonly seen in famine areas. Gorter [49] observed a similar edema in a group of children with digestive disorders who were being fed on diets of flour or rice in water. Certain authorities were inclined to regard war edema, or famine edema, as a vitamin deficiency disease, but this is unlikely. It appears to be a protein deficiency disease and may be relieved by feeding adequate amounts of protein. Nutritional edema has been produced experimentally in rats, maintained on diets deficient in protein.[50] This has been confirmed by Frisch, Mendel and Peters,[51] who have shown that in such animals a relationship exists between the reduction of serum protein and the development of edema. A similar association probably exists in war edema.

Other functions may be attributed to the plasma proteins. As will

[45] Biochem. Z., **191**, 307 (1927).
[46] Proc. Soc. Exp. Biol. Med., **26**, 173 (1928).
[47] Am. J. Physiol., **52**, 72 (1920).
[48] J. Biol. Chem., **81**, 205 (1929).
[49] Monatschr. Kinderheilk., **251**, 211 (1925).
[50] Kohman, E. A., Am. J. Physiol., **51**, 378 (1920).
[51] J. Biol. Chem., **84**, 167 (1929).

be shown later, they play a part in the buffer mechanism of the blood. A relationship has been suggested between the globulins and immune bodies. During infection or following the injection of a toxin or an antigen, the globulin content of the blood is increased. Muscular activity is also known to increase the serum globulin concentration of the blood.

Fibrinogen and the Clotting of Blood.—Fibrinogen resembles the globulins; indeed it may be classed with them. Like the globulins it is precipitated by half saturation with ammonium sulfate; it differs in being precipitated in a 0.75 molar solution of sodium sulfate and by half saturation with sodium chloride. In human plasma the amount of fibrinogen usually varies between 0.3 and 0.6 per cent. It is low in conditions of liver injury, and high in septicemia and pneumonia. Soon after it is drawn, blood forms a clot, a phenomenon which protects the body against the excessive hemorrhage that might otherwise result even from slight wounds. In persons afflicted with hemophilia, the blood does not clot readily. This condition is hereditary, affecting almost invariably the male members of the hemophilic family, but being transmitted by the females.

Careful examination with the aid of the ultramicroscope has revealed that when a fibrin clot is being formed the fibrin first separates in the form of needle-like crystals, later assuming the appearance of threads. The immediate cause of clotting is the change from *fibrinogen* to *fibrin*, but certain other factors enter in. In the first place, clotting does not occur in the absence of *calcium*, as can be shown by removing the calcium as the oxalate or citrate. The significance of calcium is as important here as in the curdling of milk by rennin. However, fibrinogen and calcium in themselves do not form a clot; another substance is essential. This other substance is believed to be a protein or a proteose and is called *thrombin*. It may be extracted from water-washed fibrin with an 8 per cent solution of sodium chloride.

From a consideration of these facts, one is naturally led to inquire why the blood does not clot in the blood vessels. To explain this, it has been assumed that thrombin does not exist as such in the blood but is present in an inactive form which has been named *thrombogen* or *prothrombin*. The cause for the change from prothrombin to thrombin has long been sought without success; it is known, however, that blood in contact with dead or injured tissue or with disintegrated blood corpuscles, and especially with broken-down blood platelets, clots more readily than otherwise. Howell has suggested that when blood platelets or tissue cells disintegrate, a substance is liberated which hastens the coagulation process and for which he suggested the name *thromboplastin*. This constituent is soluble in fat-solvents and appears to be cephalin.

The function of the cephalin in the clotting process is not known definitely, but it is believed by Howell,[52] that it neutralizes the effect of *heparin*, a constituent of the blood which in some way prevents the transformation of prothrombin into thrombin. Heparin is a substance isolated from the liver which is believed to be responsible for maintaining the normal fluidity of the blood. Its effect is said to be to inhibit the transformation of prothrombin to thrombin, a property formerly attributed to a substance termed *antiprothrombin*. Another possibility is that heparin combines with some other substance to form *antithrombin* and that this too is neutralized by cephalin. It is to be admitted that our conception of the process of clotting is not necessarily clarified by the introduction of so many terms to represent alleged substances, or rather effects. However, for convenience the process of clotting may be briefly summarized as follows (Howell's theory). There are found in the blood fibrinogen, prothrombin, calcium salts, cephalin, heparin and antithrombin. When blood is shed, owing to the disintegration of the blood platelets or in some other fashion, cephalin is liberated. This combines with heparin and possibly antithrombin, with the result that prothrombin is liberated and in the presence of the calcium salts is converted into thrombin. The thrombin then transforms the fibrinogen into fibrin.

A number of other views have been suggested. According to Bordet, the plasma contains fibrinogen and proserozyme, the latter being converted to serozyme by the action of calcium salts. The tissues, and particularly the platelets, contain a lipoprotein which Bordet [53] named cytozyme and which he believed to be lecithin, but which really belongs to the group of cephalins. Cytozyme unites with the serozyme to yield thrombin, which transforms fibrinogen to fibrin.

That there are two distinct mechanisms concerned with the coagulation of blood seems likely from the observations of Mills [54] and others. In addition to the thrombin mechanism, clotting may be produced by the action of tissue fibrinogen on plasma or plasma fibrinogen, even in the absence of thrombin or prothrombin. There are probably many different tissue fibrinogens in the different organs and tissues of the body.

The clotting time of blood varies in different species. In man it is usually $2\frac{1}{2}$–3 minutes. Delayed clotting of blood may be caused by several factors. Hemophilic blood may require more than two hours for clotting. In this disease the number of platelets is normal but they

[52] Bull. Johns Hopkins Hospital, **42**, 199 (1928).

[53] Ann. de l'Institut Pasteur, **34**, 561 (1920).

[54] Am. J. Physiol., **57**, 395 (1921).

exhibit an abnormal resistance to disintegration. Thus, the cephalin does not become readily available for the process of clotting. This theory has been advanced by Minot and Lee.[55] The addition of normal platelets or cephalin to hemophilic blood hastens its clotting.

In conditions of severe liver damage, such as occur in chloroform and phosphorus poisoning and in acute yellow atrophy, there is deficient formation of fibrinogen, as a result of which its content in the blood may fall to very low values. To this reduction of fibrinogen is attributed the delayed clotting time and hemorrhagic tendency in these conditions.

As has been mentioned, the removal of calcium from the blood as the oxalate or citrate prevents its clotting. However, physiologically, diminished clotting of the blood is not frequently attributable to a diminished calcium content of the blood. It is perhaps a factor in obstructive jaundice, for the " coagulability " of the blood of patients with this disease may be increased by the administration of calcium salts.

In purpura hemorrhagica and other hemorrhagic diseases, the blood may have a normal clotting time, although the clot is usually soft and fails to retract, but the bleeding time is greatly prolonged. Normally after a slight puncture there is rapid diminution in the intensity of the hemorrhage, successive drops of the blood being smaller and smaller, and soon the bleeding stops altogether. Cessation of the hemorrhage is brought about by the formation of small thrombi in the injured capillaries, the thrombi being made up essentially of aggregations of blood platelets. In the hemorrhagic diseases referred to, there is no prompt checking of the hemorrhage, which may continue for hours, the drops showing little or no tendency to diminish in size. This prolongation in the bleeding time is attributed to a deficiency of platelets, which in severe hemorrhagic diseases have often been found to be as low as 10,000 per cubic millimeter.

The coagulability of the blood may be experimentally inhibited by the injection of oxalate, peptone, hirudin, heparin and other substances. Hirudin is the active substance of the extract prepared from the head of the medicinal leech.

There are a number of circumstances which favor the clotting of blood in the blood vessels, such clots being called *thrombi* and the process, *thrombosis*. Intravascular clotting may result from the slowing down of the blood flow, such as may occur in heart disease, or where there is an abnormal dilatation of the blood vessels, as in varicose veins, or where there is some obstruction in the vessel. A thrombus also tends to form around an area of injury, such as an atheromatous patch in an artery, or on a damaged heart valve. Infections, such as typhoid fever, predispose

[55] Arch. Int. Med., **18**, 474 (1916).

to thrombus formation and there are other causes which are described in textbooks of pathology.

The formation of intravascular clots differs from clotting outside the blood vessels. At first, there is a clumping together of blood platelets, forming a framework, which becomes infiltrated by white blood cells. At this stage, the thrombus is whitish in color and is called a white thrombus. When its size is sufficient, it causes obstruction of the blood vessel, the flow of blood is stopped and a red blood clot, the red thrombus is formed. This consists of fibrin and all the blood elements. It fills the obstructed blood vessel to the point of its nearest anastomosis with some other vessel.

Hydrogen-ion Concentration of the Blood.—There is normally only slight variation in the reaction of the blood. This is very fortunate if we consider that the processes which normally take place in the living cell can occur only within certain limits of hydrogen-ion concentration. The blood is slightly alkaline in reaction, varying ordinarily between *p*H 7.35 and 7.43. The *p*H of the serum is somewhat higher than that of the corpuscles. According to Earle and Cullen,[56] the *p*H of normal serum is usually between 7.4 and 7.5. It varies during the day, increasing from early morning, before rising, to late evening. The total increase varies from 0.01 to 0.07 *p*H and is not constant, being interrupted by fluctuations due to digestion, exercise and other factors. Recent work also shows that there is very little difference in reaction between arterial and venous blood. According to Parsons,[57] the difference in *p*H is 0.02, being lower in venous blood. The data of Peters, Barr, and Rule [58] show variations of 0.01 to 0.04; and those of Doisy [59] and associates, differences ranging from 0.013 to 0.037. After strenuous exercise, there is a temporary increase in the hydrogen-ion concentration. Barr, Himwich and Green [60] have recorded values as low as 7.05. An increase in the alkalinity of the blood may be produced physiologically by forced breathing. In this way, Davies, Haldane and Kennaway [61] obtained values as high as *p*H 7.85. Ordinarily, any marked divergence from the normal is indicative of serious disturbance in the acid-base equilibrium of the body. A value of *p*H 7.55, for example, indicates a condition of alkalosis. It may be due, as will be shown presently, either to an uncompensated excess of alkali or to an uncompensated deficit of carbon

[56] J. Biol. Chem., **83**, 539, 545 (1929).
[57] J. Physiol., **51**, 440 (1917).
[58] J. Biol. Chem., **45**, 489 (1920–1).
[59] *Ibid.*, **54**, 305 (1922).
[60] *Ibid.*, **55**, 495 (1923).
[61] J. Physiol., **54**, 32 (1920).

dioxide. On the other hand, a *p*H below 7.3 indicates a condition of acidosis, due either to an uncompensated deficit of alkali or to an uncompensated excess of carbon dioxide. Values lower than *p*H 7.0 are rarely observed in individuals who later recover. Such values are indicative of extreme acidosis and have been observed almost exclusively in cases of diabetic or uremic coma which terminated fatally.

Carbon Dioxide in the Blood.—In man, the arterial blood normally contains about 50 volumes per cent of carbon dioxide. Venous blood usually contains from 55 to 60 volumes per cent. If the amount of carbon dioxide ordinarily found in arterial blood were dissolved in an equivalent volume of water, the hydrogen-ion concentration of the resulting solution would be 3.1×10^{-5}. If the blood were to become as acid as this, life would cease immediately. Actually, the hydrogen-ion concentration of the blood is only about one-thousandth of this value. How, then, is the blood able to carry so much carbon dioxide and yet remain an alkaline solution? In attempting to answer this question, we become involved in another very important problem which has to do with the mechanism whereby the blood maintains its hydrogen-ion concentration within fixed limits.

There are four forms in which carbon dioxide can exist in solution: (1) as free anhydrous carbon dioxide; (2) as carbonic acid (H_2CO_3); (3) as bicarbonate; and (4) as carbonate. It may be assumed that, in water, a constant amount, if not all, of the free CO_2 would change into H_2CO_3, particularly as the water concentration remains constant. It is also apparent that, in the presence of carbonic acid, carbonates are changed to bicarbonates as follows:

$$B_2CO_3 + H_2CO_3 = 2BHCO_3,$$

where B is used to represent a monovalent base such as sodium or potassium. The conditions in the blood are such that only H_2CO_3 and $BHCO_3$ can possibly exist. It is very important that both of these be present in certain more or less definite proportions. As Van Slyke [62] points out, if the CO_2 were all present as H_2CO_3, the blood would be a thousand times more acid than it is. If the CO_2 were all present as bicarbonate, the blood would be hundreds of times too alkaline. There is normally a definite balance or ratio between the amount of CO_2 present as H_2CO_3 and the amount present as $BHCO_3$. Because there is a mechanism which makes this balance possible, the blood is able to carry large amounts of carbon dioxide from the tissues to the lungs, to be excreted, without the production of any very marked change in its hydrogen-ion concentration. We shall now examine the nature of this mechanism.

[62] Physiol. Reviews, **1**, 141 (1921).

The Nature of Buffers.—Buffer action may be defined as the resistance to change in hydrogen-ion concentration. Solutions may be prepared of mixtures of acids or bases with an excess of alkali salts in the case of the acids, and, in the case of the bases, an excess of their salts with strong acids. Such solutions are termed "buffer solutions" and are distinguished by the fact that upon dilution, or the addition of moderate amounts of acid or base, but little effect upon their hydrogen-ion concentration is produced.

Buffer action may be illustrated by the following example. If, to a liter of pure water of *p*H 7.0, we should add 1 cc. of 0.01 N hydrochloric acid, the hydrogen-ion concentration of the resulting solution would become equivalent to a *p*H of about 5.0. If, however, instead of using pure water, we should add the same amount of acid to water containing potassium acid phosphate (KH_2PO_4) and sodium hydroxide in such proportion as to give the solution a *p*H value of 7.0, the resulting change in hydrogen-ion concentration would be hardly measurable.

The behavior of sodium acetate as a buffer is similar. When added to acetic acid, sodium acetate reduces the ionization of the acid and consequently the C_H of the solution. If to a solution of acetic acid and sodium acetate, hydrochloric acid is added, little effect is produced on the hydrogen-ion concentration, as the hydrogen ions combine with acetate ions to form the almost un-ionized acetic acid. On the other hand, the same amount of hydrochloric acid added to water or to a solution of sodium chloride would cause a marked increase in hydrogen ions. The sodium acetate-acetic acid solution would also be effective against hydroxyl ions. The addition of a base like sodium hydroxide would result in its reaction with the acetic acid to give sodium acetate.

Fernbach and Hubert,[63] who first studied the power of certain solutions to resist changes in reaction, compared their action to that of a tampon. Sørensen translated this by the German word "puffer." In translating this into English, the word "buffer" has been adopted. Various analogies have been employed to explain buffer action. A buffer has been compared to a sponge having the capacity of "soaking up" hydrogen and hydroxyl ions. It has also been likened to a shock absorber. Just as a shock absorber blocks the transmission of the full force of the impact, so a chemical buffer resists the change in H^+ ion concentration which tends to occur when acid or alkali is added.

The Buffers of the Blood.—The buffers of the blood are salts of weak acids. These are the bicarbonates, phosphates, and alkali salts of the proteins, including both hemoglobin and oxyhemoglobin. In each case, however, part of the buffer is present as the free acid, the remainder

[63] Compt. rend., **131**, 293 (1900).

as the salt of the weak acid with a strong base. We thus have a number of what we might term buffer pairs. These are:

$$\frac{H_2CO_3}{BHCO_3'} \quad \frac{BH_2PO_4}{B_2HPO_4'} \quad \frac{HHbO_2}{BHbO_2'} \quad \frac{HHb}{BHb'} \quad \frac{H\ Protein}{B\ Protein}.$$

B is used here to indicate any monovalent base, such as sodium or potassium, $HHbO_2$ the free oxyhemoglobin, $BHbO_2$ the alkali salt of oxyhemoglobin, HHb the free or acid hemoglobin, BHb the basic salt of hemoglobin, H Protein the free protein, and B Protein the alkali proteinate, respectively.

The maintenance of the acid-base balance of the blood is not dependent upon any one buffer pair, but rather upon the total effect of several such pairs. The advantage of such an arrangement in providing security against acidosis or alkalosis may be likened to the advantage of having five sentinels on duty instead of one. It is obviously unsafe to leave a single individual to safeguard a treasure or to hold and defend a mountain pass against an enemy. No matter how well-armed he may be, he is always in great danger of being overcome. Five guards strategically placed would constitute a much more solid defense. If one or two of the guards were overcome there would still be a few left to hold the enemy back for a time, perhaps even until help arrived. This analogy, crude as it may be, is quite apropos if we suppose the enemy to be H^+ or OH^- ions, and the mountain pass the outer limits of the normal *p*H range of the blood. Of course, all five guards in our analogy might be overcome. Translated into terms of acid-base balance, this is what happens when coma or tetany develops.

General Laws.—There are two general laws which may be applied to buffer solutions:[64]

1. The hydrogen-ion concentration of a buffer solution is proportional to the ratio $\frac{\text{free buffer acid}}{\text{buffer salt}} = \frac{HA}{BA}$, where A represents the acid radical and B a monovalent base.

2. A given buffer mixture is most efficient in maintaining constancy of *p*H when the ratio $\frac{HA}{BA}$ is equal to 1, and when H^+ approximates K, the dissociation constant of the free acid forming one of the buffer pairs.

Relationship between *p*H and the Ratio $\frac{HA}{BA}$.—The proof of the first law will be considered at this point. The dissociation of an acid HA

[64] L. J. Henderson, Ergebnisse d. Physiol., **8**, 254 (1909), see also Van Slyke,[62] previous citation.

may be represented by the equation $HA = H^+ + A^-$. From the law of mass action, it follows that, when equilibrium is reached,

$$K \times HA = H^+ \times A^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where K is the dissociation constant of the acid. Therefore,

$$H^+ = K\frac{HA}{A^-} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

However, in buffer mixtures of the type with which we are concerned, there is to be considered not only the weak acid, but the salt of the acid as well. No matter which of the buffer pairs we may select, the dissociation of the free acid is negligible as compared with the dissociation of the salt BA (salt of a weak acid and a strong base). It is to be noted here that the salts are present in the blood in low concentration, which means that they are, relatively speaking, highly dissociated (60–90 per cent). The degree of dissociation, which may be represented by λ, will not vary appreciably for any given base over the range of its concentration in the blood. The concentration of the anions A^- is therefore equal to λBA. Substituting in Equation (2),

$$H^+ = K\frac{HA}{\lambda BA}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

As λ remains practically constant, the above equation may be simplified by substituting K_1 for $\frac{K}{\lambda}$. Thus,

$$H^+ = K_1\frac{HA}{BA}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Expressed in terms of pH, Equation (4) becomes

$$p\text{H} = -\log K_1 - \log\frac{HA}{BA}. \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The symbol pK_1 may be used to signify the logarithm of the reciprocal of K_1. Hence Equation (5) may be written

$$p\text{H} = pK_1 + \log\frac{BA}{HA}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

For any given buffer pair, pK_1 remains practically constant. It is 6.1 for $BHCO_3 : H_2CO_3$ (Hasselbach corrected by Van Slyke). For the phosphates it is 6.8. By substituting these values for pK_1 in Equation (6), it becomes possible to calculate the ratio of a buffer mixture for any given pH, or, if the ratio is known, the pH may be calculated.

Efficiency of Buffers.—According to the second generalization, the maximum efficiency of a buffer is obtained when $\frac{BA}{HA} = 1$, and $[H]^+ = K_1$. The bicarbonate : carbonic acid ratio is 1 at a *p*H of 6.1, while at a *p*H of 7.4 it is equal to $\frac{20}{1}$. This means that the bicarbonate, as a buffer, does not act with its maximum efficiency in the blood, where the *p*H is about 7.35. At the same *p*H the ratio $Na_2HPO_4 : NaH_2PO_4$ is 3.55, whereas at 6.8 the ratio is 1. This means that the closer the reaction of the blood approaches the danger zone, the more efficient is the buffer action of the phosphates.

Two factors contribute to the buffer mechanism of the blood. The first is the buffer action of the bicarbonate and proteins of the plasma, and of the bicarbonate, phosphates, and proteins of the cells. The second factor depends upon the property of hemoglobin to change from a weak acid to a relatively strong one when it changes from the reduced to the oxidized form. The dissociation constant of reduced hemoglobin (of the horse) is approximately 1/29 of that of oxyhemoglobin.

The Transportation of Carbon Dioxide and the Neutralization of Acids.—Intimately connected with the buffer action of these substances is their rôle in the transportation of carbon dioxide. According to the definition given by Van Slyke, a carbon-dioxide carrier is a constituent of the blood that increases the amount of carbon dioxide which may be taken up by arterial blood with a change in reaction equal only to the normal *p*H difference between arterial and venous blood. The maintenance of the reaction of the blood at a constant level and the transportation of carbon dioxide are both due to the giving up, by the buffer salts, of part of their reserves of alkali for the purpose of neutralizing any acid, including carbonic acid.

All buffers act in essentially the same manner in neutralizing acid. Let us assume that a large amount of lactic acid is being formed which requires neutralization. The sodium bicarbonate will react with it as follows:

$$NaHCO_3 + \text{Lactic acid} = \text{Na·lactate} + H_2CO_3.$$

The basic phosphates would react similarly:

$$B_2HPO_4 + \text{Lactic acid} = \text{B·lactate} + BH_2PO_4.$$

The plasma proteins would neutralize a portion according to the equation:

$$\text{B·Protein} + \text{Lactic acid} = \text{B·lactate} + \text{H·Protein}.$$

The remaining buffers would act in the same way.

The Action of Buffers as Carriers of Carbon Dioxide.—All buffers act in essentially the same manner as carriers of carbon dioxide. For the purpose of illustration, we shall consider the behavior of the phosphates and calculate the changes that occur when the H_2CO_3 is increased sufficiently to lower the *p*H from 7.35 to 7.25. Of course, it is to be clearly understood that changes of this magnitude do not actually occur in the blood. This marked shift, which is several times the normal *p*H difference between arterial and venous blood, is selected mainly in order to make the calculations somewhat more significant and the illustration somewhat clearer.

In these calculations it will be assumed that the concentration of total phosphate ($Na_2HPO_4 + NaH_2PO_4$) is 0.05 M; and of $NaHCO_3$, 0.03 M.

Recalling the relation between the ratio of a buffer pair and *p*H, and substituting 6.80 for pK_1 in the equation representing this relation, we have

$$\log \frac{Na_2HPO_4}{NaH_2PO_4} = 7.35 - 6.80 = 0.55,$$

or

$$\frac{Na_2HPO_4}{NaH_2PO_4} = 3.55.$$

As the total concentration of PO_4 is 0.05 M, the concentration of NaH_2PO_4 is obviously $0.05 - Na_2HPO_4$. Calculating, we find that at *p*H 7.35,

$$\frac{Na_2HPO_4}{NaH_2PO_4} = \frac{0.0390}{0.0110} = 3.55.$$

The ratio changes with *p*H, being but 2.82 at *p*H 7.25. Calculating as before, we find that at *p*H 7.25,

$$\frac{Na_2HPO_4}{NaH_2PO_4} = \frac{0.0369}{0.0131} = 2.82.$$

That the change in *p*H is accompanied by the release of a certain amount of base can be seen from the fact that, in the equation above, NaH_2PO_4 has one less sodium than Na_2HPO_4. The amount of alkali which is thus set free to combine with H_2CO_3 to form bicarbonate is calculated as follows:

$$\begin{array}{r} 0.0390 \text{ M } Na_2HPO_4 \\ - \ 0.0369 \text{ M } Na_2HPO_4 \\ \hline \text{Difference} = 0.0021 \text{ M Na set free to form } NaHCO_3. \end{array}$$

We began with an initial bicarbonate concentration of 0.03 M. At the higher *p*H,

$$\log \frac{NaHCO_3}{H_2CO_3} = 7.35 - 6.10 = 1.25;$$

hence

$$\frac{NaHCO_3}{H_2CO_3} = 17.8.$$

It therefore follows that

$$H_2CO_3 = \frac{0.03}{17.8} = 0.00169 \text{ M}.$$

At *p*H 7.25 the ratio $NaHCO_3 : H_2CO_3$ is equal to 14.1. Had the $NaHCO_3$ concentration remained constant, the H_2CO_3 would have been

$$H_2CO_3 = \frac{0.03}{14.1} = 0.00212.$$

This is not the case, however, for it has just been shown that, with the change in *p*H from 7.35 to 7.25, the $NaHCO_3$ concentration increased to 0.0321 M. Therefore, at *p*H 7.25,

$$H_2CO_3 = \frac{0.0321}{14.1} = 0.00228 \text{ M}.$$

The difference between 0.00212 and 0.00169 = 0.00043 M represents the amount of free H_2CO_3 that is added to 0.03 N $NaHCO_3$ solution with a change in *p*H from 7.35 to 7.25. The difference between 0.00228 and 0.00212 = 0.00016 M represents the amount of free H_2CO_3 added because of the buffer effect of the phosphates and the consequent increase of the $NaHCO_3$ concentration. Thus, the additional amount of carbon dioxide that can be carried on account of the presence of phosphates is

$$\begin{array}{l} 0.00210 \text{ M } CO_2 \text{ as } NaHCO_3, \\ + \ 0.00016 \text{ M } CO_2 \text{ as } H_2CO_3 \\ \hline 0.00226 \text{ M } CO_2 = CO_2 \text{ capacity of the phosphates} \end{array}$$

between *p*H 7.35 and 7.25.

The remaining buffers of the blood contribute, in like manner, their quotas of base, in order that the removal of carbon dioxide may proceed at a more or less constant rate. Except for the difference in direction, there is here, as in the oxygen-carrying mechanism, a pressure gradient. The outside atmosphere contains about 0.04 per cent of carbon dioxide; this is equivalent to about 0.3 mm. of mercury, partial pressure. The partial pressure of carbon dioxide in the alveoli of the

lungs, as well as that in arterial blood, is about 40 mm. of mercury. It is somewhat greater in venous blood and still greater in the tissues. Carbon dioxide passes from points of higher to points of lower concentration.

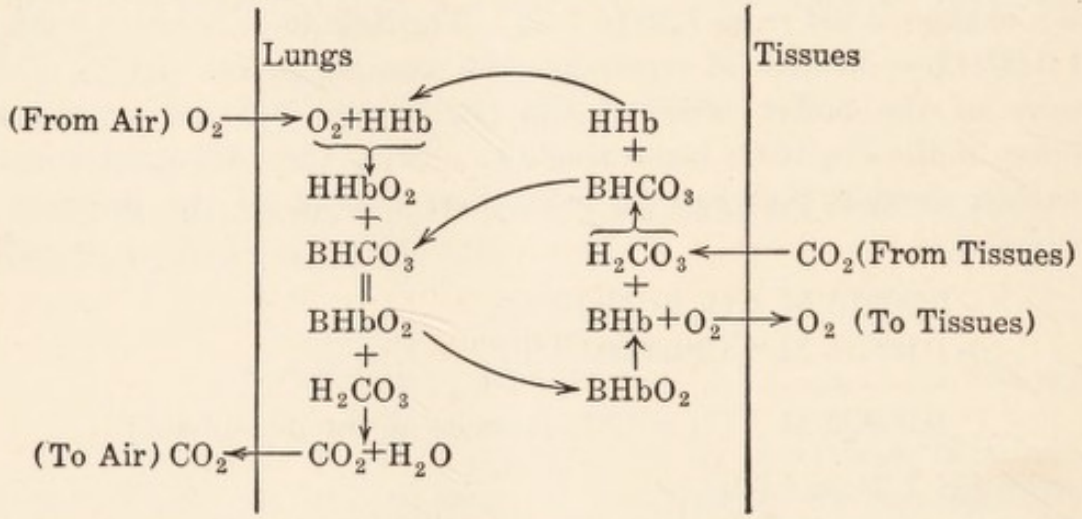
The Hemoglobin-oxyhemoglobin Change.—Oxyhemoglobin is a stronger acid than reduced hemoglobin, as evidenced by the following values for their dissociation constants:[65]

$$\text{K for oxyhemoglobin} = 1.35 \times 10^{-7}$$
$$\text{K for reduced hemoglobin} = 4.68 \times 10^{-9}$$

Both the reduced and the oxidized forms exist partly as free acids and partly as the salts of strong bases. The tendency of oxyhemoglobin to form salts is greater, however, than that of reduced hemoglobin, and hence, at a given *p*H, the proportion of salt to acid will be greater in the former case than in the latter.

$$\frac{BHbO_2}{HHbO_2} > \frac{BHb}{HHb}.$$

This is very significant, for, in changing to the reduced form in the tissues, oxyhemoglobin liberates sufficient alkali to neutralize a considerable part of the carbon dioxide that is present. Subsequently, when the reduced hemoglobin reaches the lungs and is oxidized, it reacts with bicarbonate, with the consequent liberation of carbon dioxide. The cycle may be represented as follows:



Estimations have been made of the approximate distribution of the carbon-dioxide carrying power among the buffers of the blood. The calculations of Van Slyke, based on the most reliable data available in 1921, show that the base furnished by hemoglobin accounts for 84–94

[65] These values have been calculated from the *p*K values obtained by Hastings for oxyhemoglobin and for reduced hemoglobin (of the horse) in solutions containing 30 millimols of cation (Na or K). For details see the papers of Hastings and coworkers, J. Biol. Chem., **60**, 89 (1924), especially p. 151; **61**, 317 (1924).

per cent of the total carbon dioxide absorbed. For details, the student is referred to the reviews of Van Slyke [62] and Wilson [66] as well as to L. J. Henderson's monograph.[67] Suffice it to say here that in the transportation of carbon dioxide, hemoglobin plays a dual rôle. Owing to its action as a buffer, it contributes materially to the carbon-dioxide capacity of the blood; but even more important than this is the fact that half or more of the carbon dioxide is carried in combination with the base liberated as a result of the isohydric change of oxyhemoglobin to hemoglobin.

Doisy, Briggs, Eaton, and Chambers [68] have obtained fairly close approximations of the carbon dioxide carried by various buffer systems in the blood. Their results, which are somewhat lower than those obtained by others, are presented in Table XXXIV and show that about 53 per cent of the total carbon dioxide carried is due to the change

$$BHbO_2 \rightleftarrows HHb,$$

and that about 20–30 per cent more is carried by the base liberated from hemoglobin as a result of the change in *p*H. That the most important buffer of the blood is isolated within the corpuscles is a matter worthy of note.

Buffer Effect of Plasma and Corpuscles.—Although the major part of the buffer effect of the blood resides in the corpuscles, both the plasma and the corpuscles take part in the transportation of carbon dioxide. The plasma, according to the observations previously mentioned of Bock, Field and Adair,[38] carries about 60 per cent, and the corpuscles about 40 per cent, of the carbon dioxide which arterial blood takes on when it becomes venous blood.

When plasma or serum is in contact with red cells (" true " plasma or serum), its capacity for taking up carbon dioxide is much greater than in the absence of cells (" separated " plasma or serum). This is because the cells pass on their buffer effect to the plasma in accordance with the mechanisms that have been discussed. Some features of these mechanisms still remain to be considered, however.

Zuntz [69] and Schmidt [70] found that they could increase the titratable alkalinity of serum by subjecting the blood to high tensions of carbon dioxide. Zuntz concluded that the carbonic acid passed into the corpuscles, where it split off alkali from the cell proteins, and that the alkali then diffused into the serum. That the corpuscles are freely permeable to carbonic acid has been well established. The transfer of

[66] Physiol. Reviews, **3**, 295 (1923).

[67] L. J. Henderson, Blood, A Study in General Physiology, New Haven, 1928.

[68] J. Biol. Chem., **54**, 305 (1922).

[69] Centr. med. Wiss. (1867), 529; Dissertation, Bonn (1868).

[70] Ber. k. sächs. Ges. Wiss., Math.-phys., **19**, 30 (1867).

cations between the corpuscles and the plasma or serum seems very improbable.

TABLE XXXIV

CARBON DIOXIDE CARRIED BY BUFFER SYSTEMS OF THE BLOOD *

	E. A. D.		W. H. C.		J. M.	
	Volumes, Per Cent	Per Cent of Total	Volumes, Per Cent	Per Cent of Total	Volumes, Per Cent	Per Cent of Total
Total CO_2 carried for R. Q. of 0.75...	2.32	.....	4.23	.....	5.08	
$BHCO_3$ carried isohydrically.....	1.233	53.1	2.262	53.5	2.72	53.5
$BHbO_2 \rightleftharpoons HHb$						
$BHCO_3$ carried by change of *p*H:						
By hemoglobin: $BHbO_2 \rightleftharpoons HHbO_2$						
$BHb \rightleftharpoons HHb$...	0.439	18.9	1.070	25.3	1.384	27.2
By B_2HPO_4 in cells.....	0.010	0.43	0.012	0.3	0.013	0.25
By separated serum.....	0.089	3.84	0.198	4.7	0.142	2.8
CO_2 physically dissolved.....	0.249	10.7	0.511	12.1	0.657	12.9
Sum, per cent of total.....	2.020	87.0	4.053	96.0	4.196	97.0
Per cent of total CO_2 carried by hemoglobin.....	72.0		78.8		80.7	
	*p*H		*p*H		*p*H	
Arterial blood (*p*H values recalculated)	7.296		7.310		7.281	
Venous blood (*p*H values recalculated)	7.283		7.280		7.244	
Difference.....	0.013		0.030		0.037	

* Compare, Doisy, Briggs, Eaton and Chambers, J. Biol. Chem., **54**, 322 (1922); Wilson, Physiol. Reviews, **3**, 304 (1923).

Chloride Shift.— In seeking an explanation for the increased titratable alkalinity of the serum when carbon dioxide is passed through blood, Gürber [71] studied the ash of the serum and determined that no sodium or potassium diffused from the corpuscles. These observations have been confirmed by many workers. Gürber found, however, that sufficient chloride passed into the corpuscles to account for the increase in serum bicarbonate. That this is not entirely correct has been shown by Van Slyke and Cullen,[72] who were able to account for only 72 per cent of the alkali increase of the plasma on the basis of the chloride shift. Other

[71] Maly's Jahresb., **25**, 165 (1895).

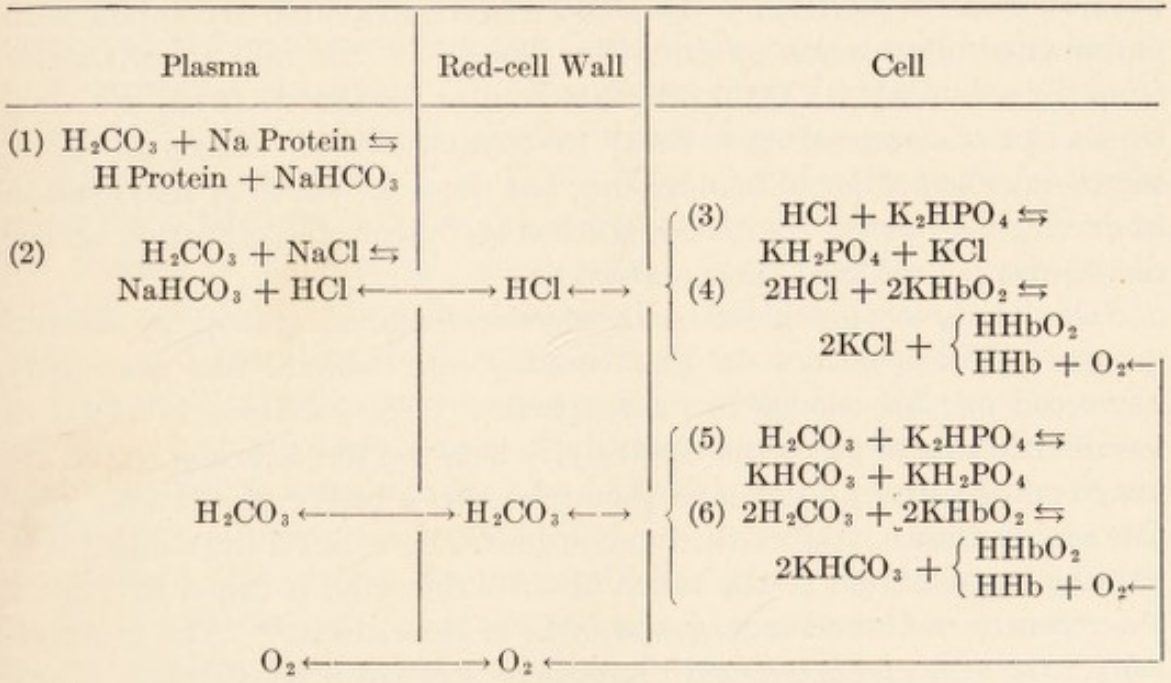
[72] Cited by Van Slyke. Physiol. Rev., **1**, 161 (1921).

anions, SO_4 and PO_4, are also capable of migration through the red-cell membrane. When the reaction of the blood is changed artificially from 7.45 to 7.25 by the absorption of carbon dioxide, the base furnished to form the additional plasma bicarbonate comes from the following sources, according to the calculations of Doisy, Eaton, and Chouke:[73]

1. Due to non-migrating serum buffers (plasma proteins, amino acids, and organic acids) ..... 16 per cent
2. Due to migration of Cl into corpuscles..... 80 per cent
3. Due to the migration of other acid radicals such as SO_4 (de Boer [74]), and PO_4 (Doisy and Eaton [75])... 4 per cent

That the transfer of chloride between plasma and corpuscles under the influence of changing tensions of carbon dioxide occurs *in vivo* has been demonstrated by numerous workers who compared arterial with venous blood and found more chloride in the plasma of the former than in that of the latter.

The reactions which may be supposed to occur in the plasma and corpuscles, when CO_2 and O_2 are either absorbed or given off, have been summarized in a diagram which is reproduced below.[76] In interpreting this diagram it is to be appreciated that HCl and H_2CO_3 do not diffuse through the membrane as such, but as H^+, Cl^- and HCO_3^- ions.



[73] J. Biol. Chem., **53**, 61 (1922).

[74] J. Physiol., **51**, 211 (1917).

[75] J. Biol. Chem., **47**, 377 (1921).

[76] Austin, Cullen, Hastings, McLean, Peters, and Van Slyke, J. Biol. Chem., **54**, 121 (1922).

Electrolyte and Gas Equilibria.—The distribution of electrolytes in the blood, including the transfer of chloride, hydrogen ions, carbon dioxide, and water between the plasma and corpuscles, can be explained partly on the basis of Donnan's theory of membrane equilibrium, by assuming that the membrane of the red cell is the semi-permeable membrane which separates the plasma from the fluid in the corpuscles. This membrane, as we have seen, is impermeable to proteins and cations, with the exception of H^+ ions. It is permeable to HCO_3^- and other anions (Cl, SO_4 and PO_4). Electrolytes that are present on either side of the membrane will tend to distribute themselves equally on the two sides. This tendency will be opposed, however, by the attractive forces of the non-diffusible ions, with the result that when equilibrium is reached there will be an uneven distribution of ions on the two sides of the membrane.

As is evident from what has been stated earlier in this chapter, the equilibria established between the cells and serum (or plasma) are due in a large measure to the base binding power of hemoglobin and its variation with changing *p*H and oxygenation. These properties explain the following facts, as summarized by Van Slyke:[77] (1) The cells contain more base in proportion to water than serum, but much less Cl and HCO_3; (2) the cell contents are more acid than serum; (3) the cells carry most of the buffer alkali (as BHb) of the blood which is available to combine with carbonic or other acids entering the blood; (4) the cells absorb water from the serum when CO_2 or other acids enter the blood; (5) at the same time Cl passes from serum to cells; (6) oxygenation of reduced blood, by increasing the acidity of hemoglobin, has the same effect as acidification in driving CO_2 out of the blood, but has the effect of alkalization on the distribution of diffusible ions and water.

The study of the blood as a physico-chemical system by several groups of investigators in this country and abroad has materially advanced our knowledge of its properties. The relations involved in the distribution of gases and electrolytes between the cells and serum are very complex and do not lend themselves to a brief formulation. For this reason they cannot be further considered here, but if the student is to gain an appreciation of the scope of the subject he is urged to consult the recently published monograph of L. J. Henderson.[78] The composition of the cells and serum with respect to any given constituent, at any stage in the respiratory cycle is quantitatively dependent on practically

[77] D. D. Van Slyke, Factors Affecting the Distribution of Electrolytes, Water and Gases in the Animal Body, Philadelphia and London, 1926, p. 28.

[78] L. J. Henderson, Blood, A Study in General Physiology, Yale University Press, New Haven, 1928.

all the other constituents. In other words, each is a variable and the relations of all the variables may be mathematically formulated and graphically represented. An idea may be gained of the complexity of the situation if some of the variables are listed. These are:

1. Concentration of Cl in serum (Cl_s).
2. Concentration of Cl in cells (Cl_c).
3. The per cent of total blood chloride or bicarbonate present in the cells (A).
4. Volume of cells (V).
5. Per cent H_2O in cells.
6. The base combined with cell protein (BP_c).
7. The combined carbonic acid of the cells ($[BHCO_3]_c$).
8. The combined carbonic acid of the serum ($[BHCO_3]_c$).
9. Total CO_2 of the blood.
10. Free CO_2 of the blood.
11. Base bound by protein of serum (BP_s).
12. *p*H in the serum.
13. *p*H in the cells.
14. Oxygen pressure.
15. Combined oxygen (HbO_2).

Alkali Reserve.—The alkali reserve refers to the amount of base combined as bicarbonate and not to all of the base stored in the blood, as is sometimes supposed. This is apparent from the methods used in its determination, one of the simplest of which being the method introduced by Van Slyke and Cullen[79] in 1917. This procedure is based upon the assumption that the CO_2-combining power of the blood depends upon the amount of alkali which is available. The essential features of the method consist in saturating the blood, after it is drawn, with carbon dioxide, and liberating the CO_2 in a definite amount of blood by treating it with acid *in vacuo* in an apparatus devised for this purpose. In *acidosis*, which may result from the generation of acids and the consequent removal of base from the blood, the CO_2-combining capacity of the blood is low. Where the reserve alkali is above normal, as in conditions of *alkalosis*, the carbon dioxide-combining power is abnormally high.

Both in acidosis and alkalosis, the normal relations of acids and bases in the blood, or the " acid-base balance," are disturbed.

The rate of respiration is governed ordinarily by the oxygen requirements of the tissues and by the amount of carbon dioxide formed. During exercise, for example, one breathes at a more rapid rate than

[79] J. Biol. Chem., **30**, 289 (1917). This method has been modified so that the CO_2 liberated is measured manometrically (J. Biol. Chem., **61**, 523, 575 (1924)).

normally. A decrease in the alkali reserve of the blood must therefore increase the frequency of pulmonary ventilation, as would be expected from the fact that under these conditions the carbon-dioxide capacity of the blood is less than normal. This is a matter of frequent clinical observation, particularly in diabetic and nephritic acidosis. This effect is well illustrated experimentally by the symptoms developed by J. B. S. Haldane,[80] whose method of producing acidosis consisted in swallowing considerable amounts of ammonium chloride. The effect this had on respiration can be well imagined from the fact that in walking, even at a leisurely pace, he panted so hard as to attract the attention of everyone who saw him.[81]

Acid-base Balance of the Blood.—In considering the normal and abnormal variations in the acid-base equilibrium of the blood, Van Slyke has shown that nine conditions are theoretically possible. The blood bicarbonate may be high, low, or normal, and in each of these cases the *p*H may be high, low, or normal. Van Slyke[82] has represented all the possible variations in the form of a chart which is reproduced on p. 237.

For the purpose of the present discussion, we may accept *p*H 7.3–7.5 as the limits of the normal *p*H range for the blood although actually the normal *p*H values fall within a much narrower range. While occasional reports have been made of *p*H values higher than 7.8 in severe alkalosis and lower than 7.0 in marked acidosis, we may nevertheless regard the maximum tolerated range to be between *p*H 7.0 and 7.8. The total carbon dioxide of the blood, at a tension of 40 mm. of mercury and 38° C., usually falls between 43 and 55 volumes per cent; for the plasma the values range between 51 and 63 volumes per cent. This determination can be made accurately by the method of Van Slyke and Cullen, mentioned above, or by the more recently devised manometric method of analysis. In the chart, four variables are represented, namely, the total carbon dioxide, the CO_2 tension, *p*H, and H_2CO_3. To locate any point in the figure, two of the variables must be known. Thus, if the CO_2 tension and the total $BHCO_3$ are known, the *p*H may be readily determined. Knowing the *p*H and CO_2 content of the blood, one may obtain a fair idea of the acid-base balance which exists. This follows from what has been said previously concerning the relation between *p*H and the ratio $BHCO_3 : H_2CO_3$. Since *p*H is a function of this ratio, when $BHCO_3$ is represented as ordinates and H_2CO_3 as abscissae, the

[80] J. Physiol., **55**, 265 (1921).

[81] See Y. Henderson, Physiological Regulation of Acid-Base Balance, Physiol. Reviews, **5**, 131 (1925).

[82] J. Biol. Chem., **48**, 153 (1921).

curve plotted will be a straight line for all points corresponding to a constant ratio. Reference to the chart will make this clear. The slope of the line will depend on whether the ratio is large or small, being steeper in the former case than in the latter.

As represented in the chart, nine areas are clearly defined. These will be considered briefly.

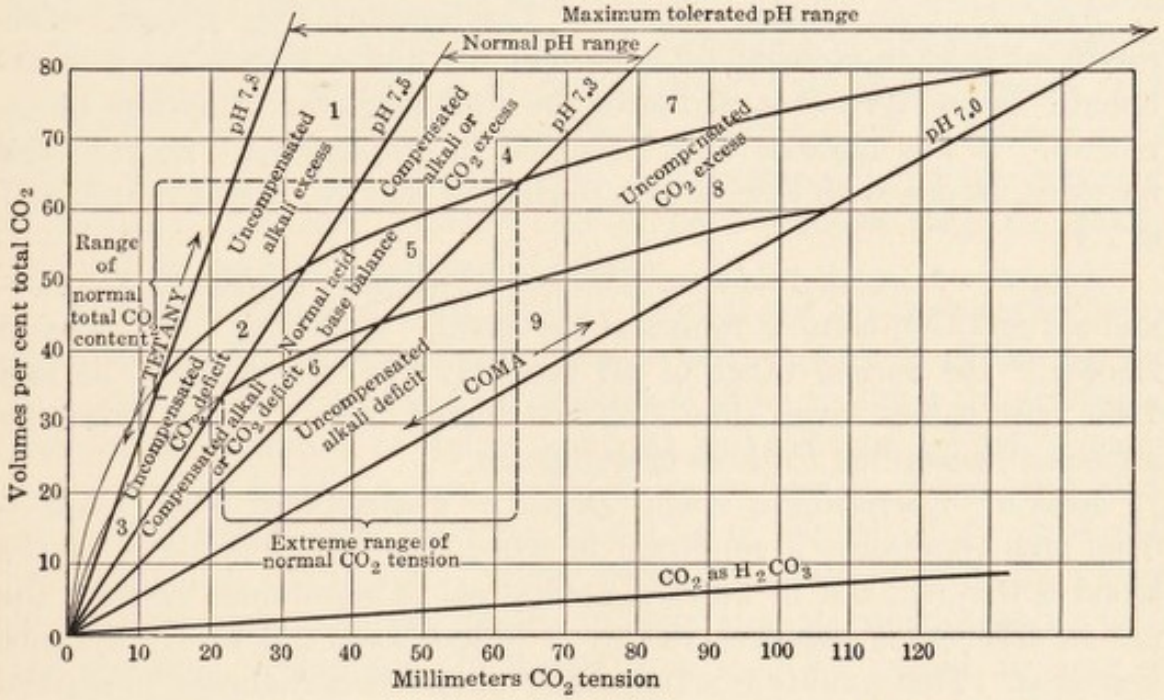


FIG. 36.—Normal and abnormal variations of the [$BHCO_3$], [H_2CO_3], CO_2 tension, and *p*H in oxygenated human whole blood drawn from resting subjects at sea-level. The bicarbonate CO_2 at any point is obtained by subtracting from the total CO_2 the relatively small amount present as H_2CO_3 indicated by the slanting line near the bottom of the figure. (After Van Slyke.)

Area 1. Uncompensated Alkali Excess.—This condition is characterized by a high *p*H and a high blood and plasma bicarbonate, due to an increase in the ratio $NaHCO_3 : H_2CO_3$. Alkalosis due to uncompensated alkali excess may be brought about by the administration of excessive amounts of sodium bicarbonate. It is also encountered as a result of loss of hydrochloric acid through persistent vomiting, as in intestinal obstruction (p.189). Myers [83] in his review cites a number of observations which fall into this group. The highest *p*H recorded is 7.60, occurring in a case of diabetes following the administration of $NaHCO_3$. The CO_2 content of this plasma was 75. Exposure to X-rays or to radium may be followed by an uncompensated excess of alkali in the blood (Hussey).[84] If the uncompensated alkali excess is sufficient, symptoms of tetany develop. Whether tetany is caused by alkalosis, or

[83] Physiol. Reviews, **4**, 314 (1924).

[84] J. Gen. Physiol., **4**, 511 (1922); see also review by Myers.

is merely associated with it, is not definitely known. Greenwald [85] believes that the symptoms of tetany may be due to "sodium poisoning."

Areas 2 and 3. Uncompensated CO_2 Deficit.—Here we have an abnormally high *p*H with a normal or low CO_2 content. This condition may result from voluntary or involuntary over-ventilation of the lungs. If the CO_2 deficit is sufficient, symptoms of tetany may develop.

Area 4. Compensated Alkali or Compensated CO_2 Excess.—This condition is characterized by a normal *p*H and a high plasma bicarbonate. It has been observed after the administration of sodium bicarbonate. It has likewise been observed in emphysema. Scott [86] has recorded, in one such case, a CO_2 content of 82.7 cc. with a normal *p*H of 7.4.

Area 5. Normal Acid-base Balance.—This area represents the normal *p*H and CO_2-content ranges of the blood. According to Myers and Booher,[87] the normal range of *p*H probably lies between *p*H 7.35 and 7.43. *p*H values lower than 7.32 and higher than 7.47 are definitely abnormal, according to these investigators.

Area 6. Compensated Alkali Deficit or Compensated CO_2 Deficit.—This area represents a condition in which the available alkali of the blood is lowered, but in which a normal *p*H is maintained because the fall in $BHCO_3$ is compensated by a proportional fall in H_2CO_3 and *vice versa*. This condition is frequently observed in diabetes, in nephritis, and in the diarrheal acidoses of infants. At low oxygen tensions or at high altitudes, a condition of CO_2 deficit may be brought about. Under these conditions, the CO_2 content of the alveolar air is lowered, and hence the CO_2 content of the blood is diminished. As the amount of CO_2 formed by the tissues does not change, the rate of respiration is increased. When the individual becomes adjusted to the high altitude, it is often found that the alkali reserve of the blood is lower than that observed at atmospheric pressure.

Areas 7 and 8. Uncompensated CO_2 Excess.—The conditions represented by these areas are characterized by a low *p*H and a high or normal CO_2. Uncompensated CO_2 excess results when the respiratory excretion of CO_2 is retarded. It has been observed in cases of cardiac decompensation accompanied by dyspnea. Peters and Barr [88] have recorded *p*H values for venous blood as low as 7.17 in the cases that they studied. This condition is also encountered in morphine narcosis.

Area 9. Uncompensated Alkali Deficit.—Here we have both a low

[85] J. Biol. Chem., **54**, 285 (1922).
[86] Arch. Internal Med., **26**, 544 (1920).
[87] J. Biol. Chem., **59**, 699 (1924).
[88] J. Biol. Chem., **45**, 537 (1921).

*p*H and a low plasma bicarbonate. The condition is one of "uncompensated acidosis" and is observed most frequently during the premortal period in cases of diabetes and nephritis. In diabetes, as the result of faulty fat metabolism, large amounts of aceto-acetic and hydroxy-butyric acids are formed. These are eliminated in combination with base, thus depleting the alkali reserve of the body. Acidosis may likewise result from the failure of the kidney to eliminate acids such as NaH_2PO_4. Alkali deficit and carbonic-acid retention occur in ether anesthesia, according to the work of Van Slyke, Austin, and Cullen.[89] Certain cardiac cases likewise exhibit an uncompensated alkali deficit. It has recently been demonstrated by many workers, including Cullen and Jonas,[90] that diabetic acidosis may be readily relieved by the administration of insulin.

The Water Balance of the Body.—This subject has been reviewed by Rowntree[91] and by Marriott.[92] Water balance may be defined as the daily relation between the total amount of water entering the organism, through the ingestion of liquids and food, and the total output of water lost from the body by way of the kidneys, bowels, lungs, and skin. The water that results from the oxidation of the foodstuffs must also be included in the intake. Water plays a number of important rôles in vital processes, and the supply, in most animals, must keep up with the demand. Voluntary abstinence from food for periods as long as two months has been endured by man, but deprivation of water for much shorter periods brings on serious effects. Rowntree cites the case of Viterbi, an Italian political prisoner who refrained from food and drink for eighteen days and died as a result. He is said to have suffered but little from hunger after the first day, but to have experienced terrible thirst until he died. Death from thirst, in the case of individuals who are lost in the desert, is believed to result after 36 to 72 hours of water deprivation.

It is estimated that from 7500 to 10,000 cc. of water per day are excreted into various parts of the alimentary tract as saliva, gastric juice, pancreatic juice and intestinal juice. Nearly all of this water, which is about two to three times the usual water intake, is reabsorbed. Water is lost to the body chiefly by way of the skin, respiratory tract, and kidneys. The kidneys are the most important, except under unusual conditions of heat and exercise, when the amount of water lost as sweat may exceed the amount in the urine.

[89] Proc. Soc. Exp. Biol. and Med., **17**, 169 (1919–20).
[90] J. Biol. Chem., **57**, 541 (1923).
[91] Rowntree, L. G., Physiol. Rev., **2**, 116 (1922).
[92] Marriott, W. McKim, Physiol. Rev., **3**, 275 (1923).

Numerous experiments have been performed in which the attempt was made to alter the composition of the blood by forced water administration. For example, in the experiment of Haldane and Priestley [93] 5500 cc. of water was taken in a period of four hours. Marked diuresis followed, so that at one time the rate of urine secretion was 2500 cc. per hour. And yet they observed no evidence of blood dilution. Similar results were obtained by Adolph.[94] These experiments show the remarkable control exercised by the kidneys in maintaining the normal water balance.

However, more recently, Greene and Rowntree [95] succeeded in producing blood dilution in the dog by forced administration of water.

There are many phases to the problem of water exchange, of which only a few can be referred to even briefly. The relation between water equilibrium and acid-base balance has been emphasized by a number of investigators recently.[96] Acidosis and dehydration are often associated phenomena, and alkalosis may be accompanied by sufficient water retention to show an increase in body weight. In another connection mention has been made of the effects resulting from the loss of water and hydrochloric acid in intestinal obstruction (p. 189).

Water retention due to lowered barometric pressure has been observed in rats and dogs by C. S. Smith.[97] Associated with this disturbance in water balance is marked restlessness, which has led to the interesting speculation as to the possibility of a similar relationship existing in those animals which are capable of anticipating a change in weather.

Larson, Weir and Rowntree [98] have described a form of water intoxication which may be produced in dogs, cats, rabbits and guinea pigs by the administration of excessive amounts of water. The symptoms are nausea, vomiting, salivation, convulsions, stupor and coma; death ensues if water administration is continued after the onset of the convulsions. On the other hand, the convulsions may be prevented by the injection of hypertonic saline when the first symptoms of the intoxica-

[93] J. Physiol., **50**, 296 (1915–16).

[94] *Ibid.*, **55**, 114 (1921).

[95] Am. J. Physiol., **80**, 209 (1927).

[96] Stieglitz, E. J., Arch. Int. Med., **41**, 10 (1928); Marriott, W. McKim, and Hartmann, A. F., J. Am. Med. Assoc., **91**, 1675 (1928); Schoenthal, L., Am. J. Dis. Child., **37**, 244 (1929); Editorials in J. Am. Med. Assoc., **90**, 1294, 1378; **91**; 2066 (1928); **92**, 148, 744, 898 (1929).

[97] Am. J. Physiol., **87**, 200 (1928).

[98] Cited by Rowntree ([91]).

tion become apparent. The convulsions are believed to be cerebral in origin.

The following description by Haldane[99] illustrates the effects of derangement of the salt and water balance:

" Perhaps the hottest place in England is about a mile under Salford, where the coal-miners work in boots and bathing-drawers, and empty the sweat from their boots at lunch—or snapping-time. One man sweated eighteen pounds in the course of a shift, and it is probable that even this figure has been exceeded. This sweat contained about an ounce of salt—twice what the average man consumes in all forms per day. The salt loss was instinctively made up above ground by means of bacon, kippers, salted beer and the like. And as long as they did not drink more than a quart of water underground, no harm came to the miners. But a man who has sweated nearly two gallons is thirsty, and coal-dust dries the throat, so this amount was often exceeded, and the excess occasionally led to appalling attacks of cramp, often in the stomach, but sometimes in the limb or back. The victims had taken more water than was needed to adjust the salt concentration in their blood, and the diversion of blood from their kidneys to their muscles and skin was so great that they were unable to excrete the excess. The miners in question were offered a solution of salt in water which was about the composition of sweat, and would be somewhat unappetizing to the average man. They drank it by quarts and asked for more. And now that it has become their regular beverage underground there is no more cramp, and far less fatigue. It is almost certain that the cramp of stokers, and of iron and glass workers, which is known to be due to excessive water-drinking, could be prevented in the same way."

It was shown by Weed and McKibben[100] that the intravenous injection of strong hypertonic solutions of salt or glucose produces a marked lowering of the cerebrospinal fluid pressure, together with a diminution in the volume of the brain. These effects, which are brought about by the withdrawal of water from the brain, as well as from other tissues, may also be obtained by the intra-intestinal administration of hypertonic solutions.[101] Clinical application of these physiological observations has been made, particularly in brain surgery, where occasionally a mod-

[99] J. B. S. Haldane, Possible Worlds, Harper, New York and London, 1928, p. 82. A fairly full account of "Some effects of high air temperatures and muscular exertion upon colliers" is given by K. N. Moss, Proc. Roy. Soc., B, **95**, 181 (1924). See also, E. F. Adloph, J. Physiol., **55**, 114 (1921).

[100] Am. J. Physiol., **48**, 512, 531 (1919).

[101] Foley and Putnam, *ibid.*, **53**, 464 (1920).

ification of the pressure of the cerebrospinal fluid or a diminution of the brain bulk is desired.

Water and Heat Regulation.—Rowntree states that water regulates heat distribution and dissipation because of its mobility and thermal properties. The high specific heat of water favors the storage of heat. The high caloric demands for the evaporation of water permit rapid elimination of heat when necessary. The high heat conductivity provides rapid equalization of heat within the tissues of the body, according to Barbour.[102] Rowntree points out that the latent heat of vaporization of water is of universal significance in relation to the dissipation of body heat, because of the fact that evaporation occurs at all temperatures.

Severe diuresis, owing to the depletion of water, produces a febrile condition in man and animals. In this way, Balcar, Sansum and Woodyatt[103] produced a fever as high as 125.6° F. in dogs through the intravenous administration of concentrated solutions of glucose. An elevation in temperature also develops in infants deprived of food and water, but in new-born puppies Pucher[104] obtained an opposite effect, namely a rapid fall in temperature. In dogs, Greene and Rowntree[105] have observed a fall in temperature to result from the forced administration of water, even though the water given was at a temperature equal to or slightly above that of the body.

The Lymph.—A number of difficulties present themselves in a consideration of the exchange of material between the blood and tissue cells. One obstacle in the way of a more lucid understanding of the subject than we possess at present is due to the uncertainty which still remains concerning the relation between the lymphatics and the tissue spaces. According to one school of anatomists, the lymphatics are continuous with the tissue spaces. If we accept this view, it follows that the contents of the lymph vessels and the fluid in the tissue spaces are identical in composition. If, on the other hand, we accept the hypothesis that the lymphatics form a closed system, it naturally follows that in the transference of material from the gastro-intestinal tract to the tissues, three fluids are concerned, namely, the blood, the lymph, and the tissue fluid. In view of our inability to differentiate chemically between lymphatic lymph and tissue lymph, it would serve no real purpose to introduce any distinction between them in this brief discus-

[102] Physiol. Reviews, **1**, 295 (1921).
[103] Arch. Int. Med., **24**, 116 (1919).
[104] J. Biol. Chem., **76**, 319 (1928).
[105] Am. J. Physiol., **80**, 230 (1928).

sion of the subject. The author, therefore will use the term lymph in a collective sense, to apply both to the fluid within the vessels and to that in the interstitial spaces.

The Composition of Lymph and Transudates.—Lymph and transudates in general resemble plasma but are more variable in composition. The water content is usually about 94–96 per cent. The solids constitute 4–6 per cent. There is a small amount of fibrinogen and both albumin and globulin. Of the solids, the protein fraction is the most abundant. There is, however, less protein in lymph than in plasma. White corpuscles are likewise present in lymph. When allowed to stand, lymph clots slowly.

In the accompanying table are outlined the results obtained by several investigators who have analyzed lymph from various sources. Analyses 1 and 2 are those of Gubler and Quevenne who obtained the lymph from the upper part of the thigh of a woman aged thirty-nine. Analysis 3 was made by von Scherer on lymph collected from the lymphatic vessels of the spermatic cord. Analysis 4 is that of C. Schmidt who obtained the lymph from the neck of a colt.

TABLE XXXV

Composition of Lymph.* The Results are Expressed in Parts per 1000

	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
Other proteins	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

* Compare Hammarsten-Mandel, A Textbook of Physiological Chemistry, John Wiley and Sons, 1914 edition, pp. 347–8.

Cajori and Pemberton [106] have compared the composition of blood plasma with that of synovial fluid in cases of arthritis with joint effusion and found almost identical values for non-protein nitrogen, urea nitrogen and amino acid nitrogen. Less protein was present in the synovial fluid than in the plasma and the globulin-albumin ratio was slightly

[106] J. Biol. Chem., **76**, 471 (1928).

higher in the synovial fluid than in the plasma. The sodium chloride content was somewhat higher in the synovial fluid. As pointed out by Fremont-Smith and Dailey,[107] in a similar study, these results may be explained by assuming that a simple membrane equilibrium exists between blood plasma and synovial fluid. Below are tabulated the average results of Cajori and Pemberton.

TABLE XXXVI

CONCENTRATION OF DIFFUSIBLE CONSTITUENTS OF SYNOVIAL FLUID AND PLASMA IN CASES OF JOINT EFFUSION, IN MG. PER 100 CC. (AVERAGE OF 9 CASES)

Non-protein N		Urea N		Amino Acid N		Sodium Chloride	
Plasma	Fluid	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid
26.2	26.1	15.8	15.8	5.6	5.9	565	595

TABLE XXXVI*a*

ALBUMIN AND GLOBULIN CONTENT OF SYNOVIAL FLUID AND PLASMA IN CASES OF JOINT EFFUSION, IN PER CENT (AVERAGE OF 10 CASES)

Total Proteins		Albumin		Globulin		Albumin/Globulin	
Plasma	Fluid	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid
7.00	4.97	3.76	3.00	1.97	1.47	1.9	2.0

Normal cerebrospinal fluid is a clear, colorless, alkaline fluid with a specific gravity of about 1.006 to 1.008 and is apparently an almost protein-free ultra-filtrate from plasma. The chloride content (expressed as NaCl) is 0.73 to 0.75 per cent; sugar 0.05 to 0.08 per cent; and the quantities of other diffusible constituents are comparable to the concentrations in the plasma. The protein content is very low, being about 0.02 per cent, as compared with about 7.0 per cent in the plasma.

The intra-ocular fluids are similar in composition. Duke-Elder[108]

[107] *Ibid.*, **70**, 779 (1926).

[108] Duke-Elder, W. S., Biochem., J., **21**, 66 (1927); J. Physiol., **62**, 315 (1927); Brit. J. Ophthal., Monograph series, iii, 1927; and particularly Recent Advances in Ophthalmology, Blakiston's Son & Co., Philadelphia, 1929, p. 189–212.

gives the following figures for comparison between the composition of the blood serum and the aqueous and vitreous humors:

	Quantities of Various Constituents in Grams per 100 cc.		
	Aqueous	Vitreous	Serum
Water	99.6921	99.6813	99.3238
Total protein	0.0201	0.0403	7.3692
Urea	0.028	0.029	0.027
Reducing substances estimated as glucose	0.0983	0.0973	0.091
Na	0.2787*	0.2731	0.3351‡
Cl	0.4371†	0.4168	0.3664§

* 121 millimols per liter.
† 123 millimols per liter.
‡ 145.6 millimols per liter.
§ 103 millimols per liter.

Duke-Elder has shown that the aqueous humor is formed by a process of dialysis and that its composition can be explained on the basis of Donnan's theory of membrane equilibria (p. 30). Indeed, this is, thanks to the work of Duke-Elder and others, one of the most clear-cut illustrations of the application of this theory to vital phenomena. Considering the distribution of the sodium and chloride ions between the blood and the aqueous humor, the theoretical relationship is:

$$[Na^+]_{aqueous} \times [Cl^-]_{aqueous} = [Na]_{serum} \times [Cl]_{serum}$$

Substituting the respective values for these concentrations, in terms of millimolar equivalents, the relationship becomes:

$$121 \times 123 = 145 \times 103$$

$$14{,}883 = 14{,}935$$

Duke-Elder points out that the close agreement in these results froms a strong argument that the formation of the aqueous humor is by a process of dialysis.

The lymph as it flows away from the lacteals is rich in fat and other absorbed material. As the fat is in a highly emulsified condition, the fluid has a milky appearance, and for this reason was called, by the earlier physiologists, " lacteal fluid," a name later replaced more or less generally by the term chyle. Except for a higher solid content, the chyle is very similar in composition to the lymph in other parts of the body.

The composition of human chyle has been determined by Owen-Rees who obtained it of a recently executed person. Hoppe-Seyler

made a similar analysis of chyle obtained in a case of rupture of the thoracic duct. These analyses are tabulated below.

TABLE XXXVII

COMPOSITION OF CHYLE. THE RESULTS ARE EXPRESSED IN PARTS PER 1000

	Owen-Rees	Hoppe-Seyler
Water	904.8	940.72
Solids	95.2	59.28
Fibrin	Traces	
Other proteins	70.8	36.67
Fat	9.2	7.23 2.35 soaps 0.83 lecithin
Remaining organic bodies	10.8	1.32 cholesterol 3.63 alcohol extractives 0.58 water extractives
Salts	4.4	6.80 soluble salts 0.35 insoluble salts

A comparative study of the chemical composition of blood serum and thoracic lymph of the dog has been made by Arnold and Mendel,[109] whose results show that both normally and abnormally (after exclusion of the kidney, thyroparathyroidectomy, etc.), there is an interrelationship between the composition of the lymph and plasma. An interchange of material is continually taking place and whenever any fluctuations in the concentrations occur, the diffusible constituents pass easily and rapidly between the blood, lymph, and the tissues. The normal relations are indicated in the following table:

TABLE XXXVIII

	Total Solids	Chlorides	Calcium	Phosphorus	Sugar	Non-protein Nitrogen	Protein Nitrogen
	Gm. per 100 cc.	Mg. per 100 cc.	Mg. per 100 cc.	Mg. per 100 cc.	Mg. per 100 cc.	Mg. per 100 cc.	Gm. per 100 cc.
Serum	8.3	392	10.4	4.3	123	27.2	0.9
Lymph	5.2	413	9.2	3.6	124	27.0	0.57

[109] J. Biol. Chem., **72**, 189 (1927).

CHAPTER IX

PHYSIOLOGICAL OXIDATIONS

An oxidation reaction is most simply defined as one in which a given substance combines chemically with oxygen. By convention, the removal of hydrogen is likewise called oxidation. We also speak of the oxidation of a ferrous salt to a ferric salt, even though oxygen does not enter into the reaction. In terms of the theory of electrolytic dissociation, an increase in the positive ionic charge or a decrease in the negative charge of ions is called oxidation, whereas a decrease in the positive charge or an increase in negative charge is termed reduction.[1]

Oxidations *in Vitro*.—Tissue extracts may be allowed to act on certain substances *in vitro*, and the changes which they undergo may be followed. Much information has been obtained by studying the effect of chemical reagents, such as hydrogen peroxide, on glucose, fatty acids, amino acids, etc. The application of knowledge gleaned from experiments of this type to physiological processes is often possible.

The oxidation of a substance occurs in stages. Taking as an example the very simple reaction in which carbon monoxide is oxidized to carbon dioxide, it can be shown that the first step is a reaction with water, yielding formic acid. This and the succeeding steps may be represented as follows:

$$CO + H_2O = HCOOH$$
$$HCOOH = CO_2 + H_2$$
$$H_2 + O_2 = H_2O_2$$
$$H_2O_2 = H_2O + \tfrac{1}{2}O_2$$

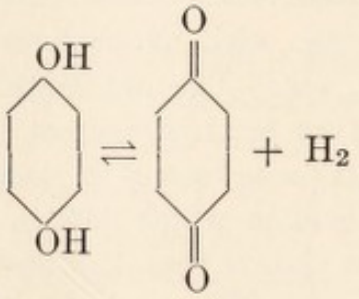
The second stage in the reaction involves the decomposition of the formic acid into carbon dioxide and hydrogen. This reaction proceeds

[1] The use of the terms "reduction" and "oxidation" in the broad sense is not without objection. Oxidation is not so much a process of the addition of oxygen as it is of the removal of an electron or electrons from an element. The contrary effect is obtained in reduction, which involves the addition of an electron or electrons to an element. Accordingly, E. C. Franklin (personal communication) and H. P. Cady and R. Taft [Science, **62**, 403 (1925)] suggest the term "electronation" for "reduction," and "de-electronation" for "oxidation."

as indicated in the above scheme because of the presence of oxygen. The oxygen acts as a "hydrogen acceptor"; the hydrogen peroxide which is formed is then broken down to water and oxygen. As Dakin [2] points out, the above scheme for the burning of carbon monoxide is not a figment of the chemist's imagination but is based on the actual isolation, under suitable experimental conditions, of formic acid, hydrogen, and hydrogen peroxide as intermediate products.

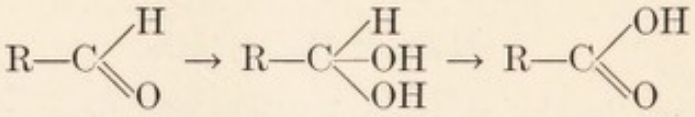
It is a matter of much significance that the oxidation of carbon monoxide will take place in the absence of oxygen but not in the absence of water. In the above equations, the hydrogen which is split off unites with, or is "accepted" by, free oxygen, to form a peroxide. In the presence of palladium black, which is capable of taking up a certain amount of hydrogen, the reaction also occurs. The most important change, therefore, in the oxidation of carbon monoxide, is the removal of hydrogen, a process which has been termed dehydrogenation.

Wieland's Theory of Oxidation.—The view that has just been expressed is at the basis of Wieland's [3] theory of oxidation. Wieland has shown, for example, that the oxidation of hydroquinone can be brought about in the complete absence of oxygen.



Hydroquinone Quinone

The liberated hydrogen may be taken up to some extent by palladium black. In the absence of other hydrogen acceptors the reaction soon comes to a standstill. The oxidation of acetaldehyde to acetic acid may be represented as follows:



Aldehyde Aldehyde-hydrate Acid

Here, as in the case of carbon monoxide, the first step in the oxidation is hydration; the second step is one of dehydrogenation (removal of hydrogen). Wieland has postulated the existence of enzymes which are assumed to catalyze dehydrogenation reactions by activating organic

[2] Oxidations and Reductions in the Animal Body, 1922 edition, p. 4.

[3] Ber., **45**, 484, 679, 2606 (1912); **46**, 3327 (1913); **47**, 2085 (1914).

compounds to give up their hydrogen more readily and which have therefore been called " dehydrases."

Bach-Engler Theory.—In opposition to Wieland's theory is that of Bach [4] and Engler, who have independently advanced an hypothesis for tissue oxidation, according to which molecular oxygen is believed to react with some constituent of the protoplasm (oxygenase) to form a peroxide. The latter is broken down by an enzyme, peroxidase, yielding active oxygen which is capable of oxidizing the metabolites in the tissues.

The formation of peroxides as intermediate products of oxidation is a familiar phenomenon. For example, in the oxidation of benzaldehyde to benzoic acid, there is first formed benzoyl-hydrogen peroxide

$$(C_6H_5 \cdot CHO + O_2 = C_6H_5 \cdot CO \cdot O \cdot OH).$$

This substance is a powerful oxidizing agent and reacts with another molecule of benzaldehyde to form two molecules of benzoic acid

$$(C_6H_5 \cdot CO \cdot O \cdot OH + C_6H_5 \cdot CHO = 2C_6H_5 \cdot COOH).$$

In the presence of substances that are readily oxidized, such as indigo, the oxygen of the peroxide is taken up by the indigo and there is formed but one molecule of benzoic acid and the oxidation products of the indigo. That similar reactions may occur in tissues is likely. This does not conflict with or overthrow Wieland's theory, as some physiologists seem to think. There is actually no reason for assuming that there is only one mechanism concerned in physiological oxidations. Dakin points out that Wieland's theory, taken in conjunction with, and not to the exclusion of, the peroxide theories of oxidation, may throw much light on the mechanism of biochemical reactions.

Peroxidase.—The function of peroxidase in biological oxidations is believed to be the transfer of oxygen, linked as a peroxide, to oxidizable substances. Gum guaiac or its constituent, guaiaconic acid, when mixed with potato scrapings, turns blue. The change in color is due to the oxidation of the guaiaconic acid by active oxygen. Taken by itself, this observation is of little significance; but when considered in conjunction with certain observations of Bach and Chodat and of Bach,[5] it offers a clue to the mechanism involved in the oxidation of the guaiaconic acid.

Bach and Chodat approached the peroxidase problem by studying extracts prepared from the roots of the horseradish. These extracts,

[4] Ber., **46**, 3864 (1913).

[5] Various papers in the *Berichte*, beginning in 1903.

when treated with guaiaconic acid, did not give a blue reaction; but when hydrogen peroxide was also added, the guaiaconic acid was oxidized, with the production of the blue color characteristic of the test. It is to be noted that the addition of hydrogen peroxide to the horseradish extracts did not result in the liberation of oxygen gas. Moreover, hydrogen peroxide alone did not produce any effect on the gum guaiac. Bach and Chodat therefore concluded that the horseradish extracts contained a substance capable of activating hydrogen peroxide, and as this constituent showed the properties of an enzyme (it was destroyed by heating, precipitated by alcohol, etc.), it was called " peroxidase." The guaiac reaction is not specific, however, for peroxidase. Alsberg [6] has shown, for example, that a large variety of substances, including hemoglobin, hemocyanin, ferrous sulfate, the chlorides of cobalt, nickel and copper give the guaiac test.

As is well known, a cut surface of a potato blackens on exposure to air. This is usually attributed to the oxidation of the amino acid tyrosine, and possibly of compounds related to it, for it has been shown by Bach that fresh potato juice oxidizes tyrosine rapidly and that it therefore contains tyrosinase. When the juice is treated with alcohol, a precipitate is formed which no longer exhibits this property unless hydrogen peroxide is also added. As the hydrogen peroxide, by itself, has no effect on tyrosine, we may assume that the added peroxide serves merely to replace a similar substance which was present in the cell and in the fresh potato juice, but which was not precipitated by the alcohol.

What is the significance of these observations? According to Bach's theory, the oxidation of guaiaconic acid by potato scrapings, and of tyrosine by potato juice, may be explained on the following basis. In the first case, we have the enzyme peroxidase; in the second, we have as one of the constituents of the tissues an auto-oxidizable substance (Bach's oxygenase). The splitting of the peroxides, and hence the transfer of oxygen from the peroxides to the substrate (guaiaconic acid and tyrosine in the above examples), is accelerated by the enzyme peroxidase. In the presence of free oxygen, the auto-oxidizable constituent may be reoxidized to the peroxide form. According to Gallagher,[7] the auto-oxidizable constituent in the potato is a lecithin-like substance.

Certain inorganic salts behave like " peroxidases." The oxidation of lactic acid by hydrogen peroxide is an extremely slow process. This reaction may be accelerated, however, by the addition of a peroxidase (extract of horseradish) or a trace of ferrous sulfate. Not only the salts of iron but also the salts of copper and manganese, metals which

[6] Archiv. f. exper. Pathol. u. Pharmakol., Supplement-Band, 39 (1908).

[7] Biochem. J., **17**, 515 (1923); **18**, 29 (1924).

are capable of existing in two different states of valence, are effective in this regard. That peroxidases are really only the active forms of these metals has been claimed by a number of workers, among whom may be included Bertrand and Perrin. Bayliss [8] states that a peroxidase is, in all probability, a peculiarly active form of the colloidal hydroxide of manganese, iron, or copper, preserved in this active state by the presence of an emulsoid colloid, such as gum or albumin. It is to this stable colloid that the enzyme owes its coagulation by heat, its precipitation by alcohol, and possibly any degree of specificity that it may possess. On the other hand, Bach [9] reports that he has succeeded in preparing " oxidase " preparations free from both manganese and iron. The importance of iron in oxidations will be referred to again, in connection with the work of Warburg.

Catalase.—Catalase is widely distributed both in plants and in animals. It has the property of decomposing hydrogen peroxide into water and oxygen, the latter being given off as a gas. Numerous attempts have been made to show that catalase is directly concerned in the oxidative processes of the tissues, but we are still uncertain of its full significance if it has any. Its chief function may be the decomposition of hydrogen peroxide that is formed as a by-product in physiological oxidations. Dixon [10] has shown that when purine bases are oxidized by molecular oxygen in the presence of xanthine-oxidase as a catalyst, the oxidase is progressively destroyed during the course of the reaction by the hydrogen peroxide that is formed. Dixon found that he could prevent destruction of the oxidase by the addition of catalase. Thus, catalase seems to have a protective function in animal tissues.

Specific Oxidizing Enzymes.—Certain other enzymes which are believed to take part in specific oxidations, may be mentioned briefly in this connection. One of these, indophenol oxidase, believed to oxidize cytochrome, will be considered later (p. 262). Another is the enzyme tyrosinase, which is of widespread occurrence both in plants and in animals and is obtained especially from certain mushrooms, such as *Russula delica* and *Russula nigricans*. Bertrand [11] found that on broken mushrooms (*R. nigricans*), the red coloration turns black, a change which he has attributed to the action of the oxidizing enzyme tryosinase. The oxidation and coloration of the juice of the beet, of potatoes, and of

[8] Bayliss, Principles of General Physiology, 1924 edition, p. 586.

[9] Ber., **43**, 364 (1910).

[10] Biochem., J., **19**, 507 (1925).

[11] See Effront-Prescott, Biochemical Catalysts in Life and Industry, John Wiley and Sons, 1917, p. 299.

dahlias are among the familiar examples of the action of this enzyme. The oxidation of tyrosine to melanin by the action of tyrosinase has been studied by Raper and his associates.[12]

Of importance in relation to purine metabolism are the enzymes xanthine-oxidase and uricase. The former, discovered by Shittenhelm [13] is concerned in the transformation of hypoxanthine to xanthine and of xanthine to uric acid. In man, this compound is the end-product of purine metabolism. Man and certain anthropoid apes do not have in their tissues the enzyme uricase which is capable of oxidizing uric acid. Uricase is present, however, in many other animals, including the dog. In the dog, allantoin is the end-product of purine metabolism, this compound being derived as a result of the action of uricase on uric acid. An exception to the rule that dogs excrete allantoin and not uric acid was discovered by Benedict,[14] who found that the Dalmatian coach hound normally excretes uric acid in the urine. The liver of this animal does not contain uricase, as might be expected.

Luciferase.—Some organisms, among which may be included certain fungi, bacteria, protozoa, medusae, insects, molluscs, and fish, are capable of emitting light by a process of chemi-luminescence. This is due to oxidative changes in which the chemical energy is converted directly into light energy. The process differs widely, therefore, from that involved in artificial light production, where most of the energy formed is lost as heat. One of the constituents concerned in the production of light is an oxidizable substance, luciferin, which is thermostable. The other constituent is thermolabile and is believed to be an enzyme, hence the name luciferase.

The indications are, according to recent work of Harvey,[15] that luciferase catalyzes the oxidation of luciferin. In turn the energy of oxidation of the luciferin excites the luciferase. The excited molecules of luciferase emit light on returning to the normal state.

Dehydrogenation and Hydrogen Acceptors.—In accordance with Wieland's hypothesis, it is to be expected that, in the oxidation of metabolites, hydrogen will be given off to any easily reducible substances that may be present. Such substances are called hydrogen acceptors. Certain dyes, such as alizarin blue, indophenol blue, and methylene blue, behave as hydrogen acceptors.

[12] Raper, H. S., and Wormall, A., Biochem., J., **17**, 454 (1923); **19**, 84 (1925); **19**, 92 (1925); **20**, 69, 735 (1926); **21**, 89, 1370 (1927); see also H. S. Raper, The Aerobic Oxidases, Physiol. Rev., **8**, 245 (1928).

[13] Z. physiol. Chem., **45**, 121, 152, 161 (1905).

[14] Harvey Lectures, 1915–1916, 346.

[15] J. Biol. Chem., **78**, 369 (1928); see also E. N. Harvey on "The Nature of Animal Light." Philadelphia and London, 1920.

In 1921, Hopkins [16] made an important contribution to our knowledge of physiological oxidations by isolating from plant and animal tissues a hydrogen acceptor which he named glutathione.

Glutathione.—The historical background of Hopkins' discovery begins with the work of de Rey-Pailhade [17] who observed, in 1888, that many tissues have the property of reducing sulfur to hydrogen sulfide. The strong reducing power of tissues he believed to be due to one of their constituents. What the chemical nature of this constituent was he did not know, but he thought it was a "hydride of protein" and therefore assigned to it the name "philothion."

By means of Mörner's sodium nitroprusside test, which is given by the sulfhydryl (SH) group, a number of workers (Heffter,[18] Arnold [19]) were able to show that many tissues contain free SH groups. Arnold believed the SH groups to belong to free cysteine. He did not, however, isolate this amino acid from the tissues and tissue extracts with which he worked. Heffter, on the other hand, had a more correct appreciation of the significance of the sulfhydryl-containing constituent of the tissues. Although he had no knowledge of the chemical nature of this substance, he expressed the view that it was responsible, at least in part, for the reducing properties of protoplasm. He also believed that this constituent might take part in cell oxidation by undergoing alternate oxidation (to S—S compounds) and reduction.

In an attempt to determine the chemical nature and physiological significance of the tissue constituent which de Rey-Pailhade had named philothion, Hopkins made an exhaustive fractionation of tissue extracts. He succeeded in isolating, from yeast, muscle, and mammalian liver, the substance responsible for the nitroprusside reaction. At the time it seemed to be a dipeptide of cysteine and glutamic acid and exhibited the properties of de Rey-Pailhade's philothion. Although the concentration was found to be low (0.01–0.02 per cent of the fresh tissue), nevertheless, Hopkins emphasized its importance in the chemical dynamics of the cell.

It has been known for a long time that cysteine may be readily oxidized to cystine. In fact, this conversion occurs spontaneously in slightly alkaline solutions of cysteine exposed to the air, as shown by Mathews and Walker.[20] And, in turn, the reverse process, the reduction of cystine occurs almost as readily in the presence of even mild reducing agents. Accordingly, a system composed of cysteine and cys-

[16] Biochem. J., **15**, 286 (1921).
[17] Compt. rend., **106**, 1683 (1888); **107**, 43 (1889).
[18] Med. Naturwiss. Arch., **1**, 81 (1908); Maly's Jahresb. (1908).
[19] Z. physiol. Chem., **70**, 300 (1911).
[20] J. Biol. Chem., **6**, 21 (1909).

tine may be regarded as an auto-oxidizable system, the cysteine being oxidizable by molecular oxygen, the cystine being reducible by hydrogen. Such a system could conceivably play a part in tissue oxidations. The reactions involved would be essentially as represented by the equation:

$$O + \begin{matrix} COOH\cdot CHNH_2\cdot CH_2SH \\ COOH\cdot CHNH_2\cdot CH_2SH \\ \text{Cysteine} \end{matrix} \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftharpoons}} \begin{matrix} COOH\cdot CHNH_2\cdot CH_2S \\ | \\ COOH\cdot CHNH_2\cdot CH_2S \\ \text{Cystine} \end{matrix} + H_2O$$

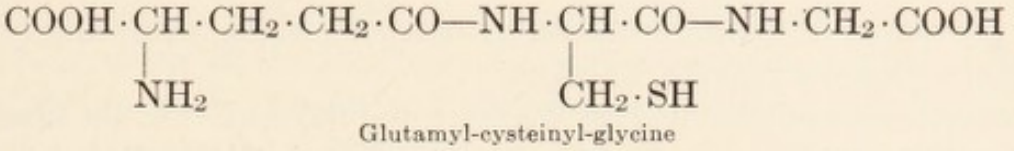
The properties which cysteine and cystine exhibit to a limited degree were much more evident in the case of glutathione, the tissue constituent isolated by Hopkins. Subsequently Stewart and Tunnicliffe [21] synthesized the dipeptide, cysteine-glutamic acid, which was thought to be identical in constitution and properties with the reduced form of glutathione. When this was oxidized to cystine-glutamic acid, it appeared to be identical with the oxidized form of glutathione. This seemed to confirm the idea that glutathione, in its reduced form was the dipeptide cysteine-glutamic acid having the formula:

$$SH\cdot CH_2\cdot \underset{\displaystyle COOH}{\underset{|}{CH}}\cdot NH\cdot CO\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH$$

However, it was pointed out by Hunter and Eagles [22] that various preparations of glutathione which they obtained did not analyze in agreement with the formula ascribed to it; that their preparations contained more than the theoretical amount of nitrogen and less sulfur, and therefore that glutathione could not be a dipeptide. In a reinvestigation of the problem, Hopkins [23] confirmed the work of Hunter and Eagles and reached the conclusion that glutathione is a tripeptide yielding on hydrolysis cystine, glutamic acid and glycine. Incidentally, the technique of isolation was improved and a yield of 1 gram of glutathione, and upwards, per kilogram of yeast was obtained. At about the same time Kendall, McKenzie and Mason [24] published similar results. Additional work reported by Kendall and associates somewhat later [24a] indicated that

[21] Biochem., J., **19**, 207 (1925).
[22] J. Biol. Chem., **72**, 147 (1927).
[23] J. Biol. Chem., **84**, 269 (1929); see also Pirie and Pinhey, *ibid.*, **84**, 321 (1929).
[24] *Ibid.*, **84**, 657 (1929).
[24a] Proc. Staff Meetings of the Mayo Clinics, **4**, 359 (1929).

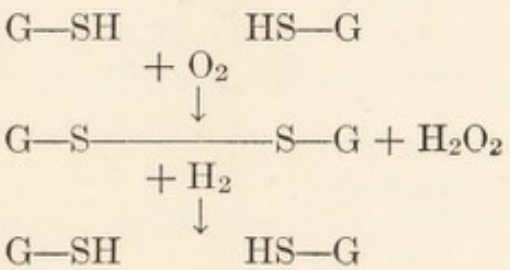
in glutathione, the glutamic acid is attached to cysteine and that the latter is in turn attached to the amino group of glycine, as represented by the following formula:



Glutamyl-cysteinyl-glycine

The Rôle of Glutathione in Tissue Oxidation.—We shall now consider briefly some of the physiological properties of glutathione. In his first paper, Hopkins pointed out certain relations in the behavior of fresh tissues toward methylene blue and glutathione. Fresh tissues, as is well known, reduce methylene blue. This dye is likewise reduced by the reduced form of glutathione. However, tissues are capable of reducing the oxidized form of glutathione. From these observations, it is to be concluded that the reduction potential of tissues is greater than that of glutathione, which in turn has a greater reduction potential than methylene blue. These relations are not always fixed, but, according to Hopkins' first experiments, are closely dependent on the hydrogen-ion concentration of the medium. In any event, at a *p*H of 7.4, Hopkins found the rate of reduction of methylene blue by washed tissues to be greatly accelerated upon the addition of small amounts of the oxidized glutathione.

The explanation lies in the fact that the S—S group of the oxidized tripeptide acts first as a hydrogen acceptor. The hydrogen taken up is then transferred to the methylene blue. It would appear, therefore, that the two reactions, namely the transfer of hydrogen to the tripeptide and its subsequent transfer to the methylene blue, together run faster than the single reaction involving the direct transfer of hydrogen from the tissues to the dye. Of course, some other hydrogen acceptor can take the place of the methylene blue. In physiological oxidations, we must therefore consider the relation of glutathione to oxygen, the most available of hydrogen acceptors. The changes which glutathione undergoes in oxidation may be represented as follows:



These equations need but little explanation. They represent glutathione in the position of an intermediary, removing hydrogen from the tissues, or more properly from the metabolites, and passing it on to the oxygen. The hydrogen peroxide is formed as a by-product and is perhaps prevented from accumulating by catalase, which, according to this scheme, assumes a definite function.

Attention was first called by Warburg and Sakuma [25] to the observation that the SH group of thiol compounds is oxidized by molecular oxygen only in the presence of minute traces of iron. This seems to hold true, likewise, in the case of glutathione, according to the observations of Harrison.[26] It appears that the auto-oxidation of the reduced form is very much accelerated by the addition of Fe as ($FeCl_3$) but is relatively very slow in the absence of all but irremovable traces of iron. However, as Hopkins [27] has pointed out, this relationship with iron does not remove any significance from the presumed function of the sulfhydryl group. The metal is but another link in the chain.

In 1922, Hopkins and Dixon [28] presented evidence to show that a tissue that has been washed until it no longer " respires " will, upon the addition of glutathione, again take up oxygen and yield carbon dioxide. Muscle may be extracted with boiling water, washed with alcohol, dried *in vacuo*, and powdered, and yet exhibit in the presence of glutathione a marked capacity for taking up oxygen. This property is not exhibited, however, in the absence of glutathione. It is apparent, therefore, that the thermostable, water- and alcohol-insoluble tissue residue must contain something which acts in conjunction with glutathione and which ordinarily has a higher reduction or lower oxidation potential than glutathione. In the experiments of Hopkins and Dixon, the tissue preparations were suspended in phosphate buffer solutions. Under these conditions, the oxygen uptake was found to be about 400 cmm. per gram of dried powder. It was later discovered, however, that the high concentrations of phosphate which were used limited, for some reason, as yet unknown, the utilization of oxygen. On substituting Ringer's solution or pure water, it was found that the uptake of an average preparation, at a *p*H of 7.6, was of the order of 2000 cmm. per gram.

Much light has been shed on the problem by the brilliant researches of Meyerhof.[29] As early as 1918, he showed that dead yeast cells (acetone-washed yeast) lost the property of respiration after being

[25] Pflüger's Archives, **200**, 203 (1923).
[26] Biochem., J., **18**, 1009 (1924).
[27] *Ibid.*, **19**, 787 (1925).
[28] J. Biol. Chem., **54**, 527 (1922).
[29] Pflüger's Arch., **170**, 367, 428 (1918).

exhaustively washed with water. By means of qualitative reactions, he determined that the washings contained something which had free SH groups. He discovered that he could restore the respiratory properties of the dead yeast by the addition of substances containing the sulfhydryl group, such as thioglycolic and thiolactic acid.

The relation of the thermostable, water-insoluble residue of tissues to oxidation in the presence of glutathione (Hopkins and Dixon) was likewise discovered by Meyerhof.[29a] He showed that the behavior of these residues was due to the lecithin fraction, and more particularly to the unsaturated fatty acids of the lecithin molecule.

Lecithin itself may be regarded as an auto-oxidizable constituent of tissues. The unsaturated fatty acids are readily oxidized on exposure to air or oxygen, especially in the presence of certain inorganic salts, such as those of iron, cobalt, copper or nickel. Oxidation of these fatty acids is accompanied by the disappearance of double bonds, as can be shown by making iodine-number determinations. What probably happens to the double linkage is indicated as follows:

$$\begin{array}{l} \mid \\ \mathrm{CH} + \mathrm{O} \\ \| \\ \mathrm{CH} + \mathrm{H_2O} \\ \mid \end{array} \quad = \quad \begin{array}{l} \mid \\ \mathrm{CHOH} \\ \mid \\ \mathrm{CHOH} \\ \mid \end{array}$$

In the oxidation of linolenic acid, only two of the three double linkages disappear. Nevertheless, for every molecule of the acid, two molecules of oxygen are taken up. This can be explained by assuming that an additional oxygen atom is used up in oxidizing the secondary alcohol groups, as shown by the following equation:

$$\begin{array}{l} \mid \\ \mathrm{CHOH} \\ \mid \\ \mathrm{CHOH} \\ \mid \end{array} \quad + \mathrm{O} = \quad \begin{array}{l} \mid \\ \mathrm{CHO} + \mathrm{H_2O} \\ \mid \quad \mid \\ \mathrm{CHO} \\ \mid \end{array}$$

Meyerhof has shown that in systems containing lecithin and thioglycolic acid or linolenic acid and thioglycolic acid, oxidation of the fatty acids occurred readily. The reaction most favorable to oxidation was found to be *p*H 3.0–4.0. According to Meyerhof, glutathione may function as an oxygen carrier, transferring oxygen to oxidizable constituents in the tissues, such as fatty acids, amino acids, etc. This point of view is shared by Sir Frederick Hopkins who has shown that glutathione promotes the oxidation of both fats and proteins, but that, for some reason that is not clear, as yet, carbohydrate oxidation is not catalyzed by

[29a] Pflüger's Arch., **199**, 531 (1923).

glutathione. The effect of hydrogen ions is an important factor, as will be appreciated on examining some of Hopkins' results. Hopkins measured the utilization of oxygen by mixtures of linolenic acid and reduced glutathione. In an acid medium (*p*H 3.0–4.0), the oxygen uptake is many times in excess of the amount of oxygen required to oxidize the SH to S—S. The amount of oxygen taken up is diminished very noticeably near the neutral point as well as on the alkaline side of neutrality. According to Meyerhof, the oxidation of fat under the influence of thioglycolic acid is completely inhibited in a neutral solution. Hopkins, however, does not find this to be the case with regard to glutathione. The capacity of fat to combine with oxygen is greatly diminished, but not abolished, in an alkaline medium. Hopkins' experiments show that under these conditions the oxygen uptake of linolenic acid is equal, or approximately equal, to the amount of oxygen required to oxidize the SH. He therefore has postulated that in neutral or alkaline systems, during the oxidation of the SH group, fatty acids are simultaneously oxidized in such a way that equipartition of oxygen occurs. This is brought out by the following data:

Material Employed, 10 Mg. as Emulsion in Distilled Water	*p*H	Time for Uptake, Hours	Total Uptake, Cmm.	O_2 Equivalent of SH Present, Cmm.	O_2 to Fat, Cmm.
Linolenic acid	3.5	6	210	32	178
	3.8	6	225	41	184
	7.6	6½	40	26	14
Lecithin	7.6	6½	732	390	342

In the experiment with lecithin, a relatively large amount of glutathione was added, sufficient to combine with 390 cmm. of oxygen. It should be pointed out that in the first two experiments, oxygen uptake was still progressing at the end of the period of observation, whereas, in the last two experiments cited, the uptake was complete at the end of the period of observation.

The oxidation of a protein seems to depend upon its possessing an SH group (as shown by the nitroprusside reaction). Protein is oxidized under the influence of glutathione in a neutral or faintly alkaline solution, but not in an acid medium. Hopkins states that proteins that do not exhibit an SH group (e.g., gelatin) show no tendency to reduce G_2S_2 (oxidized glutathione). During oxidation of proteins, the SH group of the protein is oxidized. The amount of oxygen taken up amounts to about ten times the oxygen equivalent of the sulfhydryl

present. After the SH has disappeared, the "oxidized" protein can be reduced by contact (as a solid phase) with cysteine, thioglycollic acid, or glutathione. After this reduction the protein again exhibits free SH groups and is again able to take up ten times the amount of oxygen with which it would be expected to combine on the basis of the content of SH.

The Rôle of Iron in Oxidation Reactions.—It has been shown by Warburg [30] that charcoal shaken in aqueous solutions of amino acids causes chemical changes resembling reactions of metabolism. In an approximately neutral solution, at body temperature, leucine yields ammonia, carbon dioxide and valeric acid. From cystine are formed ammonia, carbon dioxide and sulfuric acid, in addition to some undetermined products of incomplete combustion. It has been suggested by Warburg that these substances are oxidized at the surface of the charcoal and that iron present in the charcoal plays an essential part in the reactions involved by facilitating the transference of oxygen. Iron-free charcoal, such as may be prepared from pure sucrose, does not have this effect, but if impregnated with iron salts, or with organic compounds, containing iron, and heated to glowing, it becomes activated.

Mention has been made of the relation of iron to the oxidation of glutathione. In the case of cysteine also, auto-oxidation to cystine does not occur in the absence of iron. Mathews and Walker [31] have made the observation that the conversion of cysteine to cystine is inhibited in the presence of cyanides, an effect which they have attributed to combination of the cyanide with the sulfhydryl group.

Warburg, however, has pointed out that one molecule of prussic acid inactivates 1000 and more molecules of cysteine. It would therefore be more logical to assume that the effect of the cyanide was to combine with the iron that was present as an impurity in the cysteine which Mathews and Walker used. When specially prepared cysteine, containing almost no iron, is used, the oxidation of the cysteine progresses very slowly. The addition to these preparations of small amounts of iron greatly increases the velocity of the oxidation.

Warburg's charcoal model is likewise inhibited by cyanides. If sufficient cyanide is added to combine with all of the iron contained in the charcoal, the latter completely loses its capacity to oxidize amino acids. The same effect on the iron presumably present in the tissues has been suggested as the underlying cause of the inhibiting action of cyanides on biological oxidations in general. Warburg advanced the view that in respiring cells there is a cycle in which molecular oxygen reacts with

[30] Pflüger's Arch., **148**, 295 (1912); Ergebnisse d. Physiol., **14**, 253 (1914); Science, **61**, 577 (1925).

[31] J. Biol. Chem., **6**, 21 (1909).

bivalent iron, with the result that iron in a higher state of oxidation is formed. The oxidized iron is then supposed to react with organic substances, being thus reduced to bivalent iron, whereas the organic material is, at the same time, oxidized. Then the cycle is repeated.

Not all substances undergo oxidative changes in the presence of activated charcoal. Only amino acids are attacked in this way. The failure of the charcoal to act upon carbohydrates and fatty acids may be due to the fact that these substances must first be changed into some other form. The observation of Spoehr [32] that glucose is oxidized by molecular oxygen in the presence of iron pyrophosphate is suggestive. Equally significant are the observations of Warburg that fructose disappears from a neutral oxygenated solution in sodium phosphate at body temperature. This does not happen in solutions of fructose containing other salts, nor does it happen to other sugars. As a result of this oxidation, carbon dioxide is formed in the proportion of one molecule of carbon dioxide to three molecules of oxygen used, showing that the oxidation of fructose is incomplete. Warburg believes that even this reaction is due to the presence of iron as an impurity in the reagents. Spoehr has likewise adduced evidence to show that the addition of minute amounts of iron greatly facilitates the oxidation of sugars. Retardation of sugar oxidation may be obtained on the addition of hydrocyanic acid.

The oxidation of sugars may be accelerated by adding small amounts of copper or manganese. In a weakly alkaline solution, Meyerhof [33] found that 1–100 mg. of copper, when added to 100 mg. of fructose, accelerated the oxidation 140 per cent. A similar amount of iron caused an increase of 70 per cent.

Working with Meyerhof, Warburg has shown that iron controls the respiration or oxygen consumption of unfertilized, cytolyzed sea urchin eggs. In their experiments, these investigators determined the iron content as well as the oxygen consumption of the eggs, and, from these data, calculated the reactivity of the iron by dividing the oxygen consumed by the iron content. The eggs were then dissolved; 1–100 mg. of iron was added to 1 gram of the egg substance and the increase in oxygen utilization determined. It was found that the iron originally present in the intact egg had the same effect as the iron that was added. In both cases, each milligram of iron aided in the taking up of the same amount of oxygen per hour, namely, 7000 cmm.

Thunberg [34] has shown that lecithin is readily oxidized in the presence

[32] Carnegie Institute Yearbook, **22**, 55 (1924). J. Am. Chem. Soc., **46**, 1494 (1924).

[33] Cited by Warburg in Science, **61**, 575 (1925).

[34] Skand. Arch. Physiol.. **24**, 90 (1911).

of iron. This observation may be correlated with that of Warburg who found that the oxidation of linolenic acid is influenced similarly by iron. Moreover, Warburg found that the active material in the sea urchin eggs was lipid in character. He was able to show that, upon the addition of iron, the extracted lipids consumed an amount of oxygen equivalent to the oxygen consumption of a corresponding amount of egg substance.

To sum up what has been considered thus far, it may be stated that iron accelerates certain oxidation reactions. It is apparently an important factor in Warburg's charcoal model; it is involved in the auto-oxidation of such substances as cysteine and glutathione; it also seems to catalyze the oxidation of unsaturated fatty acids and certain sugars. The inhibiting effect of cyanides in these reactions may be attributed to the withdrawal of iron as an effective agent. This very interesting and useful information does not, however, explain the mechanism of oxidation in the tissues. Not that iron does not play an important rôle in physiological oxidations, for indeed it does, but it is iron in a totally different form than the inorganic iron contained in charcoal, for example. This point should be clear before proceeding to a brief consideration of the part which cytochrome plays in physiological oxidations.

The Rôle of Cytochrome in Physiological Oxidations.—As has been stated previously (p. 210), cytochrome is an intracellular pigment widely distributed in nature. Its presence has been established in aerobic bacteria, yeasts and in the higher plants and animals. Cytochrome is not a single substance but is composed of three heme compounds, which Keilin [35] has provisionally designated a', b', c'. These substances originate from heme, the first step being the formation of a hemochromogen from the free intracellular heme, which is contained in all aerobic cells, and some undetermined nitrogen compound. Component b' of cytochrome somewhat resembles this hemochromogen. In some cells (*B. coli* and other facultative aerobes) the latter is the only visible heme compound. This hemochromogen, then, is the precursor of all three components of cytochrome, being converted into these presumably as a result of repeated oxidations and reductions.

Keilin has shown that the heme constituents of the cell are responsible for the thermostable peroxidase reaction. The test for peroxidase is shown by the oxidation of benzidine, guaiacum, paraphenylenediamine and other chromogens, in the presence of hydrogen peroxide. A large variety of animal tissues have been shown to give this reaction before, and particularly after, boiling and the intensity of the reaction has been found to be proportional to the cytochrome and free hemin content of the tissues.

[35] D. Keilin, Proc. Roy. Soc., B, **98**, 312 (1925); **100**, 129 (1926); **104**, 206 (1928–29).

Of the three components of cytochrome, a' and c' are not auto-oxidizable. On the contrary, component b', the free heme, and the hemochromogen precursor of cytochrome are auto-oxidizable. The oxidation of cytochrome in the tissues, particularly of components a' and c', is brought about by a specific oxidizing enzyme, *indophenol oxidase* (oxidizes Nadi's reagent, consisting of dimethyl-paraphenylene-diamine-hydrochloride and α-naphthol, to indophenol blue). Anything which inhibits or destroys this enzyme, such as KCN, H_2S, CO at high partial pressure in the dark, acetone, alcohol, heat (when the cells are warmed above 70° C., or dried in air), inhibits or abolishes the oxidation of cytochrome. Thus, indophenol oxidase is intimately connected with cytochrome in the processes of cellular respiration.

The reduction of cytochrome (the oxidized form) is brought about as follows, according to the views of Keilin: The organic constituents of the tissues, subject to the reactions of metabolism, are activated by *dehydrases* and thus become hydrogen donators. Any factors which inhibit or destroy these enzymes, such as cold (below −2° C.), heat (above 52° C.), narcotics, such as alcohol and ethyl urethane, also inhibit or prevent the reduction of oxidized cytochrome.

On the basis of a careful study, Keilin has reached the conclusion that in addition to the peroxidase effect that has been mentioned, cytochrome acts as a carrier between two types of activating mechanisms in the cell: (1) the dehydrases, activating the hydrogen of organic molecules; and (2) the indophenol oxidase, activating oxygen. Cytochrome thus acts as a hydrogen acceptor which is specifically oxidized by the indophenol oxidase.

The Rôle of Heme as a Catalyst in Oxidations.—Component b' of cytochrome shares with components a' and c' the functions that have just been described, but in addition, being an auto-oxidizable substance it may act more directly as a carrier between the hydrogen donators and molecular oxygen, without the intervention of an oxidase. The hemochromogen precursor of cytochrome and the free heme have similar properties. All three (heme, hemochromogen, cytochrome component b'), in addition, are also capable of acting as direct catalysts, promoting the oxidation of substances which are not activated by dehydrases.

Concerning the importance of the compounds of heme in cellular oxidations there can be little question. The functions of heme as a "respiratory ferment" have been discussed by Warburg[36] and by Anson and Mirsky,[37] but perhaps the most striking observations that

[36] Die Naturwissenschaften, **16**, 345 (1928); Science, **68**, 437 (1928).

[37] Science, **68**, 647 (1928).

have been made so far are those of Kuhn and Meyer.[38] These workers have shown that hemin is very effective in accelerating the oxidation of unsaturated fatty acids, sterols and plant pigments. Not all fatty acids are similarly affected; the reactions appear to be highly specific. Thus crotonic acid, $CH_3—CH=CH—COOH$, is oxidized very slowly, whereas sorbic acid, $CH_3—CH=CH—CH=CH—COOH$, is oxidized twenty times more rapidly. This is not attributed to the difference in unsaturation but rather to the difference in the molecular configuration of the two compounds. Perhaps a better illustration of this is in the case of the isomers, oleic and elaidic acids. At a *p*H of 7.6, in the presence of hemin as a catalyst, the former is oxidized fifty times faster than the latter. Other differences in the molecule exert their influence. Thus, ethyl oleate is less rapidly oxidized than olive oil, which in turn is less rapidly oxidized than the free acid. Linoleic acid is very rapidly oxidized, but its isomer stearolic acid, with a triple bond, which might be supposed to be highly reactive, actually remains unchanged.

To illustrate the effect of hemin on the oxidation of olive oil and linseed oil, the following data may be given:

TABLE XXXIX

(After Kuhn and Meyer)

I. 0.8 cc. of citrate buffer, *p*H 6.68, + 0.1 cc. H_2O + 0.1 cc. solution of pyridine-hemin* + 0.3 cc. olive oil.

II. As in I, except that 0.3 cc. linseed oil was used instead of olive oil.

III. 0.8 cc. of citrate, *p*H 6.68 + 0.1 cc. H_2O + 0.1 cc. of 5 per cent pyridine + 0.3 cc. linseed oil.

IV. 0.8 cc. of citrate buffer, *p*H 6.68, + 0.1 cc. of 5 per cent pyridine + 0.3 cc. linseed oil + 0.1 m/650 Fe, as $FeCl_3$.

Oxygen Consumption, in Cubic Millimeters of Oxygen

Time in Hours	I. Olive Oil + Hemin	II. Linseed Oil + Hemin	III. Linseed Oil Alone	IV. Linseed Oil + $FeCl_3$
1	8	1030	0	0
$2\frac{1}{2}$	37	2695	0	0
$3\frac{1}{2}$	60	3415	0	0
6	113	4780	1	3
9	177	6210	2	5

* The hemin was prepared from cattle blood and recrystallized from pyridine and chloroform. The pyridine-hemin solution was prepared by dissolving 50 mg. of hemin in 2.5 cc. of pyridine, and diluting to 50 cc. with water.

[38] Z. physiol. Chem., **185**, 193 (1929).

Hemin itself does not take up any oxygen. It is to be noted that under the conditions of the experiment, oxidation of the linseed oil, in the absence of hemin, was very slight and that iron added as an inorganic salt did not exert an appreciable catalytic effect.

Anaerobic Oxidations.—There are organisms that live in the absence of free oxygen. Among these may be included certain bacteria (both obligatory and facultative anaerobes), yeasts, fungi, snails, intestinal worms, and leeches. Since the liberation of free energy is necessary for life, it is of interest to see how these organisms manage to exist. Their energy is obtained chiefly from carbohydrate and fat. Very obviously, the oxidation of these substances cannot go to completion, and there are therefore formed, in addition to carbon dioxide and water, a variety of other substances, such as lactic acid and alcohol. The intestinal worm, *Ascaris*, seems to oxidize carbohydrates according to the following equation:

$$\underset{\text{Glucose}}{4C_6H_{12}O_6} = 9CO_2 + \underset{\substack{\text{Valerianic}\\ \text{acid}}}{3C_5H_{10}O_2} + 9H_2$$

Wieland's dehydrogenation hypothesis offers a basis for explaining anaerobic oxidations and more precise information has been provided by Quastel [39] and his associates. These have shown that the metabolism and growth of bacteria under anaerobic conditions are possible only when the organisms possess a system capable of activating some constituent of the medium to act as a hydrogen acceptor. The energy derived in anaerobic oxidations is small as compared with that obtained when oxygen is available. Thus, yeasts, in converting glucose into carbon dioxide and alcohol, obtain only 5 per cent of the energy which is made available when the glucose is burned completely to carbon dioxide and water.

Reduction of Indicators.—Anaerobiosis, or life without oxygen, is possible to these organisms because of their extraordinary ability to reduce substances in their environment. Their reduction capacity can be demonstrated by their action on dyes, such as the indophenols and methylene blue. Living tissues in general have this reducing property.

Among the earlier attempts to measure the oxidation-reduction intensities of tissues by means of indicators were the experiments of Ehrlich. He injected intravenously, into living animals, suspensions of alizarin blue and indophenol blue, and subsequently examined the organs for the presence of these dyes, either in the colorless or reduced form, or in the oxidized or blue form. Of the two dyes, indophenol

[39] Biochem., J., **18**, 365 (1924); **19**, 652, 660 (1925).

blue is more easily reduced. Ehrlich [40] found that certain organs (heart, gray matter of the brain) which presumably have a high oxygen potential, did not reduce either dye. Most of the tissues reduced indophenol blue but had no effect on alizarin blue. The reduction potential of still other organs (lungs, liver, fatty tissue, Harderian gland) is apparently very high as even alizarin blue was reduced by these organs in Ehrlich's experiments.

Methylene blue has been used very extensively in demonstrating the reducing action of protoplasm.

Within the last few years, interest in this problem has been revived and its scope greatly extended by a group of active workers, the most prominent of whom is W. Mansfield Clark.[41] He and his associates have developed a sound theoretical basis for the measurement of oxidation-reduction potentials in organic systems and some progress has been made in studying the significance of the reduction intensity and capacity of living cells.[42] The further development of knowledge in this direction will undoubtedly increase our comprehension of many physiological processes. Just as the ability to measure hydrogen ions has made it possible for us to attack numerous fundamental problems in physiology and biochemistry, so it is not at all unlikely—it is, indeed, very probable—that with the advent of suitable methods for measuring the oxidation-reduction potentials of tissues, we shall have gained the necessary tools for the precise study of many of the problems in physiology and pathology with which we are now struggling feebly and ineffectively.

[40] P. Ehrlich, Das Sauerstoffbedürfniss des Organismus, Berlin, 1885.

[41] Recent Studies on Reversible Oxidation-Reduction in Organic Systems, Chem. Reviews, **2**, 127 (1925).

[42] Cannan, Cohen and Clark, United States Health Service Publications, Supp. 55A, 1009 (1926); Needham and Needham, Proc. Roy. Soc., B, **99**, 173 (1926); Cohen, Chambers and Reznikoff, J. Gen. Physiol., **11**, 585 (1928).

CHAPTER X

INTERMEDIARY METABOLISM OF CARBOHYDRATES

In tracing the fate of carbohydrates in metabolism, we are primarily concerned with the chemical changes which glucose undergoes after absorption. The carbohydrates of the diet do not constitute the only source of glucose, for glycerol and certain of the amino acids are convertible into sugar and glycogen during metabolism. In 100 grams of protein there is a sufficient amount of the so-called sugar-forming amino acids to yield about 58 grams of glucose. As we shall see later (Chapter XII), many stages in the intermediary metabolism of these amino acids are similar to those which we encounter in carbohydrate metabolism. The actual formation of glucose from protein can be demonstrated when little or no carbohydrate is fed, during starvation, in diabetes and other conditions. A severe form of renal glycosuria may be produced experimentally by injecting phlorhizin (see p. 65) into animals. Dogs made diabetic in this way excrete large amounts of sugar even after the glycogen of the liver has been depleted. In these animals the proteins of the tissues are partly converted to glucose, and, if the disturbance is severe enough, for each gram of nitrogen excreted in the urine, 3.65 grams of glucose are also eliminated. Each gram of nitrogen in the urine represents the metabolism of about 6.25 grams of protein.

Another possible source of glucose is the glycerol part of the fat molecule. The bulk of experimental evidence favors the view that there is no conversion of fatty acids into sugar. Chambers and Deuel [1] have recently shown a practically complete conversion of glycerol to glucose in a number of the phlorhizinized dogs with which they have worked. Normally, glycerol is burned to carbon dioxide and water. The intermediary stages of this combustion are identical with the final stages of glucose oxidation. The fatty acids, however, follow a different path in metabolism.

Fructose, when fed in large amounts, is rapidly changed into glycogen. Galactose is not stored as readily, and if given in excessive doses is partly excreted in the urine. It is interesting to note, however, that

[1] J. Biol. Chem., **65**, 21 (1925).

the sugar which is lost in this way is not altogether galactose but some other sugars which appear to be isomeric with it. Deuel and Chambers [2] express the view that both fructose and galactose may first be broken down into trioses and then synthesized into glucose. They have been able to show in phlorhizinized dogs a quantitative conversion (100 per cent) of fructose into glucose. The conversion of galactose to glucose is not quantitative, as shown by the fact that Deuel and Chambers were able to recover only about 88 per cent of the theoretical amount in the urine of these animals.

Herbivorous animals obtain a certain amount of their energy from pentosans. These polysaccharides are acted upon by bacteria in the alimentary tract. By this action a variety of substances, such as organic acids, are produced which the animal is able to utilize. The synthesis of lactose from the fermentation-digestion products of the pentosans has also been suggested. However, in man, the pentosans are not utilized at all, and if pentose sugars are fed, they are excreted unchanged in the urine.

Glycogen Synthesis.—The sugar that enters the portal circulation is carried to the liver and there stored as glycogen. Considerable amounts of glycogen are also stored in the muscles and utilized in muscular activity. These facts were established over fifty years ago by the famous French physiologist, Claude Bernard. Bernard found also that if starving animals are fed proteins, glycogen is formed. The liver is ordinarily able to store about 300 grams of glycogen.

The process of glycogen synthesis, or glycogenesis, in addition to conserving the food material, aids in regulating the sugar concentration of the blood. In the normal individual, whenever the sugar concentration exceeds 0.10–0.11 per cent, the excess sugar is rapidly removed for storage by the liver and other tissues. The regulation of the concentration of sugar in the blood, as well as the oxidation of glucose, is believed to be under the control of the pancreatic hormone (insulin, p. 294).

Regulation by Kidneys.—Accumulation of sugar in the blood is prevented by the regulatory effect of the kidneys. Normally, the amount of sugar in the urine is very small, not exceeding 0.02–0.04 per cent. However, the excretion of sugar becomes much more marked when the sugar concentration in the blood reaches 0.16–0.18 per cent. This value is called the *renal threshold.* It varies in different individuals, being low in the condition known as renal diabetes. The renal threshold is frequently higher than 0.18 per cent, especially in nephritis and in diabetes of long standing.

[2] J. Biol. Chem., **65**, 7 (1925).

Glycogenolysis.—To meet the requirements of the tissues when no carbohydrate is being absorbed from the alimentary tract, the glycogen of the liver is converted into glucose—a process termed glycogenolysis. This is believed to be at least partly under the control of the sympathetic nervous system and adrenalin. The glycogen of the liver does not appear to be completely exhausted even after prolonged starvation, for in addition to the glycogen there is still another important source of sugar, namely the tissue proteins. The various points that have

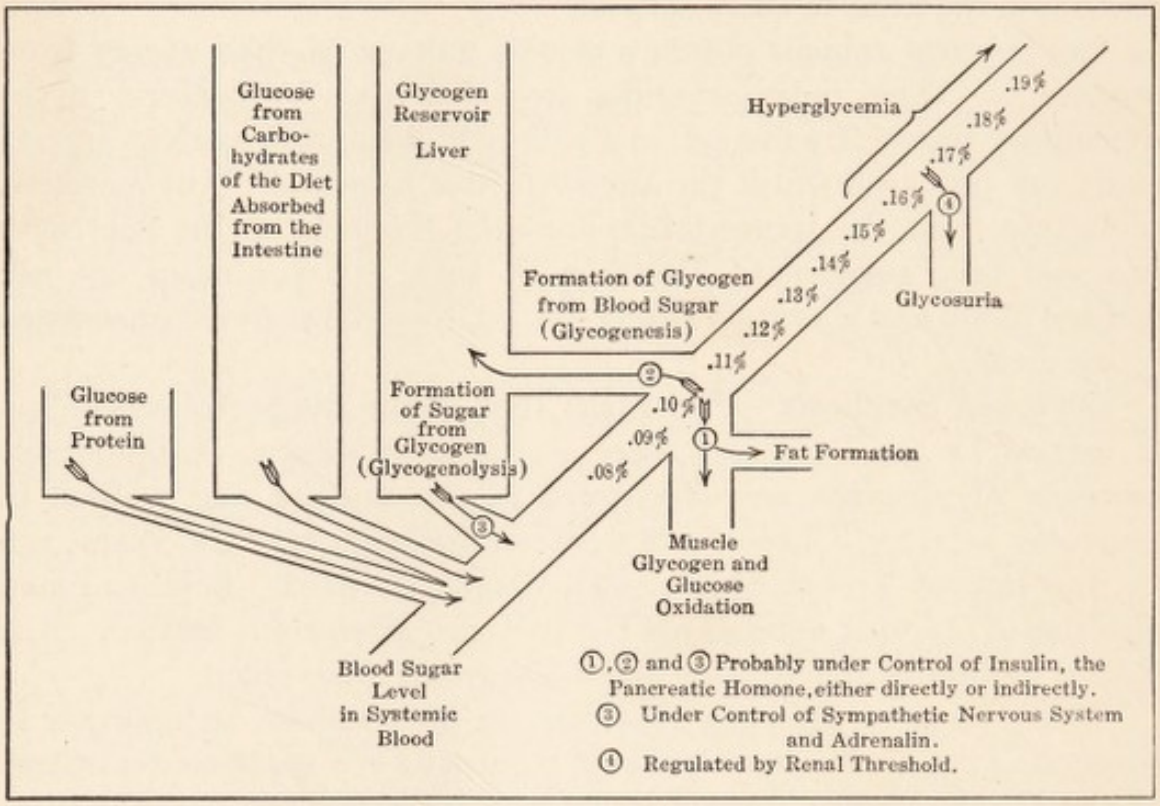


FIG. 37.—Schematic illustration of some of the factors which regulate the sugar concentration of the blood. (After Ringer and Baumann, with slight modifications, Endocrinology and Metabolism, edited by L. F. Barker, 1922, Appleton & Co., vol. 3, p. 252.)

been mentioned in the last few paragraphs are represented in the above diagram (Fig. 37) drawn by Ringer and Baumann and modified slightly to conform more nearly to the facts as they are known at present.

Carbohydrate Tolerance.—The capacity of the body to assimilate carbohydrates has long been the subject of intensive study. Prior to the advent of modern methods of blood analysis, this was measured by determining the amount of sugar which it was necessary to feed an individual before sugar appeared in the urine. A healthy person can tolerate 100–200 grams of glucose at a single dose without developing glycosuria. This was taken to indicate the efficiency to the tissues in removing the absorbed sugar from the blood. It must be borne in mind, how-

ever, that individual variations in renal threshold may introduce an error in this method, for in the case of a high threshold no sugar will appear in the urine even though the accumulation of sugar in the blood may be considerable, whereas in an individual with a low renal threshold, marked glycosuria may develop even with a moderate increase in the sugar content of the blood.

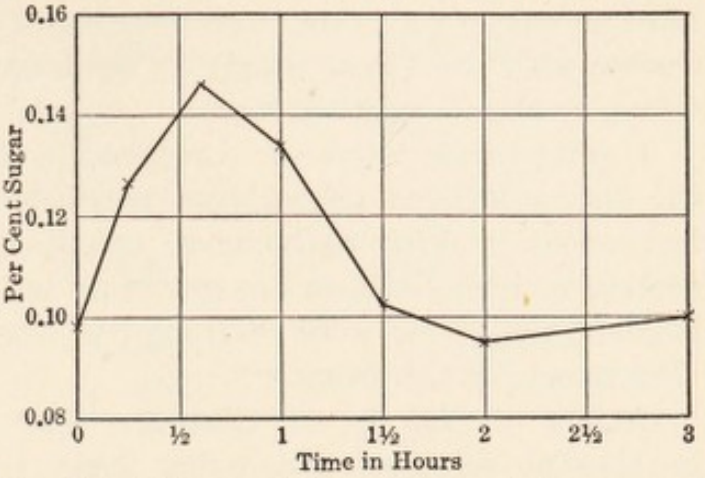


FIG. 38.—Glucose tolerance curve of a normal individual. Ordinates = per cent sugar in the blood; abscissæ = time after the ingestion of 100 g. of glucose.

With the introduction of simple methods for the quantitative measurement of blood sugar, the disappearance of glucose from the blood has been taken as the basis for determining carbohydrate tolerance. The blood is analyzed before and at certain intervals after giving a definite amount of glucose, usually 100 grams. Normally, the blood sugar rises to a maximum during the first half-hour or hour and returns to normal levels by the end of the second hour. The analytical data obtained may be plotted on coordinate paper as ordinates, and the time intervals as abscissae. The maximum height of the curve, the time at which this maximum occurs, and the time required for the curve to return to normal levels are all taken into account in interpreting the results. Variations in the rate of absorption may have a modifying

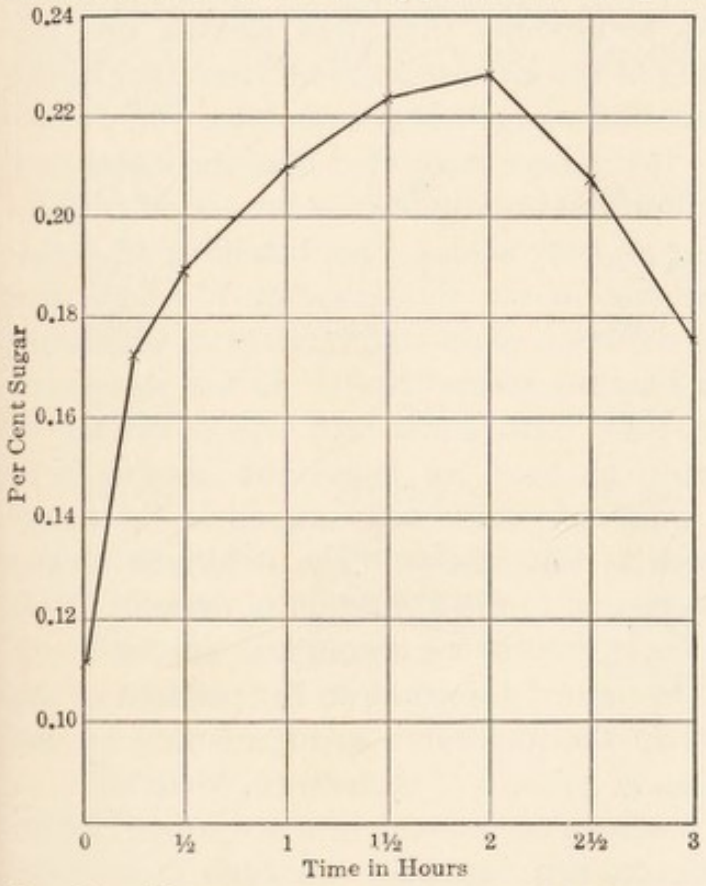


FIG. 39.—Glucose tolerance curve in the case of a diabetic individual. Ordinates = per cent sugar; abscissæ = time after the ingestion of 100 g. of glucose.

effect on the sugar-tolerance curve. Cajori, Crouter and Pemberton [3] have recently shown that sugar tolerance is altered as a result of interference with the blood supply to the large muscles which may be produced by elevation of the legs.

Carbohydrate tolerance is reduced in diabetes, in conditions where the liver is injured (phosphorus and chloroform poisoning), and to a less extent in other pathological conditions. An abundant literature dealing with this subject has grown up in the past ten or twelve years, beginning with the work of Bang [4] in Germany and of Hamman and Hirschman [5] in this country.[6]

Action of Alkali on Glucose.—The sugars are more reactive in alkaline solution, undergoing many transformations both in the presence and absence of oxygen and yielding a large variety of degradation products. These changes have been carefully studied for many years partly because it was hoped that in this way some knowledge might be gained regarding the changes of glucose in metabolism. In a weak alkaline solution α, *d*-glucose readily changes to β, *d*-glucose. Even more remarkable than this change are the intra-molecular rearrangements of the glucose molecule, resulting in the formation of six isomeric hexose sugars, including *d*-mannose and *d*-fructose (Nef [7] and Glattfield [8]). It has been suggested that the instability of sugars in alkaline solution is primarily due to the formation of salts of the sugars, the sugars acting as weak acids. The instability of *d*-glucose is increased with an increase in the concentration of alkali, the glucose breaking up into 116 different compounds, many of which are sugars containing 2, 3, 4, and 5 carbon atoms (Nef).[7] In the absence of oxygen these dissociation products form lactic acid and a variety of other substances. These reactions have an important bearing here because they yield products similar to those encountered in the intermediary metabolism of glucose in the tissues. The weakness of the glucose molecule is believed to be due to the formation of di-enols (Nef) as intermediate products. These substances decompose almost spontaneously. The di-enols may be named according to the position of the double bond—the weak point in the molecule—as represented by the following formulas:

[3] J. Biol. Chem., **66**, 89 (1925).

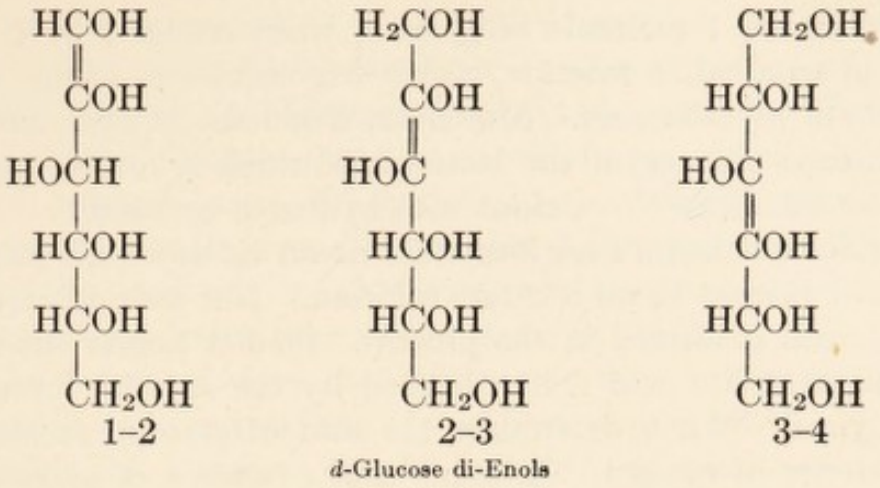
[4] Bang, I., Die Blutzucker, Wiesbaden, 1913.

[5] Arch. Int. Med., **20**, 761 (1917).

[6] The subject of blood sugar has been reviewed by Macleod, J. J. R., Physiol. Rev., **1**, 208 (1921), and by Stammers, A. D., *ibid.*, **6**, 630 (1926).

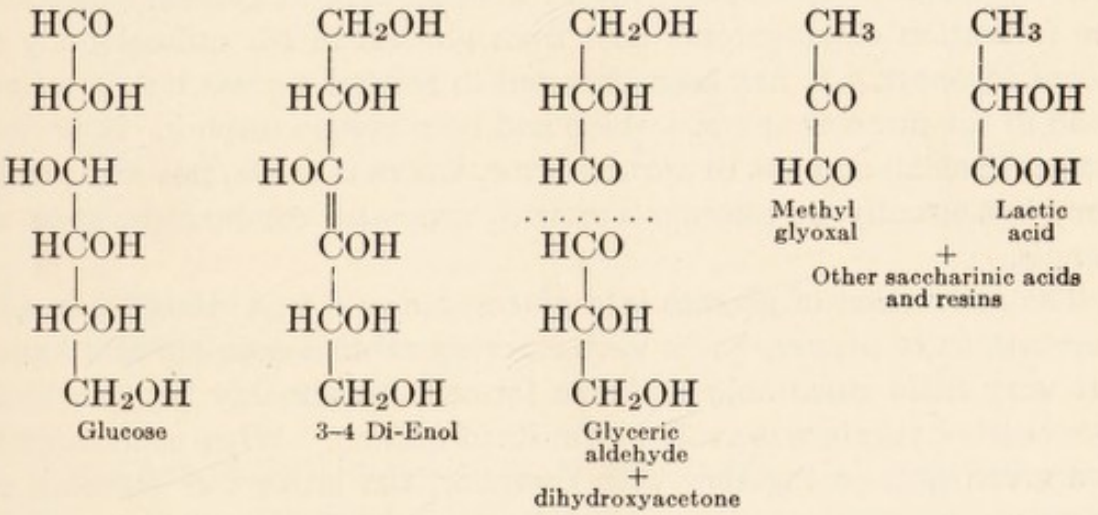
[7] Liebig's Ann., **403**, 204 (1913).

[8] Amer. Chem. J., **50**, 135 (1913).



d-Glucose di-Enols

A quantitative study of the formation of lactic and other acids has recently been made by Shaffer and Friedemann.[9] From their work it appears that in 0.5 N alkali and in the absence of oxygen, glucose is mainly converted into 3–4 di-enol, which in turn is decomposed to glyceric aldehyde, methyl glyoxal, and lactic acid. The intermediate formation of dihydroxyacetone as a result of the splitting of 3–4 di-enol is also likely.



The above, according to Shaffer, is an outline of the main reactions which occur when glucose is decomposed in half-normal alkali in the absence of oxygen. However, the oxidation of glucose in an alkaline solution seems to follow a different path. In the presence of oxygen, glucose is not broken down by way of lactic acid. According to Shaffer

[9] J. Biol. Chem., **61**, 585 (1924). P. A. Shaffer, Intermediary Metabolism of Carbohydrates, Physiol. Reviews, **3**, 394 (1923).

and Friedemann, 1 molecule of glucose, when oxidized by hydrogen-peroxide in an alkaline solution, yields 4 molecules of formic acid and 1 molecule of glycolic acid. Moreover, if glucose is first decomposed in the absence of oxygen, the lactic acid which is formed cannot be oxidized by subsequent treatment with hydrogen-peroxide.

This rules out lactic acid formation as an intermediate step in the oxidation of glucose in an alkaline solution. Nor does it appear that methyl glyoxal is formed in the process. Shaffer points out that the production of lactic acid is not limited by the rate of formation of methyl glyoxal. What determines the rate of glucose decomposition is the presence of oxygen. It seems that oxidation of glucose begins even before the production of glyceric aldehyde. Evidence for this may be found in Friedemann's observations, which show that the products of the simultaneous oxidation and dissociation of glucose and glyceric aldehyde are different. Glyceric aldehyde yields more formic acid and little or no glycolic acid, whereas a considerable amount of glycolic acid is formed from glucose. In the absence of oxygen, where we have merely the dissociation of the substance by the alkali, glucose and glycolic acid yield the same products.[10]

Glucuronic Acid.—Before taking up the main path of carbohydrate metabolism, we shall consider briefly another type of glucose oxidation. The formation of glucuronic acid from glucose in the animal body is known to occur. It has been detected in several tissues but was first found in the urine of animals which had been given camphor. It is also present in small amount in normal urine, where it exists, not in the free form, but usually in combination with aromatic compounds, such as phenols.

The conversion of glucose into glucuronic acid was indicated in the observations of Mayer,[11] who gave starving rabbits camphor and found that very little glucuronic acid was formed, presumably because little stored carbohydrate was available for its formation. When such animals were given glucose together with camphor, the latter was excreted as the glucuronate.

Different results were obtained by Mandel and Jackson,[12] who administered camphor to starving dogs and noted the excretion of glucuronic acid. When glucose was given to these animals, the protein metabolism fell, and with it the glucuronic acid excretion, but when chopped meat was fed there was an increase in glucuronic acid elimina-

[10] A recent review of the mechanism of carbohydrate oxidation is that of W. L. Evans, Chem. Reviews, **6**, 281 (1929).

[11] Biochem. Centr., **1**, 377 (1903).

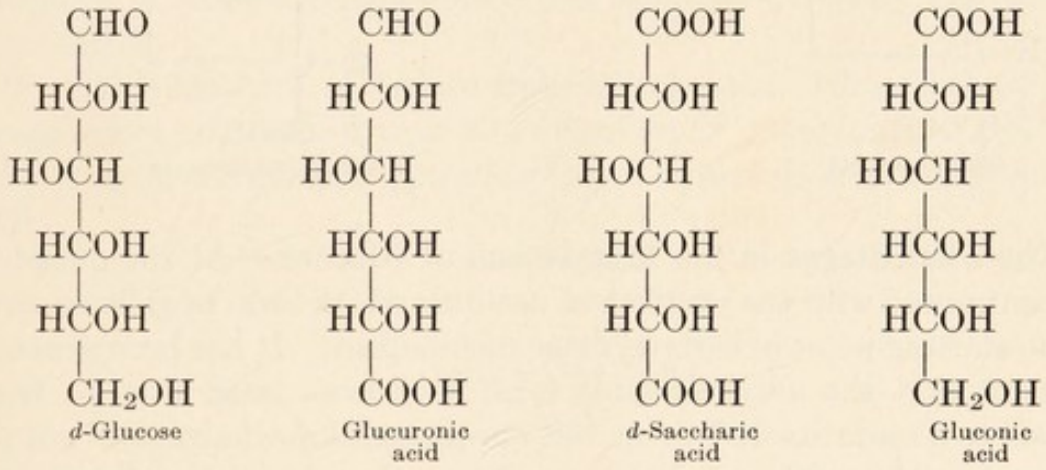
[12] Am. J. Physiol., **8**, p. xiii (1902).

tion. From these results, it has been inferred that glucuronic acid is produced solely in the intermediary metabolism of protein (Lusk).[13]

Quick [14] believes that glucuronic acid may arise, not from glucose, *per se*, but from glycogen and the sugar-forming amino acids. When there is a demand for it, the capacity for producing glucuronic acid is not diminished in the dog after removal of the pancreas. Under these conditions the production of glucuronic acid is accompanied by a corresponding decrease in the excretion of sugar in the urine.

It may be noted here that glucuronic acid is formed in greater abundance in herbivorous than in carnivorous animals.

Although the evidence for the formation of glucuronic acid from glucose is plausible, it does not appear that further oxidation to saccharic acid and oxalic acid takes place in the animal body, as Mayer thought. Glucuronic acid is very resistant to oxidation and when given to animals is excreted unchanged. This is true, likewise, of gluconic and saccharic acids. These substances cannot be regarded, therefore, as intermediate products in carbohydrate metabolism, despite the close structural relationship which is suggested by their formulas:

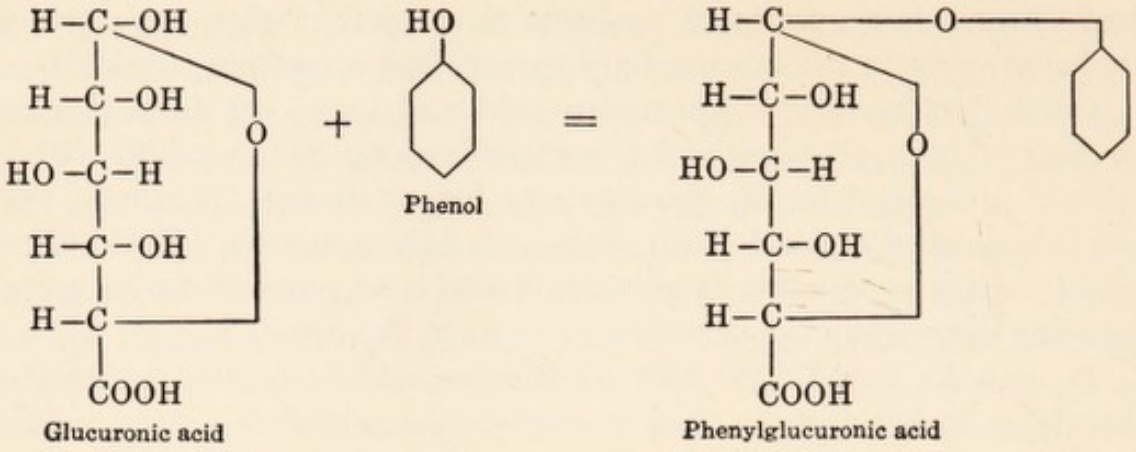


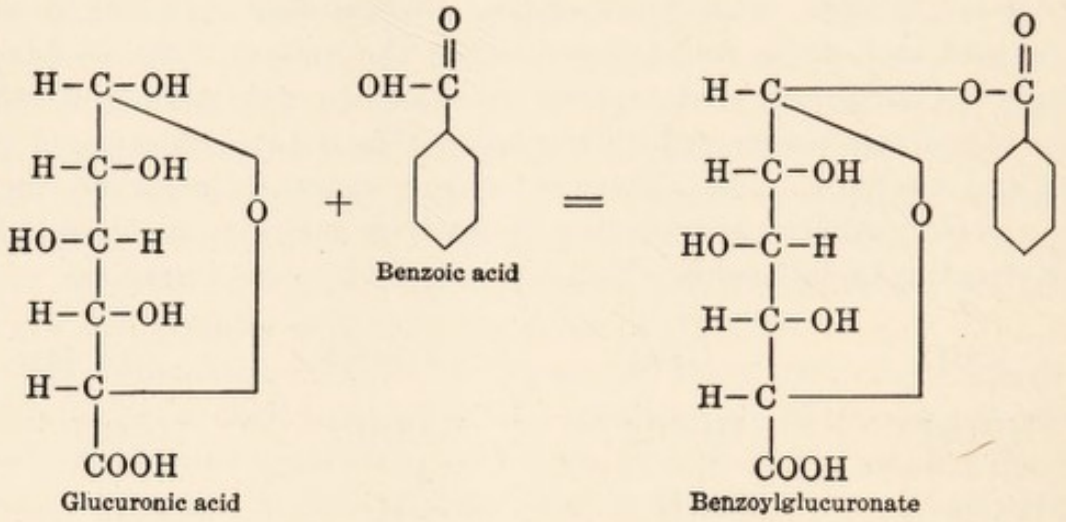
The physiological significance of glucuronic acid is found in its relation to the process of detoxication of both aliphatic and aromatic compounds. Tertiary alcohols combine even more readily than primary and secondary alcohols to form glucuronates. At least two different forms of combination are known. Glucuronic acid may combine to form a glucoside type of linkage, or it may react to form an ester as shown by the following equations: [15]

[13] Lusk, The Science of Nutrition, 4th edition, 683 (1928).

[14] J. Biol. Chem., **70**, 59, 397 (1926).

[15] See C. P. Sherwin, The Fate of Foreign Organic Compounds in the Animal Body, Physiol. Reviews, **2**, 268 (1922).



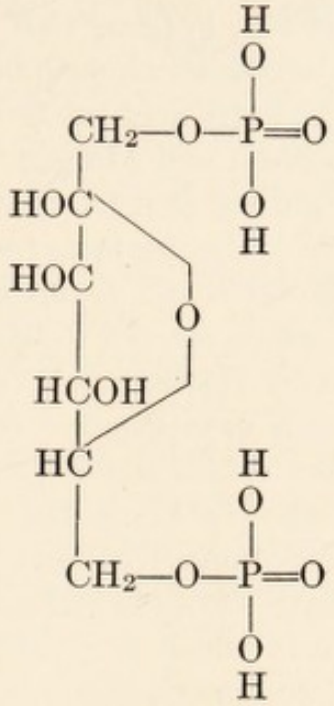


The First Stages in the Metabolism of Glucose.—At the outset we are confronted with the problem of deciding which form of glucose serves as the starting point in carbohydrate metabolism. It has been generally assumed that the amylene-oxide form of glucose must first be transformed to a more reactive form, but our present knowledge does not permit us to point with conviction to any single isomeric modification of glucose as the form which is directly involved in metabolic processes. As a working hypothesis, we may however accept Levene's conception of an " active " glucose, to which reference has been made (page 52). Accordingly, it may be assumed that the first step in metabolism is the conversion of glucose to the more reactive form, " active glucose."

The next step is also beset with elements of uncertainty. Ever since Harden and Young [16] showed that phosphates accelerate the fermentation of sugars by yeast and discovered hexose-diphosphoric acid in the products resulting from such fermentation, the relation of phosphates to carbohydrate metabolism has been seriously considered. Hexose-

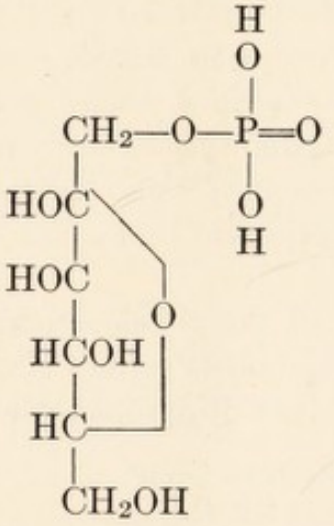
[16] Biochem., Z., **32**, 173 (1911); Proc. Roy. Soc., B, **81**, 528 (1909).

diphosphoric acid, according to Morgan and Robison,[17] is α-fructose-1 : 6-diphosphoric acid:



The work of Morgan and Robison has been confirmed by Levene and Raymond.[18]

Partial hydrolysis of hexose-diphosphoric acid yields hexose-monophosphoric acid, first obtained by Neuberg.[19] Morgan and Robison and Levene and Raymond agree that this is α-fructose-6-monophosphoric acid:



A second hexose-monophosphoric acid is formed along with the diphosphoric acid ester in yeast fermentation, as discovered by Robison,[20]

[17] Biochem. J., **22**, 1270 (1928).
[18] J. Biol. Chem., **80**, 633 (1928).
[19] Biochem. Z., **88**, 432 (1918).
[20] Biochem. J., **16**, 809 (1922).

in 1922. It differs from Neuberg's hexose-monophosphoric acid and, according to recent work, may be a mixture of glucose- and fructose-monophosphoric acids. It also appears that by varying the conditions of fermentation different hexose-monophosphoric acids may be formed, and, in fact, not only phosphoric acid esters of monosaccharides but of disaccharides as well. Robison and Morgan [21] have discovered that in the fermentation of either glucose or fructose with dried yeast, trehalose-monophosphoric acid is one of the products formed.

From muscle tissue, Embden and Zimmerman [22] isolated in 1924 a hexose-diphosphoric acid, which seemed to be identical with the hexose-diphosphoric acid isolated by Harden and Young. Embden and Zimmerman named this substance *lactacidogen*. Subsequently by a modified method they obtained from rabbit muscle a hexose-monophosphate.[23] This ester has also been studied by Pryde and Waters,[24] who believe that the normally occurring phosphoric acid ester in muscle is a monophosphoric acid ester. They have shown in addition that this ester is probably a mixture, 90 per cent of which is glucose-monophosphate and the remaining 10 per cent fructose-monophosphate.

The hexose mono- and diphosphoric acid esters are not the only phosphorus compounds of muscle. In recent years, several other substances, probably of great physiological importance, have been discovered. Eggleton and Eggleton [25] discovered a labile organic phosphate in muscle which they called phosphagen. This has been shown to be (for the most part) creatine-phosphoric acid by Fiske and Subbarow.[26] Rapidly following this discovery was that of Meyerhof and Lohmann [27] who have shown that crustacean muscle contains another guanidine-phosphoric acid (this is a generic name employed by Meyerhof for phosphagens), namely arginine-phosphoric acid. Another important contribution is that of Lohmann,[28] who has recently reported the occurrence in muscle of pyrophosphoric acid which is enzymically hydrolyzed to orthophosphoric acid.

A precise formulation of the physiological rôle of the various phosphates in carbohydrate metabolism cannot be made at present, especially in view of the recent discovery of pyrophosphoric acid which may neces-

[21] Biochem. J., **22**, 1277 (1928).

[22] Z. physiol. Chem., **141**, 225 (1924).

[23] *Ibid.*, **167**, 114 (1927).

[24] J. Soc. Chem. Ind., **46**, 1182 (1927); **47**, 1346 (1928); Annual Reports, **25**, 249 (1928).

[25] Biochem. J., **21**, 190 (1927).

[26] Science, **65**, 401 (1927); J. Biol. Chem., **81**, 629 (1929).

[27] Biochem. Z., **196**, 22, 49 (1928).

[28] Naturwiss., **16**, 298 (1928); **17**, 624 (1929).

sitate a revision of the conceptions that have developed in the last few years. For the details of the processes involved one should therefore rely more on the reports that are likely to appear from the laboratories of those engaged in studying the problem. Pryde,[29] writing in 1928, states: " It would now appear to be a certainty that the primary point of attack in carbohydrate cleavage in the muscle—as in yeast fermentation—is to be sought in the esterification of sugar with phosphoric acid."

A relation of phosphate to carbohydrate metabolism is indicated by the work of numerous investigators.[30] After the ingestion of sugar there is a decrease in the elimination of phosphates in the urine, as well as a fall in the inorganic phosphates of the blood. This relation of glucose ingestion to decreased phosphate elimination is observed in normal individuals, but in diabetes, when carbohydrate metabolism is impaired, the relation is not observed. In completely depancreatized dogs, there is no appreciable fall in phosphates unless insulin is administered. According to Bolliger and Hartman, the demand for inorganic phosphates by the tissues is apparent only when the pancreatic hormone is available and when carbohydrate is being utilized.

Carbohydrate Metabolism in Relation to Muscular Activity.—Muscular contraction does not depend on the oxidation of fuel and is not accompanied by the taking up of oxygen or liberation of carbon dioxide. During this phase of muscular activity there is the formation of lactic acid, the amount of which depends on the vigor of the contraction, a maximum of lactic acid being obtained in heat rigor when the contraction is likewise at a maximum. In the formation of 1 gram of lactic acid from glycogen outside the body, and in the neutralization of this with phosphate or bicarbonate, 190 calories of heat are evolved. The production of the same amount of lactic acid in intact muscle yields 370 calories, but in hashed muscle only 190 calories. This difference in behavior between living and dead muscle has not been entirely accounted for, although Meyerhof has obtained some evidence to show that the extra 180 calories may represent the heat of dissociation of the proteins of the sarcoplasm in the presence of the acid. Approximately this amount of heat is obtained when 1 gram of lactic acid is allowed to penetrate muscle or when it is added to a buffered mixture of amino acids.

When muscle is kept in an atmosphere free from oxygen, the lactic acid accumulates up to a certain point, after which the muscle loses

[29] J. Pryde, Recent Advances in Biochemistry, Philadelphia (1928), p. 182.

[30] Fiske, J. Biol. Chem., **49**, 71 (1921); Perlzweig, Latham and Keefer, Proc. Soc. Exp. Biol. and Med., **21**, 33 (1923–24); Bolliger and Hartman, J. Biol. Chem., **64**, 91 (1925).

its irritability and no further heat production is manifested. If such muscle is then exposed to oxygen, the lactic acid disappears. Here again, much depends on the condition of the muscle, for, as shown by Fletcher and Hopkins,[31] the oxidative removal of lactic acid does not take place in hashed or injured muscle. With the disappearance of the lactic acid, the muscle recovers. During this stage there is again an evolution of heat, the amount of which has been measured by Hill [32] and found to be about 1.5 times the heat production during the phase of contraction; the total energy liberated in the production and subsequent oxidative removal of 1 gram of lactic acid therefore amounts to about 900 calories. As this is equivalent to only about 25 per cent of the heat of combustion of 1 gram of lactic acid or of a corresponding amount (0.9 gram) of glycogen, it at once became evident to some, though not to all, physiologists that the formerly accepted view of complete oxidation of lactic acid to CO_2 and H_2O is untenable as it does not explain the low heat production during muscular recovery.

The problem was enshrouded in a maze of contradictions when Meyerhof [33] began his epoch-making researches on the mechanism of muscular activity. In 1919 he showed that, during the phase of recovery, the volume of CO_2 produced is almost equivalent to the volume of oxygen used. These observations pointed definitely to the combustion of a substance like lactic acid or some carbohydrate. He was next able to show that whenever lactic acid appears, as during muscular contraction, a corresponding amount of glycogen disappears. Moreover, he found a conversion of lactic acid into glycogen during the recovery phase. These observations explain the liberation of only 900 calories, instead of about 3700, in the oxidative removal of 1 gram of lactic acid. Only part of the energy liberated by oxidation appears as heat; the rest is apparently absorbed in some physico-chemical process, restoring the muscle to its original condition (Meyerhof). It is important to realize that we are not at all certain that it is lactic acid which is burned. It may very well be that all of the lactic acid undergoes synthesis and that some intermediate product, perhaps one closely related to glucose itself, is the substance actually burned. This would have much significance if it were found to be the case, for it would necessitate a modification of the prevailing ideas of carbohydrate metabolism, as we shall see presently.

[31] J. Physiol., **35**, 247 (1907).

[32] Physiol. Reviews, **2**, 310 (1922).

[33] Pflüger's Arch., **175**, 88 (1919); **182**, 284 (1920); **185**, 11 (1920); J. Gen. Physiol., **8**, 531 (1927).

Considering the evidence before us, the following series of events appears to be probable:

1. The recovery process consists of an oxidation (either of lactic acid or of carbohydrate), of which part of the energy appears as heat, and part is absorbed in restoring the muscle to its original condition of readiness for muscular activity.

2. In the initial process of contraction, glycogen, or some product of glycogen, is changed explosively into lactic acid.

3. In the recovery process the glycogen (or its product) is restored and the lactic acid removed. The oxygen used in recovery is employed in oxidizing carbohydrate (or lactic acid) in an amount equivalent to about one-quarter of the lactic acid removed.[32]

Taking into account the intermediate formation of hexose-monophosphate (as the potassium salt), these ideas may be more formulated as follows:[33a]

I. Anaerobic break-down

$$5/n(C_6H_{10}O_5)_n + 5H_2O + 4K_2HPO_4 \rightarrow$$
$$\rightarrow 4C_6H_{11}O_5(K_2PO_4) + C_6H_{12}O_6 + 4H_2O \rightarrow$$
$$\rightarrow 8C_3H_6O_3 + 4K_2HPO_4 + C_6H_{12}O_6$$

II. Oxidative recovery

$$8C_3H_6O_3 + 4K_2HPO_4 + C_6H_{12}O_6 + 6O_2 \rightarrow$$
$$\rightarrow 4C_6H_{11}O_5(K_2PO_4) + 6CO_2 + 10H_2O \rightarrow$$
$$\rightarrow 4/n(C_6H_{10}O_5)_n + 4K_2HPO_4 + 6CO_2 + 10H_2O$$

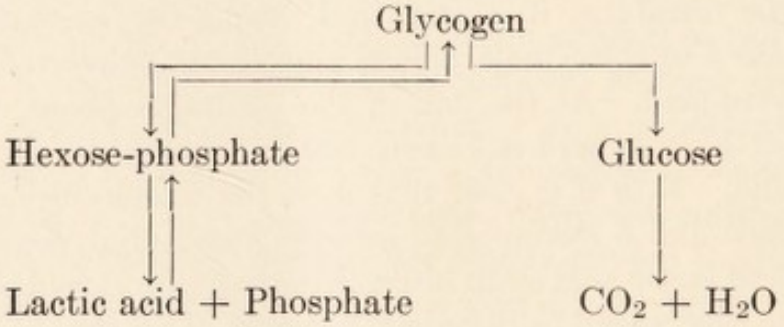
From the foregoing, it is seen that during the anoxidative or contractile phase 4 of the 5 molecules of glucose are converted into 8 molecules of lactic acid. At the end of the oxidative phase, we recover 4 molecules of glucose and sufficient carbon dioxide to account for the fifth molecule. Now it is clear that if, of the 8 molecules of lactic acid, 2 were burned and 6 reconverted into glucose and glycogen, we should have to accept lactic acid as an intermediate step in carbohydrate metabolism. The case would be different, however, if we should discover that the fifth glucose molecule was burned. Recent work has tended to support the view that lactic acid formed is not oxidized further, but is reconverted possibly through hexose-phosphate to glycogen. As emphasized by Shaffer in his review, a number of years ago, this is a very fundamental point, for if all of the lactic acid formed were reconverted to

[33a] See Pryde, Recent Advances in Biochemistry, Philadelphia (1928), p. 184.

glucose, lactic acid would not be a stage in the path of glucose oxidation in muscle. Of course, the possibility exists that the oxidation of the fifth glucose molecule may involve the intermediate formation of lactic acid, but as we shall see presently there is no proof of this.

There is a fundamental difference in the mechanics of a combustion engine and of muscle. In the former, the spark ignites the fuel; combustion occurs, with the result that heat, water (steam), and carbon dioxide are formed. The gases, being under pressure, cause compression; mechanical work and heat energy become manifest. On the other hand, in muscle, presumably as a result of a nervous stimulus, lactic acid is formed from its precursors. The acid, by causing a contraction of the muscle, sets up a tension which yields both heat and mechanical energy. It is only after the contraction is over that most of the oxidation occurs. In the engine, mechanical work follows the combustion of fuel, whereas in muscle, the production of energy precedes the combustion of fuel.

The Products of Glucose Oxidation.—Thus far we have considered mainly the reversible transformation of glycogen to lactic acid which is associated with muscular activity. At present, the more generally accepted view is that the conversion in either direction involves the intermediate formation of a hexose-phosphate and that the lactic acid, formed in the anaerobic, or contractile phase, is not oxidized but reconverted to glycogen during the period of recovery. Whatever oxidation occurs is supposed to be due to approximately one-fifth of the glucose derived from the glycogen during the period of contraction. These relations may be represented tentatively as follows:



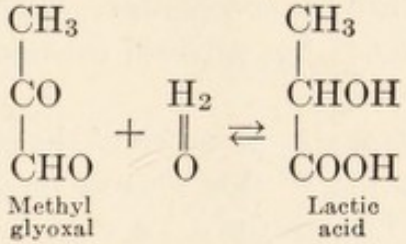
We are now to consider the oxidation of glucose. The prevailing view is that the six-carbon molecule is broken down to fragments, having three carbon atoms each, the chief fragment being glyceric aldehyde. This idea seems logical for a number of reasons. As shown by Sansum and Woodyatt,[34] and Ringer and Frankel,[35] when glyceric aldehyde is

[34] J. Biol. Chem., **24**, 327, 343 (1916).

[35] *Ibid.*, **18**, 233 (1914).

given to phlorhizinized dogs, it is almost completely converted into glucose. This is true also for dihydroxyacetone, which may be formed from or along with glyceric aldehyde in the break-down of glucose. Perfusion of dog liver with *d-l*-glyceric aldehyde yields both *d*-sorbose and *d*-glucose, according to Embden, Schmitz and Wittenberg.[36] The formation of glycogen by perfusing tortoise livers with glyceric aldehyde has been observed by Parnas.[37] Moreover, glyceric aldehyde is oxidized in the body quite readily,[38] although perhaps not as rapidly as might be expected. Sansum and Woodyatt [39] found the tolerance of *d*-glyceric acid to be relatively low. When incubated with blood corpuscles it yields lactic acid just as glucose does. Since glyceric aldehyde is convertible both into glucose and into lactic acid, it may very well be a product in the intermediary metabolism of glucose.

Because of the position which it occupies in relation to lactic acid and glucose, methyl glyoxal is considered the next possible link in carbohydrate metabolism. It is converted into glucose, as shown by Dakin and Dudley [40] who, after giving a phlorhizinized dog 9 grams of methyl glyoxal, recovered 7 grams of extra sugar in the urine. Rapid conversion into lactic acid occurs when methyl glyoxal is subjected to the action of many tissues. According to Dakin and Dudley,[41] this is brought about by the enzyme "glyoxalase." As represented in the following equation, this change is an internal Cannizzaro reaction, part of the molecule (aldehyde group) being oxidized while another part (carbonyl group) is reduced. For this reason Neuberg [42] prefers the name "keto-aldehyde mutase" for the enzyme involved in this reaction.



Dakin [43] holds that in the conversion of *l*-lactic acid into *d*-glucose there must be some intervening reaction which involves the loss of

[36] Z. physiol. Chem., **88**, 210 (1913).
[37] Zentralblatt f. Physiol., **26**, 671 (1912).
[38] Neuberg, C., Arch. Anat. u. Physiol., Physiol. Abt. 571 (1904).
[39] J. Biol. Chem., **24**, 343 (1916).
[40] J. Biol. Chem., **15**, 127 (1913).
[41] *Ibid.*, **14**, 155, 423 (1913).
[42] Biochem. Z., **51**, 484 (1913).
[43] H. D. Dakin, Oxidations and Reductions in the Animal Body, 2d edition, Chapter IV.

asymmetry and hence of optical activity of the lactic acid. Methyl glyoxal or dihydroxyacetone, being optically inactive, might fulfill this requirement. Nevertheless, Dakin is cautious in emphasizing the fact that it is impossible at the present time to reach a definite decision as to the rôle of glyceric aldehyde, dihydroxyacetone, and pyruvic aldehyde in carbohydrate metabolism.

Even greater difficulty is encountered in considering the transformation of lactic acid into glucose, where we do not have as many *in vitro* analogies to guide us. It has been suggested (Parnas and Baer)[44] that this may take place by way of glyceric acid, β-hydroxypyruvic acid, and glycol-aldehyde. Although the formation of glucose from glycol-aldehyde has been definitely established, the above scheme has not been generally accepted for the reason that it does not account for the quantitative conversion, in diabetic animals, of all the carbon atoms of lactic acid into glucose. Nor is this objection met by assuming that the lactic acid goes to glucose by way of pyruvic acid and acetic aldehyde. The more generally accepted view postulates the reversal of all the intermediate reactions encountered in the conversion of glucose into lactic acid. For evidence in support of this idea, we have mainly Dakin and Dudley's demonstration [45] of the formation, *in vitro*, of methyl glyoxal from lactic acid. The formation of hexoses from trioses is also a possibility, but the remaining links are missing, for we have no evidence of the conversion of methyl glyoxal into glyceric aldehyde. In considering the question of muscular contraction, we have seen that the conversion of lactic acid into glycogen requires oxygen. This does not fit very well into the mechanism which has just been described. For this reason, Shaffer [46] has advised caution in the adoption of this hypothesis.

Essentially in agreement with much of the earlier work just described is the suggestion of Fischler [47] that glucose may be converted into either glyceric aldehyde, or through the 1 : 2 enol form to dihydroxyacetone, and that these give rise to methyl-glyoxal. There is no certainty as to what happens next, but there is a strong possibility that formic acid and acetaldehyde are produced. Lusk [48] states that given aerobic conditions, if acetaldehyde were formed, it would be immediately oxidized to acetic acid. Under anaerobic conditions methyl glyoxal is reduced to

[44] Biochem. Z., **41**, 386 (1912).

[45] J. Biol. Chem., **14**, 555 (1913).

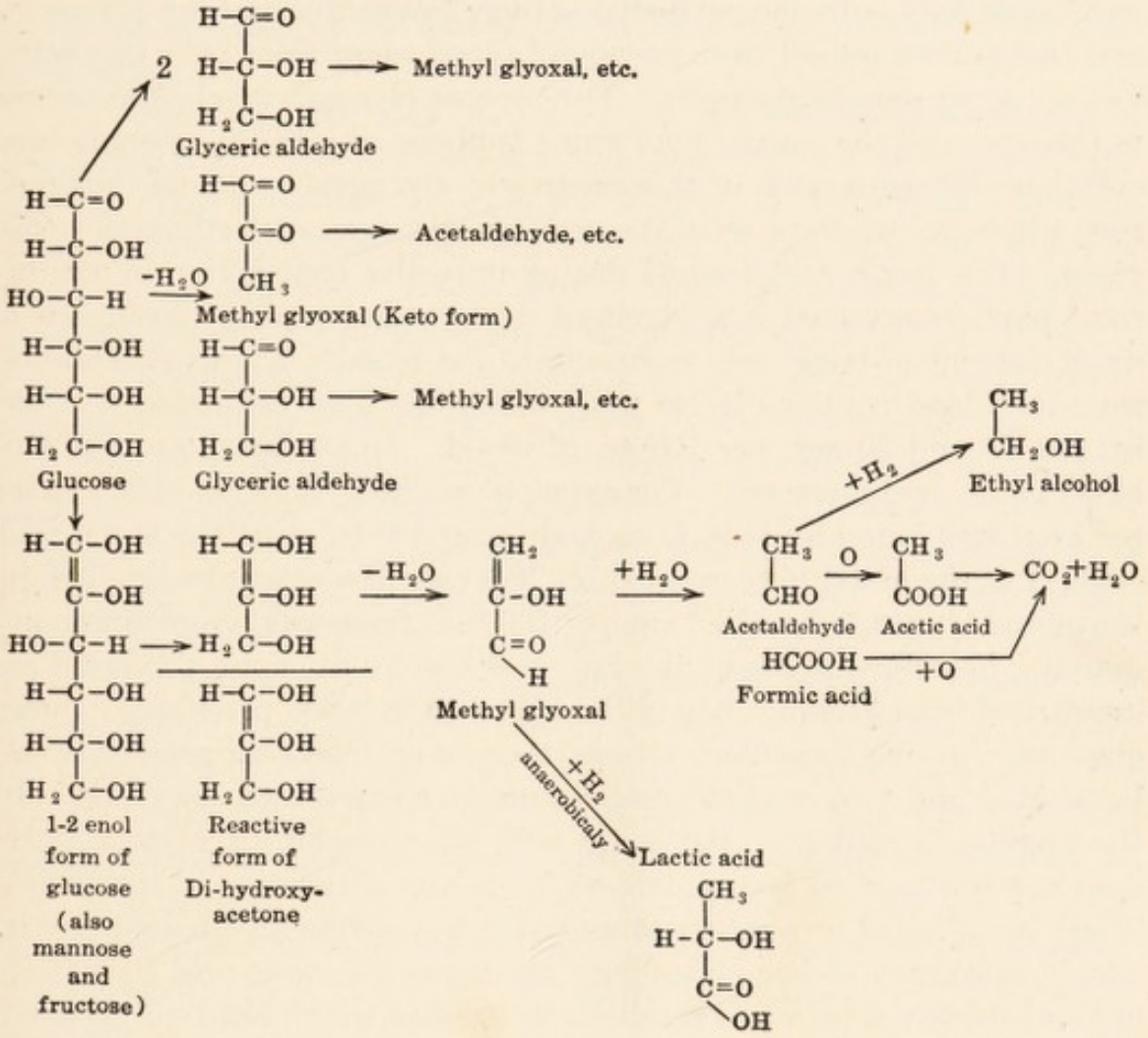
[46] P. A. Shaffer, Intermediary Metabolism of Carbohydrates, Physiol. Rev., **3**, 419 (1923).

[47] Z. physiol. Chem., **165**, 53, 68 (1927).

[48] Graham Lusk, The Elements of the Science of Nutrition, Philadelphia, 1928, p. 349.

lactic acid which is reconverted into glucose before it can be oxidized. It is further stated by Lusk that if anaerobic conditions were present and acetaldehyde were produced, this would be at once converted into ethyl alcohol as in alcoholic fermentation.

The more plausible speculations together with the few facts which we have regarding the intermediary metabolism of glucose may perhaps be best summarized as follows:



It will be noted that the formation of ethyl alcohol from acetaldehyde, anaerobically, is indicated in the preceding chart. This is based on the observation of Taylor [49] that small amounts of alcohol are formed in animal tissues. Taylor sought to answer an objection to this view, which had been raised because the possible origin of alcohol in intestinal fermentation was recognized. He therefore removed the entire alimentary tract of a dog which had been previously starved for about twenty-four hours. Eighteen hours after the operation, the animal was killed. Prompt analysis of the tissues revealed the presence of minute

[49] J. Biol. Chem., **15**, 217 (1913).

amounts of alcohol. The formation of ethyl alcohol is to be regarded as a side reaction which may take place particularly in asphyxia. The production of ethyl alcohol in large amounts is limited by the fact that under anaerobic conditions, as has been stated, methyl-glyoxal would be reduced to lactic acid, which must first be converted to glucose before it is again available for oxidation (Lusk).[48]

Further Observations on the Interrelationship of Glycogen, Glucose and Lactic Acid.—In one particular is there less confusion than formerly, and this is with regard to the origin of blood sugar from liver glycogen, but not from muscle glycogen. The process glycogen $\rightleftarrows$ glucose occurs in the liver and the sugar thus formed is liberated into the systemic circulation. Reconversion of this sugar into glycogen occurs in the muscles, where, as we have seen, the reaction glycogen $\rightleftarrows$ lactic acid takes place. The lactic acid formed during muscular contraction is for the most part reconverted into glycogen during the recovery phase, but a small amount of lactic acid escapes into the blood. Even in a state of rest, the blood contains lactic acid, which has been estimated to vary between 5 and 20 mg. per 100 cc. of blood. In moderate exercise the blood lactic acid increases. For example, walking at a rate of 3.5 miles per hour was found by Hill, Long and Lupton [50] to cause the lactic acid content of the blood to increase from 20.9 mg. (the concentration before the period of exercise) to 36.6 mg. per 100 cc. Somewhat more strenuous exercise, namely walking at the rate of 4.1 miles per hour, produced an increase of from 21.4 mg., the initial value, to 58.9 mg. per 100 cc. Quite obviously, during muscular exercise, there is an increased production of lactic acid and a somewhat greater amount escapes into the blood. If the exercise is moderate, the lactic acid, after reaching a certain level, does not continue to accumulate either in the muscles or in the blood. There is sufficient respiratory stimulation to provide an adequate extra supply of oxygen to keep pace with the increased lactic acid formation and in this way a balanced condition is reached which has been referred to as the *steady state*. This means that there is a steady rate of oxygen utilization, and that the lactic acid content of the muscle, though above normal, is nevertheless maintained at a constant level.

The situation is different when the exercise is more violent in character. First, with regard to the lactic acid in the blood, there is a marked increase. In an experiment of Hill and his associates, the subject ran in a standing position for four minutes (breathing pure oxygen). At rest, the lactic acid content was 20 mg. per 100 cc., whereas immediately after the exercise it was 86 mg. In another experiment, the subject ran in

[50] Proc. Roy. Soc., London, B, **96**, 438; **97**, 84, 155 (1924).

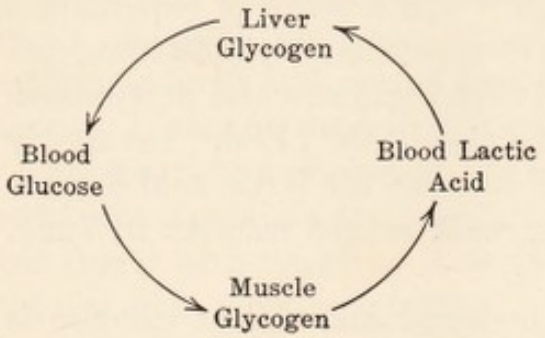
place at 239 steps per minute for 9.5 minutes, breathing air. The lactic acid rose from 8.5 to 204 mg. per 100 cc. A similar effect was observed by Barr, Himwich and Green.[51] In a series of experiments they subjected a number of individuals to approximately 3500 kilogram-meters of work, performed in a period of 3.5 minutes and determined, among other things, the change in lactic acid in the blood. Invariably there was an increase. The difference between the lactic acid concentration at rest and at the end of the exercise ranged between 31.7 mg. to 85.8 mg.

The essential difference between moderate and violent exercise is that in the latter there is a considerable accumulation of lactic acid in the muscle. This is because the supply of oxygen, after reaching a limiting value, can not be increased any further and does not keep pace with the lactic acid production. When light or moderate exercise is stopped, there is a prompt return to normal of the gaseous exchange and the lactic acid concentration in the blood begins to fall. Not so when violent exercise is suddenly terminated. For some minutes thereafter the lactic acid content of the blood continues to increase (see for example Barr and Himwich [52]), before the drop sets in, and the oxygen utilization continues at a high level for a considerable period. During violent exercise when the oxygen supply is inadequate, the tissues go into "oxygen debt," and a long period of recovery, during which the oxygen utilization continues at a high level, is required before the debt is paid. Thus, in the experiments of Hill, Long and Lupton, a subject, after running 3 meters per second for five minutes, took 9.5 minutes to recover (i.e., before his oxygen intake returned to normal) and his oxygen debt was 1.7 liters. After running in place for twenty seconds as violently as possible, he went into debt 5.5 liters of oxygen and took fourteen minutes to recover. A quarter of a mile run, followed by severe gymnastic exercise, resulted in an oxygen debt of 12.4 liters, the subject taking forty-four minutes to recover.

Now, what we are primarily interested in here is the fate of the lactic acid which escapes into the blood, for, it is seen, this occurs to some extent even in the resting condition and is much increased during exercise. A small portion of the lactic acid is excreted in the urine and the amount lost in this way may be considerable during violent exercise, but the larger proportion is returned to the liver, there to be resynthesized into glycogen. Thus, as Cori and Cori [53] have pointed out, a sugar molecule can go through a complete cycle in the body; it can in turn be liver glyco-

[51] J. Biol. Chem., **55**, 495 (1923).
[52] J. Biol. Chem., **55**, 539 (1923).
[53] J. Biol. Chem., **81**, 389 (1929).

gen, blood sugar, muscle glycogen, blood lactic acid, and again liver glycogen. This cycle may be represented as in the diagram to the left.



An abundance of evidence has accumulated in support of this idea, but only a small portion of the literature can be referred to here. For example, the question as to whether muscle glycogen is converted directly into glucose has been virtually settled. When the liver is removed, the blood sugar rapidly falls to very low levels, as shown by the work of Bollman, Mann and Magath.[54] As the muscle contains considerable amounts of glycogen and as this does not prevent the hypoglycemia and does not disappear to any great extent, it is concluded that muscle glycogen is not readily converted into glucose.

It is well known that epinephrin, ether anesthesia and asphyxia produce hyperglycemia. But, as has been shown by Soskin,[55] if the abdominal viscera, including the liver, are removed, hyperglycemia does not develop under these conditions despite the fact that there is glycogen in the muscles.

There remains to be considered some of the evidence for the transformation of lactic acid into glycogen in the liver. It is to be supposed that in severe liver damage, as in phosphorus poisoning, the liver would lose its ability to convert lactic acid into glycogen and that there would be an increased excretion of lactic acid in the urine. This is actually the case. More direct evidence is that of Parnas and Baer,[55a] who observed glycogen synthesis in the turtle liver, perfused with sodium lactate. Similar observations have been made by many others, although occasionally negative results have been reported. Abramson, Eggleton and Eggleton [56] were unable to demonstrate the synthesis of glycogen in the liver from racemic sodium lactate in dogs under amytal anesthesia. On the other hand, Izume and Lewis [57] observed glycogen deposition in the liver of fasting rabbits injected subcutaneously with sodium lactate, and more recently Cori and Cori,[53] working with rats, found that if sodium *d*-lactate is fed by mouth or injected subcutaneously, glycogen is deposited in the liver. Sodium *l*-lactate, though absorbed from the intestine at the same rate as the *d*-isomer, hardly formed any liver glyco-

[54] Am. J. Physiol., **74**, 238 (1925).
[55] Am. J. Physiol., **81**, 382 (1927).
[55a] Biochem. Z., **41**, 414 (1912).
[56] J. Biol. Chem., **75**, 763 (1927).
[57] J. Biol. Chem., **71**, 51 (1926–27).

gen. Cori and Cori state that of the *d*-lactate absorbed in three hours, 40–95 per cent was retained as liver glycogen and none was excreted in the urine, whereas 30 per cent of the *l*-lactate absorbed was recovered in the urine.[58]

The Metabolism of Nerves.—The significant contributions to our knowledge of nerve metabolism are of very recent origin.[59] It has been established that activity in nerve is accompanied by an increased consumption of oxygen and production of carbon dioxide, and the development of a small amount of heat. The respiratory quotient of resting nerve varies between 0.75 and 0.80. Such values would be consistent with the utilization of a mixture of fats, proteins and carbohydrates. During activity, the respiratory quotient of nerve rises. If the respiratory quotient of the extra metabolism is computed, it is found to vary between 0.95 and 1.0. This corresponds to the values which would be obtained if only carbohydrate were being oxidized, or if protein were being utilized in such a way as to form ammonia, rather than urea, as the end product of the nitrogenous metabolism. It has been shown that ammonia increases in amount when nerve is stimulated.

Holmes, Gerard and Solomon[60] have shown that during rest in oxygen there is no change in the glycogen or lactic acid content of nerve. The "free sugar," on the contrary, decreases in amount with time. In rabbit nerves, at 37° C., the decrease is 36 mg. per cent for the first hour and progressively less during later periods. In bullfrog nerves, at 22° C., the rate of fall of "free sugar" is constant for at least nine hours at 6 mg. per cent, per hour. This is 50 per cent more than can be oxidized by all the oxygen used by the nerve at rest. The fate of this sugar is unknown.

Conversion of Carbohydrate into Fat.—The synthesis of fat from carbohydrate in the animal organism is an established fact and a matter of common knowledge and experience. The synthesis involves the formation from glucose of both glycerol and fatty acids. From their chemical relationship, the origin of glycerol from glyceric aldehyde is quite obvious. Indeed, it has been observed that during liver perfusion with glyceric aldehyde and dihydroxyacetone, small amounts of glycerol are formed. The origin of the fatty acids is a somewhat more debatable point. These are probably formed from acetic aldehyde by aldol con-

[58] For a more extensive review refer to C. F. Cori, The Harvey Lectures, 1927–1928, p. 76, Baltimore (1929).

[59] For a review of the literature on the metabolism of nerves, see W. O. Fenn, Harvey Lectures, Series 23, Baltimore, 1929, p. 115. A concise summary of nerve metabolism is given by R. W. Gerard, Science, **66**, 495 (1927).

[60] Personal communication from R. W. Gerard. See also Holmes and Gerard, Biochem. J., **23**, 738 (1929).

densation. Other fragments of the glucose molecule (glycolic aldehyde, glyoxal) have also been suggested as possible precursors of the fatty acids. According to Smedley and Lubrzynska [61] there is a condensation of acetic aldehyde with pyruvic acid, $CH_3 \cdot CO \cdot COOH$, derived by the oxidation of methyl glyoxal (pyruvic aldehyde), yielding a ketonic acid which is converted by the splitting off of carbon dioxide into an aldehyde having one carbon atom less than the ketonic acid. The aldehyde then condenses with another molecule of pyruvic acid and again gives off a molecule of carbon dioxide. By the repetition of this process, long carbon chains may be built up. This type of synthesis has been observed *in vitro* in the case of butyl aldehyde and pyruvic acid. When the fatty acid chain is built up, it very likely undergoes a certain amount of oxidation yielding intermediate compounds containing unsaturated linkages. One stage in the synthesis may be represented as follows:

$$CH_3CHO + CH_3CO \cdot COOH \rightarrow CH_3CH : CH \cdot CO \cdot COOH + H_2O.$$

$$CH_3CH : CH \cdot CO \cdot COOH \rightarrow CH_3CH : CH \cdot CHO + CO_2.$$

Tissue Constituents Containing Sugars.—It should now be pointed out that certain amounts of sugar and other foodstuffs are used in the construction and repair of tissue and that these processes also constitute a phase of metabolism, called anabolism. The reverse process, which is the dissimilation of tissue constituents, is called catabolism. The metabolism of tissue constituents proceeds at a rather constant rate, because tissue break-down in health is usually constant and may be broadly referred to as endogenous metabolism, in contradistinction to the direct metabolism of foodstuffs, which is called exogenous metabolism. However, these terms are customarily used in a more restricted sense, as we shall see later in considering purine and creatine metabolism.

Carbohydrates enter into the synthesis of the nucleic acids and of closely related constituents of cells. One of the products obtained on hydrolyzing nucleic acids, of animal origin, is levulinic acid formed from a desoxypentose, $CHO \cdot CH_2 \cdot CHOH \cdot CHOH \cdot CH_2OH$, present in the nucleic acid molecule. Plant nucleic acids contain a pentose sugar (see p. 362). Closely related to the nucleic acids are two substances, inosinic and guanylic acids, which on hydrolysis yield a pentose sugar, *d*-ribose. The presence of *d*- and *l*-arabinose in the urine has been reported. The condition is very rare and is known as idiopathic pentosuria.

The conversion in the body of glucose into galactose is indicated by the synthesis of lactose in the mammary glands of lactating mammals.

[61] Biochem. J., 7, 364, 375 (1913).

Galactosamine has been reported present along with glucosamine in various protein combinations. Moreover, galactose exists in combination with lipids in nervous tissue (galactolipids or cerebrosides), and together with glucose in the mucoproteins. Glucose and mannose are also known to occur in other proteins.

Cartilage, bone, tendons, and fascia contain chondroitin, an amino polysaccharide conjugated with sulfuric acid.

These are among the better-known tissue constituents containing carbohydrate in combination. Their synthesis in the body must therefore be considered as part of carbohydrate metabolism. What becomes of these substances in catabolism is uncertain. Perhaps a certain amount of these carbohydrates is reconverted into glucose and shares its fate in metabolism. There is also a likelihood that the small amounts of pentose, galactosamine, and glucosamine, etc., which may be formed in tissue catabolism are excreted in the urine unchanged.

Glycosuria.—The presence of sugar in the urine is termed glycosuria (glucosuria) or mellituria. Glycosuria may be entirely physiological, but is often a symptom of diabetes, a disease in which the metabolism of carbohydrates is impaired. Diabetes has been known for many years, and it is to the intensive study of this pathological condition that we owe much of our knowledge of the intermediary metabolism of the foodstuffs.

When sugar is found in the urine, it is nearly always glucose. Rarely, fructose and pentose may be present. During the last stages of pregnancy, particularly during the last few days before delivery, as well as during lactation, there is an excretion of lactose. The factors involved in lactosuria have been recently studied by Watkins.[62] Following the injection or rapid absorption of disaccharides (sucrose, maltose) these may appear in the urine.

Sugar appears in the urine either when its concentration in the blood is high and exceeds the renal threshold, or when the kidneys are unusually permeable to this substance. The concentration of glucose in the blood may increase as a result of insufficient glycogenesis or because of an excessive amount of glycogenolysis. When large amounts of carbohydrate are being absorbed and the rate of conversion into glycogen does not keep pace with the rate of absorption, there is a piling up of sugar in the blood (hyperglycemia) and the excretion of part of it in the urine. When the appearance of sugar in the urine is due to the ingestion of excessive amounts of carbohydrate, the condition is termed *alimentary glycosuria*. This is purely a physiological phenomenon and may be produced in normal individuals.

[62] J. Biol. Chem., **80**, 33 (1928).

Deficient glycogenesis occurs in conditions of acidosis and of liver injury (alcoholism, cirrhosis, phosphorus poisoning) and is associated with the excretion of sugar in the urine.

Glycosuria and hyperglycemia occur in mechanical asphyxia and in carbon monoxide poisoning, obviously as a result of increased glycogenolysis, for it has been shown that no hyperglycemia or glycosuria develops in animals in which the circulation through the liver is excluded by means of an Eck fistula. Hyperglycemia and glycosuria occur in ether and chloroform anesthesia and in morphine and strychnine poisoning.

Puncture Glycosuria.—In a classical experiment, Claude Bernard discovered that puncturing the medulla of the brain in the region of the floor of the fourth ventricle resulted in glycosuria. This form of experimental glycosuria has been named *la piqûre* or puncture diabetes. It is important to bear in mind that the intensity and duration of the glycosuria produced in this way depends on the amount of glycogen present in the liver at the beginning of the experiment. If the glycogen has been removed by previous starvation, glycosuria does not occur, or is very slight.[63]

A similar effect may be obtained by stimulating the sympathetic nerve supply to the liver (electrical stimulation or injection of adrenalin or epinephrin). These forms of glycosuria are believed to result from increased glycogenolysis.

Possibly related to these conditions are various forms of transitory glycosuria due to nervous disturbances. It is well known that a blow on the head may lead to glycosuria (traumatic glycosuria). Fright, agitation, or struggling causes hyperglycemia and the consequent excretion of sugar in the urine (psychic or emotional glycosuria). Folin [64] examined students before and after a difficult examination and found sugar in the urine of over 15 per cent. Similar observations were made by Cannon, but others have been unable to confirm these observations. Some clinicians have attempted to establish a relationship between nerve strain and the incidence of diabetes. While the importance of nervous factors in the etiology of diabetes has probably been exagger-

[63] Hiller and Tannenbaum (Arch. Neurol. and Psychiat., **22**, 901 (1929)) have recently reported that the hyperglycemia associated with the *piqûre* operation is due to various incidental factors, such as manipulation of the animal, fright, partial asphyxia, anesthesia, etc. Indeed when these factors were eliminated, or accounted for, the increase in blood sugar attributable to the *piqûre* was found to be very slight. Hiller and Grinker (*ibid.*, **22**, 919 (1929)) have challenged the conception that there is a definite center in the medulla which controls glucose metabolism through the sympathetic system.

[64] Folin, Denis, and Smillie, J. Biol. Chem., **17**, 519 (1914).

ated, nevertheless Macleod points out the frequent occurrence of diabetes in those predisposed to neurotic conditions, or in those whose daily habits entail much nerve strain.

Renal Glycosuria and Phlorhizin Diabetes.—Glycosuria due to increased renal permeability is perhaps more frequent than was imagined before it became possible to analyze the blood for its sugar content. Errors in diagnosis were no doubt frequent when the clinician was forced to rely solely on urine analyses. Renal diabetes is distinguished by the low concentration of sugar in the blood. The experimental production of a somewhat similar condition was accomplished in 1886 by von Mering [65] upon injecting into animals phlorhizin, a glucoside which is found in the root bark of the cherry, apple, pear, and plum trees. Just as in renal diabetes, the sugar concentration of the blood in phlorhizin diabetes frequently falls to 0.07–0.08 per cent. The animal organism, in attempting to maintain the sugar concentration of its blood within normal levels, uses up a large proportion of its stored glycogen, and when this is depleted the proteins of the tissues are called upon to supply the needed sugar. The sugar-forming amino acids are converted into glucose, but no sooner is it formed than it is excreted by the kidneys. Thus, in severe phlorhizin diabetes, after the glycogen has been used up, 3.65 grams of glucose are excreted for every gram of nitrogen. The ratio between glucose and nitrogen excretion is called the D : N or G : N ratio and has been found to be a most valuable criterion in determining the severity of the condition both in phlorhizin diabetes and in true pancreatic diabetes. When no food is taken, a D : N ratio of 3.65 is called the fatal ratio, for it indicates a complete failure in the utilization of glucose.

As an experimental method, phlorhizin diabetes has proved itself most useful. In the hands of the distinguished American physiologist, Graham Lusk, his numerous students, and a number of others, this was developed into a powerful tool by means of which many difficult problems in intermediary metabolism were solved. The quantitative character of these studies enhances their value considerably. The author will again have occasion to refer to these investigations in discussing the intermediary metabolism of the proteins.[66]

It is important to bear in mind that the various forms of glycosuria described in the preceding paragraphs are usually transitory and, moreover, are not true forms of diabetes.

Pancreatic Diabetes.—One of the most fascinating chapters in the history of medicine began in the year 1889 when von Mering and Min-

[65] Verhandl. d. V. Cong. f. inn. Med., 1886, p. 185.

[66] An excellent review on the subject of Phlorhizin Diabetes has been written by T. P. Nash, Physiol. Reviews, **7**, 385 (1927).

kowski [67] removed the pancreas from dogs and discovered that this produced a condition similar to the diabetes observed in man. It had previously been suspected that the pancreas might be related in some way to this condition as lesions in this organ were occasionally found in severe cases. As in human diabetes, the most prominent symptoms observed in the experimental form of pancreatic diabetes were the appearance of sugar in the urine, thirst, voraciousness, emaciation, and death in coma.

Some years later, Minkowski [68] grafted a piece of pancreas under the skin of a dog and subsequently removed the pancreas of the animal, leaving the grafted piece which by this time had established circulation with the blood. It was found that in this way diabetes could be prevented or delayed for several months. Similar experiments were performed by Hédon.[69]

Among the numerous experiments which ultimately led to the adoption of the idea that the action of the pancreas in regulating carbohydrate metabolism was due to a hormone, may be mentioned that of Forshbach,[70] who made an anastomosis of the blood supply of two dogs and then removed the pancreas of one of the animals. Neither dog developed diabetes. Interpreted in the light of our present knowledge, the pancreas of the unoperated dog obviously supplied sufficient hormone to take care of the metabolism of both animals.

An equally ingenious experiment is that of Carlson,[71] who depancreatized a number of pregnant bitches and observed no glycosuria in these animals, presumably because of the functional activity of the pancreases of the fetuses. Lusk,[72] however, cites some experiments of Murlin, who found that such dogs have diabetic respiratory quotients (0.69). Murlin suggests that the absence of glucose from the urine may be due to the retention of carbohydrate by the fetuses.

The observations of Knowlton and Starling [73] were likewise suggestive of a hormone mechanism. These workers demonstrated an increase in the consumption of glucose by a heart taken from a depancreatized animal and perfused with blood from the same animal, when there was added to the blood an extract prepared from the pancreas.

The Islands of Langerhans.—Pancreatic tissue contains certain structures consisting of clumps of cells which differ in appearance and

[67] Arch. f. exp. Path. und Pharm., **26**, 371 (1890).

[68] *Ibid.*, Supplementary volume, 1908, p. 399.

[69] Arch. de Physiol., 65^{e} série, 269 (1894).

[70] Archiv. f. exp. Path. und Pharm., **60**. 131 (1909).

[71] J. Biol. Chem., **17**, 19 (1914).

[72] Lusk, The Science of Nutrition, 1917 edition, p. 453.

[73] J. Physiol., **45**, 146 (1912).

staining reactions from the remaining acinous or secreting epithelium. Because of their insular appearance, these structures have been named the islands of Langerhans.[74] Even before there was any certainty that the pancreatic hormone was formed in these islands, the belief grew up that this was the case. In fact, the hormone was named insulin by Sir Edward A. Schäfer in 1916 when its existence was still hypothetical. However, there was available some evidence to show that the lesions of the pancreas in severe diabetes were limited to the islets of Langerhans, but the value of these observations was more or less neutralized by contradictory observations. The degeneration of the acinous tissue of the pancreas, with relatively little injury to the insular cells, may be brought about by ligating the ducts from the pancreas. This was accomplished by several workers as early as 1900 and is of historical interest in connection with the important experiments of Banting to be described shortly.

Fifteen years ago the outstanding problem of diabetes was clearly defined. It was a question of isolating and of determining the chemical nature of the pancreatic hormone. The first part of the problem was solved in 1922 by a group of active students in Macleod's laboratory at the University of Toronto. Before speaking of the achievements of these men, it is fitting to make some mention of the efforts of other workers who attempted to isolate the pancreatic hormone. In this country, Scott,[75] Clark,[76] Kleiner,[77] Murlin and Kramer,[78] and others performed experiments which suggested the probable usefulness of pancreatic extract in relieving the symptoms of diabetes. One of the main obstacles in the way of obtaining active preparations appeared to be the destructive action of trypsin.

Guided by the work of Zuelzer,[79] W. G. MacCallum,[80] Bensley,[81] and others, Banting and Best [82] proceeded to prepare more active preparations in which the effect of trypsin would be eliminated. By ligating the pancreatic ducts in dogs they succeeded in producing considerably more degeneration of the acinous cells than of the insular tissue. After a

[74] The first to describe these structures was Langerhans (Inaugural Diss., Berlin, 1869).

[75] Am. J. Physiol., **29**, 306 (1911).

[76] Johns Hopkins Hosp. Rept., **18**, 229 (1917).

[77] J. Biol. Chem., **40**, 153 (1919).

[78] J. Biol. Chem., **15**, 365 (1913); **27**, 481, 517 (1916).

[79] Z. exper. Path. u. Therap., **5**, 307 (1908–09).

[80] Johns Hopkins Hosp. Bull., **20**, 265 (1909).

[81] Am. J. Anat., **12**, 297 (1911); Harvey Lectures, 1914–15, p. 250.

[82] Am. J. Physiol., **59**, 479 (1922); J. Lab. and Clin. Med., **7**, 251 (1922); **8**, 464 (1922). (Numerous other papers.)

few weeks the dogs were depancreatized. Extracts prepared from these pancreases, when injected subcutaneously or intravenously into normal and diabetic animals, proved to be very effective in causing a reduction of the blood sugar and in otherwise relieving the symptoms of diabetes. At about this time, Collip joined Banting and Best in their work and in a very short time developed methods for the preparation of extracts suitable for use in human diabetes. Thus was inaugurated a new era in the treatment of diabetes.

Insulin.—It is beyond the scope of this book to review in detail the huge amount of work that has been done on insulin since its discovery by Banting and his associates; nor is it possible to enter here into a discussion of the clinical aspects of the problem. A review of the subject has been prepared by J. J. R. Macleod,[83] and further information may be found in the current medical and biochemical literature. Nevertheless, some of the more important features of the problem will be considered briefly.

Source and Preparation.—Insulin is widely distributed in both vertebrates and invertebrates. The main source at present is the pancreas of cattle. Fetal pancreases, lacking in functional acinous tissue, yield active preparations of insulin. Collip's [84] method for the preparation of insulin consists in fractional precipitation with alcohol. Various modifications have been proposed, among which may be mentioned that of Doisy, Somogyi, and Shaffer,[85] which consists in the further purification of insulin by precipitation at the isoelectric point (*p*H 5.0–6.0). Another method is that devised by Dudley.[86] It consists in precipitating the insulin from solution with picric acid as the picrate. It is subsequently converted to the hydrochloride and washed free from fat and certain other impurities with acetone and ether in which insulin hydrochloride is insoluble. Dodds and Dickens [87] have modified Dudley's procedure. According to their method the finely minced pancreas is mixed with picric acid. This combines with the insulin to form the picrate, which is extracted with acetone. The extract is evaporated and the residue again extracted with ether to remove the fat and picric acid. The picrate is then converted to the hydrochloride and further purified as in Dudley's method.

Collip [88] has prepared insulin from clams and other invertebrates,

[83] Physiol. Reviews, **4**, 21 (1924); Carbohydrate Metabolism and Insulin. Longmans, Green & Co., 1926.

[84] Trans. Roy. Soc. Canada, **16**, 28 (1922).

[85] J. Biol. Chem., **55**, Proc. xxxi (1923).

[86] Biochem. J., **17**, 376 (1923).

[87] Brit. J. Exp. Path., **5**, 115 (1924).

[88] J. Biol. Chem., **56**, 513 (1923); **57**, 65 (1923).

in which its presence seems to be associated with that of glycogen. Oatmeal, onions, and other plant substances contain an insulin-like constituent which differs in behavior from the insulin of animal sources in that its action in lowering the blood sugar is not immediate but is delayed for twelve hours or more. This is true, likewise, of the insulin-like substance of yeast. To distinguish this principle from insulin, Collip has named it " glucokinin."

It is reported that certain solanaceous fruits grown in Siam are used there successfully in the treatment of diabetes.[89]

Chemical Properties of Insulin.—The first work on insulin indicated that it might be a protein-like substance. Banting and Best[90] determined that insulin is readily destroyed by trypsin, an observation confirmed by Dudley,[91] who showed, moreover, that pepsin produces the same effect. This behavior explains, no doubt, the ineffectiveness of insulin when given by mouth. Equally important were the observations of Doisy, Somogyi and Shaffer,[85] whose method for preparing insulin has been mentioned, that insulin has an isoelectric point at approximately *p*H 5.0. Their purified samples gave many of the reactions characteristic of proteins. Shonle and Waldo,[92] on the basis of their observations, suggested that insulin more nearly resembles a proteose. Scott[93] made a study of the distribution of nitrogen in insulin and reported the presence of arginine, histidine, lysine, cystine, tryptophane, and tyrosine. He found, likewise, that formaldehyde and nitrous acid greatly reduce the activity of insulin and that carbon bisulfide and benzoyl chloride completely inactivate it. While these results established a resemblance of insulin to protein, the evidence was far from being conclusive. There was the possibility, for example, that the material giving the tests was itself inert and that the active principle of the pancreas was adsorbed to it.

Important advances in our knowledge of the chemistry of insulin have emanated from the laboratory of J. J. Abel[94] at Johns Hopkins University, where methods for preparing highly purified, optically active, crystalline insulin were first developed. Crystallization

[89] H. M. Smith, Science, **66**, 619 (1927).

[90] J. Lab. Clin. Med., **7**, 251 (1922).

[91] Biochem. J., **17**, 376 (1923).

[92] J. Biol. Chem., **58**, 731 (1924).

[93] J. Biol. Chem., **63**, 641 (1925); **65**, 601 (1925).

[94] Abel, J. J., Proc. Nat. Acad. Sci., **12**, 132 (1926); Abel, J. J., Geiling, E. M. K., Rouiller, C. A., Bell, F. K., and Wintersteiner, O., J. Pharmacol. Exp. Therap., **31**, 65 (1927); Du Vigneaud, V., Jensen, H., and Wintersteiner, O., *ibid.* **32**, 367, 387 (1927–28); Du Vigneaud, V., Geiling, E. M. K., and Eddy, C. A., *ibid.*, **33**, 497 (1928); Jensen, H., Wintersteiner, O., and Geiling, E. M. K., *ibid.*, **36**, 115 (1929).

occurs at a *p*H of 5.55 to 5.65, the isoelectric point of insulin. Repeated recrystallization of crude preparations of beef insulin (and recently of fish insulin, but not of pig insulin) using widely different methods have yielded a uniform product, both as to crystalline structure and physiological potency.[95] Crystalline insulin gives positive tests for protein (biuret, Millon, etc.). Tests for tryptophane, however, are negative. According to analyses, on the basis that the molecule contains but one atom of sulfur, the formula is $C_{45}H_{69}O_{14}N_{11}S$. The work of Du Vigneaud,[96] however, indicates that insulin contains a disulfide linkage and that therefore the formula should be at least twice that given by Abel. Hydrolysis of pure crystalline insulin yields the following amino acids: cystine, tyrosine, arginine, histidine, leucine and lysine. The presence of a sulfur-containing compound other than cystine has also been detected.

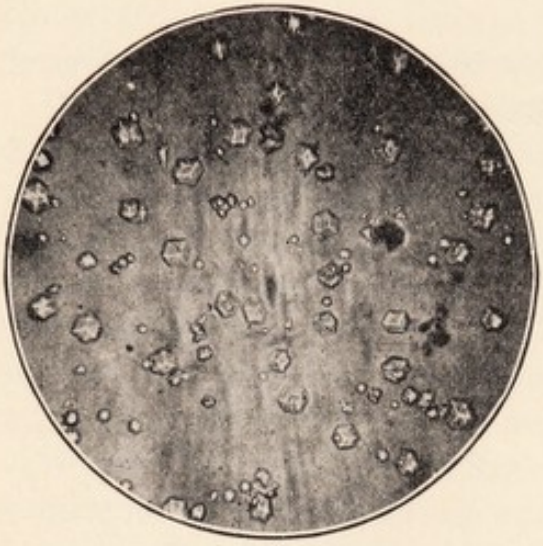


FIG. 40.—Crystalline Insulin.
(Reproduced from a micro-photograph kindly furnished by Professor John J. Abel.)

Insulin is stable in neutral and weak acid solutions, but is rapidly destroyed in the presence of alkali, the change being associated with the splitting off of sulfur.

It is possible that future work will necessitate a revision of the empirical formula that has been assigned to insulin ($C_{90}H_{138}O_{28}N_{22}S_2$, or $C_{90}H_{138}O_{28}N_{22}S_2 \cdot 6H_2O$ for the air-dried crystals).

Action.—The administration of insulin by mouth is without influence. Insulin is effective when given subcutaneously, but produces its maximum effect when injected intravenously. The introduction of insulin into the duodenum, when the stomach is not digesting, is said to diminish hyperglycemia and glycosuria in diabetic animals. Intravenous injection of insulin is followed immediately by a rapid fall in blood sugar; if a sufficient quantity is given, marked hypoglycemia

[95] See also Harington, C. R., and Scott, D. A., Biochem. J., **23,** 384 (1929). These investigators found certain commercial preparations of amorphous insulin with a potency almost as high as that of crystalline insulin. This would indicate that the preparation of the crystalline product is not a matter of isolating a highly active substance from a crude mixture, but rather a process analogous to the crystallization of a protein.

[96] Du Vigneaud, V., J. Biol. Chem., **75,** 393 (1927).

develops, and at a sugar level of 0.04–0.045 the rabbit develops convulsions. If these symptoms are not relieved at once the animal dies. The amount of reserve glycogen is a factor, and where this is present in abundance, convulsions do not develop quite as readily as in poorly nourished animals. Insulin convulsions may be relieved by injecting glucose, and to a less extent by mannose, galactose, levulose and maltose, but not by the pentoses xylose and arabinose nor by the disaccharides sucrose and lactose.

In the case of the diabetic individual, insulin relieves at once the symptoms of hyperglycemia, glycosuria, acetonuria, and acidosis. There is a marked improvement in the utilization of carbohydrates, as determined by an elevation of the respiratory quotient, and in the deposition of glycogen in the liver. With these changes there is also an improvement in fat metabolism and in the conservation of the tissue proteins.

To insure uniform potency of commercial preparations and to avoid the grave dangers of overdosage, great care is exercised in the standardization of insulin. It was recognized early that the rabbit is a suitable animal (mice are now also used) for purposes of insulin assay, and, therefore, at the first meeting of the Standardization Committee of the League of Nations, it was decided provisionally to define a unit of insulin as one-third of the amount required to lower the blood sugar of a normal rabbit, weighing 2 kg. and previously fasted for twenty-four hours, to the convulsive level (0.045 per cent) within three hours. At the present time the Health Committee of the League keeps under its auspices a preparation of insulin hydrochloride which serves as an international standard. One milligram of this standard is equivalent to 8 units. Accordingly, the definition of the international unit is the quantity (of a given preparation) which produces an effect on carbohydrate metabolism equal to that of one-eighth of a milligram of the standard preparation of insulin hydrochloride. By this definition, the mode of assay is not prescribed. It may be mentioned here that 1 mg. of crystalline insulin (as prepared by Abel and his associates) is equivalent to approximately twenty-four international units.

For a number of years before the advent of insulin, the method of treatment of diabetes which seemed to give the best results was the Allen method of controlling the diet. This idea was based upon the obvious fact that, at best, there was no point in giving the diabetic carbohydrate and other food which he was unable to utilize and was therefore forced to excrete. Periods of starvation and strict regulation of the diet therefore constituted, until several years ago, the most effective method of combating the disease. The method of treatment employed

by Allen involved wherever necessary a preliminary period of starvation lasting sufficiently long to render the urine of the diabetic free from sugar and acetone bodies. From this point on, by carefully controlling the diet, it was often possible to keep the patient free from these symptoms for long periods.

However, such a procedure was practically futile in the case of children. A few capable clinicians were in a measure able to cope with the situation and even succeeded in prolonging for a few years the lives of some of their young patients; but, as the method of treatment consisted in the strict limitation of the food intake, the growth of the children was limited. The situation is now improved; normal growth and development have been reported in many diabetic children treated with insulin.[97]

Tuberculosis is a frequent complication in diabetes. The difficulties formerly encountered in treating tuberculous diabetics may be readily imagined. To control the diabetes, periods of starvation and subsequent limitation of the diet were thought imperative, whereas to control the effects of tuberculosis, an ample diet was called for.

The mechanism involved in the action of insulin is still obscure, although several attractive hypotheses are at present under discussion.

[97] F. N. Allan and R. M. Wilder have recently compared the mortality of diabetic children before and after the discovery of insulin. (J. Am. Med. Assoc., **94**, 147 (1930).) Of a group of 30 children observed at the Mayo clinic during the period Oct. 1, 1919 to Oct. 1, 1922, the deaths numbered 22, or 73.3 per cent. During the period Oct. 1, 1922 to Oct. 1, 1928, 167 patients were studied, including the eight who survived the pre-insulin period. Of these patients, 164 have been traced and a total of 17 deaths recorded. The per cent mortality was therefore only 10.4. For the years 1925–1928, the mortality rate averaged only about 4 per cent. Of the few deaths that occurred, several might have been avoided if the patients had continued their dietary restrictions and insulin injections after returning to their homes.

CHAPTER XI

INTERMEDIARY METABOLISM OF FAT

THE changes which fats undergo while in transport to the tissues constitute the starting point in our discussion of intermediary fat metabolism. A number of observers, notably Bloor,[1] have found an increase in the concentration of lecithin in the blood, and especially in the corpuscles, during absorption. The fatty acids increase in similar fashion, and Knudson [2] and others have observed that cholesterol has a like tendency to increase during absorption. The source of the extra cholesterol is not clearly indicated. If it had its origin in the food or in the bile, then, as Leathes [3] points out, the association of cholesterol with the fat would be accidental rather than essential to the process of absorption, or to the transfer of fat from the blood to the tissues. Bloor has shown that in persistent lipemia both lecithin and cholesterol are increased along with the fat, the fat increasing first, the lecithin next, and the cholesterol last. As the lipemia subsides and the excess of fat disappears, the lecithin and cholesterol persist for some time, the latter being the last to return to normal levels.

A certain amount of fat is probably carried to the tissues as such. The remainder is perhaps transported in combination as cholesterol esters and as phospholipids. What proportion of the fatty acids is converted in this way, we do not know; nor have we any clear understanding of the source of the phosphoric acid and nitrogenous bases which enter into the synthesis of the phosphatides in question. It is interesting to point out, however, that phosphoric acid seems to play an important rôle in the initial stages of both carbohydrate and fat metabolism.

Accepting for the moment the assumption that a certain amount of the fat is carried in the blood as lecithin and cholesterol esters, we may now trace it to the liver where the fatty acids undergo desaturation or dehydrogenation as the next step in metabolism. From the liver these highly unsaturated phosphatides are transported to the tissues where they are converted into simpler substances, as we shall see later. Another

[1] W. R. Bloor, Fat Transport in the Animal Body, Physiol. Reviews, **2**, 92 (1922).
[2] J. Biol. Chem., **32**, 337 (1917).
[3] J. B. Leathes and H. S. Raper, The Fats, 1925 edition, 150.

portion of the fat may, however, follow a different path; it may be transported to certain other tissues for storage. The deposition of fat is believed to involve a reconversion of lecithin into the simpler triglycerides of the fatty acids. Conversely, when storage or depot fat is needed for fuel or energy, it is said to be changed partly into lecithin and cephalin before leaving the tissues and entering the blood.

Less is known regarding the part that cholesterol plays in fat metabolism. Conjugated with fatty acids, it may aid in their transportation. In consideration of the antagonistic action of lecithin and cholesterol, it has been suggested that the increase of cholesterol in the blood, following the accumulation of lecithin, may be for the purpose of counteracting the injurious effects of the latter compound. Obviously, we are still very much in the dark concerning the initial stages of the intermediary metabolism of fat.

Sources.—The fat in the body arises from the fat of the diet and from glucose. In the preceding chapter the conversion of glucose into fat was considered. Certain amino acids are converted into glucose and in this way may furnish an additional source of fat. Thus, a certain amount of fat may arise indirectly from the protein of the diet.

Anabolic Products of Fat Metabolism.—As in the case of the other foodstuffs, the fats have a dual fate in metabolism. A portion is used in the synthesis of certain essential constituents of the tissues (myelin of the nervous system, etc.). The phosphatides and related compounds, the lipids of the sebaceous glands, and certain fat-protein compounds or lipoproteins are products of fat anabolism. The remainder of the fat is used as fuel. It may be used directly or, if the amount present is much in excess of immediate energy requirements, it may be stored in various parts of the body for future use.

Owing to their close chemical relationship, the origin of the phosphatides and cerebrosides from fats is assumed. These substances are essential constituents of every cell and are believed to determine the permeability of cells to anesthetics and to other lipid-soluble substances. The relation of lecithin to tissue oxidation was mentioned in an earlier chapter. There are, no doubt, many other properties concerning which we know very little. The part played by fats and fat-like substances in the life of the cell has recently been reviewed in a very thorough manner by J. B. Leathes.[4]

The sebaceous lipids enable certain animals, particularly the fur- and feather-bearing animals, to shed water. The secretions on the skin also serve to diminish heat radiation. Sebum is a mixture of a number

[4] J. B. Leathes and H. S. Raper, The Fats, Chapter X, 1925 edition; also Leathes in *Lancet*, pp. 803, 853, 957, 1019 (1925).

of fatty substances and is secreted by the sebaceous glands of the skin. A similar substance is cerumen, which is formed in the sebaceous and sweat glands of the cartilaginous part of the outer ear.

Lipoproteins are present in small amounts in the blood as well as in many tissues. Taylor [5] states that if a gland like the kidney which is rich in lipoproteins be completely extracted with fat solvents and then digested with trypsin, a subsequent extraction will yield a goodly amount of fatty substance. Some of this will be found to consist of phosphatides and sterols and the remainder of neutral fat.

There is no satisfactory evidence for the formation of cholesterol and other sterols from fat, the view generally held being that cholesterol is formed in the body from the sterols of the diet. However, Channon [6] and Randles and Knudson [7] have reported the synthesis of cholesterol in rats maintained on a diet poor in, or free from, sterols.

Storage of Fat.—The storage of fat may occur in many regions of the body, but especially in the superficial fascia under the skin where it may be present as a layer an inch or more in thickness. This layer of fat is called the panniculus adiposus. Large amounts of fat occur, likewise, in the intermuscular connective tissue, omentum and mesentery and in the internal organs, such as the lungs, heart, kidneys and liver. The fat in the adipose tissue of any given species is normally characteristic of that species, but the deposition of fat foreign to an animal may occur under certain conditions. A classical experiment showing this is that of Lebedev,[8] who starved two dogs until their reserve fat was nearly used up. One dog was then fed mutton tallow and the other linseed oil, with the result that the fat deposited in the adipose tissue of the first dog resembled mutton fat, whereas the fat laid down in the second animal was liquid at 0° C. and contained larger amounts of unsaturated fatty acids than is normal for dog fat. In a similar experiment, Munk [9] fed a previously starved dog rape-seed oil and was able to demonstrate the deposition of the triglyceride of erucic acid ($C_{22}H_{42}O_2$). Eckstein [10] has also shown that the nature of the fat deposited is influenced by the fats of the diet and he has been able to demonstrate the deposition of the myristyl radical both in the hides and carcasses of rats fed with myristic acid. However, the fat deposited on diets containing tributyrin or tricaproin did not contain the butyryl or caproyl radical, although it differed from the fat synthesized from fat-free precursors,

[5] A. E. Taylor, Digestion and Metabolism, 1912, p. 347.

[6] Biochem. J., **19**, 424 (1925).

[7] J. Biol. Chem., **66**, 459 (1925).

[8] Pflüger's Arch., **31**, 11 (1883).

[9] Arch. path. Anat. u. Physiol., **95**, 407 (1884).

[10] J. Biol. Chem., **81**, 613 (1929); **84**, 353 (1929).

namely protein and carbohydrate. The difference was in the degree of unsaturation rather than in the saponification numbers, as might be supposed. On a practically fat-free diet Eckstein's rats deposited fat having on an average an iodine number of 68 and a saponification number of 194, whereas on the tricaproin diet these values were 59 and 191, respectively. Brominized and iodized fats, obtained by treating fats containing unsaturated fatty acids with iodine or bromine, have been fed previously starved animals and later recovered in their bodies.

These facts have an important bearing in relation to stock-raising. In certain parts of this country, the diet of hogs is composed largely of meal prepared from cotton seed or peanuts. This food may so modify the consistency of the fat laid down by these animals as to affect the marketability of the lard and other products. This subject has been studied experimentally in hogs by Ellis and Isbell [11] and in rats by Anderson and Mendel.[12] Ellis and Isbell were able to modify the composition of the pork fat very markedly by varying the diet. For example, the lard from peanut-fed hogs contained similar percentages of the various fatty acids as are present in peanut oil. In Anderson and Mendel's experiments, the diet was very carefully controlled and only the fat, or other nutrients available for fat formation, were varied. They found that when soy bean oil, cottonseed oil or peanut oil was fed, the resulting body fat resembled the food fat. When butter fat or cocoanut oil were fed, the deposited fat differed from the food fat, having a somewhat higher iodine number. These results agree with the later observations of Eckstein, to which reference has been made, that the lower fatty acids are built up into higher fatty acids, and these are synthesized into a fat which is softer than the hard body fat normally formed from carbohydrates (corn starch, etc.) or proteins (casein, cottonseed globulin, etc.). Anderson and Mendel point out that even after a particular type of " soft " body fat has been developed, it is possible to alter its chemical make-up by changing to a " hardening " diet rich in carbohydrate. This process is apparently due first to the gradual depletion of the soft fat and the subsequent deposition in its place of the harder variety. This process is relatively slow, but it may be materially hastened if prior to changing the diet the fat reserves are partly used up by a short period of fasting. Over twenty years ago, Henriques and Hansen [13] made similar though not as extensive observations on hogs.

[11] J. Biol. Chem., **69**, 219, 239 (1926); see also Ellis, N. R., and Hankins, O. G., *ibid.*, **66**, 101 (1925).

[12] J. Biol. Chem., **76**, 729 (1928).

[13] Skand. Arch. f. Physiol., **11**, 151 (1901); also described in monograph by Leathes and Raper, p. 100.

In one of their experiments, they fed a hog on barley and another on maize. The fat laid down in the connective tissue of the former had an iodine number of 57.7 and a melting-point of 27.4°, whereas the fat of the corn-fed hog had an iodine value of 75.6 and a melting-point of 23°. Despite the profound effect which diet has on the nature of the fat deposited, it is nevertheless true that the fat in any given species is fairly constant in composition. This is due in large part, no doubt, to the similarity in the type of food consumed by animals of the same species.

The composition of adipose-tissue fat varies in different parts of the body. In their experiments on hogs, Henriques and Hansen showed that subcutaneous fat has a higher iodine number and a lower melting-point than perirenal fat, which in turn has a higher iodine number and a lower melting-point than omental fat. These differences may be due to temperature, for these workers have shown that the temperature of subcutaneous tissue in the hog, 1 cm. from the surface, is 33.7°; at 2 cm., it is 34.8°, and at 4 cm., 39° C. The composition of fat has been modified by altering the temperature of the environment. In one experiment, Henriques and Hansen kept three pigs from the same litter at different temperatures; the first was kept at 30–35° C. and the second at 0°; the third was also kept at 0° but was covered and kept warm with a sheepskin coat. After two months, the pigs were killed and the fat analyzed. It was found that the fat of the pig kept at 0° C. without any cover showed the highest iodine number (72.3). The pig kept at 30–35° had deposited fat having an iodine number of 69.4, whereas the clothed pig, kept at 0° C., had fat which showed the lowest iodine number of the series (67.0). There is, as yet, no satisfactory explanation which accounts for these variations. One can readily see, however, the advantage of having a relatively solid fat, of high melting-point, in the region of the back of some animals, which is so often exposed to the relatively high temperatures of the sun's rays.

It is important to bear in mind that not all the fat in the body has the same physiological significance. An apparently clear-cut and sound distinction has been made by a number of workers (Mayer, Schaeffer,[14] Terroine,[15] and others) between the so-called *élément constant* and *élément variable*. The tissues of animals that have starved to death still contain a certain amount of fat, which seems to be fairly constant for any given species. In the mouse, for example, about 23 per cent of the dry weight of the animal consists of fatty acids, whereas in the chicken the fatty acids constitute about 25 per cent of the dry weight. It is

[14] J. de Physiol. et Path. gen., **15**, 510, 535, 773, 984 (1913); **16**, 1, 23 (1914).

[15] E. F. Terroine, J. de Physiol. et Path. gen., **16**, 384, 212 (1914); Physiologie des Substances Grasses, Paris (1919).

believed that a certain amount of fat is an essential component of protoplasm, and that this cannot be reduced without causing death. This is the *élément constant.* On the other hand, the reserve fat is variable in amount, depending on the state of nutrition and other factors. The fatty acids in reserve or storage fat are present in combination with glycerol as neutral fat. Such fat, because it varies in amount, has been called the *élément variable* by the group of workers mentioned above.

It appears that the kidney, spleen, lung and heart contain no *élément variable* or storage fat but only the *élément constant,* or indispensable fat. This is demonstrated by comparing the composition of these organs in overfed, normal, and starved animals. Leathes cites data for the kidney, in which 11.1, 11.9, and 13.4 per cent of fatty acids were found in overfed, normally fed, and starved animals, respectively. If anything, these data, instead of showing that fat is stored on a high-fat diet, indicate that there is a greater migration of fat to this organ during starvation than normally. In muscle, the figures obtained in the conditions just mentioned were 17.6, 11.3, and 4.6 per cent, showing unmistakably the possibility of the storage of fat in muscle tissue. Finally, the values given for the liver are 12.9, 10.5, and 11.3 per cent. Thus, the liver does not seem to play as important a part in fat storage as is often supposed. Occasionally, the amount of fat in the liver may increase considerably, usually as a result of sudden fat mobilization, but even then the storage of fat in this organ is believed to be transitory.

Interchange of Fat in the Body.—Although the energy requirements of the liver are much less than those of the heart or kidney, the liver takes up more fat than either of these organs. The liver, however, does not mobilize this fat for its own use. As we have just seen, fat is not stored in the liver, except temporarily; nor is it completely oxidized in this organ. The part which the liver plays in fat metabolism is an exceedingly important one and consists in converting the fatty acids that it takes up into more highly unsaturated fatty acids, by a process of dehydrogenation. The fats are thus rendered more useful to the other tissues, because the ease with which fatty-acid chains are broken up and oxidized is presumably determined by the number of double linkages. Moreover, in the repair of protoplasm, highly unsaturated fatty acids are required.

A partial insight into the sequence of events during the earlier stages of fat metabolism may be gained by comparing the composition of the lipids of various tissues. Storage or depot fat, which is relatively resistant to oxidation, consists almost entirely of the triglycerides of fatty acids, only a small proportion of which are highly unsaturated. The amount of phospholipid is small and the iodine number varies between

60 and 70, being usually about 65.[16] In contrast to this is the composition of the lipids present in the liver. Bloor,[17] who has made a detailed study of the tissues of the beef, gives 87 for the average iodine number of the mixed fatty acids of the fat (acetone-soluble) fraction of the liver. The mixed fatty acids of the cephalin and lecithin fractions have higher iodine numbers, these being 119 and 108, respectively. Somewhat higher values are given by Theis,[18] whose results show a constant relation of phospholipid to neutral fat in the liver, as indicated by the following figures:

	Phospholipid, Per Cent	Neutral Fat, Per Cent
Beef liver	55	45
Rabbit liver	55–65	35–45
Human liver	60	40

However, if the liver is damaged or diseased, this relation is altered; the proportion of phospholipid is greatly diminished apparently because of failure to convert neutral fat to phospholipid. In liver injury the desaturation of the fatty acids is not carried as far as normally, for according to the data given by Theis the iodine number of the various lipid fractions is markedly diminished.

This evidence would indicate strongly that the liver takes part in the conversion of fat to phospholipid and in the desaturation of the fatty acids. In further support of the desaturation hypothesis may be cited a remarkable experiment of Leathes and Wedell.[19] These workers fed certain animals (rats, cats) highly unsaturated fats, such as cod-liver oil, and showed that the iodine number of the fatty acids isolated from the livers was higher than of the fatty acids that had been fed and still higher than the iodine number of the fatty acids normally found in the livers of these animals. An equally interesting experiment is that of Raper,[20] who found that if cocoanut oil is fed to an animal the volatile fatty acids subsequently recovered from the liver were more unsaturated than the volatile fatty acids of the original oil.

As to the degree of unsaturation of the fatty acids of the liver lipids,

[16] Eckstein, H. C., J. Biol. Chem., **64**, 797 (1925).

[17] J. Biol. Chem., **56**, 711 (1923); **59**, 543 (1924); **68**, 33 (1926); **72**, 327 (1927); **80**, 443 (1928).

[18] J. Biol. Chem., **76**, 107 (1928); **77**, 75 (1928); **82**, 327 (1929).

[19] Cited by Leathes and Raper, The Fats, pp. 187, 193.

[20] J. Biol. Chem., **14**, 117 (1913).

it would seem from the work of Brown [21] that arachidonic acid, $C_{20}H_{32}O_2$ (4 double bonds), is probably the most highly unsaturated fatty acid present in any significant amount.

In comparing various muscles of the beef, Bloor found that the more active the muscle, the higher its per cent content of phospholipid and unsaponifiable substance. A similar relation can be made out for different organs (kidney, pancreas, lung, brain), for according to Bloor, the arrangement of the organs in the order of their phospholipid content gives a series which represents also the order of their functional activity.

Oxidation of Fatty Acids.—Normally, the fatty acids are completely oxidized to carbon dioxide and water. Of the intermediate steps in the process, little was known until Knoop [22] published the results of his investigations in 1904. Earlier attempts had been made to trace the fate of fatty acids in metabolism, by feeding the lower members to animals and then examining the urine for end-products. These experiments were unsuccessful because the fatty acids were either completely oxidized or partly excreted unchanged. Knoop, however, conceived the idea of feeding phenyl derivatives of the lower fatty acids.

Benzoic acid is not oxidized in the body, and when fed, is excreted in the urine in combination, partly with glycine as hippuric acid. The reaction is represented by the following equation:

$$C_6H_5 \cdot COOH + CH_2NH_2 \cdot COOH = C_6H_5CONHCH_2COOH + H_2O$$

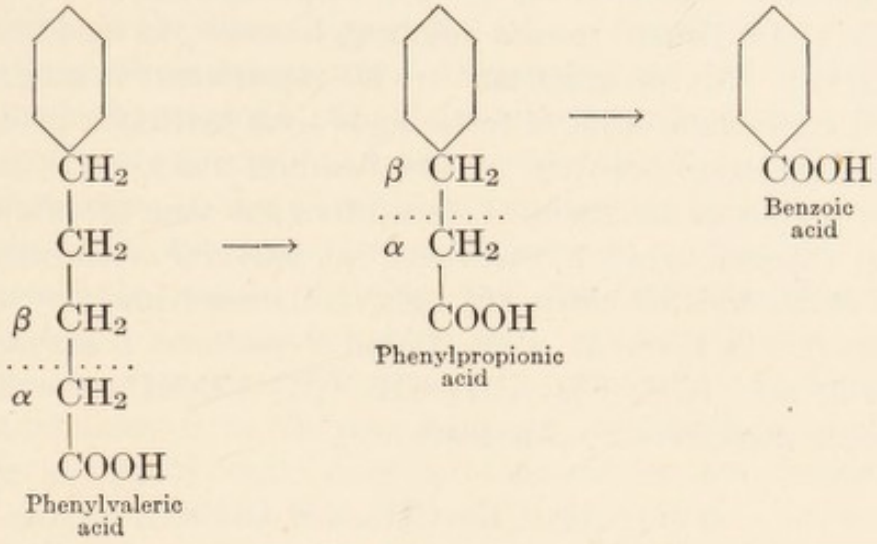
Phenylacetic acid is likewise resistant to oxidation and is detoxified to form phenaceturic acid, in which form it is excreted. In man, according to Thierfelder and Sherwin,[23] phenylacetic acid is conjugated to form phenacetyl-glutamine. The formation of phenaceturic acid may be written:

$$C_6H_5CH_2COOH + CH_2NH_2 \cdot COOH$$
$$= C_6H_5CH_2CO \cdot NH \cdot CH_2COOH + H_2O$$

However, on feeding phenylpropionic acid, Knoop found hippuric acid in the urine, showing that two carbon atoms of the side chain had been removed by oxidation. Phenylbutyric acid yielded phenaceturic acid, and phenylvaleric acid gave rise in the body to benzoic acid, which was in turn converted into hippuric acid and excreted. Knoop

[21] *Ibid.*, **80**, 455 (1928).
[22] Beiträge Z. Chem. Physiol. u. Path., **6**, 150 (1904).
[23] Ber., **47**, 2630 (1914).

therefore concluded that the oxidation of a fatty-acid chain occurs at the β-carbon atom. The oxidation of phenylvaleric acid may be represented as follows:



Other evidence is available in support of Knoop's hypothesis of β-oxidation. In diabetes, the oxidation of fat is incomplete, with the result that products of incomplete oxidation are found in the urine. These are β-hydroxybutyric acid and acetoacetic acid. In normal metabolism, these are further oxidized to carbon dioxide and water; but in diabetes, or more correctly, in the absence of glucose metabolism, they are partly converted into acetone, the remainder being excreted unchanged. Collectively, they are called the acetone bodies. A better term would be " acetone substances " or " acetone compounds." Both hydroxybutyric and acetoacetic acids are excreted partly as salts. This accounts for the loss of fixed base from the body when these substances are formed in metabolism. The formation of acetone bodies cannot be explained otherwise than by assuming that the long-chain fatty acids, of an even number of carbon atoms, are oxidized successively at the β-carbon atom.

Ringer [24] has shown that when propionic acid is given to phlorhizinized dogs, it is completely converted into glucose. When valeric acid is given, oxidation apparently occurs at the β-carbon atom, yielding two fragments, one of which is propionic acid. This in turn is converted into glucose, so that three-fifths of the carbon of valeric acid may be accounted for in the extra sugar of the urine.

Further evidence in support of Knoop's theory is to be found in the work of Dakin, who, after administering phenylpropionic acid, isolated in the urine not only hippuric acid but also a number of other

[24] J. Biol. Chem., **12**, 511 (1912); **14**, 43 (1913).

substances, including β-phenyl-β-hydroxypropionic acid, benzoylacetic acid and acetophenone, these being obviously intermediate products of the oxidation of phenylpropionic acid and analogous to the acetone bodies. Dakin [25] extended his studies to phenyl derivatives of other fatty acids and obtained results pointing, likewise, to oxidation at the β-carbon atom. No less important are his experiments *in vitro*, in which he treated ammonium salts of fatty acids with hydrogen peroxide and found that oxidation occurred at the β-carbon atom, with the partial formation of ketones having one carbon atom less than the original acids.

Knoop's theory, while it postulates the removal of successive pairs of carbon atoms does not throw any light on the mechanism by which it is accomplished. In a recent study Dakin [26] perfused a surviving liver with caproic acid ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$) as well as with the following derivatives: αβ-hexenic acid

$$(CH_3 \cdot CH_2 \cdot CH_2 \cdot CH{=}CH \cdot COOH),$$

β-hydroxycaproic acid ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CHOH \cdot CH_2 \cdot COOH$), and β-ketocaproic (butyrylacetic) acid ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CO \cdot CH_2 \cdot COOH$).

The results with caproic acid showed a pronounced formation of acetoacetic acid and β-hydroxybutyric acid, but the intermediate formation of β-hydroxycaproic acid could not be definitely established.

Perfusion with αβ-hexenic acid resulted in the formation of acetoacetic acid and probably β-hydroxybutyric acid. There were also indications for the intermediate formation of some β-hydroxycaproic acid, but not of β-ketocaproic acid.

Perfusion with β-hydroxycaproic acid gave rise to both acetoacetic acid and β-hydroxybutyric acid, with indications of the possible intermediate formation of β-ketocaproic acid.

Perfusion with β-ketocaproic acid resulted in the production of considerable amounts of acetoacetic and β-hydroxybutyric acids, with indications of the reduction of the β-ketocaproic acid to β-hydroxycaproic acid.

Dakin states that no definite answer can be given to the question whether an unsaturated, β-hydroxy, or β-ketonic acid is formed first in the oxidation of caproic acid. He suggests the probability that all these acids are in a readily shifting equilibrium with each other and are easily interconvertible.

Quick [27] has made a careful study of the metabolism of the phenyl

[25] J. Biol. Chem., **4**, 77, 227, 419 (1908); **5**, 173, 303 (1908); **6**, 203, 221 (1909).
[26] J. Biol. Chem., **56**, 43 (1923).
[27] J. Biol. Chem., **77**, 581, **80**, 515 (1928).

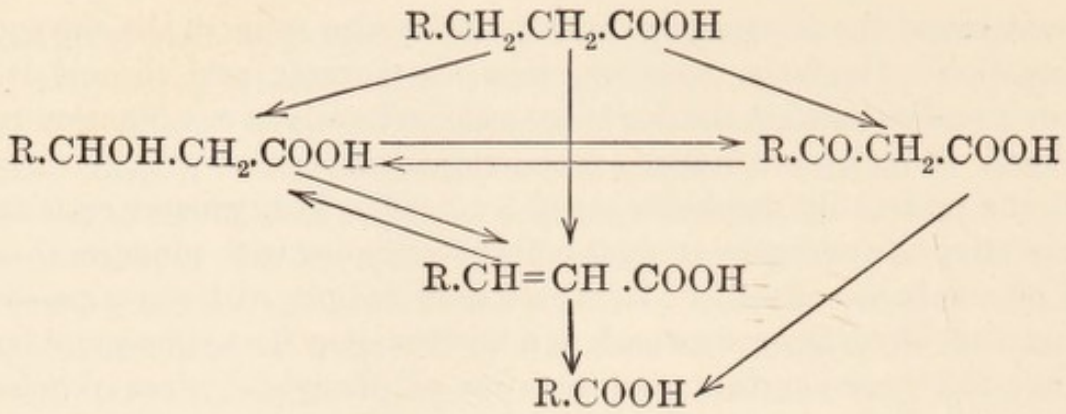
derivatives of the fatty acids and his results also support the theory of β-oxidation. He finds, however, that the benzoic acid formed from phenylpropionic acid in the dog is not excreted solely in combination with glycine. In fact, a much larger proportion is conjugated with glucuronic acid, the ratio in the dog being about 3 : 1. Similarly phenylacetic acid, irrespective of its source in metabolism, combines with glucuronic acid and glycine in the ratio of 1 : 2. Quick does not regard the intermediate formation of hydroxy compounds as a likely step in the oxidation of fatty acids. For example, the administration of phenyl-β-hydroxypropionic acid results in the excretion of about 75 per cent, unchanged.

We cannot be certain therefore that the oxidation of butyric acid occurs by way of β-hydroxybutyric acid, acetoacetic acid, and acetic acid, in the order named. When either butyric acid or β-hydroxybutyric acid is administered to diabetic animals, aceotacetic acid is formed. Yet there is equally valid evidence that the reverse transformation, namely, that of acetoacetic acid to β-hydroxybutyric acid, may occur. This question may also be considered in the light of Wieland's theory of oxidation. We may assume dehydrogenation to be the first step in the oxidation of butyric acid, and hydration the second step. The third step is again dehydrogenation. The acetoacetic acid thus formed may then react with water to yield two molecules of acetic acid. Another possible decomposition is suggested by the work of Dakin, who treated sodium acetoacetate with hydrogen peroxide at room temperature and obtained acetic, glyoxylic, and formic acids and carbon dioxide. The reactions may be represented as follows:

$$\begin{array}{lcl} CH_3 & & CH_3 \\ | & & | \\ CO & & COOH \\ | & \rightarrow & \\ CH_2 & & CHO \\ | & & | \\ COOH & & COOH \end{array} \rightarrow H\cdot COOH + CO_2$$

There is perhaps a somewhat greater amount of evidence that either the unsaturated acids or the β-ketonic acids, rather than the β-hydroxy acids, are the initial products of the oxidation of fatty acids. Nevertheless, the following diagram (after Dakin)[28] is a fairly accurate representation of our present knowledge of the relations that have been considered thus far:

[28] H. D. Dakin, Oxidations and Reductions in the Animal Body, Longmans, Green & Co., 1922 edition, p. 42.



There is little doubt that β-oxidation is the predominant reaction of fatty acids in metabolism. The question, however, has been raised whether this is the only mode of oxidation. Clutterbuck and Raper [29] treated the ammonium salts of various fatty acids, from caproic to stearic, with hydrogen peroxide and obtained evidence of oxidation occurring at the α- and δ-carbon atoms. In a later study, Raper and Wayne [30] administered normal phenylpropionic, phenylbutyric, phenylvaleric and phenylcaproic acids to dogs, and the results of their analyses showed that the fatty acid side chains were oxidized in accordance with the theory of β-oxidation. Under the same conditions, phenylnonoic (9 carbon atoms in the side chain) and phenyldecoic (10 carbon atoms in side chain) acids yielded smaller amounts of benzoic acid and phenylacetic acid, respectively, than would be expected if there were quantitative β-oxidation of the side chain. They suggested therefore that, in addition to β-oxidation, some other mode of oxidation takes place. The nature of this additional mode of oxidation has not been determined.

The Utilization of Fat in the Production of Energy.—There can be no doubt of the utilization of fat by the animal body for the production of heat. The mere oxidation of the fatty acids to carbon dioxide and water would account for that. On the other hand, we are in complete darkness with regard to the mechanism involved in the conversion of fat into muscular work. In the first place, we may consider the evidence for the utilization of fat by muscles. This is afforded largely by the experiments of Zuntz,[31] Benedict, and Cathcart,[32] and of Krogh and Lindhard.[33] If muscular work is accomplished at the expense of

[29] Biochem. J., **19**, 385 (1925).

[30] *Ibid.*, **22**, 188 (1928).

[31] N. Zuntz, Die Quellen der Muskelkraft, Oppenheimer's Handbuch Biochem., **4**, p. 826.

[32] F. G. Benedict and E. P. Cathcart, Muscular Work, Carnegie Inst. Publ. (1913).

[33] Biochem. J., **14**, 290 (1920).

carbohydrates, the respiratory quotient is high, whereas, if fats are burned, the quotient is low. This will be considered later in greater detail; for the time being, it is sufficient to state that, by measuring the respiratory quotient of animals during muscular exercise, a fair idea may be had regarding the kind of material which is being burned. This is therefore one method of studying the problem in question.

The results of Zuntz and those of Benedict and Cathcart show that fats and carbohydrates are about equally well utilized in the production of muscular work. Krogh and Lindhard, on the contrary, were unable to account for about 11 per cent of the potential energy of fat. No satisfactory explanation has been offered to account for this discrepancy. Nevertheless, the fundamental fact remains that fat is utilized in muscular work.

Fat as a Source of Carbohydrate.—The conversion of glycerol into glucose is probable. In the diabetic animal this conversion has been demonstrated by Chambers and Deuel,[34] who administered glycerol to a phlorhizinized dog and recovered 97 to 98 per cent as extra glucose in the urine.

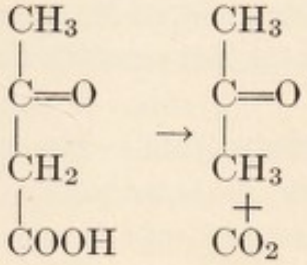
Whereas the conversion of carbohydrate into fatty acids is an established fact, most of the available information seems to point to the view that fatty acids are not convertible into carbohydrate. Nevertheless a certain amount of evidence has been offered in support of the fatty acid → glucose conversion. Chaikoff and Weber,[35] for example, injected epinephrine repeatedly into a depancreatized dog and observed an extra excretion of glucose in the urine which could not be accounted for as being derived from the glycogen stores of the liver, glycerol or tissue protein.

Low respiratory quotients, below 0.7, have been frequently reported for animals during hibernation. It is stated that during this period of winter sleep the glycogen does not disappear completely and that the nitrogen excretion is too low to make it seem probable that protein is the exclusive source of carbohydrate. The only remaining source of the energy requirements for this period would appear to be the fat reserves. Many of these observations have been criticized on the ground of the faulty methods employed, so that in summarizing the available data Lusk[36] and Macleod[37] essentially agree, the former confirming his view that there is no evidence for the transformation of fatty acids into carbohydrate, the latter admitting that "neither in the starving, depan-

[34] J. Biol. Chem., **65**, 21 (1925).
[35] *Ibid.*, **76**, 813 (1928).
[36] "Science of Nutrition," 4th edition, Philadelphia, 1928, pp. 209, 405, 639–643.
[37] "Carbohydrate Metabolism and Insulin," London, 1926, p. 130.

creatized nor in hibernating animals have we so far been able to observe respiratory quotients which would unquestionably prove that carbohydrate has been formed from fat."

Ketogenesis.—Reference has been made to the formation of acetone bodies (ketogenesis or ketosis) and their excretion in the urine (ketonuria or acetonuria) in conditions of faulty fat metabolism. Small amounts of these substances appear normally in the urine, particularly acetone, which may be regarded as the product of a side reaction. It has its origin in acetoacetic acid as represented by the following equation:



Ketosis occurs in diabetes, in starvation, during the early stages of phosphorus poisoning, during anesthesia, in children during infections, and in other conditions. When the question is examined carefully, it becomes obvious at once that the complete oxidation of fatty acids is in some way dependent on carbohydrate metabolism. In diabetes, the failure to oxidize the fatty acids completely can be related directly to faulty carbohydrate metabolism. When carbohydrate utilization is improved, as by the administration of insulin, the formation of acetone bodies ceases. Similarly, during fasting, as long as glycogen is present, fat metabolism proceeds normally; but just as soon as the reserve carbohydrate is depleted and the starving animal has only fat and protein to draw on for its metabolic needs, the products of incomplete fatty-acid oxidation appear. This does not continue indefinitely, however, for a point may be reached in starvation when, perhaps as a result of some metabolic adjustment, the process of utilizing fat becomes more efficient. The interrelationship between carbohydrate and fat metabolism is indicated by the oft-repeated statement that " fats burn in the flame of carbohydrates," and by the following statement of Macleod:

> If the carbohydrate fires do not burn briskly enough, the fat is incompletely consumed; it smokes, as it were, and the smoke is represented in metabolism by the ketones and derived acids.

In addition to the fatty acids there is another potential source of acetone bodies, namely, the amino acids, leucine, tyrosine and phenylalanine. In conditions of carbohydrate deprivation or in severe dia-

betes, these substances are ketogenic (that is, give rise to ketones), as we shall see when we study their metabolism in the next chapter.

Anti-ketogenesis.—In 1921, Shaffer [38] described certain experiments in which he demonstrated that the velocity of oxidation of acetoacetic acid by hydrogen peroxide in an alkaline solution is greatly accelerated in the presence of glucose. The accelerating effect of glucose, thus demonstrated *in vitro*, was accepted as analogous to the well-known fact that, in the body, the oxidation of glucose facilitates the oxidation of fatty acids. This effect, according to Shaffer, may perhaps be explained by assuming the formation of a highly oxidizable compound from acetoacetic acid and some intermediate product of glucose metabolism.[39] Another suggestion is of especial interest because of its possible bearing on the subject of intermediary carbohydrate metabolism. It postulates the formation of glucosone, $CHO—CO—(CHOH)_3CH_2OH$, as one of the early products of the intermediary metabolism of glucose. This substance has a marked anti-ketogenic effect.[40]

In his subsequent studies, Shaffer [41] determined certain relations in regard to the ketogenic : anti-ketogenic balance in man. The substances that may give rise to acetone bodies in metabolism are the fatty acids and certain of the amino acids. The substances that have an opposite effect, i.e., the anti-ketogenic substances, are the carbohydrates, the sugar-forming amino acids, and glycerol.

Shaffer's results show that the oxidation of acetoacetic acid occurs only when there is oxidized simultaneously one molecule of glucose for each two molecules of the acid. The inference is that two molecules of acetoacetic acid react with one molecule of glucose. It is assumed that one fatty-acid molecule gives rise to one molecule of acetoacetic acid, and that therefore, in the body, the metabolism of one glucose molecule insures the complete oxidation of two molecules of fatty acid. Glucose may be replaced by other anti-ketogenic substances. For example, the anti-ketogenic value of one glycerol molecule is one-half of that of a glucose molecule; from this it follows that a molecule of fat, to burn completely, requires but one molecule of glucose. The latter takes care of two fatty acids, while the glycerol makes possible the oxidation of the third fatty acid. Although in the body the anti-ketogenic or ketolytic value of 1 molecule of glycerol or glyceric aldehyde appears to be equivalent to one-half that of glucose, the behavior *in vitro* is such

[38] J. Biol. Chem., **47**, 433 (1921).

[39] Shaffer, P. A., and Friedemann, T. E., J. Biol. Chem., **61**, 585 (1924); Friedemann, T. E., *ibid.*, **63**, p. xxi (1925); West, E. S., *ibid.*, **66**, 63 (1925); **74**, p. xlii (1927).

[40] Friedemann, *loc. cit.*, see also Hynd, Proc. Roy. Soc., B, **101**, 244 (1927).

[41] J. Biol. Chem., **47**, 449 (1921); **49**, 143 (1921); **54**, 399 (1922).

as to indicate that the ketolytic value of glycerol, glyceric aldehyde, glycol aldehyde and glyoxal is the same as that of glucose; that is, *in vitro*, a single molecule of any one of these substances will make possible the oxidation of two molecules of acetoacetic acid. By taking into account the ketogenic and anti-ketogenic values of the amino acids, it becomes possible to calculate the ketogenic : anti-ketogenic ratio $\left(\frac{K}{A}\right)$. This is

$$\frac{K}{A} = \frac{\text{sum of keto-substances from fat and from protein}}{\text{sum of anti-ketogenic substances from glucose and from protein}}.$$

In a clinical study of the problem, Woodyatt [42] found that for the complete oxidation of 1.5 grams of fatty acid, 1 gram of glucose must be utilized. The fatty acid : glucose ratio $\left(\frac{FA}{G}\right)$, according to Woodyatt, is therefore 1.5. On a molecular basis, this ratio signifies that 1 molecule of glucose is anti-ketogenic or ketolytic for 1 molecule of fatty acid. When Woodyatt's ratio of 1.5 (the threshold for ketosis) is exceeded, acetonuria appears. Individual variations undoubtedly occur, and in fact this has been demonstrated recently in an obese individual by McClellan and associates.[43] The obese individual showed a ketosis threshold of about 2.4. This observation is interpreted as indicating a greater efficiency in the utilization of fats than in normal man. Perhaps the Eskimos are also better able to utilize fat, as suggested by Heinbecker,[44] who showed that Eskimos excrete but very small amounts of acetone bodies during starvation.

For further details on the clinical aspects of the problem of ketosis, the student is referred to original sources.[45]

Cause of Ketogenesis.—We may now return to a brief consideration of the cause of ketogenesis. It is obvious, of course, that when carbohydrate is not available for metabolism, the organism is forced to burn fat in much larger amounts than normally. The oxidation of fat under these altered conditions becomes the most prominent reaction of metabolism and, as Leathes puts it, there is a flooding of the " metabolic mill " with fat. This may conceivably result in the incom-

[42] Arch. Int. Med., **28**, 125 (1921).

[43] J. Biol. Chem., **80**, 639 (1928).

[44] *Ibid.*, **80**, 461 (1928).

[45] Wilder, R. M., and Winter, M. D., J. Biol. Chem., **52**, 393 (1922); Ladd, W. S., and Palmer, W. W., Am. J. Med. Sci., **166**, 157 (1923); McCann, W. S., Hannon, R. R., Perlzweig, W. A., and Tompkins, E. H., Arch. Int. Med., **32**, 226 (1923); Mason, E. H., J. Clin. Invest., **4**, 93 (1927).

plete oxidation of the fat and the production of acetone bodies. There are many examples of chemical reactions in which the intermediate stages can be detected only when one part of a given reaction is exaggerated. For instance, in severe muscular exercise, the formation of lactic acid is more rapid than its removal (either by oxidation or resynthesis into glycogen). The result is that lactic acid accumulates in the blood and a certain amount of it escapes in the urine. Many circumstances suggest a close parallelism between this and ketogenesis, the latter probably being the result of a one-sided metabolism in which excessive amounts of fat are burned. According to this view, the anti-ketogenic effect of glucose would be due to its fat-sparing action.

Ketogenesis and Acid-base Balance.—The relation of acetone bodies to the acid-base balance of the blood has already been referred to. The acetone bodies are excreted partly in combination with fixed base and partly in combination with ammonia. This accounts both for the depletion of the alkali reserve of the body and for the increased formation of ammonia in conditions of acidosis.

The formation of acetone bodies is perhaps the most important manifestation of deranged fat metabolism.

Obesity.—Obesity is usually the result of overnutrition, lack of exercise, or both. It is a matter of common observance, however, that certain individuals increase in weight despite an apparently moderate diet, while others remain thin in spite of all efforts to gain weight by overeating. Then there is the average individual who makes no conscious attempt to control his diet but whose weight remains fairly constant over a period of many years. Obesity is very common and appears to be hereditary. The view which is gaining headway at present associates obesity with derangements of the endocrine organs. Disease of the hypophysis, castration, the menopause, myxedema, and other physiological and pathological disturbances are usually, but not invariably, accompanied by the deposition of an abnormal amount of fat. A distinction has been pointed out by Grafe [46] between so-called exogenous obesity and the endogenous or constitutional type, the former being due to laziness and overnutrition, whereas the latter is believed to be in some way associated with endocrine disturbance.

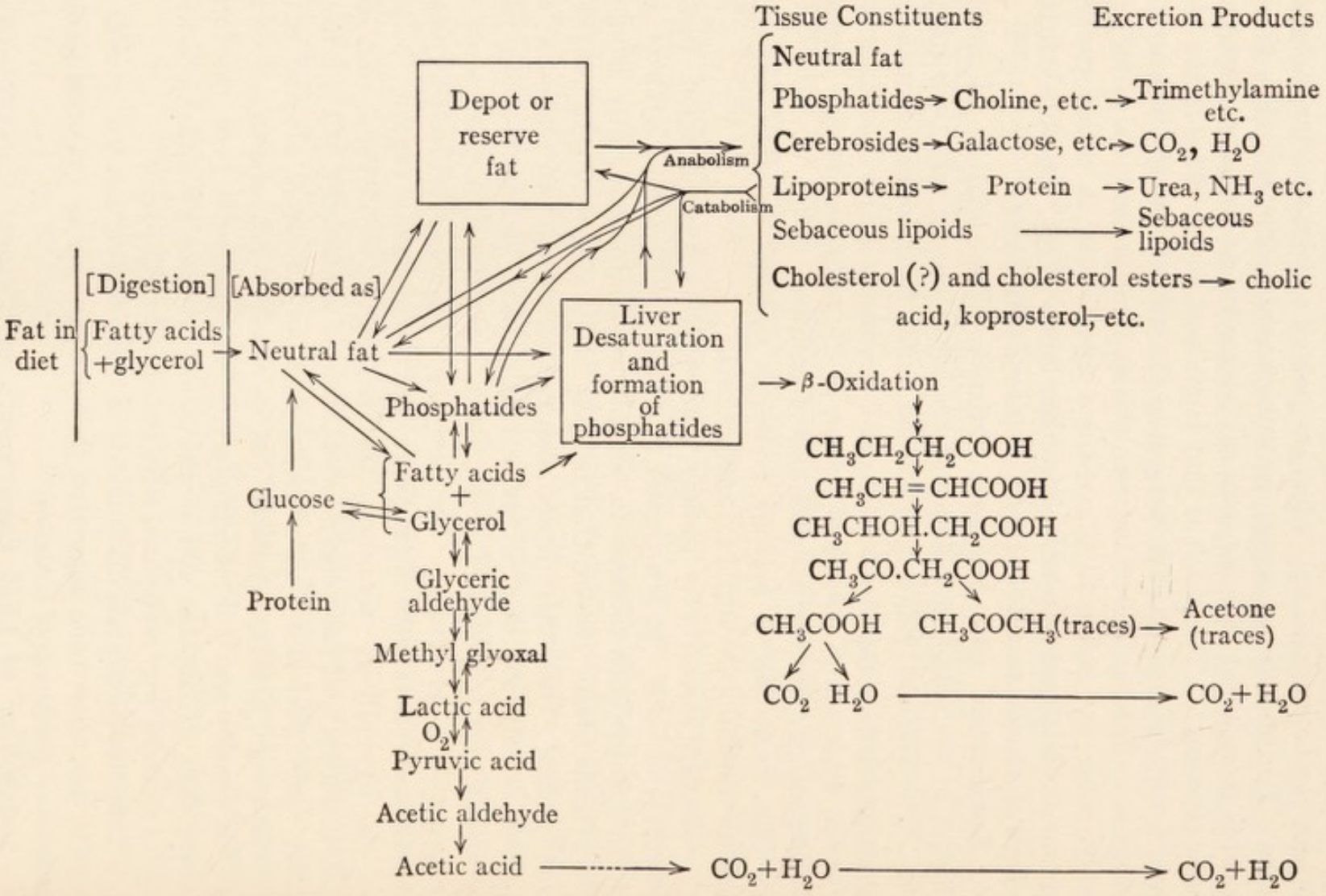
Diabetes occurs not infrequently in obese individuals past middle age and it has often been suggested that obesity may be regarded in many cases as a precursor of diabetes.[47]

Catabolism of Lipid Constituents of Tissues.—Lipoproteins are very probably broken down into their respective lipid and protein resi-

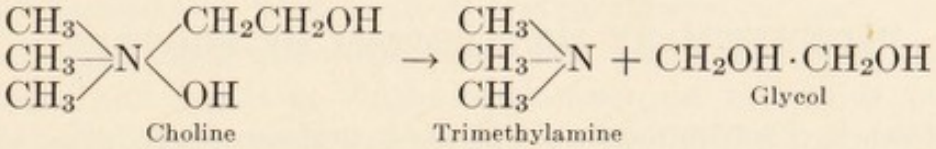
[46] Ergeb. der Physiol., **21**, part 2, 197, 282 (1923).

[47] Adams, J., J. Nutrition, **1**, 339 (1929).

SUMMARY OF NORMAL FAT METABOLISM



dues. It is assumed that these follow the usual paths of protein and fat metabolism. The lecithin is presumably broken down to glycerol, fatty acids, phosphoric acid, and choline. The last-named substance is believed to give rise to trimethylamine, small amounts of which occur in urine. The formation of trimethylamine is represented by the following equation:



The galactose of the cerebrosides follows the usual path of carbohydrate metabolism. The fate of cholesterol in metabolism is obscure. A portion is excreted unchanged in the bile. Cholic (cholalic) acid probably has its origin in cholesterol, there being a close chemical relationship between the two compounds. Cholic acid is conjugated in the body with glycine and taurine to form the two bile acids, glycocholic and taurocholic.

Summary.—The main facts of fat metabolism are outlined in the diagram given on the preceding page.

CHAPTER XII

INTERMEDIARY METABOLISM OF PROTEIN

THERE is a continuous and relatively uniform degradation of protein in the animal body, which is often referred to as the wear and tear of the tissues. It is obvious that this loss must be made up, for otherwise the animal would gradually waste away. As there is ordinarily no storage of protein in the body of an adult animal, the actual protein needs are not in excess of the amount required to replace the protein lost. However, should the supply of carbohydrate and fat be inadequate to meet the caloric requirements of the animal, the body may be forced to depend on the protein of its own tissues for the supply of energy. At such times an excessive amount of tissue break-down occurs.

Not all the absorbed amino acids are resynthesized into protein in the tissues. It is obvious that if the amino acids of the proteins of the diet are not present in the same proportions as in the proteins of the animal, there must be an excess of some amino acids which are not utilized in protein synthesis and hence must be disposed of in some other way. Moreover, the protein intake is usually in excess of the anabolic needs of the organism. Consequently, a certain amount of the amino acids derived from the diet is burned directly or converted into sugar and fat. Exogenous metabolism is the metabolism of all protein ingested in excess of that required by the tissues for maintenance and growth. Endogenous metabolism, on the other hand, usually refers to the metabolism which produces as end products creatinine, neutral sulfur, and the part of the uric acid not derived from the food.[1]

Nitrogen Equilibrium.—By comparing the intake of nitrogen as protein with the total elimination of nitrogen (in the urine, feces, and perspiration), it is possible to determine whether the body is gaining or losing protein. If such studies are carried on over a short period, such as twenty-four hours, there may appear to be a retention of nitrogen. This is more often due to a lag in the elimination of the nitrogenous end-products of protein-metabolism than to a synthesis of protein. For

[1] For an excellent summary of the theories of endogenous and exogenous protein metabolism the student is referred to H. H. Mitchell and T. S. Hamilton, The Biochemistry of Amino Acids, Chemical Catalog Co., New York, 1929, Chapter IX.

accurate work, studies in nitrogen equilibrium should be carried on over a period of several days, separate analyses being made of the food and excreta of each day. In the adult, there is normally no retention of nitrogen; that is, the nitrogen intake as protein is equivalent to the nitrogen elimination. This is a condition of nitrogen equilibrium, or nitrogen balance.

The statement that in an adult individual there is normally no retention of nitrogen needs to be qualified. It is well known that muscular development and a gain in weight result when an individual performs muscular work over a period of several weeks or months, provided he is supplied during this time with an adequate amount of food, especially protein. Bornstein [2] investigated this question and found that nitrogen retention occurred (positive nitrogen balance) when the increase in muscular activity was accompanied by an increase in the protein intake.

Negative nitrogen balance occurs when the endogenous protein metabolism exceeds the protein intake. This occurs in malnutrition, starvation, fevers, and other wasting diseases. In fevers, the patient is often given large amounts of sugar for the purpose of sparing the tissue proteins as much as possible. The more essential organs, such as the heart and brain, are spared even in prolonged starvation. During convalescence there is regeneration of the tissues and hence a retention of nitrogen.

In growing animals the excretion of nitrogen is less than the corresponding protein intake, a portion of the amino acids of the diet being used in the synthesis of new tissue protein.

Anabolism.—To meet the anabolic needs of the body a certain minimum of protein is required. This question will be considered in somewhat greater detail in other connections, but for the purpose of the present discussion it is sufficient to say that the actual needs of the body seem to be far less than the amount formerly considered necessary. The older physiologists believed that an adequate diet should contain about 120 grams of protein, a value which was based on German statistical data and which also corresponds closely to the statistical data for the average daily protein intake in the United States. Chittenden, on the other hand, has shown in a long series of experiments on individuals engaged in various occupations, including athletes and soldiers, that one-half this amount is adequate and that even less may be sufficient. There are observations on record to show that nitrogen equilibrium may be maintained under special conditions on as little as 2.2 grams of nitrogen per day, or about 15 grams of protein, provided sufficient carbohydrate is given at the same time.

[2] Arch. f. ges. Physiol., **83**, 540 (1901).

Catabolism.—When the tissues break down, the protein molecules are apparently hydrolyzed, probably with the aid of tissue enzymes, yielding mainly amino acids. There is no reason for assuming that the metabolism of these differs from that of the amino acids derived directly from the diet. The study of intermediary protein metabolism is essentially the study of the fate of the individual amino acids. This is the main purpose of the present chapter.

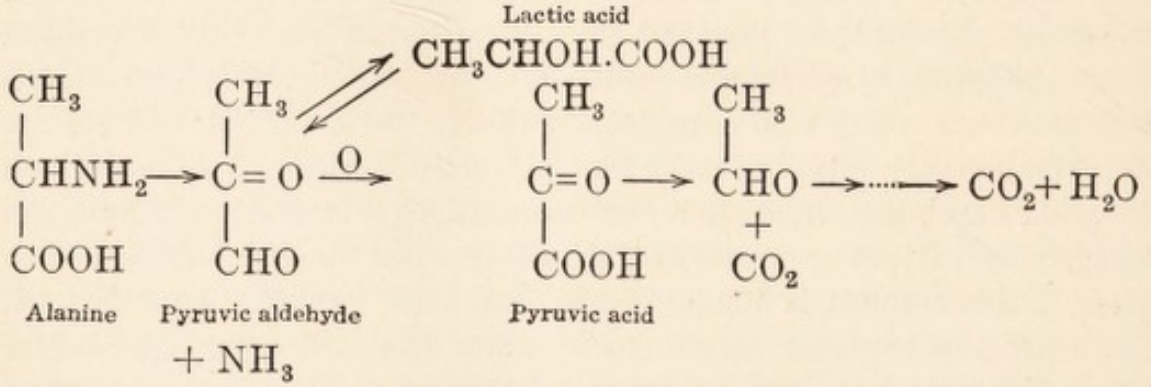
General Course of Amino-acid Metabolism.—Oxidation of amino acids occurs in the α position and is accompanied by the removal of the amino group. Much attention has been devoted to the problem in an attempt to determine the intermediate steps of the process.

Neuberg and Langstein [3] fed a starving rabbit alanine and obtained lactic acid in the urine. Embden [4] perfused a surviving liver with alanine and likewise obtained lactic acid. It was therefore supposed that the formation of α-hydroxy acids was the immediate result of hydrolysis as represented by the following equation:

$$R—CH_2 \cdot CHNH_2 \cdot COOH + HOH = R—CH_2 \cdot CHOH \cdot COOH + NH_3$$

This view is not generally accepted, however, the bulk of the evidence pointing to an oxidative deamination of the amino acids with the intermediate formation of α-ketonic acids. In general, α-amino acids and the corresponding α-ketonic acids share the same fate in metabolism and are oxidized with approximately the same rapidity, whereas the α-hydroxy acids are oxidized less readily. It is very likely that the α-hydroxy acids are not formed directly from amino acids but from the corresponding ketonic acids or aldehydes.

Dakin [5] has suggested that, in the conversion of amino acids into ketonic acids, the corresponding glyoxals are formed as intermediate products. Taking alanine as an example, these ideas may be represented as follows:

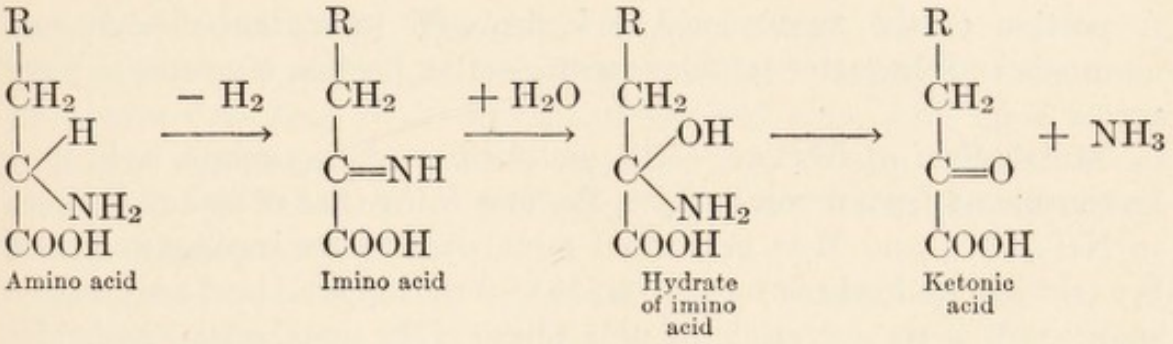


[3] Arch. Physiol., Physiol. Abt., Supplementband, 514 (1903).

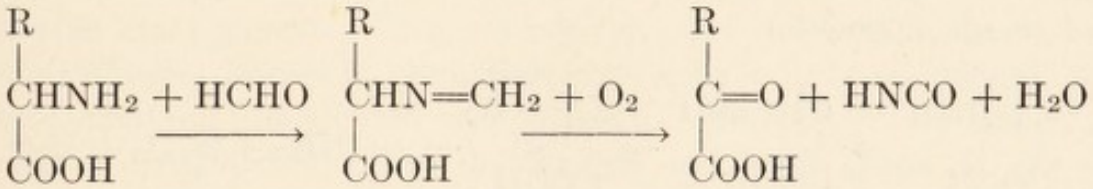
[4] Cited by Dakin in Oxidations and Reductions in the Animal Body, 1922 edition, p. 67.

[5] H. D. Dakin, Oxidations and Reductions in the Animal Body, 2d edition, Chapter III.

According to Knoop [6] hydrates of imino acids are formed in the deamination of amino acids. This is very suggestive, for we may then assume the following sequence of events: The first step in the metabolism of an amino acid may be taken to be one involving the loss of hydrogen by dehydrogenation, resulting in the formation of the corresponding imino acid. Presumably the next step would be one of hydration, yielding a hydrate which, Dakin states, would undoubtedly be very unstable. It would part with its ammonia, thus yielding a ketonic acid. These changes are represented as follows:



The *in vitro* oxidation of amino acids is favorably influenced in the presence of aldehydes, such as glucose or formaldehyde, as shown by Fearon and Montgomery.[7] These authors have, therefore, suggested that the following reactions are involved in the removal of the amino group:



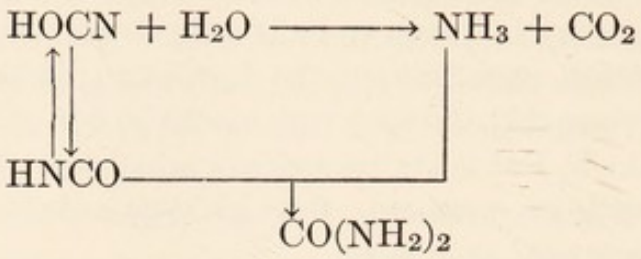
Fate of Ammonia.—The formation of urea and its physiological significance will be considered in detail later. For the present, in order not to leave too many loose ends in our discussion of the intermediary metabolism of the individual amino acids, it is sufficient to state that in mammals, and particularly in man, the ammonia which is formed in the deamination of the amino acids is converted almost quantitatively into urea. Urea is the principal end-product of protein metabolism, the quantity present in the urine being determined largely by the amount of protein ingested. The utilization of ammonia in neutralizing acid will be considered in a later section.

If the intermediate formation of cyanic acid is accepted in accordance

[6] Z. Physiol. Chem., **67**, 489 (1910); **148**, 294 (1925).

[7] Biochem. J., **18**, 576 (1924); see also Physiol. Reviews, **6**, 399 (1926).

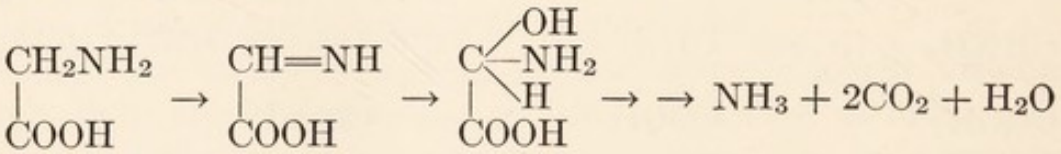
with the view of Fearon and Montgomery, its conversion into ammonia and urea may be assumed to take place as follows:



A portion of the cyanic acid is hydrolyzed to carbon dioxide and ammonia and the latter combines with another portion of cyanic to yield urea.

Metabolism of Glycine.—The metabolism of this amino acid may be considered from several angles, the first being that of its break-down to NH_3, CO_2 and H_2O in normal metabolism. Its conjugation with benzoic acid and other aromatic acids to form hippuric acid and related compounds is to be considered as a phase of its metabolism, as is also the formation of glycocholic acid by conjugation with cholic acid. We also have to include the synthesis of sugar from glycine in the diabetic animal as well as its possible formation in the normal individual.

The steps in the break-down of glycine in the course of normal metabolism are not definitely known. However, from what has been said of the general course of amino-acid metabolism, the following changes seem possible:



Evidence for the synthesis of glycine in the body is to be found in the numerous experiments in which benzoic acid or benzoates have been fed to animals and hippuric acid found in the urine.[8] Hippuric acid is a normal constituent of the urine of horses, cattle, and other herbivorous animals. In small amounts, it is likewise present in human urine. Its formation was mentioned in discussing Knoop's experiments in the preceding chapter. Not only does the body use any preformed glycine that may be present either in the diet or in the tissues, but it is at times forced to synthesize this amino acid in large amount for the purposes of detoxication. While there is no doubt that this occurs, we do

[8] See, for example, the recent papers of Griffith, W. H., and Lewis, H. B., J. Biol. Chem., **57**, 1 (1923); Griffith, J. Biol. Chem., **69**, 197 (1926); **82**, 415 (1929).

not know how it is brought about. Knoop as well as Dakin[9] have suggested that the formation of glycine in the body may result from the oxidation at the β-carbon atom of α-amino β-hydroxy acids such as serine. Dakin has shown that phenylserine does not behave like phenylalanine in the body, for it yields benzoic acid. Thus, it appears to be oxidized at the β-carbon atom as represented in the equation:

$$C_6H_5 \cdot CHOH | CHNH_2 \cdot COOH + O \rightarrow C_6H_5 \cdot COOH + CH_2NH_2 \cdot COOH$$

The conversion of glycine into sugar in the animal body has been definitely established. The relationship between amino acids and glucose has been studied, especially by Lusk and his pupils, the general procedure consisting in feeding phlorhizinized dogs with these amino acids and analyzing the urine for nitrogen and glucose. To bring out the significance of these experiments, it may be well to describe them in somewhat greater detail.

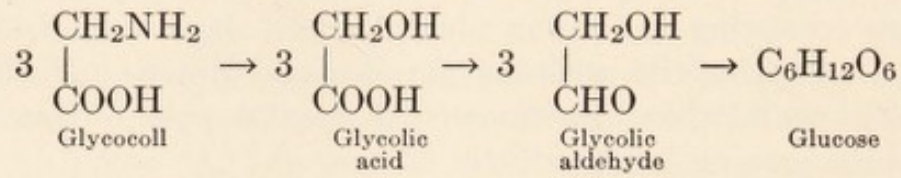
Suppose we have a dog which has been rendered completely diabetic with phlorhizin. We collect the urine of the animal for a given period and analyze it for glucose and nitrogen. This gives us the necessary data for calculating the glucose : nitrogen or D : N ratio. Let us assume that it is found to be 3.65, which means that the dog is completely diabetic and is unable to utilize any sugar, no matter what its source may be. Now suppose we give the dog 16 grams of glucose and set out to determine whether any or all of it may be recovered in the urine as extra glucose. The urine is therefore collected a second time for a sufficient period to assure complete excretion of the glucose and again analyzed for glucose and nitrogen. Suppose we now find the urine to contain 2.87 grams of nitrogen and 25.92 grams of glucose. Assuming that the D : N ratio had not changed in the meantime, there should have been excreted 3.65 grams of glucose for every gram of nitrogen, both having had their origin in the tissue proteins which were broken down during this interval. $3.65 \times 2.87 = 10.49$. The difference between this value and the total sugar elimination, $25.92 - 10.49 = 15.43$ grams, is the amount of extra sugar excreted. In other words, practically all of the sugar that was given to the dog was recovered in the urine.

We may now analyze the results obtained on feeding glycine. For this purpose an experiment described by Lusk[10] may be selected. A phlorizinized dog, having a D : N ratio of 3.38, was given 20 grams of

[9] H. D. Dakin, *loc. cit.*

[10] G. Lusk, The Science of Nutrition, 4th edition, 1928, p. 228, Ringer and Lusk, Z. physiol. Chem., **66**, 106 (1910).

glycine, and the urine collected during the next fourteen hours was analyzed and found to contain 47.42 grams of glucose and 12.84 grams of nitrogen. Since 20 grams of glycine contain 3.73 grams of nitrogen, the difference between 12.84 and 3.73, or 9.11 grams represents the protein metabolism during this interval. Therefore, $9.11 \times 3.38 = 30.79$ grams of glucose had its origin in the protein metabolized. The difference between 47.42 grams, the total sugar excreted, and 30.79 grams, that is, 16.63 grams, must have been formed, therefore, from the ingested glycine. This accords well with the calculated yield (16 grams) based on the assumption that all of the carbon of glycine is converted into glucose. This conversion may be represented as follows:



If this represents the sequence of events, glycolic acid and aldehyde should yield glucose when given to diabetic animals. Sansum and Woodyatt [11] showed that this actually occurs. As much as 75 per cent of the glycolic acid which they had administered slowly to a phlorhizinized dog escaped oxidation and appeared in the urine as extra glucose. Further evidence in support of this idea is to be found in an experiment of Neuberg and Rewald [12] who were able to show the polymerization of glucose from glyceric aldehyde in an aqueous solution.

Metabolism of Alanine.—Earlier in this chapter reference was made to the possible conversion of alanine into lactic and pyruvic acids. All of these substances are related to each other and to glucose in metabolism. The complete conversion of alanine into glucose in phlorhizin diabetes has been shown by Ringer and Lusk [10] and by Dakin and Dudley.[13] Mandel and Lusk [14] proved the transformation of lactic acid into glucose and Dakin and Dudley [15] recovered the methyl glyoxal which they administered to a diabetic dog, in the urine, as glucose. The evidence for the complete conversion of pyruvic acid is, on the other hand, not very striking and open to doubt. Dakin has suggested the possibility that only such pyruvic acid as undergoes reduction to lactic acid is converted into glucose.

[11] J. Biol. Chem., **17**, 521 (1914).
[12] Biochemisches Handlexicon, **2**, p. 266.
[13] J. Biol. Chem., **17**, 451 (1914).
[14] Am. J. Physiol., **16**, 129 (1906).
[15] J. Biol. Chem., **15**, 127 (1913).

Pyruvic aldehyde (methyl glyoxal) is believed to occupy an especially important position in these conversions. Dakin points out that both *d*- and *l*-lactic acids and *d*- and *l*-alanines are quantitatively converted in the diabetic organism. It is therefore reasonable to assume the formation of an intermediate compound which is optically inactive. Either pyruvic aldehyde or dihydroxyacetone would fulfill this requirement.

A certain amount of confusion is inevitable in attempting to picture the sequence of events from the known facts. The assumption that both pyruvic and lactic acids lie in the path of glucose formation from alanine is not altogether in harmony with the ideas (1) that methyl glyoxal may be an intermediate product in the formation of pyruvic acid from alanine (Dakin) and (2) that methyl glyoxal may be formed as an intermediate product in the conversion of lactic acid into glucose (Dakin).

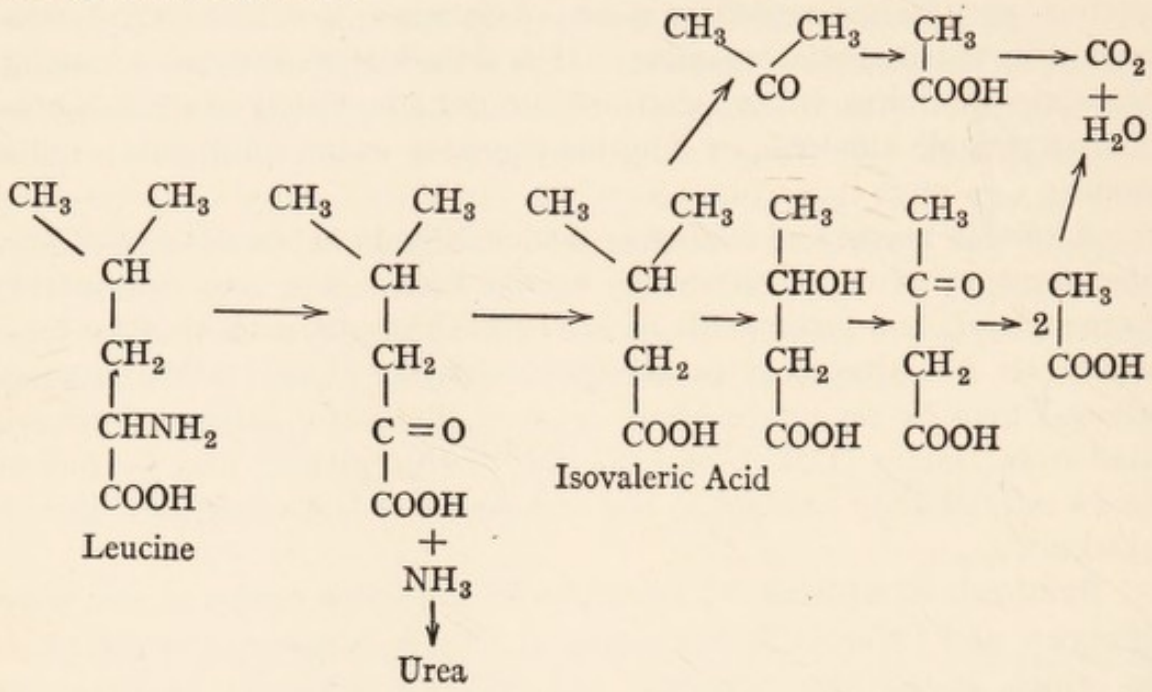
Synthesis of Alanine.—The synthesis of alanine occurs in the body. Embden and Schmitz [16] demonstrated this in a series of experiments in which ammonium pyruvate was perfused through the liver and alanine recovered in the perfusate. Glycogen may likewise serve as a source of alanine, as reported by Fellner,[17] who perfused salts of ammonia through glycogen-rich and glycogen-poor livers and observed a greater amount of alanine synthesis in the former than in the latter.

Metabolism of Valine.—The fate of valine in metabolism is not definitely known. Oxidation at the α-carbon atom should yield isobutyric acid, but Dakin [18] was not able to demonstrate this in phlorhizinized dogs. Moreover, isobutyric acid is readily converted into glucose in diabetic animals, whereas valine does not yield sugar. Nor does this amino acid give rise to acetone bodies when perfused through the liver (Embden, Salomon and Schmldt).[19]

Metabolism of Leucine.—By oxidative deamination, leucine yields the corresponding ketonic acid, which is in turn oxidized to isovaleric acid. This is believed to undergo demethylation, forming β-hydroxybutyric acid. From this point, its fate is presumably identical with that of all other fatty acids, oxidation occurring at the β-carbon atom (see Fat Metabolism). Hence, leucine may be a source of acetone bodies in the diabetic animal. A certain amount of acetone may be

[16] Biochem. Zeit., **38**, 393 (1912).
[17] Biochem. Zeit., **38**, 414 (1912).
[18] J. Biol. Chem., **14**, 321 (1913).
[19] Hofmeister's Beitrage, **8**, 129 (1906).

formed directly by the oxidation of isovaleric acid. These reactions are represented below:



Leucine is present in all proteins. Therefore, even if it were not synthesized in the body, it would probably be supplied in adequate amounts in the diet. When acted upon by bacteria it yields isovaleric acid and isoamylamine. Yeast fermentation produces isoamyl alcohol.

Metabolism of Isoleucine.—Not much information is available concerning the metabolism of this amino acid. It does not give rise to glucose and its conversion into acetone bodies is not definitely established (Dakin, p. 75).

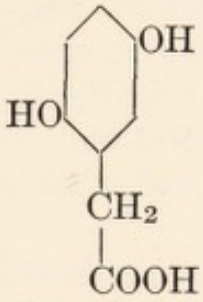
Metabolism of Phenylalanine and Tyrosine.—The first step in the metabolism of phenylalanine is believed to be its conversion into tyrosine (Embden and Baldes).[20] Dakin [21] tested this idea by injecting phenylalanine into rabbits. The urine of these animals was found to contain large amounts of phenylalanine but no tyrosine or other phenolic compounds. Para-substituted phenylalanines, such as *p*-methyl-phenylalanine, are broken down quite readily in the animal organism. According to Dakin, these observations argue against the supposition that phenylalanine is converted exclusively into tyrosine.

Concerning the immediate fate of tyrosine it is apparently first oxidized to *p*-hydroxyphenylpyruvic acid. That phenylpyruvic acid is formed from phenylalanine has neither been proved nor disproved, but there is good reason for believing on the basis of the observations of

[20] Biochem. Zeit., **55**, 301 (1913).

[21] H. D. Dakin, Oxidations and Reductions in the Animal Body, 1922, p. 86.

Kotake, Masai and Mori [22] that phenylalanine is converted into *p*-hydroxyphenylpyruvic acid. Therefore the subsequent fate of the two amino acids may be studied together. Before this is done, however, brief reference will be made to a peculiar and rare condition, called alcaptonuria, which appears to be hereditary, and in which there is apparently a derangement in the metabolism of these amino acids. Alcaptonuria occurs more frequently in males than in females. Its occurrence in a rabbit has been reported by Lewis.[23] The urine of alcaptonurics, when allowed to stand exposed to the air, absorbs oxygen and turns black, owing to the presence of homogentisic acid, which has the following formula:



Homogentisic acid

It is important to note that phenylalanine and tyrosine, when given to an alcaptonuric, are converted into homogentisic acid and excreted as such, but when given to a normal individual are oxidized completely. Moreover, when homogentisic acid itself is administered to a normal individual it is apparently oxidized, but in the alcaptonuric this is not the case. Another observation which has strengthened the view that homogentisic acid represents an intermediate stage in the normal metabolism of phenylalanine and tyrosine is that of Embden, Salomon and Schmidt,[20] who found that all three of these substances yield acetoacetic acid when perfused through a surviving liver.

Dakin, however, does not believe that homogentisic acid formation is a necessary step in the metabolism of tyrosine. There is good evidence for believing that an immediate precursor of homogentisic acid is a compound having a quinonoid structure. Dakin [24] therefore administered *p*-methylphenylalanine and *p*-methoxyphenylalanine,

$$(CH_3 \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH)$$

and

$$(CH_3O \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH),$$

[22] Z. physiol. Chem., **122**, 195 (1922).
[23] J. Biol. Chem., **70**, 659 (1926).
[24] J. Biol. Chem., **9**, 151 (1914).

to alcaptonurics and showed that these substances were completely oxidized, presumably because of their inability to form quinonoid derivatives. Fromherz and Hermanns [25] performed similar experiments with *m*-methyl-tyrosine and *p*- and *m*-methyl-phenylalanine. These substances do not undergo the quinonoid transformation and, hence, did not give rise to homogentisic acid in these experiments. Accordingly, Dakin postulates that alcaptonuria represents a condition in which there is not only an abnormal formation of homogentisic acid but also an abnormal failure to catabolize it when formed. Equations to represent these changes will be given shortly.

Alcaptonuric individuals who reach middle life frequently develop *ochronosis*, a condition in which the cartilages acquire a black pigmentation. Ochronosis is also seen in persons who over a long period of years have applied carbolic acid dressings to ulcers of the legs. A detailed account of the chemical and clinical aspects of ochronosis is given by Sir Archibald E. Garrod in his monograph on "Inborn Errors of Metabolism."

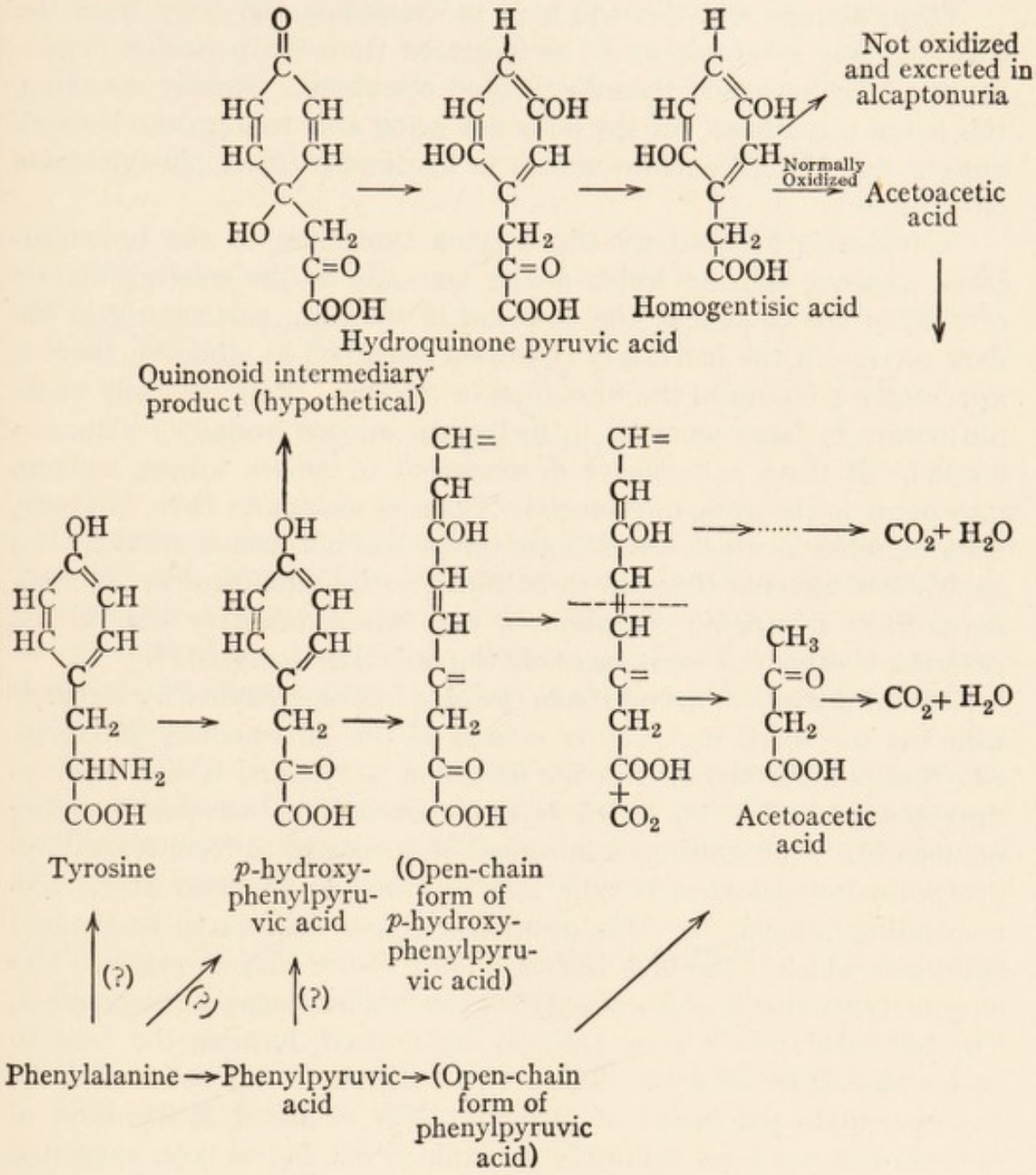
We shall now return to the consideration of *p*-hydroxyphenylpyruvic acid. As it is desirable to dispose of as many questions as possible, phenylpyruvic acid, which, as has been pointed out, is a possible derivative of phenylalanine, will be included in this discussion. In either case, we are dealing with an α-ketonic acid and it would be logical to assume the next step in metabolism to be oxidation at the α-carbon atom, resulting in the formation of phenylacetic acid and *p*-hydroxyphenylacetic acid. However, when this assumption is subjected to experimental study it is found to be incorrect. Both phenylacetic acid and *p*-hydroxyphenylacetic acid escape oxidation in the animal body. When administered to an alcaptonuric these substances do not give rise to homogentisic acid. When perfused through a surviving liver, they do not yield acetoacetic acid.

It is well known that most compounds containing the benzene ring do not undergo complete oxidation in the body, the benzene nucleus remaining intact. This does not apply, however, to the naturally occurring aromatic amino acids, phenylalanine, tyrosine, and tryptophane. In the metabolism of these substances the benzene nucleus is disrupted. Nor is this phenomenon altogether limited to these compounds. Even in the case of benzene, a certain amount of cleavage is known to take place in the animal body,[26] the resulting product being muconic acid ($COOH \cdot CH = CH \cdot CH = CH \cdot COOH$).

[25] Z. Physiol. Chem., **91**, 194 (1911).

[26] Jaffé, Z. Physiol. Chem., **62**, 58 (1909).

The metabolism of phenylalanine and tyrosine may be summarized as follows:



It is probably logical to suppose that *p*-hydroxyphenylpyruvic acid undergoes cleavage in the benezene ring, forming an open-chain compound (this is hypothetical) of nine carbon atoms; subsequently the terminal carboxyl group is removed and cleavage of the resulting product occurs at the double bond between the γ- and δ-carbon atoms. Acetoacetic acid is thus formed at the expense of two carbon atoms of the side chain and two of the benzene ring. Accordingly, both phenylalanine and tyrosine are potentially ketogenic substances. Normally, the acetoacetic acid is oxidized completely. The carbon atoms of the

open-chain remnant share a similar fate, but we have no information to serve as a basis for discussion of the intermediate steps.

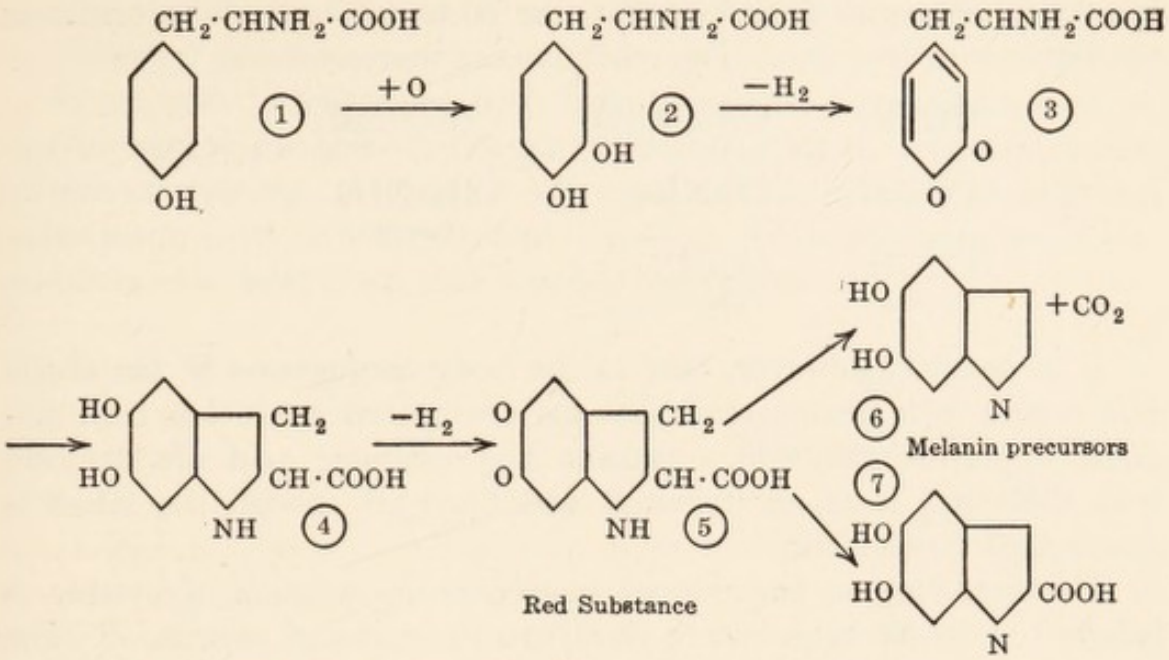
Phenylalanine and tyrosine may be formed in the body from the corresponding α-ketonic acids, as indicated from the perfusion experiments of Embden and Schmitz,[17] cited elsewhere. Strictly speaking, this is not a synthesis, for the body not being able to form the benzene nucleus *de novo* is therefore unable to synthesize either phenylalanine or tyrosine.

Tyrosine is oxidized by the enzyme tyrosinase to the brownish-black pigment melanin which occurs normally as the coloring matter of hair, in the choroid of the eye, and in the skin, particularly in the dark races. In the hereditary condition described as albinism, there is apparently a failure in the formation of melanin. Pathologically melanin occurs in large amounts in melanotic tumors (usually melanosarcomas). If there is extensive development of such a tumor, melanin may occur in the urine (melanuria). Cases of melanuria have, however, been reported in which a melanotic tumor was not demonstrable. The analyses of melanin that are to be found in the literature would seem to indicate a variable composition, but this is probably due to the presence of other cell constituents in the materials analyzed.[27]

The formation of melanin from tyrosine has been studied by Raper,[28] who has succeeded in isolating several of the intermediary products. The first product obtained in the oxidation of tyrosine ① is 3 : 4-dihydroxyphenylalanine ②, which is next oxidized to its corresponding quinone ③. This undergoes intramolecular change to form 5 : 6 dihydroxydihydroindole-2-carboxylic acid ④, which is oxidized to its corresponding quinone ⑤. This compound Raper believes to be the red substance which is the first visible product of the enzyme action. The enzyme tyrosinase is not necessary for the further stages of the reaction. The red substance is spontaneously decolorized, forming the base ⑥ or its carboxylic acid ⑦. These two compounds are believed to be the immediate percursors of melanin. The chemical constitution of melanin has not been definitely determined but it has been suggested that it may be either $(C_5H_5O_3N)_n$ or $(C_8H_7O_3N)_n$, the supposition being that many molecules of the indole combine to form the amorphous melanin. The stages in the reaction resulting in the formation of the precursors of melanin are indicated by the following formulas:

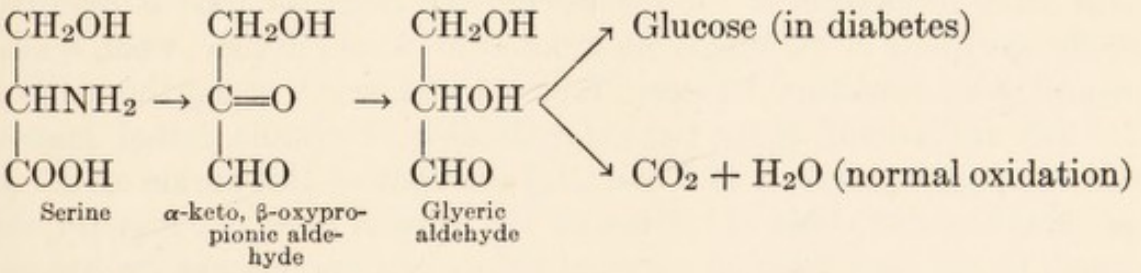
[27] For an excellent review of the subject, the student is referred to H. G. Wells, Chemical Pathology, 5th edition, Philadelphia (1925), Chapter XX.

[28] Raper, H. S., Biochem. J., **20**, 735 (1926,) **21**, 89 (1927); Physiol. Reviews, **8**, 245 (1928).



Tyrosine is closely related to and, together with phenylalanine, is probably the precursor of two additional substances of considerable physiological importance, epinephrine, or adrenaline, the hormone of the medulla of the suprarenal (adrenal) gland, and thyroxin, the hormone of the thyroid gland. These hormones will be described in a later chapter.

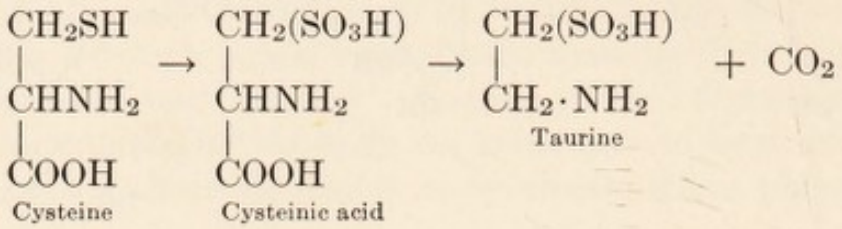
Metabolism of Serine.—The one definitely known fact concerning the metabolism of *l*-serine is its quantitative conversion into glucose in the completely diabetic animal. The intermediary metabolism of this amino acid may be represented as follows:



The close chemical relationship of serine and alanine suggests that these amino acids may be interchangeable in metabolism, and, as has been stated elsewhere, there is some evidence for the view that serine may serve as a source of glycine in metabolic processes.

Metabolism of Cystine.—The metabolism of cystine is relatively complex for it normally undergoes a variety of transformations in the animal body. It is undoubtedly the precursor of glutathione (p. 253) and taurine. Taurine occurs in combination with cholic and choleic acids in taurocholic and taurocholeic acids, respectively (p. 168). The

formation of taurine from cysteine may be brought about by oxidizing the latter with bromine. The reactions are represented as follows:



It is possible, however, that in the body conjugation of the cholic acid occurs with cysteine and that the conjugated product is then oxidized to taurocholic acid. Taurine and cysteinic acid are oxidized with difficulty, if at all (Schmidt and Clark),[29] though the latter is deaminized quite readily.

The first step in the normal metabolic degradation of cystine is believed to be its reduction to form two molecules of cysteine.[30] The next step is probably an oxidative deamination, the ammonia being presumably converted to urea. The sulfur is for the most part oxidized to sulfate and is eliminated in the urine mainly as inorganic sulfate. A smaller amount is excreted as ethereal sulfates, conjugated substances formed in the detoxication of absorbed products of intestinal putrefaction, such as phenol and indoxyl. In addition, the urine contains a certain amount of unoxidized sulfur. It is probable that most of the inorganic sulfate is derived from exogenous metabolism, for the amount varies with the total nitrogen and particularly with the urea elimination. The significance of these urinary constituents will be further considered in other connections.

A large proportion of the cystine derived from the food is required for the synthesis of various types of keratins, found in hair, wool, feathers and other epidermal tissues. Wilson and Lewis,[31] using the method of Folin and Looney[32] for the determination of cystine, found human hair to contain between 15.6 and 21.2 per cent of this amino acid. It appears, however, that the demands for protein (and cystine) for the growth of the body with its essential tissues and organs take precedence over the demands for the growth of hair. Lightbody and Lewis[33] have shown that when diets of low cystine content are fed to rats, the growth

[29] J. Biol. Chem., **53**, 193 (1922).

[30] Lewis and McGinty and Lewis, Updegraff and McGinty have shown that the administration of phenyluraminocystine and dibenzoylcystine results in the excretion of phenyluraminocysteine and dibenzoylcysteine, respectively (J. Biol. Chem., **53**, 349 (1922); **59**, 59 (1924)).

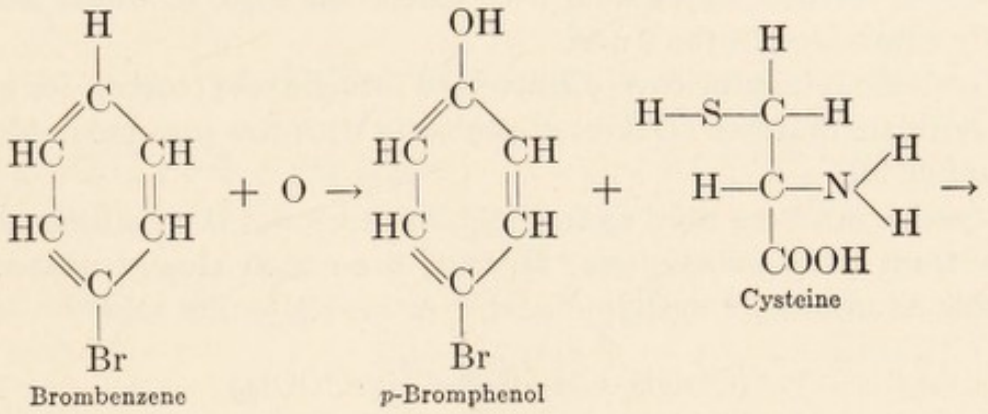
[31] J. Biol. Chem., **73**, 543 (1927).

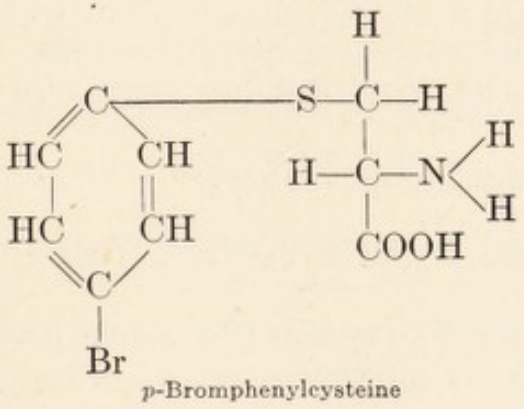
[32] *Ibid.*, **51**, 421 (1922).

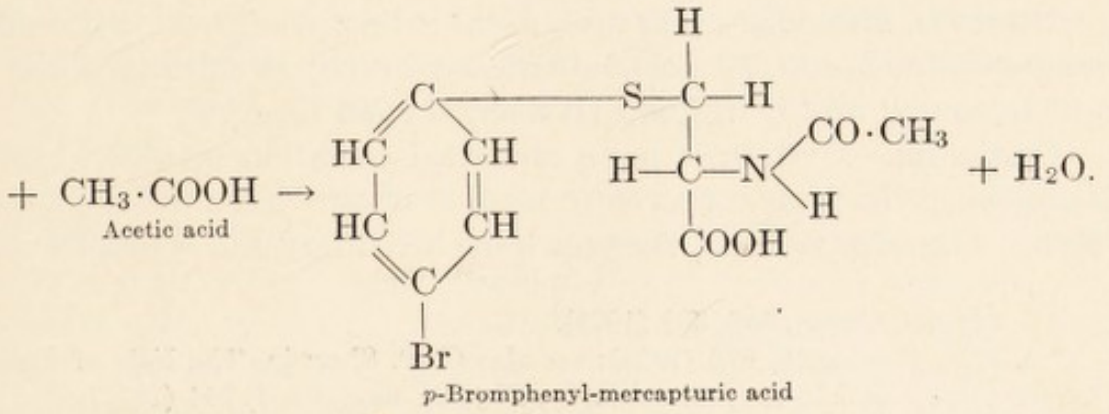
[33] *Ibid.*, **82**, 485 (1929).

of hair is much more markedly diminished than the general growth of the body.

In unusual intoxications with foreign organic compounds, cystine may be used by the organism as the detoxicating agent. Thus the monohalogen derivatives of benzene are converted into the corresponding mercapturic acid derivatives and excreted. The following reactions are believed to take place with mono-brombenzene:







It has been established by Karl Thomas [34] that the cystine for the detoxication of monobrombenzene is derived from the protein of the

[34] Thomas, K., and Straczewski, H., Arch. anat. u. Physiol., Physiol. Abt., 249 (1919).

diet and not at the expense of the tissues. When dogs are given a high carbohydrate diet and just sufficient protein to maintain nitrogen equilibrium (or the " wear and tear " level) brombenzene is not converted into *p*-bromphenyl-mercapturic acid (Kapfhammer).[35] These observations have been confirmed by Muldoon, Shiple and Sherwin.[36]

Acetylation appears to be a fairly common reaction in the animal body. The mercapturic-acid derivative described above probably undergoes further conjugation with glucuronic acid, in which form it is finally eliminated in the urine.

Cysteine is quantitatively converted into glucose (carbon for carbon) in phlorhizin diabetes (Dakin),[37] probably with the intermediate formation of serine.

Cystine is an essential amino acid, for it cannot be synthesized in the body from other substances. In fact, even such closely related compounds as taurine,[38] cysteinic acid,[39] dithiodiglycollic acid,[40]

$$(COOH \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot COOH)$$

β-dithiodipropionic acid [40]

$$(COOH \cdot CH_2 \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot CH_2 \cdot COOH)$$

and α-dihydroxy-β-dithiodipropionic acid [40]

$$(COOH \cdot CHOH \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot CHOH \cdot COOH)^{40}$$

are apparently not changed to cystine or cysteine, for it has been demonstrated that diets deficient in cystine and supplemented with these substances are inadequate for growth.

However, dithiodiglycollic acid, β-dithiodipropionic acid and α-dihydroxy-β-dithiodipropionic acid, administered orally or subcutaneously to rabbits, are all readily oxidized (Westerman and Rose).[41]

Cystinuria.—Cystinuria is an abnormal condition in which cystine is present in the urine, apparently because of some failure in its metabolism. Like alcaptonuria, it seems to be hereditary and is said to occur

[35] Z. physiol. Chem., **116**, 302 (1921).

[36] J. Biol. Chem., **59**, 675 (1924); see also C. P. Sherwin, The Fate of Foreign Organic Compounds in the Animal Body, Physiol. Reviews, **2**, 264 (1922).

[37] J. Biol. Chem., **14**, 321 (1913).

[38] Beard, H. H., Am. J. Physiol., **75**, 658 (1926); Lewis, G. T., and Lewis, H. B., J. Biol. Chem., **69**, 589 (1926); Rose, W. C., and Huddlestun, B. T., *ibid.*, p. 599.

[39] Lewis and Lewis, *loc. cit.*

[40] Westerman, B. D., and Rose, W. C., **75**, 533 (1927); **79**, 413 (1928).

[41] J. Biol. Chem., **79**, 423 (1928).

somewhat oftener in males than in females. Robson [42] has recently investigated the genealogical tree of a patient with pronounced cystinuria. There was a definite family history of this abnormality as shown by the accompanying diagram. It is to be regretted that no information was available concerning many members of this family. These are indicated by crosses. Despite these gaps, however, this family tree illustrates very clearly the hereditary nature of the disease.

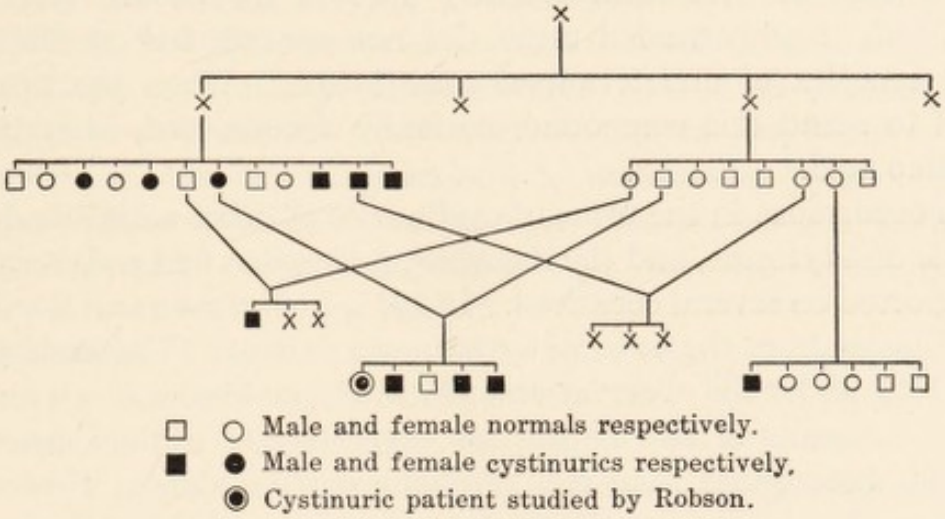


FIG. 41.—Showing the family history of a patient with cystinuria. (Courtesy of Dr. Robson).

The nature of the metabolic derangement is somewhat obscure. It has been repeatedly demonstrated that the cystinuric can utilize completely considerable amounts of the free amino acid. For example, Robson fed his patient increasing amounts of cystine, starting with 2 grams and increasing the dose by 2 grams daily until 8 grams were given. For a four-day period a total of 20 grams of cystine were administered, 85 per cent of which was accounted for in the form of the extra inorganic sulfate eliminated during that period. There was no elimination of extra cystine.

Cystinurics continue to excrete cystine even on a protein-free diet and during starvation, which would indicate that the cystine is at least partly endogenous in origin. It has also been known for some time that the excretion of cystine in cystinuria is definitely increased by increasing the quantity of protein in the diet. This would indicate an exogenous source for some of the cystine. However, Lewis and Lough [43] have made the remarkable observation that high protein diets, irrespective of their cystine content, cause an increased excretion of cystine in cystinuria.

[42] Biochem. J., **23**, 138 (1929).
[43] J. Biol. Chem., **81**, 285 (1929).

They state that the excretion of cystine varies with the total nitrogen excretion (i.e., with the protein intake) and does not appear to be related to the cystine content of the diet. It is therefore suggested that " the effect of high protein diets in increasing the urinary output of cystine in cystinuria may be due . . . to a stimulation by the protein of some processes of endogenous metabolism, which results in the production of cystine, rather than to a failure to oxidize the exogenous cystine."

In a case of cystinuria recently studied by Brand, Harris and Biloon,[44] the freshly voided urine did not contain free cystine but a cystine complex of undetermined constitution. When the urine was allowed to stand this compound gradually decomposed, liberating the free amino acid.

The occurrence in the urine of cystinurics of other amino acids, such as lysine and tyrosine, and the diamines, putrescine and cadaverine, has been reported on several occasions. In 1911, Ackermann and Kutscher[45] isolated lysine from the urine of a cystinuric patient. The same patient was in 1927 under the observation of F. A. Hoppe-Seyler,[46] who isolated arginine. Results of this nature are suggestive of a more generalized metabolic disturbance than one involving cystine alone. However, in the case studied by Robson, there was no evidence of the presence in the urine of lysine, tyrosine, putrescine or cadaverine.

Owing to its insolubility, the cystine may contribute to the formation of urinary concretions. It is usually stated that there are no other well-defined pathological symptoms. On the contrary, Robson, in referring to his patient's history, makes this statement: " Special interest was taken in the case because of the strong family history of the disease, several members having already died from the consequences of this disturbance."

Kaufman [47] describes a case of cystinuria in a twenty-one-month-old boy which came to autopsy. There were chalky deposits of cystine in various internal organs such as the kidneys, wall of the intestines, mesenteric nodes, liver, and particularly in the spleen. Abderhalden,[47a] to whom the tissues were sent for analysis, made a careful investigation of the dead child's relatives. There were two living brothers, one 14 months and the other 5½ years old. Both were cystinurics. Another brother had died at 17 months and a sister at the age of 9½ months under

[44] J. Biol. Chem., **86**, 315 (1930).

[45] Z. f. Biol., **57**, 354 (1911).

[46] Deut. Arch. klin. Med., **154**, 97 (1927).

[47] E. Kaufman, Pathology, translated by Stanley P. Reimann, Philadelphia, 1929, vol. II, p. 1413.

[47a] Z. physiol. Chem., **38**, 557 (1903).

apparently similar circumstances. It was established that the father of these children and the paternal grandfather had cystinuria. Negative results were obtained in the case of the mother, although Abderhalden states that when the urine was treated with alkali and lead acetate and heated a blackening was obtained. The nature of the sulfur-compound giving this test he was not able to determine. The paternal grandmother was negative.

Equally interesting are the two cases of cystinuria described by Lignac,[47b] one in a three-year-old boy and the other in a boy 2 years old. Both children were markedly underweight, had never learned to walk, and the older had stopped growing at the age of two years. On *post mortem* examination extensive deposits of cystine were found in all parts of the body, but especially in the kidneys. That cystinuria, particularly in the young, may be associated with a very severe and extensive pathology is clearly shown by these cases.[48]

Metabolism of Aspartic Acid.—The intermediary steps in the metabolism of aspartic acid are not definitely known. If oxidation occurred at the carbon atom attached to the amino group, keto-succinic acid would be formed. This compound is known to be converted into pyruvic acid when treated with macerated liver or muscle tissue. A different view is held by Ringer and Lusk,[49] who believe that aspartic acid is converted into malic acid and subsequently into β-lactic acid. Ackerman [50] found that aspartic acid, incubated with putrefying pancreas, produces β-alanine. This cannot, however, be the normal path of aspartic acid metabolism, for Corley [51] has shown that β-alanine is not converted into glucose in the completely phlorhizinized animal. The student will note, however, that whatever the intermediate products may be, they are in part sugar-forming. Indeed, it has been shown by Ringer and Lusk that in phlorhizinized dogs the equivalent of three carbon atoms of the four in aspartic acid can be accounted for in the form of extra glucose in the urine. Although the paths of metabolism of this amino acid in normal oxidation have not been precisely formulated, its transformation into glucose, on the basis of available knowledge, may be represented as follows:

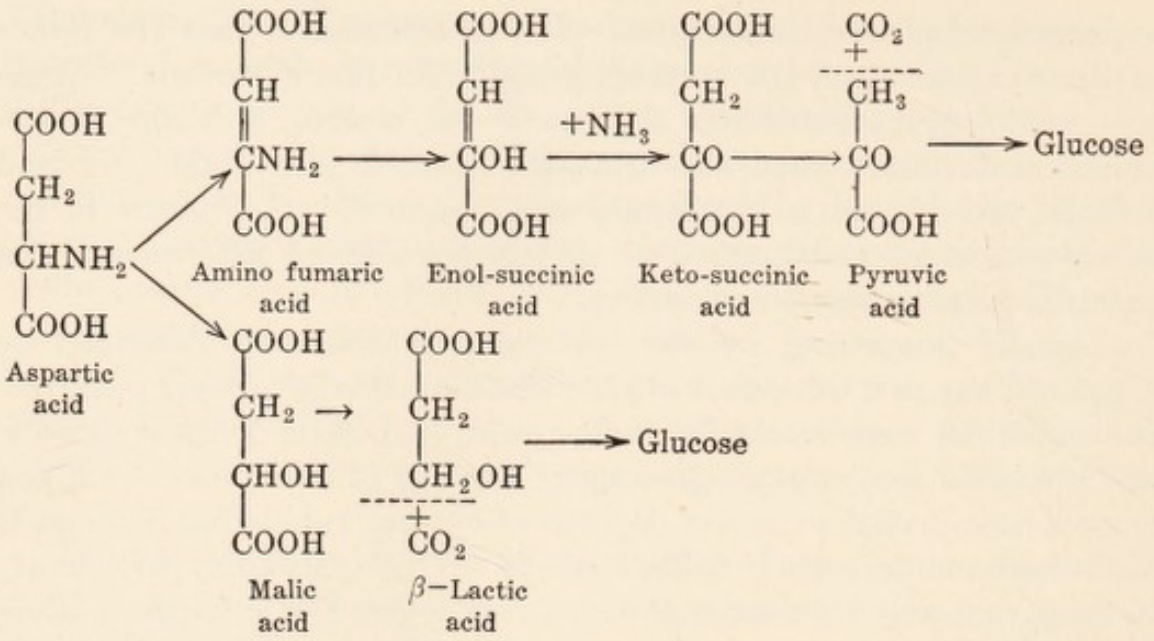
[47b] Deut. Arch. klin. Med., **145**, 139 (1924).

[48] Various aspects of the problem of cystinuria are represented in the following papers: Alsberg, C. L., and Folin, O., Am. J. Physiol., **14**, 54 (1905); Wolf, C. G. L., and Shaffer, P. A., J. Biol. Chem., **4**, 439 (1908); Looney, J. M., Berglund, H., and Graves, C. R., *ibid.*, **57**, 515 (1923); Magnus-Levy, A., Biochem. Z., **156**, 150 (1925); Rosenfeld, G., Ergeb. Physiol., **18**, 118 (1920).

[49] G. Lusk, The Science of Nutrition, 1928 edition, p. 242.

[50] Z. Biol., **56**, 87 (1911).

[51] J. Biol. Chem., **81**, 545 (1929).



Metabolism of Glutamic Acid.—Only three carbon atoms of the five in glutamic acid are converted into glucose in the completely diabetic animal (Lusk).[52] As in the case of aspartic acid, the formation of sugar has been explained in more than one way. Deamination at the α-carbon atom and subsequent oxidation at the β-carbon atom would yield glyceric acid. This, in turn, would be oxidized completely in the normal animal, or it would be converted into sugar, carbon for carbon, in the completely diabetic animal. On the other hand, it is possible to conceive that keto-glutaric acid is formed first, and that this is converted into either malic or succinic acid. Both of these compounds are non-toxic and presumably are oxidized in the body. It would be much more difficult to postulate the intermediary formation of glutaric acid,

$$(COOH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH),$$

for this compound, when injected, is severely nephropathic (Rose).[53]

Metabolism of Arginine.—The liver contains an enzyme (arginase) capable of hydrolyzing arginine with the production of urea and ornithine (Kossel and Dakin).[54] The fate of ornithine is believed to be similar to that of glutamic acid. Both ornithine and arginine yield sugar in phlorhizin diabetes, in amounts sufficient to account for three carbon atoms, or one-half the total number in arginine (Dakin).[55]

[52] Am. J. Physiol., **22**, 174 (1908).

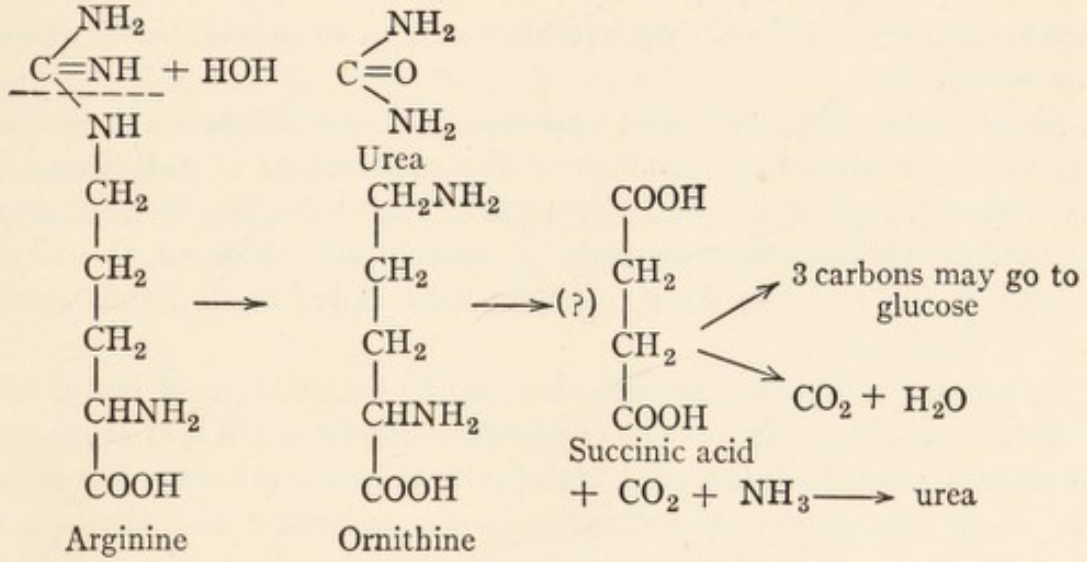
[53] J. Pharmacol. Exp. Therap., **24**, 123, 147 (1924).

[54] Zeit. Physiol. Chem., **41**, 321 (1904); **42**, 181 (1904).

[55] H. D. Dakin, Oxidations and Reductions in the Animal Body, 1922 edition, p. 81.

Another pathway of metabolism has been suggested, namely one involving the conversion of arginine into guanidine-butyric acid, $(NH_2 \cdot C(=NH) \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH)$, which by β-oxidation would yield guanidine acetic acid $(NH_2 \cdot C(=NH) \cdot NH \cdot CH_2 \cdot COOH)$. By methylation, the latter would yield creatine $(NH_2 \cdot C(=NH) \cdot NCH_3 \cdot CH_2 \cdot COOH)$. There is, however, no experimental evidence in support of this idea.[56] The conversion of guanidine-acetic acid into creatine seems to be established,[57] but arginine itself is not definitely known to be a precursor of either creatine or creatinine.[58] In experiments with rats, Rose and Cook[59] could find no relationship between the arginine content of the diet and the total creatinine elimination in the urine. More recently Hyde and Rose[59a] fed daily an amount of arginine, equivalent to 1 g. of creatine, to a male and a female subject, and although the experiment was continued for six and eight weeks, respectively, there was no evidence of an increased output of either creatine or creatinine.

A portion of the metabolism of arginine may be represented as follows:



[56] K. Thomas and Goerne, M. G. H., Z. physiol. Chem., **92**, 163 (1914); **104**, 73 (1918–19).

[57] Baumann, L., and Hines, H. M., J. Biol. Chem., **31**, 549 (1917).

[58] Compare, Thompson, W. H., J. Physiol., **51**, 111 (1917). For an excellent review of the subject the student is advised to consult Mitchell, H. H., and Hamilton, T. S., Biochemistry of Amino Acids (1929), p. 333.

[59] J. Biol. Chem., **64**, 325 (1925).

[59a] J. Biol. Chem., **84**, 535 (1929).

The chemical relationship of arginine to creatine will be shown in the next chapter.

Metabolism of Histidine.—The fate of histidine in metabolism is equally obscure. Unlike arginine, it is not a sugar-forming amino acid. It is not definitely known to give rise to acetone bodies.[60]

Leiter [61] found that histidine injected intravenously into dogs was almost completely utilized. Even when as much as 5 grams were given, the increase in urinary imidazoles could account for only about 150 mg. of histidine. On the contrary, the injection of 1 gram quantities of methyl-imidazole and methyl-imidazole-lactic acid resulted in an excretion in the first 24 hours of approximately 30 and 40 per cent, respectively. Imidazole itself was not utilized appreciably, for 93 per cent of the 0.5 gram of this substance injected was recovered in the urine. Leiter states that in every case the increased urinary imidazole output was due entirely to the presence of the same imidazole as the one injected. These results indicate that the body has a high capacity for destroying the imidazole ring when attached to a side chain, particularly as in histidine, but that it does not have this capacity in the absence of the side chain. Leiter's conclusion was that none of the compounds which he used was an intermediary in the metabolism of any of the others. In considering Leiter's data in the light of later investigations, it seems that the possibility of partial conversion of imidazole-lactic acid to histidine, in his experiments, has not been ruled out.

It has been suggested that urocanic acid, β-imidazole-acrylic acid, may be an intermediate product of the metabolism of histidine. Its occurrence in dogs' urine (Jaffe),[62] especially after feeding large amounts of histidine (Kotake and Konishi),[63] and in the urine of the coyote (Swain) [64] has been reported. Others have failed to find this constituent in the urine.

Particular attention has been devoted to the relation of histidine to purine metabolism. In a series of experiments, Ackroyd and Hopkins [65] found that when histidine and arginine were removed from the diet of rats, there was a marked reduction in the excretion of allantoin, the latter being the chief end-product of purine metabolism in rats. More-

[60] Compare Dakin, H. D., and Wakeman, A. J., J. Biol. Chem., **10**, 499 (1912); Dakin, *ibid.*, **14**, 321 (1913); Konishi, M., Z. physiol. Chem., **122**, 237 (1922).

[61] J. Biol. Chem., **64**, 125 (1925).

[62] Ber., **7**, 1669 (1874); **8**, 811 (1875).

[63] Z. physiol. Chem., **122**, 230 (1920).

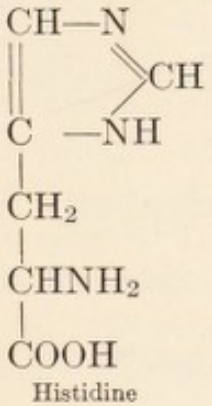
[64] Am. J. Physiol., **13**, 30 (1905).

[65] Biochem. J., **10**, 551 (1916).

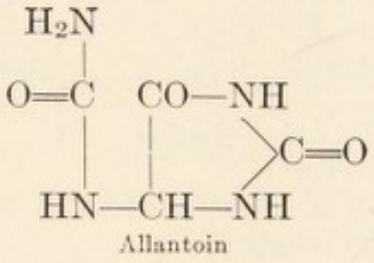
over, these amino acids are essential for growth, as we shall see in another connection. Ackroyd and Hopkins reported that the addition of either arginine or histidine, or both, to the histidine-arginine-deficient diet caused a resumption of growth as well as a return to normal values in the elimination of purine bodies in the urine. They therefore concluded that arginine and histidine were interchangeable, both in the matter of growth and as precursors of allantoin.

Contrary to the observations of Ackroyd and Hopkins, Rose and Cox [66] have shown that arginine and histidine are not interchangeable for purposes of growth, the former being without influence upon the rate of growth of rats deprived of histidine. In another investigation, Rose and Cook [59] were able to show that arginine and histidine are not interchangeable as precursors of purines. In agreement with the observations of Ackroyd and Hopkins, they found that histidine is definitely related to purine metabolism. On a diet free from histidine and arginine, Rose and Cook observed a marked reduction of both allantoin and uric acid. There was, likewise, a moderate reduction in the output of creatinine. The addition of histidine alone to the arginine-histidine deficient diet led to an increased elimination of uric acid and allantoin, as well as of creatinine, whereas the addition of arginine alone was without effect on the output of any of the urinary constituents. More recently Cox and Rose [67] have shown that imidazole-lactic acid may replace histidine in metabolism. Similar observations were reported at about the same time by Novello, Harrow and Sherwin.[68]

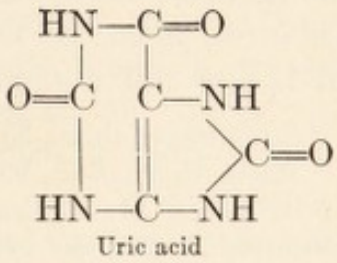
It is to be noted here that histidine is not the only source of purines in metabolism. Further than this, no attempt will be made here to trace the fate of histidine in the body. The following formulas bring out the structural relationship between histidine, allantoin and uric acid:



Histidine



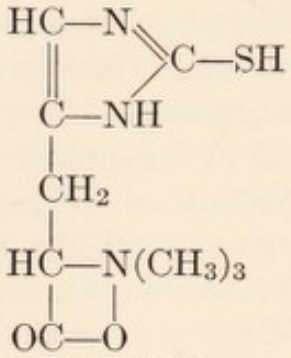
Allantoin



Uric acid

[66] J. Biol. Chem., **61**, 747 (1924); **68**, 217 (1926).
[67] J. Biol. Chem. (1926), **68**, 781 (1926).
[68] *Ibid.*, **70**, 683 (1926).

In 1909, Tanret [69] isolated a base from ergot, which Barger and Ewins [70] identified as the betaine of thiolhistidine, represented by the following structural formula:



Ergothioneine
(Thioneine)

This substance was named ergothioneine. Several years ago it became apparent to two independent groups of investigators [71, 72] that the blood corpuscles contained a hitherto unknown sulfur compound. This was eventually shown to be identical with ergothioneine, first by Newton, Benedict and Dakin [73] and later by Eagles and Johnson and Eagles and Hunter.[74] Benedict and his associates have suggested that the term ergothioneine be contracted to *thioneine*. Human blood is reported to have 10–25 mg. per 100 cc. of this substance and hog's blood, 14.5 mg. per 100 cc. However, considerable variations have been observed in the blood of pigs from different localities. Eagles and Vars [75] have reported certain experiments on pigs which they fed protein hydrolyzates and they suggest that the precursor of thioneine may be thiolhistidine.

The physiological significance of thioneine remains to be determined.

Metabolism of Lysine.—Since Dakin has shown that lysine yields neither glucose nor acetone bodies in phlorhizin diabetes, all pathways of metabolism that would give rise to either ketogenic or anti-ketogenic substances must be excluded. Corley [76] states that ϵ-aminocaproic acid is also not a sugar-former in the completely phlorhizinized dog.

[69] J. pharm. et chim., **30**, series 6, 145 (1909).

[70] Trans. Chem. Soc., **99**, 2336 (1911).

[71] Bulmer, F. M. R., Eagles, B. A., and Hunter, G., J. Biol. Chem., **63**, 17 (1925); Hunter and Eagles, *ibid.*, **65**, 623 (1925).

[72] Benedict, S. R., J. Biol. Chem., **64**, 215 (1925); Benedict, Newton, E. B., and Behre, J. A., *ibid.*, **67**, 267 (1926).

[73] Newton, E. B., Benedict, S. R., and Dakin, H. D., Science, **64**, 602 (1926); J. Biol. Chem., **72**, 367 (1927).

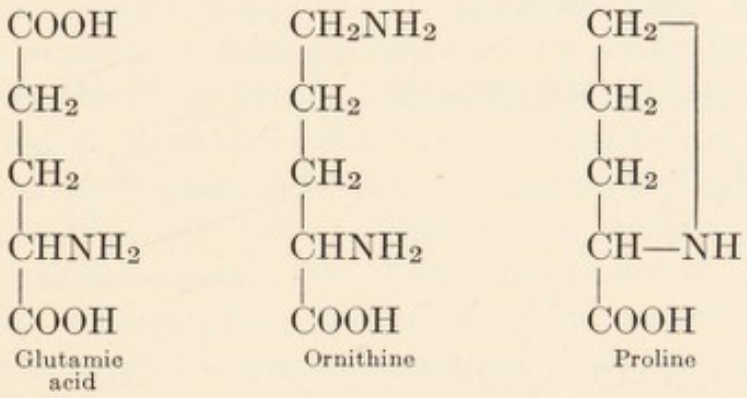
[74] Eagles, B. A., and Johnson, T. B., J. Am. Chem. Soc., **49**, 575 (1927); Hunter and Eagles, J. Biol. Chem., **72**, 123 (1927).

[75] J. Biol. Chem., **80**, 615 (1928).

[76] J. Biol. Chem., **81**, 545 (1929).

The suggestion has been made by Ringer, Frankel, and Jonas [77] that oxidation of lysine occurs by way of glutaric acid, but this idea needs more evidence to support it.

Metabolism of Proline.—The structural relationship of proline, ornithine, and glutamic acid, which is brought out by the following formulas, suggests the probability that these compounds share a common fate in metabolism.



In support of this idea may be mentioned the work of Dakin, who determined that the conversion of proline into glucose in phlorhizin glycosuria is sufficient to account for three of the five carbon atoms in proline. It will be recalled that only three carbon atoms of glutamic acid and of arginine are capable of conversion into glucose.

Moreover, there is some ground for the assumption that proline may be synthesized in the body from glutamic acid. Indeed, part of this transformation, namely the synthesis of pyrrolidone carboxylic acid ($CO \cdot CH_2 \cdot CH_2 \cdot CHNH \cdot COOH$), has been accomplished in the laboratory (Abderhalden and Kautzsch).[78]

Metabolism of Tryptophane.—One of the constituents of dog's urine is kynurenic acid. This compound is obviously derived from tryptophane and represents the only intermediate stage in tryptophane metabolism about which we know anything at all. It is very doubtful whether kynurenic acid is formed in man and in those animals in which it does not occur normally in the urine.

According to Dakin,[79] the first step in the metabolism of tryptophane is the formation of indolepyruvic acid. When this compound is injected into rabbits, it behaves like tryptophane in forming kynurenic acid. The second step is believed to involve the opening of the

[77] *Ibid.*, **14**, 539 (1913).

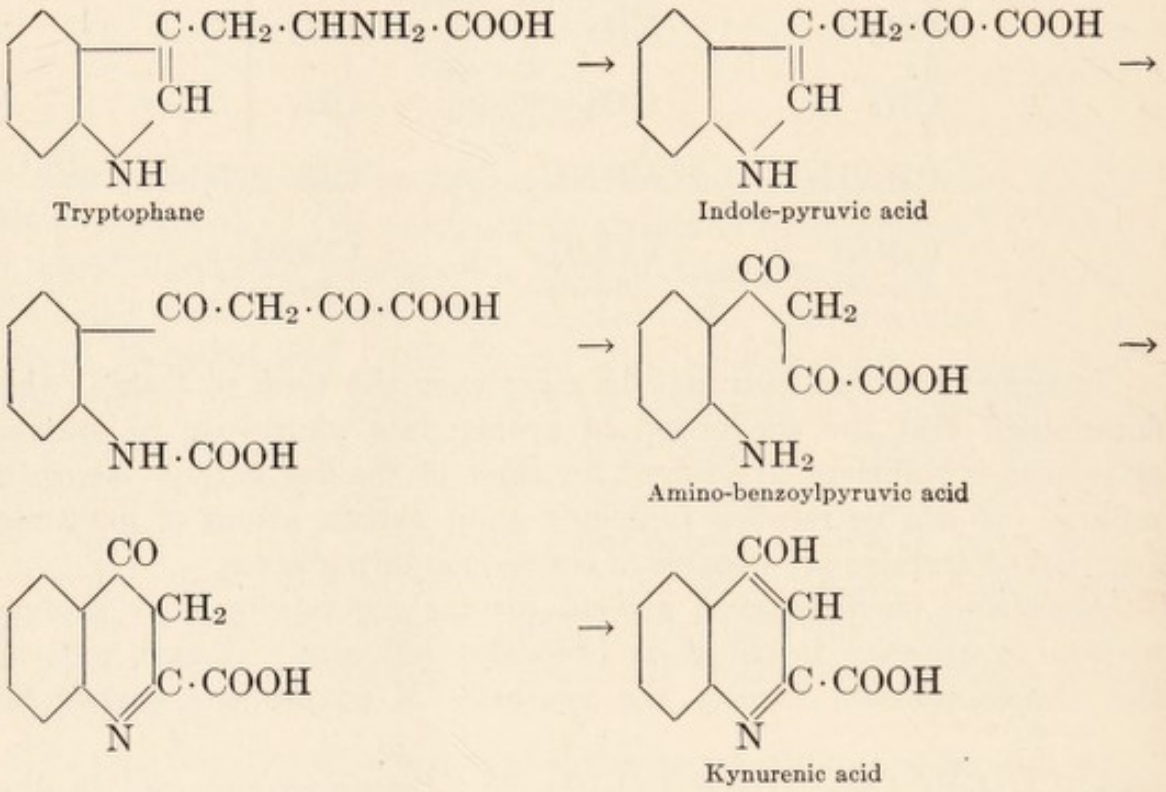
[78] Z. physiol. Chem., **68**, 487 (1910).

[79] H. D. Dakin, Oxidations and Reductions in the Animal Body, Longmans, Green & Co., 1922 edition, p. 96.

pyrrol ring with the formation of an acid, which by losing carbon dioxide yields amino-benzolypyruvic acid. By closure of the ring, this is believed to yield kynurenic acid.

What happens to the kynurenic acid is a mystery. In man, if taken by mouth, it disappears completely. However, when given subcutaneously, a portion is excreted unchanged.

The formation of kynurenic acid may be represented as follows (Ellinger and Matsuoka[80]):



Life cannot be maintained indefinitely on a diet lacking tryptophane, for the body cannot synthesize this amino acid *de novo*. However, it has been shown recently that indole-pyruvic acid can serve as a biological substitute for tryptophane.[81] The rôle of this amino acid in nutrition will be discussed in a later chapter.

Summary.—The preceding discussion of protein metabolism has been limited almost entirely to a consideration of the fate of the individual amino acids in the body. From the information which is available, it is obvious that we are far from having a complete picture of the metabolism of all the amino acids.

Without the proper scientific attitude, the student is likely to

[80] Z. physiol. Chem., **109**, 259 (1920).

[81] Jackson, R. W., J. Biol. Chem., **84**, 1 (1929); Berg. C. P., Rose, W. C., and Marvel, C. S., *ibid.*, **85**, 219 (1929).

acquire the notion that our knowledge of protein metabolism is so uncertain, so confused, as to make an attempt at accurate formulation unnecessary. This is emphatically not the case. It is to be admitted that we know very little of what happens to valine, lysine, histidine, and tryptophane in the body. It is also true that we are uncertain of many details of the metabolism of other amino acids. On the other hand, we do know that in the oxidation of certain amino acids (glycocoll, alanine, serine, cystine, aspartic and glutamic acids, proline, and arginine), intermediate products are formed which are probably identical with those obtained in the metabolism of glucose and which therefore may be expected to share a common fate. Then there are those amino acids (leucine, phenylalanine and tyrosine) which yield β-hydroxybutyric acid as an intermediate product. Thus we are enabled to correlate the metabolism of protein with that of the other foodstuffs.

Other phases of metabolism, such as the utilization of cystine in the formation of taurine, the origin of uric acid and allantoin from histidine, and the relation of homogentisic acid to tyrosine, have also been pointed out. With this knowledge to guide us, we can proceed further in the study of protein metabolism. To become well informed in the subject of biological chemistry, or for that matter in any subject, the student should ascertain what is known, what is not known, and what is uncertain. A knowledge of what remains to be discovered is often more valuable than a knowledge of established facts.

CHAPTER XIII

INTERMEDIARY METABOLISM OF PROTEIN (Continued)

UREA

In mammals, amphibia, and most fishes, the chief end-product of protein metabolism is urea; in birds and reptiles, it is uric acid. The amount of urea formed depends on the diet, being greater on a high-protein diet than on a low one. A normal adult, on a protein intake of 100 to 120 grams, excretes about 30 grams of urea, which, expressed in terms of nitrogen, is equivalent to about 15 grams. The urea nitrogen excreted in twenty-four hours usually varies between 80 and 90 per cent of the total nitrogen, but when the total nitrogen is very low (4–7 grams), the per cent in the form of urea nitrogen is much lower (about 50 or 60 per cent of the total nitrogen).

A portion of the urea has its origin in arginine, but the major part is formed in the body from the ammonia which is split off in the deamination of amino acids. It has been supposed that the ammonia combined with carbon dioxide to form ammonium carbonate which, by the loss of one molecule of water, yielded ammonium carbamate and, by the loss of a second molecule, yielded urea. These relations are indicated by the following formulas:

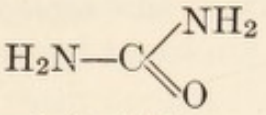
$$2NH_3+CO_2+H_2O \rightleftarrows \underset{\text{Ammonium carbonate}}{C(=O)(ONH_4)_2} \rightleftarrows \underset{\text{Ammonium carbamate}}{C(=O)(ONH_4)(NH_2)} + H_2O \rightleftarrows \underset{\text{Urea}}{C(=O)(NH_2)_2} + H_2O$$

Ammonium carbamate and abnormally large quantities of ammonia have been found in the blood and urine of dogs in which an Eck fistula had been produced. In these animals there is a communication between the portal vein and the inferior vena cava so that the blood does not pass through the liver. Consequently there is a failure in the conversion of ammonia into urea. Ammonium carbamate, conjugated in the form of uramino compounds, has been found in normal urine, but Sherwin [1] believes it very probable that most of the uramino com-

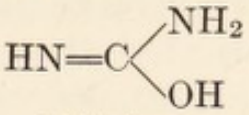
[1] Physiol. Reviews, **2**, 273 (1922).

pounds thus obtained are formed during the evaporation of the urine by the interaction of amino compounds with urea.

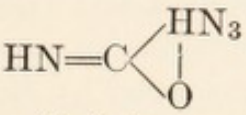
There is perhaps another and more probable method of urea formation in the body. A reaction between ammonia and carbon dioxide may be supposed to yield cyanic acid, which would yield urea when acted upon by another molecule of ammonia. It is quite likely that urea exists in several tautomeric forms, some of which may be represented as follows:



Carbamide form



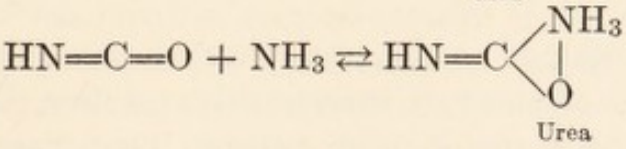
Imide form



Cyclic form

According to Werner,[2] urea exists chiefly in the cyclic form. At least in part, the formation of urea may be supposed to take place as follows:

$$CO_2 + NH_3 \rightleftarrows \underset{\text{Cyanic acid}}{HN{=}C{=}O} + H_2O;$$



Urea

In support of this idea may be mentioned the work of Fearon,[3] who has studied the mechanism of urease action. The enzyme urease is found in the jack bean, soy bean, and elsewhere, and is capable of bringing about the hydrolysis of urea to ammonia. Upon this behavior are based nearly all the methods for the determination of urea in the blood, urine, and tissues. It has been assumed that carbamic acid is formed as an intermediate product in the decomposition of urea by urease. Fearon could obtain no evidence for the formation of carbamic acid, but was able to prove the formation of cyanic acid as an intermediate product. These results are comparable with those obtained by Werner, who has shown that acid, alkali, and heat "hydrolysis" of urea, in aqueous solution, occurs in two stages (*a*) the simple dissociation of the urea molecule into ammonia and free cyanic acid, and (*b*) the hydrolysis of the cyanic acid by the solvent.

$$(a)\quad CON_2H_4 \rightarrow NH_3 + HNCO,$$
$$(b)\quad HNCO + H_2O = NH_3 + CO_2$$

In contradiction to Fearon's view, Sumner [4] does not regard cyanic

[2] Werner, E. A., Urea, Longmans, Green & Co., New York, 1923.
[3] Biochem. J., **17**, 84, 800 (1923); Physiol. Rev., **6**, 399 (1926).
[4] J. Biol. Chem., **68**, 101 (1926).

acid to be an intermediate product of the hydrolysis of urea by the enzyme urease.

Function of the Liver in the Production of Urea.—The site of the formation of urea has been the subject of numerous investigations. All possible conclusions have been reached, namely (*a*) that urea is formed exclusively in the liver, (*b*) that urea is formed by all the tissues, but chiefly by the liver, (*c*) that all the tissues contribute to the formation of urea and that the liver plays no special rôle in this regard. The experimental evidence upon which these views are based has recently been reviewed by Bollman, Mann, and Magath.[5]

Fiske and Sumner,[6] working with cats, found that the accumulation of urea per unit of mass, in the blood and tissues, after intravenous administration of amino acids, was as great (in the absence of shock) when the liver and other abdominal viscera were excluded from the circulation by ligation as when they were in their normal relations. From this they concluded that the liver was not the chief site of urea formation.

Folin and Berglund [7] employed as their criterion the relation between the concentration changes of amino acids and urea during absorption, and assumed that a rise in urea nitrogen preceding a rise in amino nitrogen would furnish positive proof that the liver was concerned in urea formation. In their experiments, however, the amino nitrogen increased first, and the urea nitrogen increased only after the amino nitrogen began to subside. From these observations they concluded that the liver had no special function in connection with deamination or urea formation.

Many similar observations, especially those of Van Slyke [8] and Morgulis,[9] fail to support this view.

A very important contribution to the subject has recently been made by Bollman, Mann, and Magath. These workers studied the effect of complete removal of the liver on urea formation in more than 90 dogs. In every case where urine was secreted after the operation, it was found to contain much less urea than normally. The urea content of the blood and tissues was likewise diminished very markedly. Assuming that no urea was being formed because of the absence of the liver, it was reasonable to argue that if the excretion of urea were prevented in some way, the urea of the blood and tissues would neither

[5] Am. J. Physiol., **69**, 371 (1924).
[6] J. Biol. Chem., **18**, 285 (1914).
[7] *Ibid.*, **51**, 395 (1922).
[8] Harvey Lecture, **11**, 146 (1915–16).
[9] J. Biol. Chem., **66**, 353 (1925).

increase nor decrease but remain constant. The correctness of this assumption Bollman [5] and his associates were able to prove in a very satisfactory manner. From certain dogs they removed both kidneys and the liver simultaneously. Because of the removal of the kidneys and the resulting anuria, there would have followed a progressive accumulation of urea in the system if any were being manufactured. Their results showed very definitely, however, that the blood urea remained at a constant level after the operation. In another series of experiments, they removed both kidneys and, after a given interval, during which there occurred a progressive increase of urea in the blood, the liver as well. Immediately after the second operation, the increase in urea ceased and its concentration remained at a fairly constant level during the remainder of the experiment. It seemed obvious, therefore, that the production of urea in the body of the dog is entirely dependent on the presence of the liver, since urea formation ceases completely as soon as the liver is removed.

Rabinowitch [10] has reported an unusual case of acute yellow atrophy of unknown origin in which the involvement of the liver was so severe that on *post mortem* examination it looked as though all the glandular epithelium had disappeared, leaving the framework only. Microscopic study of many sections revealed only isolated liver cells and the staining properties of even these were poor. Under these circumstances the liver must have been practically without function. The kidneys were likewise extensively damaged so that there was almost complete suppression of urine secretion. Before death this patient was in a condition not unlike that of the experimental animals, used by Bollman, Mann and Magath, in which both the liver and kidneys had been removed. The biochemical findings were likewise similar. The amount of urea found in the urine was practically negligible both on account of the low concentration (0.07 per cent) and the exceedingly small volume (not more than 20 cc. per 24 hours, according to a personal communication). The blood sugar concentration was 0.046 per cent before and 0.03 per cent after fermentation, which shows that only 16 mg. of glucose was present in 100 cc. of blood. There was no urea in the blood. On the contrary, the amino acid nitrogen concentration was very high, namely 216 mg. per 100 cc., which shows that there was marked retention of amino acids due to impaired renal function and practically no conversion of these into urea. These findings, unique in the literature, confirm the conclusions of Bollman, Mann and Magath that the liver is the site of urea formation.

One may recall in this connection the remarkable experiments of

[10] J. Biol. Chem., **83**, 333 (1929).

Minkowski,[11] who, on extirpating the livers of geese, discovered that the uric acid content of the urine was markedly diminished, being replaced by ammonia. These observations indicated that the tissues contributed little if anything to the conversion of ammonia into uric acid. The formation of uric acid in birds, as has already been pointed out, is the analogue of urea formation in mammals.

Conversion of Urea into Ammonia.—The question of a reversal in the body of the $NH_3 \rightarrow$ urea reaction is one that has captured the interest of many workers. It was the opinion of Wakeman and Dakin [12] that the formation of urea from ammonia in the body is an irreversible process. More recently the opposite view has received a greater amount of support, particularly at the hands of Nash and Benedict.[13]

The normal daily excretion of ammonia in the urine is quite appreciable, being usually in the neighborhood of 0.7 gram. On the contrary, the concentration of ammonia in the blood is exceedingly small, being but a few hundredths of a milligram per 100 cc. of blood. It is, of course, conceivable that the kidney may be able to concentrate ammonium salts to a greater extent than other urinary constituents, but even granting this, there would still be many facts left unexplained. The ammonia elimination may be modified, especially by the intake of acids and fixed bases. The former causes an increase in ammonia elimination, the latter a decrease. Acidosis is characterized by a high ammonia output in the urine. The significance of these changes has been determined through the study of the acid-base equilibrium in conditions of acidosis. The organism can apparently afford to lose considerable quantities of ammonia much better than it can afford to lose fixed bases (K, Na, Ca). Therefore, any ammonia which may be formed for the purpose of neutralizing acid spares an equivalent amount of fixed base. However, it is doubtful whether ammonia plays any part in the neutralization of acids transported in the blood. Now the question is, "What is the origin of urinary ammonia?"

Ammonia absorbed from the intestine would presumably be converted into urea on reaching the liver, and one is therefore forced to the conclusion that other substances, namely, either urea or the amino acids or both, may be the normal precursors of urinary ammonia. Until several years ago, it was generally assumed that ammonia formation could take place in all tissues and that the urinary ammonia had its origin in this way.

The experimental evidence upon which was based this idea of the

[11] Arch. f. Exp. Path. u. Pharm., **21**, 41 (1886).

[12] J. Biol. Chem., **9**, 327 (1911).

[13] *Ibid.*, **48**, 463 (1921); **69**, 381 (1926); Benedict and Nash, *ibid.*, **82**, 673 (1929).

vicarious formation of ammonia is no longer regarded to be adequate, and as we shall see, the experiments of Nash and Benedict, and others, make the older view of the origin of urinary ammonia open to serious doubt.

It has been discovered by Parnas and Mozolowski [14] and Embden [15] that associated with traumatic injury and any kind of rigor in muscle, ammonia is formed. More recently Parnas has stated that "muscular activity is always, without exception, associated with ammonia production." The source of the ammonia during anaerobic activity is said to be adenine nucleotide (adenylic acid), which is thus converted to inosinic acid (p. 364). When oxygen is available and muscle (frog's) is working without fatigue large amounts of ammonia accumulate, but there is no decrease of the adenine nucleotide. Parnas attributes its continual restoration under these conditions to the utilization, not of the ammonia once split off, but to the ammonia probably derived by the oxidative deamination of amino acids or their derivatives. To quote Parnas: "In living muscle the system adenine nucleotide → inosinic acid does oscillate between its two components, being discharged during activity, recharged during recovery." This conception, involving as it does ammonia formation as a phenomenon of muscular activity, is both novel and attractive and many workers have been drawn to further investigation of this important problem.

From this it does not follow that the ammonia formed in the muscle is necessarily the ammonia which is excreted in the urine. In attempting, however, to associate the two, Bliss [16] has postulated that the ammonia is transported as an amide in combination with the blood proteins and that this complex is enzymically deaminized in the kidney, resulting in the liberation of ammonia which in turn combines with an acid radical (such as lactate) and is excreted in the urine. A criticism of this view is to be found in the recent paper by Benedict and Nash.[16a]

The experiments of Nash and Benedict [13] furnish excellent evidence that the kidney is the site of ammonia formation. If the ammonia formed by the muscles or by other tissues were to be excreted as such in the urine, the effect of extirpating the kidneys or of tying off the ureters would be an accumulation of ammonia in the blood. Instead, Nash and Benedict found that these surgical manipulations produced no

[14] Biochem. Z., **184**, 399 (1927); Proc. Thirteenth International Physiol. Congress, Am. J. Physiol., **90**, 467(1929).

[15] Z. physiol. Chem., **179**, parts 4, 5 and 6 (1928).

[16] J. Biol. Chem., **81**, 137 (1929).

[16a] J. Biol. Chem., **82**, 673 (1929).

change in the ammonia content of the blood, although there was abundant evidence of retention of other non-protein nitrogenous constituents. They were also able to show that the blood collected from the renal vein in dogs contained on an average twice as much ammonia as was present in blood collected from other sources, such as the vena cava and carotid artery. It would appear, therefore, that the kidney, instead of excreting ammonia from the blood, actually forms the ammonia which it excretes, and in addition contributes a small amount of ammonia to the blood.

These observations have been confirmed by Loeb, Atchley and E. M. Benedict,[17] who likewise observed that the blood from the renal vein of the dog contained more ammonia than blood from the vena cava or femoral artery. In rabbits the situation is somewhat different, as might be expected from the fact that these animals excrete an alkaline urine which contains only traces of ammonia. Accordingly, little ammonia should be formed in the kidney and hence its content in the blood of the renal vein should not differ appreciably from the concentrations elesewhere in the circulation. This is precisely what Loeb and his associates found.

One of the first manifestations in experimental uranium nephritis is a marked reduction in the excretion of ammonia; this occurs long before there is any evidence of nitrogenous retention. Hendrix and Bodansky[18] have attributed this to renal injury, which, according to the view of Nash and Benedict, might be expected to interfere with the normal function of the kidney in producing ammonia.

It will be recalled that in hepatectomized dogs there is a cessation of urea production. Mann and Bollman[19] have observed that when the urine of dehepatized dogs becomes extremely low in urea, the ammonia content is likewise diminished. If at this time urea is injected intravenously, there is a definite increase in ammonia excretion. This would indicate that urea is probably the precursor of urinary ammonia.

CREATINE AND CREATININE

Creatine is methyl-guanidine acetic acid. It is widely distributed in animal tissues and is especially abundant in skeletal muscle. Calculations based on chemical analyses of the tissues indicate that the adult human body contains on an average about 100 grams of creatine,

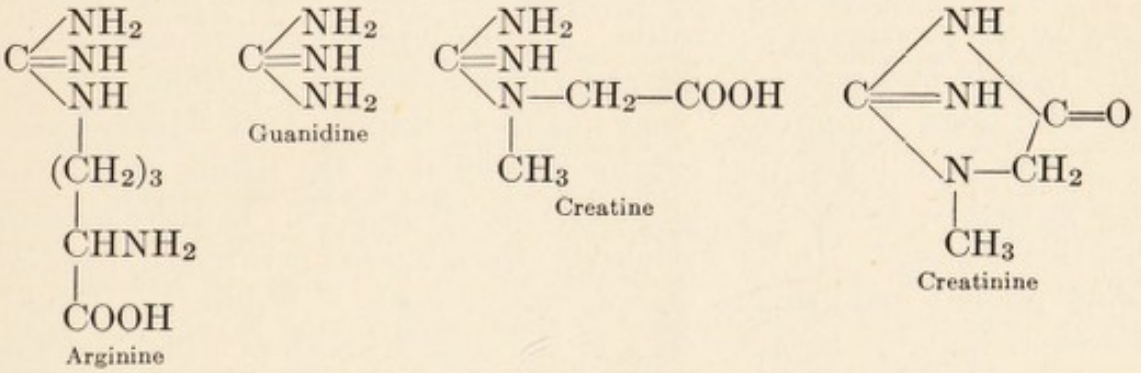
[17] *Ibid.*, **60**, 491 (1924).
[18] J. Biol. Chem., **60**, 657 (1924).
[19] Am. J. Physiol., **85**, 390 (1928).

most of which is present in the muscles where its concentration normally is about 400 mg. per 100 grams.

Creatinine is the anhydride of creatine and is a normal constituent of urine. From 1 to 2 grams of this substance is excreted daily by an adult man or woman, this amount being constant from day to day, especially in males. Normally, creatine is not found in the urine of male adults. It is occasionally found in the urine of females, and in that of children it is a normally occurring constituent. As first shown by Folin,[20] the elimination of creatinine is not influenced by the amount of protein in the diet, but is apparently a measure of endogenous protein metabolism.

When heated in acid solution, creatine is converted into creatinine; in an alkaline solution the reverse change takes place. The close chemical relationship between creatine and creatinine, and the ease with which one is changed into the other in the laboratory, would suggest that they are similarly affiliated physiologically. The subject of creatine and creatinine metabolism has engaged the efforts of numerous biochemists, and yet our knowledge of it is still incomplete.

The relation of creatine and creatinine to arginine and guanidine is indicated by the following formulas:



Origin of Creatine.—First we shall consider briefly the theories which have been proposed for the origin of creatine. According to Knoop [21] and Neubauer [22] arginine may give rise in the body to γ-guanidine-butyric acid and in turn to guanidine-acetic acid, from which, by methylation, creatine would be formed. The conversion of guanidine-acetic acid into creatine has been reported by several investigators and seems to be fairly generally accepted, but on the other hand the metabolic relationship between arginine and creatine has not been established.

[20] Am. J. Physiol., **13**, 66 (1905).
[21] Z. physiol. Chem., **67**, 489 (1910).
[22] Handlexikon d. Biochem., **4**, 386 (1911).

In the pig, Gross and Steenbock [23] observed that arginine administered orally augments creatine excretion. Hyde and Rose,[24] on the contrary, after feeding arginine for as long as eight weeks found no evidence of its conversion into creatine or creatinine in man. A similar result was obtained by Grant, Christman and Lewis,[25] who fed arginine to a dog for thirty-five days and failed to influence the urinary excretion of either creatine or creatinine. In reviewing the evidence for the origin of creatine from creatinine, Hunter [26] states, "So large a body of almost purely negative evidence leads one rather forcibly to suspect that, if creatine is related to arginine at all, its mother substance must be not the free amino-acid, but the still combined arginine of the muscle or other tissue protein."

Another theory (Jaffe,[27] Paton [28]) postulates the formation of creatine from guanidine or methyl guanidine by a process of detoxication. A certain amount of evidence has been accumulated to show that the injection of guanidine salts into rabbits and other animals causes an increased output of creatinine in the urine as well as an accumulation of creatine in the muscles. It would probably be reasonable to accept this hypothesis, if only there were proof of the formation of guanidine in metabolism.

Koch [29] and Riesser [30] have suggested that choline and betaine may be the precursors of creatine. Riesser injected choline and betaine into rabbits and noted an increased elimination of creatinine. Working with dogs, Thompson [31] was unable to confirm these observations.

It has been noted that betaine is present in the tissues of invertebrates, such as the scallop and periwinkle (Wilson [32]). In these animals creatine is absent. In the vertebrates the situation is reversed, creatine being present and betaine absent, except rarely. Wilson has shown, however, that the lamprey eel and the dogfish, both of which are low in the scale of vertebrates, contain both creatine and betaine. As Hunter [33] points out, such data of comparative biochemistry, scattered

[23] J. Biol. Chem., **47**, 33 (1921).

[24] *Ibid.*, **84**, 535 (1929).

[25] Proc. Soc. Exp. Biol. Med., **27**, 231 (1929).

[26] A. Hunter, Creatine and Creatinine, Longmans, Green & Co., London and New York, 1928, p. 227.

[27] Z. physiol. Chem., **48**, 430 (1906).

[28] Rep. Brit. Assoc. Adv. Sci., 294 (1919), cited by Hunter.

[29] Am. J. Physiol., **15**, 15 (1905).

[3] Z. Physiol. Chem., **86**, 415 (1913); **90**, 221 (1914).

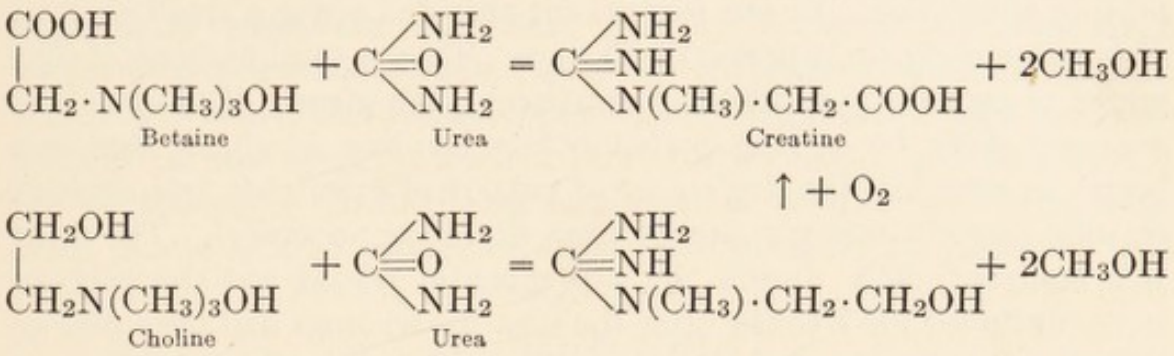
[31] J. Physiol., **51**, 347 (1917).

[32] J. Biol. Chem., **18**, 17 (1914).

[33] A. Hunter, Physiology of Creatine and Creatinine, Physiol. Reviews, **2**, 586 (1922).

and incomplete as they are, certainly suggest that betaine is a step in the evolution, and probably, therefore, in the higher forms, in the production, of creatine.

According to Riesser,[30] the formation of creatine from betaine and choline may take place as represented by the following equations:



It is to be borne in mind that these reactions are entirely hypothetical. They are included here largely because they furnish an interesting working hypothesis and one that may perhaps prove to be fruitful. As to the precursor of betaine, it has been suggested by Barger [34] that this may be glycine.

Fate of Creatine and its Conversion into Creatinine.—At the time when Folin [35] published his important studies on protein metabolism, the conversion of creatine into creatinine by the body was not questioned. Folin observed, however, that feeding creatine had no effect on the excretion of creatinine. Taken in small amounts, creatine was retained entirely; when larger quantities were ingested, only a part was retained, the remainder being excreted unchanged. As a result of these observations, Folin came to the conclusion that the organism did not possess the power of converting creatin into creatinine and that these substances were independent of each other in metabolism. Folin regarded creatine as a food and creatinine as a waste product.

Since their publication, Folin's results have been confirmed and denied by numerous workers. In 1916, Rose and Dimmitt [36] furnished evidence in support of the view that creatine is convertible into creatinine. These workers found that the ingestion of large doses of creatine in man caused an appreciable increase in the output of creatinine. Thus, in certain of their experiments, the ingestion of 10 grams of creatine caused an increase of 0.26–0.34 gram of creatinine, whereas the increases observed after taking 20 grams of creatine were between 0.30 and 0.49 gram. The greater part of the creatine, however, was excreted

[34] G. Barger, The Simpler Natural Bases, London, 1914, p. 53.
[35] Hammarsten's Festschrift, Upsala, part 3 (1906).
[36] J. Biol. Chem., **26**, 345 (1916).

unchanged. The ingestion of creatinine, on the contrary, was not followed by the appearance of creatine in the urine, from which it may be inferred that in the body the reaction creatine → creatinine is an irreversible one.

Benedict and Osterberg [37] have studied the effects of prolonged feeding of creatine. In one experiment they fed a dog a small amount of creatine daily for a period of 70 days. Thus a total amount of 32.9 grams of creatine (expressed as creatinine) was given. The urine was analyzed daily for a long period before creatine administration was begun to establish the dog's normal output of creatinine and creatine (in this case creatine was absent from the urine normally). The urine was analyzed daily during the experimental period and the analyses were continued for 7 weeks after the creatine feedings had been discontinued. Creatinuria was not observed until the tenth day after the administration of creatine was begun. From this time on, increasing amounts of creatine were present in the urine, the creatinuria continuing until the day after creatine feeding was stopped. Of the 32.9 grams of creatine fed during the 70 days, a total of 13 grams of creatine was recovered. Accordingly, 19.9 grams of creatine had been retained by the tissues. An increased daily excretion of creatinine became manifest about one week after the creatine administrations were instituted and this continued for the duration of the experiment, including the after period of 7 weeks. The extra creatinine eliminated was 5.8 grams or 29.1 per cent of the creatine retained. The difference of 14.1 grams could not be accounted for either as extra urinary creatine or creatinine.

These results are of importance because they establish (1) that creatine is converted into creatinine, (2) that this conversion is not a direct process, but apparently involves a preliminary storage of the creatine in the tissues, (3) that creatinine is probably only one of the end-products of creatine metabolism and that a proportion of the creatine may follow a different metabolic path.

Results similar to those of Benedict and Osterberg have been obtained in rats by Chanutin [38] and in man by Rose, Ellis and Helming.[39] More recently, Chanutin and Silvette [40] have approached more directly the question of why administered creatine is not fully recovered in the urine either as extra creatine or creatinine. They injected creatine into completely nephrectomized rats and analyzed the various tissues for creatine. They showed that larger amounts were stored in

[37] *Ibid.*, **56**, 229 (1923).

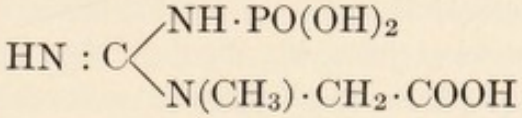
[38] *Ibid.*, **67**, 29 (1926).

[39] *Ibid.*, **77**, 171 (1928).

[40] *Ibid.*, **85**, 179 (1929); see also **75**, 549 (1927); **80**, 589 (1928); Chanutin and Beard, H. H., *ibid.*, **78**, 167 (1928).

the liver and muscles than elsewhere and moreover that not all of the creatine administered could be recovered in the tissues. In this way Chanutin and Silvette have demonstrated that creatine may be destroyed by the organism.

Phosphocreatine.—Eggleton and Eggleton,[41] on the basis of certain analyses of phosphate in muscle reached the important conclusion that muscle contains a labile (easily hydrolyzable) organic phosphate, which they called *phosphagen*. At about the same time, Fiske and Subbarow,[42] in this country, made a similar observation and showed that the substance in question was composed of creatine and phosphoric acid. Later studies confirmed these observations and indicated that phosphocreatine has the following constitution:



Traumatic damage of muscles causes an unusually rapid cleavage of the phosphocreatine which is present. Rapid hydrolysis may be produced also by acid and alkali.

Phosphocreatine participates in muscular activity, as has been shown by workers in various laboratories. For example, Dixon, Davenport and Ranson [43] stimulated a frog's gastrocnemius until it developed fatigue contracture, as a result of which the inorganic phosphate content rose from 35 to 65 mg. per 100 grams, and the phosphocreatine diminished from 54 mg. to 13 mg. per 100 grams. Eggleton and Eggleton [44] have observed that the accumulation of the free creatine which occurs in muscle in fatigue, rigor and other conditions is proportional to the amount of phosphocreatine which is decomposed. Meyerhof and Lohmann [45] have reported that crustacean muscle contains phosphoarginine (arginine-phosphoric acid). Both of these constituents are believed to undergo decomposition during the excitation stage rather than during contraction. The evidence (Meyerhof and Nachmansohn [46]) for this view is that when the nerve to a muscle is paralyzed, the decomposition of the phosphagens (creatine and arginine phosphoric acids) is hindered.

It has been reported that white muscle of various animals (rabbit, guinea pig, fowl) is richer in phosphocreatine than red muscle.[47]

[41] Biochem., J., **21**, 190 (1927); J. Physiol., **63**, 155 (1927).

[42] Science, **65**, 401 (1927); J. Biol. Chem., **81**, 629 (1929).

[43] J. Biol. Chem., **82**, 61 (1929).

[44] J. Physiol., **65**, 15 (1928).

[45] Biochem. Z., **196**, 22, 49 (1928).

[46] Naturwiss., **16**, 726 (1928).

[47] Ferdmann, D., and Feinschmidt, O., Z. physiol. Chem., **178**, 173 (1928); Palladin, A., and Epplebaum, S., *ibid.*, **178**, 179 (1928).

Creatinuria.—Creatine is not present normally in the urine of the male adult but does occur as a normal constituent in the urine of children of both sexes before the age of puberty. Creatinuria is likewise manifested in a variety of abnormal conditions, such as starvation, muscular dystrophy and other diseases of the muscular system, exophthalmic goiter, acidosis, phosphorus and choloroform poisoning, eclampsia, and diabetes. As we shall see, all forms of creatinuria cannot be referred to the same fundamental cause, although it is evident that all are due either to incomplete storage of creatine or to incomplete conversion of creatine into creatinine.

Exogenous and Endogenous Creatinuria.—In the first place, it is important to consider whether all forms of creatinuria are endogenous in origin. On this point there is much difference of opinion. According to one group of workers, notably Denis,[48] the ingestion of large quantities of protein may cause creatinuria in cases where it is absent, or increase it where it already exists, whereas the reverse effect is obtained upon a minimum protein intake.

Rose,[49] on the other hand, was unable to induce creatinuria by feeding large quantities of protein to men and women. In some of his experiments, the protein intake was so high as to result in a nitrogen excretion in the urine of as much as 35 grams. Nevertheless, there was no evidence of creatinuria in these individuals.

Hunter [33] has pointed out that the excretion of creatine on a high protein intake need not necessarily be interpreted as proof of the exogenous origin of creatine. The well-known effect of proteins in stimulating cellular metabolism (specific dynamic action) has been held responsible (Lewis, Dunn and Doisy)[50] for the increased excretion of endogenous uric acid in experiments in which excessive amounts of proteins and amino acids were fed. It may likewise have been the cause of the increased creatine formation in those cases where this has been observed. Accordingly, creatine and creatinine metabolism is to be regarded as a phase of endogenous metabolism. The only obvious exception to this occurs when creatine as such is ingested.

Creatinuria in Starvation and in Carbohydrate Deprivation.—The appearance of creatine in the urine during starvation may be considered from several angles. The excessive tissue break-down which occurs during inanition may lead to the liberation of more than the normal amount of creatine. That which is not converted into creatinine may be expected to behave like exogenous creatine, being partly retained and

[48] J. Biol. Chem., **29**, 447 (1917); **30**, 47, 189 (1917); **31**, 561 (1917); **37**, 245 (1914).
[49] J. Biol. Chem., **34**, 601 (1918).
[50] *Ibid.*, **36**, 9 (1918).

partly excreted in the urine unchanged. Accordingly, the origin of the creatine of the urine in starvation is the pre-formed creatine of the muscles. This is the more generally accepted view (F. G. Benedict,[51] Mendel and Rose,[52] Myers and Fine [53]).

Creatinuria disappears in the starving animal upon the administration of carbohydrates. It may be argued that this effect is due to the protein-sparing action of glucose, but it has also been suggested that glucose is specifically concerned in the conversion of creatine into creatinine, in a manner perhaps analogous to the interrelationship between carbohydrate and fat metabolism. The fact that proteins are nearly as effective as carbohydrates in abolishing starvation creatinuria (Rose, Dimmitt and Cheatham)[54] does not alter the situation as regards the dual explanation, for the protein may conceivably exert its effect either as a tissue sparer or by providing glucose precursors. The elimination of creatine in starvation is at the expense of creatinine.

The relationship between creatine-creatinine metabolism and carbohydrate metabolism cannot therefore be stated with certainty at present. That a relationship exists is indicated by the fact that creatinuria is characteristic of many conditions of deficient carbohydrate utilization (diabetes, phlorhizin glycosuria) as well as of conditions in which there is apparently a deficiency in glycogenic function. In the latter group are included various forms of hepatic insufficiency, such as occur in eclampsia, phosphorus and chloroform poisoning, and carcinoma of the liver. It is to be noted, however, that in none of these conditions can we exclude entirely the factor of exaggerated endogenous or tissue metabolism.

Creatinuria Due to Excessive Tissue Catabolism.—More clear-cut examples of the relation of endogenous protein metabolism to creatinuria are seen in fevers, wasting diseases, and exophthalmic goiter. In all of these an excessive amount of creatine is presumably liberated, and a portion of this is excreted into the urine unchanged. The creatinuria following parturition may likewise belong to this group.

Creatinuria in Children, Women, etc.—Infants and children normally excrete creatine in addition to creatinine, as was first shown by Rose.[55] Several explanations have been offered for this phenomenon. It has been suggested that creatinuria in children may be due to the fact that they have less ability to retain creatine because their muscula-

[51] F. G. Benedict, The Influence of Inanition on Metabolism, Washington, 1907.
[52] J. Biol. Chem., **10**, 213 (1911–12).
[53] *Ibid.*, **15**, 283 (1913).
[54] *Ibid.*, **26**, 339 (1916).
[55] *Ibid.*, **10**, 265 (1911).

ture is less developed and proportionately less abundant than in adults. The creatinuria of muscular dystrophy in adults has been attributed to the same cause. In both cases, the administration of creatine by mouth is followed by almost complete excretion, and not by partial retention as is the case in normal adults.

That creatine-creatinine metabolism may be under the control of sex hormones has also been suggested. Eunuchs, according to Read,[56] excrete creatine normally.

In women, after puberty, creatinuria is intermittent, except during pregnancy, when it is continuous. Whether creatinuria in women is to be related, as in children, to deficient muscular development or sex-glandular control, or to some other cause, is a matter that we are not able to decide at present, although the indications are that even muscularly well-developed women exhibit an occasional creatinuria.

The Significance of Creatinine.—As we have seen, creatine is, under ordinary conditions, a product of endogenous protein metabolism. In the male adult it is converted completely into creatinine, in which form it is quantitatively excreted. Creatinine is present in exceedingly small amounts in the tissues. It is a true waste product and as such is promptly removed. In the reaction creatine → creatinine, carbohydrate oxidation is apparently essential, and if for any reason the available supply of carbohydrate is deficient, the transformation is incomplete, and some creatine appears in the urine. Under these conditions, the output of creatine + creatinine is equivalent to the amount of creatinine which would have been excreted if there were no creatine. Thus, creatine is present in the urine at the expense of creatinine.

In a given individual the elimination of creatinine is constant from day to day, provided the diet is free from creatine and creatinine present as such. Creatinine is therefore an end-product of endogenous protein and more particularly of muscle metabolism. The amount of creatinine excreted daily is independent of the volume of urine, the amount of protein in the diet, and therefore of total nitrogen metabolism. It is not influenced by the amount of ordinary muscular work (Shaffer [57]). What, then, is the cause of the variations in creatinine excretion in different individuals?

The Creatinine Coefficient.—The number of milligrams of creatinine excreted in the urine in twenty-four hours per kilogram of body weight is called the creatinine coefficient. In men, the creatinine coefficient may vary between 18 and 32, with an average of about 24 or 25. In

[56] J. Biol. Chem., **46**, 281 (1921).

[57] Am. J. Physiol., **22**, 445 (1908).

women, lower values are the rule, the average being about 18, with a normal range between 9 and 26. Children have even lower values, ranging between 9 and 17 at 5–13 years (Krause [58]). The more muscular an individual, the higher is his or her creatinine coefficient. Thus, obese or muscularly under-developed men may have a very low creatinine coefficient (as low as 15–18), whereas well-developed muscular women may have as high creatinine coefficients as normal men.[59]

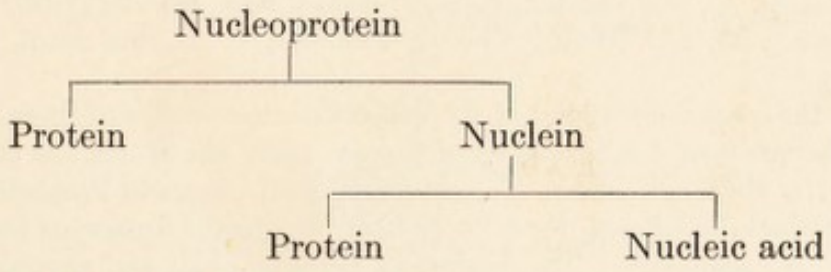
In different animal species, the creatinine coefficient is related, not only to the amount of muscle, but to the creatine content of the muscle as well. Myers and Fine [60] found the average percentages of creatine in the muscles of the rabbit, man, and dog to be 0.52, 0.39, and 0.37 per cent, respectively. The average creatinine coefficients of these are in the same order, namely, 38.4 for the rabbit, 24.2 for man, and 22.5 for the dog.

It has been suggested that creatine and creatinine may be related in some way to muscular " tone." The evidence for this supposition is, however, not conclusive.

PURINE METABOLISM

Under purine and nucleic-acid metabolism, we have to consider (1) the fate of ingested nucleoproteins and nucleic acids and (2) the anabolism and catabolism of purines and pyrimidines in the body, whether derived from exogenous or endogenous sources.

Nucleic acids are present in the nuclei of cells. It is not known with certainty that they ever occur in the cytoplasm. According to the prevailing view, the nucleic acids are combined with protein, thus constituting the so-called nucleoproteins. On partial hydrolysis, nucleoproteins yield a protein residue and a protein-nucleic acid complex which has been called nuclein. If the cleavage is carried somewhat further, the nuclein yields as cleavage products protein and nucleic acid. These changes may be represented as follows:



[58] Quart. J. Exp. Physiol., **7**, 87 (1914).

[59] See for example the data of Hodgson, P., and Lewis, H. B., Am. J. Physiol., **87**, 288 (1928).

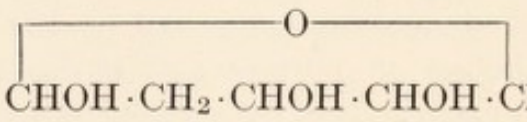
[60] J. Biol. Chem., **14**, 9 (1913).

Careful study of the nucleic acids obtained from a variety of sources has revealed the interesting fact that there are, perhaps, only two such compounds, one being characteristic of plant tissues, such as yeast, the other occurring in animal tissues. The difference between the two nucleic acids is indicated by the products which they yield on hydrolysis.

PRODUCTS OF HYDROLYSIS OF NUCLEIC ACIDS

Of Animal Origin	*Of Plant Origin*
Phosphoric acid	Phosphoric acid
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Thymine	Uracil
Levulinic and formic acids	Pentose

Similar purines are present in both nucleic acids. One of the two pyrimidines is likewise common to both. The other pyrimidine is uracil in plant nucleic acid and thymine in animal nucleic acid. The carbohydrate component in nucleic acid of plant origin, is *d*-ribose (Levene and Jacobs), whereas, in animal nucleic acid, it is believed to be *d*-2 ribodesose, a desoxypentose (Levene and London [61]),



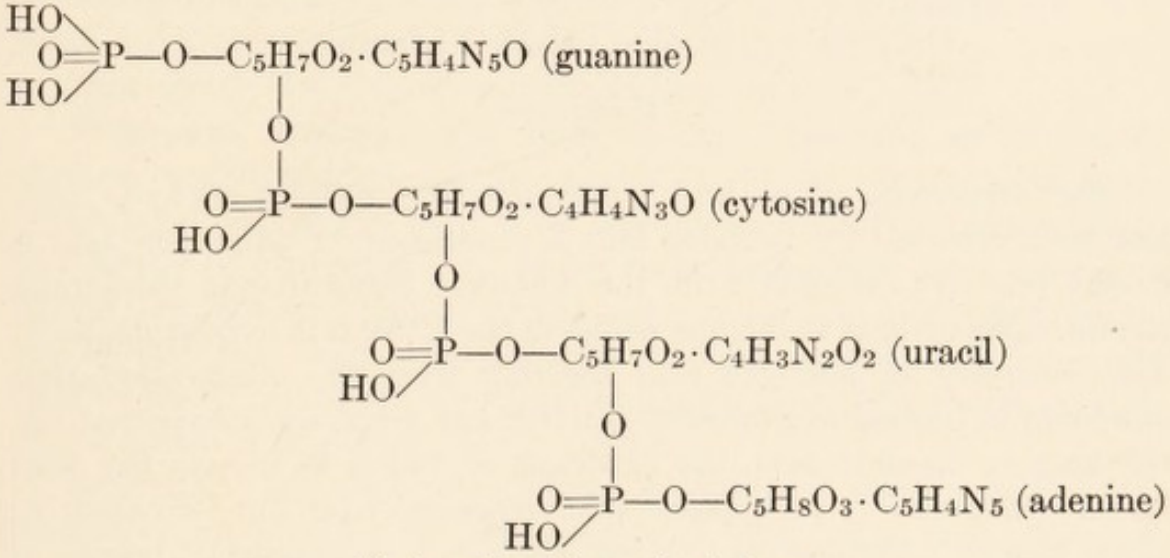
which during the process of hydrolysis is decomposed to levulinic and formic acids. With so many hydrolytic products, the complexity of the nucleic-acid molecule may be readily surmised.

Concerning the molecular configuration of the nucleic acids, certain details have been fairly well established, thanks to the labors of Levene, Jones, and others.[62] In yeast nucleic acid, for example, the purine or pyrimidine base is united directly to the sugar (*d*-ribose), the type of

[61] Levene, P. A., and London, E. S., J. Biol. Chem., **83**, 793 (1929); Levene and Mori, T., *ibid.*, **83**, 803 (1929); Levene, Mikeska, L. A., and Mori, *ibid.*, **85**, 785 (1929–30).

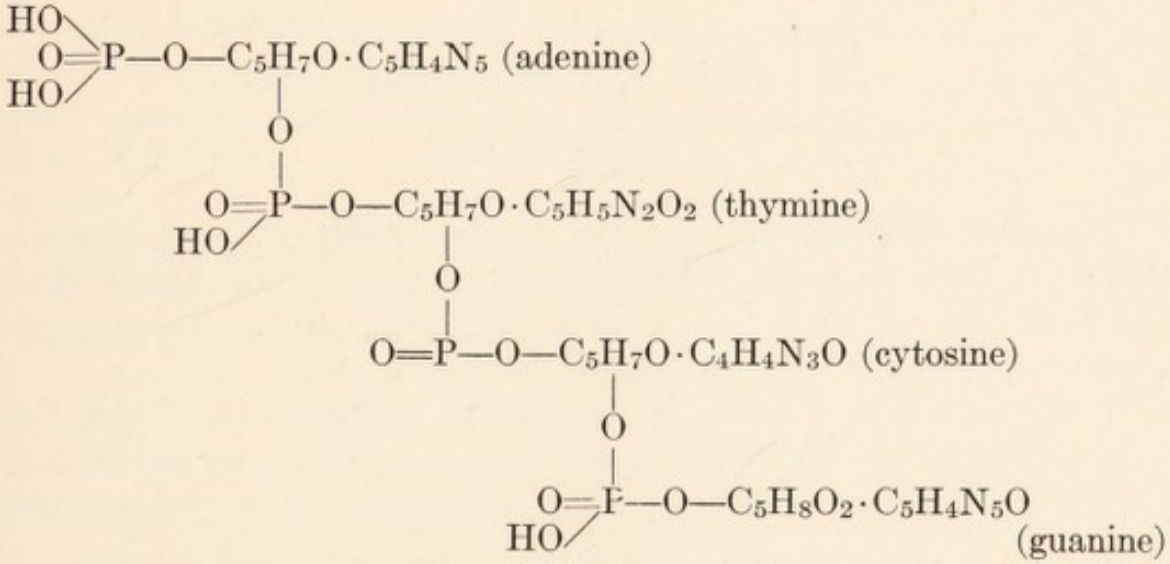
[62] Only the essential features of the subject can be dealt with here. For a more detailed discussion of the chemistry of nucleic acids, the student is referred to the monograph by Jones (W. Jones, Nucleic Acids, their Chemical Properties and Physiological Conduct, 2d edition, New York, 1920) and to the numerous papers by P. A. Levene and his associates. The distinction between plant and animal nucleic acids is probably not as sharply drawn as has been supposed. Thus, Calvery, on hydrolyzing the β-nucleoprotein prepared from chicken embryos obtained the same four pentose nucleotides that have been isolated from yeast nucleic acid (J. Biol. Chem., **77**, 489 (1928)).

linkage being that of a glucoside. Compounds of this type (sugar—purine or pyrimidine base) are called nucleosides. In turn, each nucleoside is united to a molecule of phosphoric acid (probably by an ester linkage), the point of union being between the sugar and the acid. The phosphoric acid-sugar-base compounds are called nucleotides or mono-nucleotides. Four such nucleotides constitute a tetranucleotide or nucleic-acid molecule. According to Levene,[63] yeast nucleic acid has the following molecular configuration:



Yeast nucleic acid, according to Levene

Thymus nucleic acid has a formula analogous to that of yeast nucleic acid (Levene and London [61]).

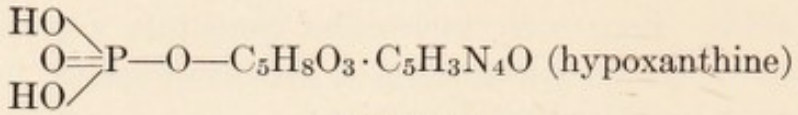


Thymonucleic acid (Levene and London)

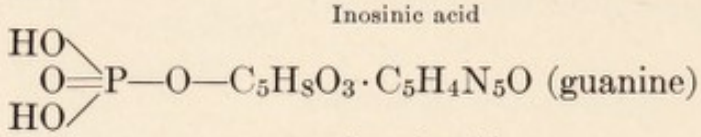
Inosinic and Guanylic Acids.—Two mononucleotides are present in animal tissues. These are inosinic and guanylic acids. The former

[63] J. Biol. Chem., **41**, 19 (1920).

was first isolated from meat extract by Liebig; the latter was discovered by Hammarsten in pancreatic tissue. On hydrolysis, inosinic acid yields phosphoric acid, hypoxanthine, and *d*-ribose. Guanylic acid is composed of phosphoric acid, guanine, and *d*-ribose.

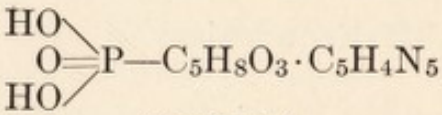


Inosinic acid



Guanylic acid

The occurrence of a third mononucleotide, namely adenylic acid, has been reported by Embden and Zimmermann.[64] Adenylic acid is closely related to inosinic acid, the difference being that in the former the nitrogenous base is adenine whilst in the latter it is hypoxanthine.[64a] The discovery of adenylic acid together with the observation that ammonia is formed in muscular activity has given an unexpected significance to inosinic acid, the existence of which in muscle has been known since 1847. It appears that accompanying the formation of lactic acid in muscular contraction, there is a simultaneous release of ammonia (p. 351), the source being adenylic acid which is thereby converted into inosinic acid. The assumption is that this is one of the buffer mechanisms of muscle.



Adenylic acid

Digestion of Nucleoproteins and Nucleic Acids.—The nucleoproteins of the food are converted by the proteolytic enzymes of the gastric and pancreatic secretions into protein and nucleic acids. These secretions take no part in the hydrolysis of the nucleic acids.

It has been shown, chiefly by Levene and Medigreceanu,[65] that the tetranucleotides are disintegrated into mononucleotides by an enzyme present in the succus entericus, called nucleinase. A second enzyme

[64] Z. physiol. Chem., **167**, 137 (1927).

[64a] Voluntary muscle of the ox, pig, rabbit and man have the power of deaminizing adenylic acid, with the formation of inosinic acid (M. V. Buell, J. Biol. Chem., **85**, 435 (1929–30)).

[65] J. Biol. Chem., **9**, 375, 389 (1911).

or group of enzymes which is present in the intestinal juice decomposes the purine nucleotides, the products being phosphoric acid and nucleosides. The pyrimidine nucleotides are probably not acted upon by the intestinal juice, but are digested by a nucleotidase present in the intestinal mucosa. Here, purine nucleotidases are likewise to be found. Finally, the purine nucleosides are hydrolyzed by a nucleosidase which is found in the intestinal mucosa. Disintegration of the pyrimidine nucleosides apparently does not occur either in the intestinal wall or elsewhere in the body. The nucleinases and nucleotidases, on the other hand, are present not only in the intestinal juice and mucosa, but occur in other organs (liver, heart, muscle, kidney) as well.

Exogenous Purines.—The body is not dependent on exogenous purines or pyrimidines for the anabolism of its own nucleic acids. Purine synthesis in the body from non-purine material is indicated by the results of experiments in which individuals, kept on a very low-purine diet, not only gained weight but eliminated much larger quantities of uric acid than could be accounted for from the purine intake (Kollmann [66]). Growth of young mammals occurs on a diet of milk, a food which is relatively poor in purines.

Benedict [67] has demonstrated the synthesis of purines in the Dalmatian coach hound. In this species of dog, as in man, the end-product of purine metabolism is uric acid and not allantoin as in other dogs. Benedict kept a Dalmatian dog on a purine-free diet, and yet this animal continued to excrete uric acid.

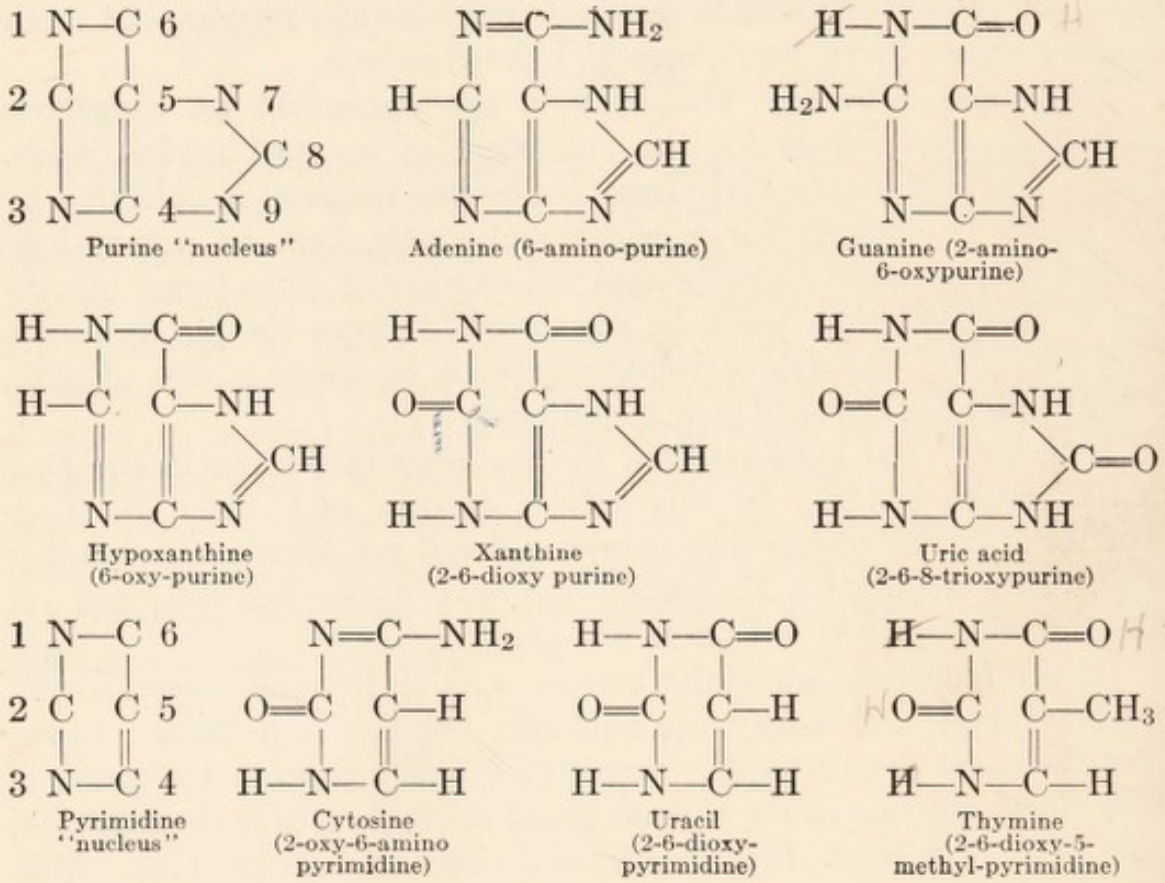
Of the foodstuffs, glandular tissues, such as thymus (sweetbreads), pancreas, liver, and kidney, are especially rich in purine bases. In the liver these are present to the extent of about ½ gram in 100 grams of fresh tissue, whereas in the thymus gland the content is in the neighborhood of 1.5 per cent. Steak contains about 0.15–0.20 per cent. Milk contains about 15 mg. of uric acid per liter, and smaller quantities of adenine and guanine. Not all vegetables are devoid of purine bases. Peas, beans, and spinach contain appreciable amounts. Smaller quantities are present, likewise, in wheat, rye, and other grains.

Coffee, tea, and cocoa contain methyl-purine derivatives, as well as amino- and oxypurines, tea being especially rich in adenine. Calvery [68] has prepared both guanine nucleotide and cytosine nucleotide from dried tea leaves and is of the opinion that a pentose nucleic acid is a natural product of tea. It is unlikely that all of the methylated

[66] Biochem. Zeit., **123**, 235 (1921).
[67] J. Lab. Clin. Med., **2**, 1 (1916); Harvey Lectures, 1915–16, p. 346.
[68] J. Biol. Chem., **72**, 549 (1927).

purines are converted into uric acid in metabolism. Some of these are probably excreted partly unchanged and partly after demethylation to mono-methyl-purine. Caffeine and theophylline, after ingestion, increase the elimination of uric acid.[67, 69] Theobromine, on the other hand, does not seem to be converted into uric acid.

Chemistry of the Purines and Pyrimidines.—The structural relationships of the purines and pyrimidines that are of special interest in metabolism are indicated below.



Anabolism of Purines.[70]—Perhaps the best example of nucleic-acid synthesis in the animal body is that first observed by Miescher,[71] who showed that the salmon, during its long migration from the sea to its spawning grounds, though abstaining from food, forms large amounts of nuclear material from its own tissues. Likewise, during the incu-

[69] Mendel, L. B., and Wardell, E. L., J. Am. Med. Assoc., **68**, 1805 (1917); Myers, V. C., and Wardell, J. Biol. Chem., **77**, 697 (1928).

[70] For a detailed discussion of purine metabolism the student is referred to the review of the subject by W. C. Rose, Physiol. Reviews, **3**, 544 (1923).

[71] Cited by Rose, p. 555.

bation of an egg, there is a progressive increase in the content of purine bases (Mendel and Leavenworth [72]). It may be noted here that there is a similar formation of creatine during incubation.

Further evidence is to be found in the experiments of Burian and Schur,[73] who compared the purine content of new-born rabbits and puppies with the concentrations in the tissues after varying periods of growth on a diet limited to the milk of the mother. Much greater increases were found than could be accounted for on the basis of the purine content of the milk consumed during the periods of the experiments. The experiments of Kollmann [66] and Benedict [67] previously referred to, furnish additional evidence in support of the view that the body is able to synthesize purines, and, hence, nucleic acids, from precursors other than the purines and pyrimidines derived from exogenous sources.

Indeed, it has often been questioned whether exogenous purines are ever anabolized into nucleic acids. As shown by a number of workers (Mendel and Brown,[74] Mendel and Lyman,[75] etc.), the feeding of purine bases or of nucleic acids is followed very promptly by an increased elimination of either allantoin or uric acid. However, the excretion is not quantitative. Koehler [76] has observed that after the ingestion of uric acid, the concentration of this constituent in the blood does not increase and only about one-half of the amount administered is recovered in the urine. It is not known what happens to the retained purines. Koehler suggests that uric acid may be destroyed in the body. Another possibility is that a portion may be used for anabolic purposes. That the body may utilize the purines of its own tissues over and over again during starvation has been suggested by Rose.[77]

As to the non-purine precursors, attention has already been called (p. 341) to the work of Ackroyd and Hopkins and of Rose on the relation of histidine and arginine to purine metabolism. The synthesis of purines from histidine in the body is apparently an established fact.

Catabolism of Nucleic Acids.—It is probably correct to assume that exogenous and endogenous purines share an identical fate in metabolism. In catabolism, the nucleic acids undergo changes similar to

[72] Am. J. Physiol., **21**, 77 (1908).
[73] Z. physiol. Chem., **23**, 55 (1897).
[74] J. Am. Med. Assoc., **49**, 896 (1907).
[75] J. Biol. Chem., **8**, 115 (1910).
[76] *Ibid.*, **60**, 721 (1924).
[77] *Ibid.*, **48**, 575 (1921).

those which take place during digestion. Enzymic hydrolysis yields two pyrimidine and two purine nucleotides. The pyrimidine nucleotides are converted into nucleosides.

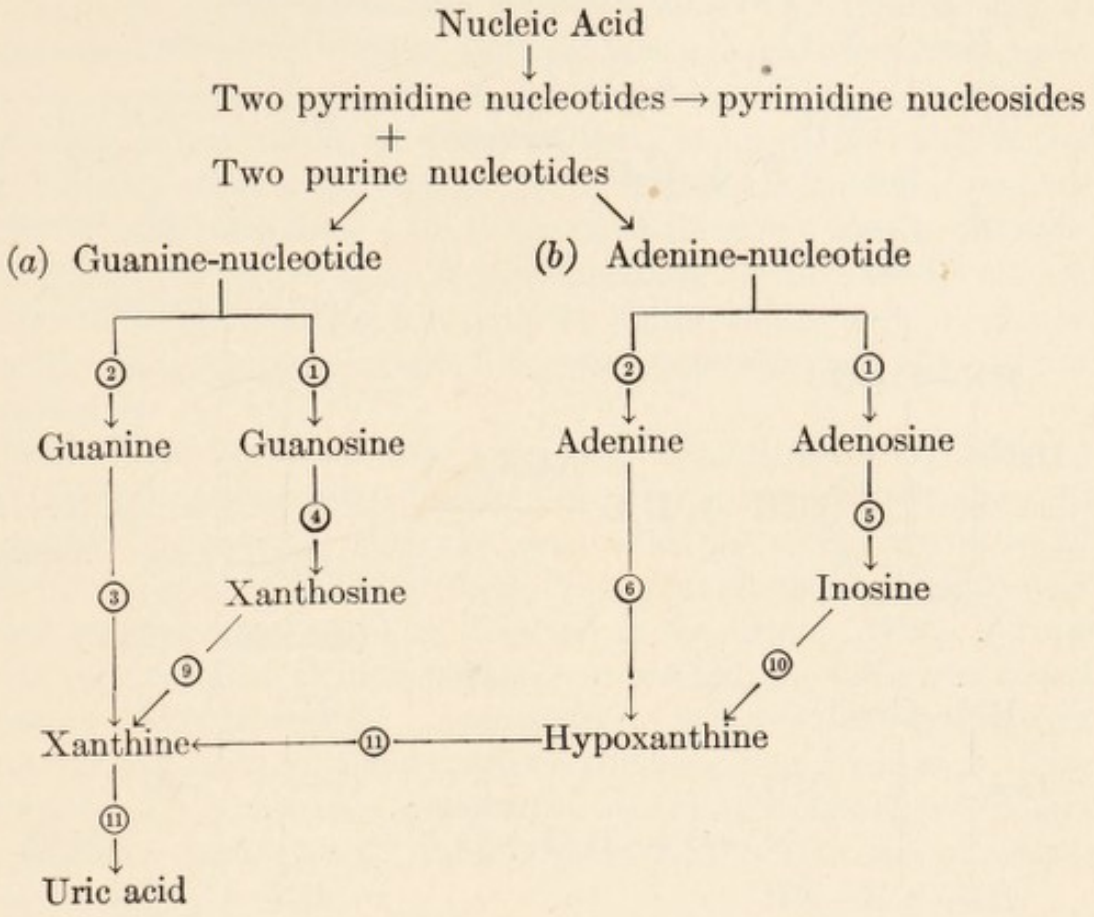
There is evidence that the purine nucleotides may undergo two types of cleavage. The phosphoric acid may be broken off, leaving the nucleoside, or the purine base may be removed, leaving a sugar-phosphoric acid complex. The first type of cleavage is accomplished by the enzyme nucleotidase, or, as it is called by Amberg and Jones,[78] " phospho-nuclease." The second type of hydrolysis is due to the enzyme " purine-nuclease."

It is not unlikely that cleavage of the purine nucleosides is incomplete and that in addition to the free purines, considerable amounts of the purine nucleosides are absorbed. There is some evidence in fact that purines in combination as nucleosides are more readily metabolized than when present in the free form. As to the fate of the sugar part of the nucleic acid molecule, it is presumably the same as that of any other carbohydrate. It is also probable that the body utilizes as much of the phosphoric acid as it needs, the remainder being excreted in the urine in combination with base. On a high-purine diet (liver, thymus, pancreas, etc.), the excretion of phosphates and of acids (NaH_2PO_4) in the urine is increased.

We shall consider first the fate of the purines, guanine and adenine, and of the purine nucleosides, guanosine and adenosine.[79] By hydrolysis, (guanosine-hydrolase or nucleosidase), guanosine may be converted into guanine, or, by deamination, it may yield xanthosine. Guanine, by the action of guanase, is changed into xanthine, whereas the enzyme xanthine-hydrolase (Jones), acting on xanthosine, yields the same product. Adenosine, on hydrolysis, yields adenine, or it may form inosine by deamination. The former is converted by adenase into hypoxanthine, whereas the same compound is formed from inosine by the action of a nucleosidase (inosine-hydrolase of Jones). Hypexanthine is converted into xanthine by xanthine-oxidase. In man, xanthine is finally converted into uric acid. These transformations and relations are outlined on the next page.[78] The numbers refer to the enzymes.

[78] Compare Amberg and Jones, Z. physiol. Chem., **73**, 408 (1911) and Rose, Physiol. Reviews, **3**, 564 (1923).

[79] The nucleosides are named after the purine or pyrimidine base which they contain. Thus the nucleoside of guanine is guanosine, of adenine, adenosine, of cytosine, cytidine, and of uracil, uridine.



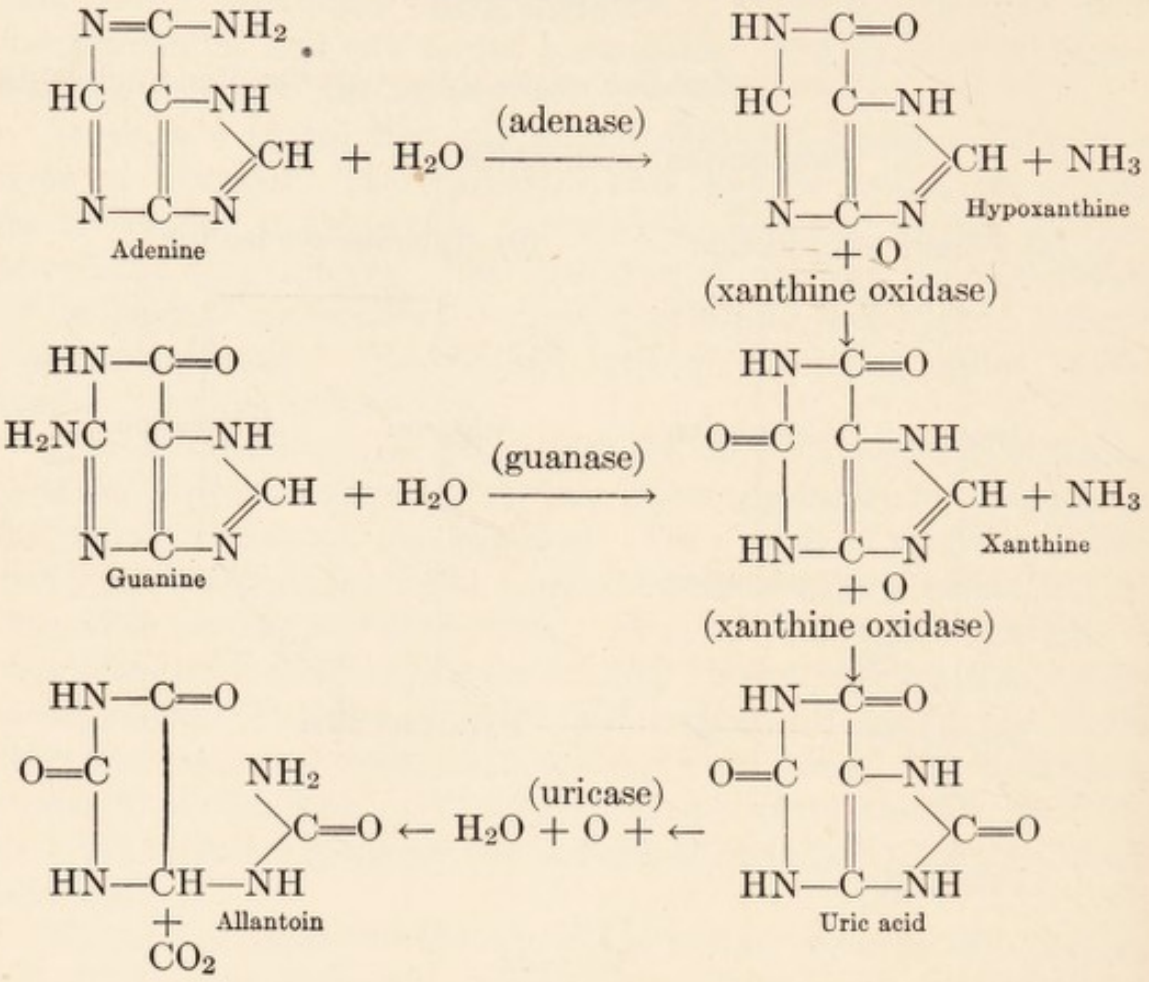
ENZYMES

1. Phospho-nuclease (Jones) or nucleotidase (Levene)
2. Purine-nuclease
3. Guanase
4. Guanosine-deaminase
5. Adenosine-deaminase
6. Adenase
7. Guanosine-hydrolase (Jones) or nucleosidase (Levene)
8. Adenosine-hydrolase (Jones) or nucleosidase (Levene)
9. Xanthosine-hydrolase or nucleosidase
10. Inosine-hydrolase or nucleosidase
11. Xanthine-oxidase

The presence of adenase in human tissues has been questioned by Jones and Winternitz.[80] Accordingly, it may be necessary to assume that, in man, deamination of adenine occurs before it is liberated from its nucleoside. Adenase is very widely distributed in the tissues of other animals. With these facts in mind the genesis of uric acid from adenine and guanine may nevertheless be represented as follows:

[80] Z. physiol. Chem., **44**, 1 (1905).

FATE OF ADENINE AND GUANINE



In marsupials, rodents, carnivora, ungulates, and other animals, including monkeys, but excluding the anthropoid apes and man, allantoin is the chief end-product of purine metabolism, its formation from uric acid being indicated above. A most interesting exception, and one cited in an earlier connection, is the excretion of uric acid by the Dalmatian coach hound. This exception applies only to pure-bred animals, for a dog that is only part Dalmatian excretes both uric acid and allantoin.

Bollman, Mann, and Magath [81] have shown that the destruction of uric acid and its conversion into allantoin in the dog is dependent on the liver, since complete extirpation of this organ results in the excretion of uric acid.

Our knowledge of the fate of the pyrimidines in metabolism is less complete than that of the purines. Sweet and Levene [82] found that if they fed thymine to a dog, more than half could be recovered in the urine, but if the same amount of thymine was fed in the form of nucleic acid,

[81] Am. J. Physiol., **72**, 629 (1925).
[82] J. Exp. Med., **9**, 229 (1907).

none could be recovered in the urine. Wilson [83] performed similar experiments with uracil. When administered as such, uracil was quantitatively excreted unchanged, but with uracil combined in the form of a nucleoside or nucleotide, very little of the uracil could be found in the urine. Nearly all of it had apparently undergone metabolism, the end-product of which was urea. On the basis of these and similar observations, Wilson suggested that the intermediary metabolism of nucleic acids involves radical changes in both the purine and pyrimidine groups before the relatively complex combinations (nucleosides and nucleotides) are broken up.

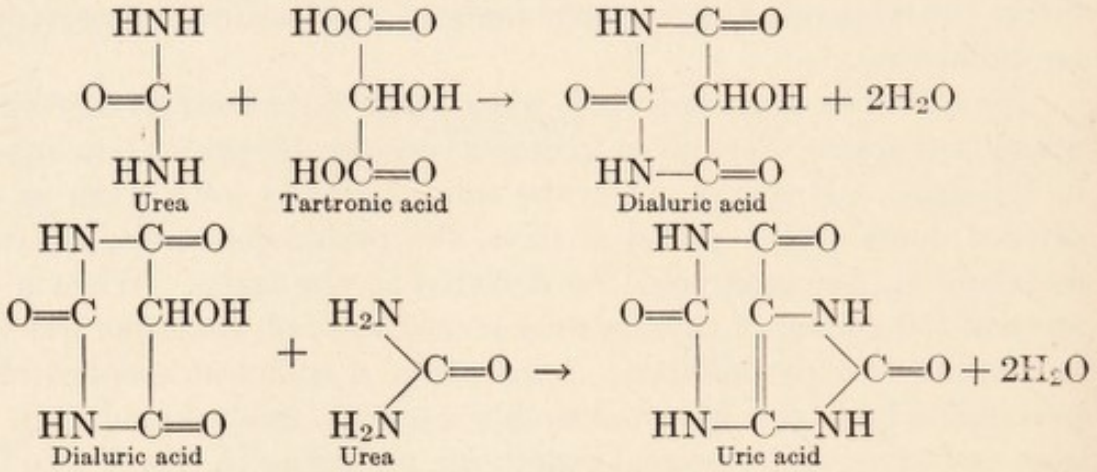
Deuel [84] has observed that when large quantities of thymine or uracil (1–3 grams) were given to dogs a considerable proportion appeared in the urine. However, when the same amounts were given in small divided doses over a period of days, the pyrimidines were apparently metabolized, for none could be detected in the urine. When a large amount (50 grams) of thymus nucleic acid was fed, the urine was found to contain free pyrimidines. Apparently, a sufficient amount of free pyrimidine had been liberated so that a portion escaped oxidation. This does not occur, under normal conditions, according to Deuel, who found it impossible to isolate even a trace of pyrimidine in 150 liters of human urine.

Results similar to those of Deuel were obtained by Cercedo [85] with uracil and thymine. When fed in small amount to dogs, these were metabolized, the predominant end-product being urea. Cytosine, on the other hand, escaped oxidation, being partly excreted unchanged and partly deaminized to uracil. In a later experiment, Emerson and Cercedo confirmed the observation that cytosine, administered as such, is not utilized directly, but that when given in combination as the nucleoside, is completely metabolized.

When the observations just recorded are considered together, the following conclusions seem justified: (1) uracil and thymine when present in small amount as the free base are readily utilized, (2) cytosine as such is not utilized directly, being partly excreted unchanged and partly converted into uracil, (3) pyrimidines in nucleoside combination, particularly cytosine nucleoside, are more readily and more completely metabolized than the free pyrimidines, (4) normally the urine contains little, if any, pyrimidines; possibly when large quantities of nucleoproteins are fed, the urine may contain a small amount of unchanged pyrimidines.

[83] J. Biol. Chem., **56**, 215 (1923).
[84] *Ibid.*, **60**, 749 (1924).
[85] *Ibid.*, **75**, 661 (1927); Proc. Soc. Exp. Biol. Med., **27**, 203 (1929).

Purine Metabolism in Birds and Reptiles.—In birds and reptiles, uric acid is not only the end-product of purine metabolism, but it is likewise the chief end-product of protein metabolism. It is believed that in these animal forms the catabolism of amino acids results first in the formation of urea, and that this is the precursor of uric acid. Wiener [86] has suggested that lactic acid may be oxidized in the liver to tartronic acid, which, by combining with urea, may yield uric acid. These reactions are represented by the following equations:



[64] Beitr. chem. Physiol. Path., **2**, 42 (1902).

Excretion of Uric Acid.—Uric acid is excreted in combination with sodium, potassium, and ammonium in the form of urates. In addition to uric acid, there are probably smaller amounts of other purines in the urine, such as adenine, xanthine, hypoxanthine and methyl-purine derivatives. The daily excretion of uric acid is subject to considerable variation, being influenced by diet and other factors.[87] On a purine-free diet, the normal daily excretion is between 0.2 and 0.4 gram. The content of uric acid in the blood usually varies between 1 and 3 mg. per 100 cc. Marked retention of uric acid occurs in nephritis, in gout, and, as recently shown by Lennox,[88] during starvation. According to Lennox uric-acid retention is in some way associated with ketosis. In conditions such as leukemia and pneumonia, which are associated with marked destruction of nuclear material, the uric-acid content of the blood and urine increases appreciably. The excretion of uric acid is not influenced by muscular activity. Certain organisms of the alimentary tract are said to be capable of synthesizing purines and even uric acid. Accordingly, it has been suggested by McDonald and Levine [89] that in addition

[86] Beitr. chem. Physiol. Path., **2**, 42 (1902).

[87] For a detailed discussion of the uric acid problem and the factors influencing its excretion see Folin, O., Berglund, H., and Derick, C., J. Biol. Chem., **60**, 361 (1924).

[88] J. Biol. Chem., **66**, 521 (1925).

[89] Am. J. Physiol., **78**, 437 (1926).

to the exogenous and endogenous sources of urinary uric acid a portion may owe its origin to bacterial synthesis in the bowel.

PHOSPHORUS

The phosphorus required by the body is derived from a variety of sources, the following being the most important:

1. Phosphoproteins (casein of milk, vitellin of egg yolk, etc.), nucleproteins and lecithoproteins of the diet.
2. Phospholipids of the diet.
3. Organic phosphorus-containing compounds of the diet other than 1 and 2. Phosphorus occurs in combination in starch, grains, etc.
4. Inorganic phosphates of the diet. These are present in animal tissues and particularly in bone.

While there is a possibility that a certain amount of phosphorus in organic combination is assimilated, most of the phosphorus is probably absorbed in the form of inorganic phosphates, for, in the process of digestion of the organic phosphorus-containing compounds of the diet, the phosphoric acid is liberated. At least it is certain that, even in the growing animal, the phosphorus requirement may be supplied exclusively from inorganic sources (Osborne and Mendel [90]).

In the adult organism there is an equilibrium between the intake and the excretion of phosphates, but in the growing animal the retention of phosphorus is a well-known phenomenon. Considerable quantities are used by the growing animal in the formation of bone, in which calcium phosphate is the chief constituent. Birds require a large amount of phosphorus in the production of eggs. In the lactating mammal, phosphorus is needed in the formation of casein and other milk constituents. Meigs, Blatherwick and Cary [91] state that phosphatides and inorganic phosphates are probably the only phosphorus compounds present in normal blood plasma. In the lactating cow, the phosphatide content of the plasma is much higher than normal, and this constituent is probably the precursor of nearly all the fat and phosphorus contained in the milk.

Phosphates are utilized in maintaining the acid-base balance of the blood and in the synthesis of such important cell constituents as the phospholipids and nucleoproteins. The presence in muscle and other tissues of phosphoric acid in combination with creatine, arginine, glu-

[90] *Ibid.*, **34**, 131 (1918).
[91] J. Biol. Chem., **37**, 1 (1919); **40**, 469 (1919).

cose, etc., suggests the probability that it takes part in numerous reactions of physiological importance.

It is probably correct to assume that the phosphorus in bone is in equilibrium with its environment and that, even in the adult organism, any loss which may occur by solution is promptly made good. The phosphorus content of the skeleton would thus remain, under normal conditions, approximately constant. For the most part, the excretion of phosphorus is dependent on the intake. Accordingly, on a high-purine diet, the increase in uric-acid elimination is parallel to the increase in phosphorus excretion. This parallelism holds similarly in conditions of exaggerated nuclear metabolism.

Muscular activity increases the excretion of phosphorus, this being associated with increased carbohydrate metabolism and the liberation of phosphoric acid from its combination as hexose-phosphate. The same thing is believed to occur when insulin is given to a normal individual. During the period of hypoglycemia there is a marked decrease in phosphorus, both in the blood and in the urine, which may be attributed to the taking up of hexose-phosphate by the tissues.[92] When the carbohydrate is used up, the phosphoric acid is liberated, enters the blood and is excreted.

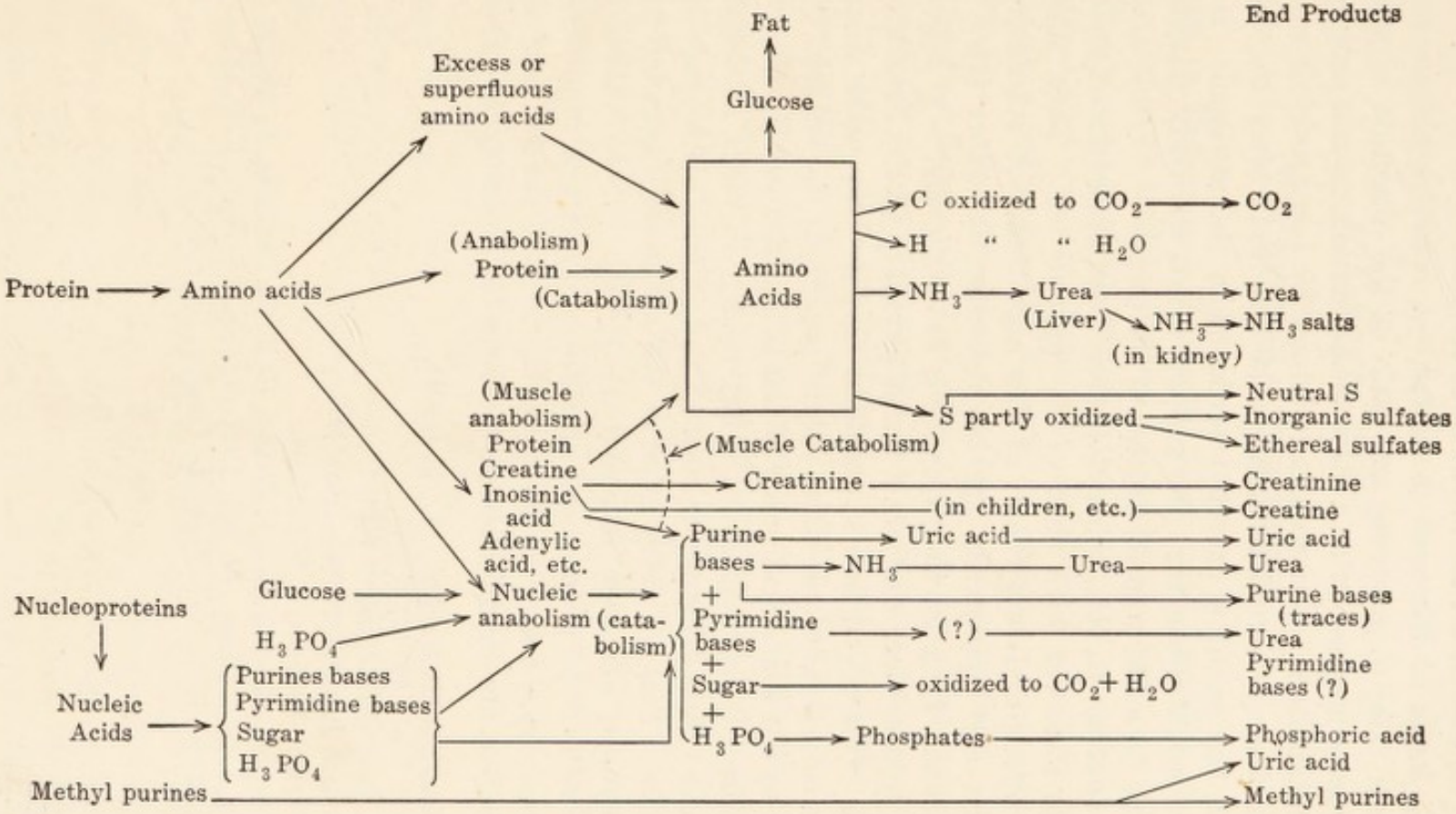
SULFUR

The main features of sulfur metabolism were discussed in the preceding chapter in connection with the fate of cystine in the body. Cystine represents the most important source of sulfur in metabolism and is apparently essential for maintenance. There is, however, another sulfur-containing amino acid, methionine. A small amount of sulfur is probably derived from the sulfur-containing lipids or sulfatides of the diet. Inorganic sulfates are apparently of no importance from a nutritional standpoint.

Among the more familiar physiological constituents, other than protein, that contain sulfur, may be mentioned glutathione, taurine, insulin, and the sulfolipids contained in the tissues of the central nervous system and in the secretions of the skin. Sulfur is removed from the body in several ways. In the growth of hair, considerable quantities are lost in the form of cystine, which is especially abundant in the albuminoid, keratin. The bile contains sulfur compounds which, if not reabsorbed, are excreted in the stools. The saliva, urine, and bile contain thiocyanates, probably formed in the detoxication of small amounts of CN arising in metabolism. The urine is the most important channel of excretion of the end-products of protein metabolism, including those

[92] See, for example, Blatherwick, Bell and Hill, *ibid.*, **61**, 241 (1924).

SUMMARY OF PROTEIN AND NUCLEIC-ACID METABOLISM



containing sulfur. These have been referred to previously and will receive further attention in the next chapter.

The Ratio of Nitrogen to Sulfur.—A considerable amount of work has been done in studying the ratio of the excretion of sulfur to the excretion of nitrogen with a view to elucidating certain problems in protein metabolism. The results obtained by one group of workers (von Wendt,[93] etc.) have led them to believe that after the ingestion of protein, the sulfur is excreted earlier than the nitrogen. If this were true, it would mean that the protein which the body retains is poorer in sulfur than the original protein of the diet.

In contrast to this, others (Gruber,[94] etc.) have found that the N : S ratio is identical with that of the protein fed. Then there are the observations of Lewis[95] and of Fay and Mendel,[96] who have shown a frequent and apparently specific retention of sulfur after periods of starvation. Because the evidence is so conflicting, it is difficult to subject it to a critical analysis. For a summary of the literature, the student is referred to Lewis' excellent review[97] on sulfur metabolism as well as to several of the more recent papers on the subject.[98]

As to the numerical value of the starvation N : S ratio, von Wendt determined it to be about 9.3, but nearly all other investigators have obtained values ranging between 13 and 16 with an average of about 14. This means that for every gram of sulfur normally excreted in the urine, the nitrogen excretion is about 14 grams. These values correspond rather well to the proportions of sulfur and nitrogen in muscle proteins.

Summary.—Some of the main features of protein and nucleic-acid metabolism are summarized in the diagram given on the preceding page.

[93] Skand. Arch. Physiol., **17**, 211 (1905).

[94] Z. f. Biol., **42**, 407 (1901).

[95] J. Biol. Chem., **26**, 61 (1916).

[96] Am. J. Physiol., **75**, 308 (1926).

[97] Physiol. Reviews, **4**, 394 (1924).

[98] Fay and Mendel, *loc. cit.*, Wilson, H. E. C., Biochem. J., **19**, 322 (1925); Morgulis, S., J. Biol. Chem., **77**, 627 (1928); see also Cathcart, E. P., The Physiology of Protein Metabolism, New York and London, pp. 105, 123.

CHAPTER XIV

EXCRETION: THE URINE

Channels of Excretion.—The removal of waste products from the body is accomplished largely, but not solely, by the lungs and kidneys. The former are concerned mainly with the excretion of gaseous waste products, the latter with the elimination of solids in solution. Among the other organs which may be regarded as at least in part excretory are the liver and the gall-bladder. These are concerned with the removal of cholesterol, bile pigments, and other substances. The intestinal epithelium excretes inorganic constituents, especially those foreign to the body. The saliva contains small quantities of nitrogenous and other waste products. Not only water, but also compounds such as urea and sodium chloride are excreted by way of the skin. The daily output of perspiration contains about 0.2–0.3 gram of nitrogen.[1] An additional 0.1 gram of nitrogen is lost to the body daily in the growth of hair and nails. In careful studies of nitrogen balance, it is desirable to take into account all the channels of excretion, so as to allow for all losses. Due allowance must also be made for the excretion of nitrogenous material by the intestinal epithelium, as this usually amounts, even in starvation, to about 0.5 gram per day. Thus the extra-renal excretion of nitrogen may be estimated at about 1.0 gram.

Formation of Urine.—Textbooks of physiology and histology contain descriptions of the kidney. For a proper understanding of what is to follow, the student should be familiar with the microscopic anatomy of this organ.

Various theories have been proposed to explain kidney secretion. According to the theory advanced by Ludwig in 1844, the glomeruli filter from the blood its non-colloidal constituents. Large amounts of fluid thus pass into the tubules. During its passage through the tubules, the fluid becomes concentrated by the reabsorption of water.

According to the theory postulated by Bowman and Heidenhain, water and salts are secreted by the glomerulus, whereas the organic constituents are added to the urine as a result of the secretory activity of the renal tubular epithelium.

[1] G. A. Talbert and his associates have published a series of papers on the composition of sweat: Am. J. Physiol., **81**, 74 (1927); **82**, 153, 639 (1927); **84**, 577 (1928).

Cushny [2] in his monograph "The Secretion of the Urine," reviews the evidence for and against these theories, and advances what he terms the "modern theory," according to which water and the non-colloidal constituents of the plasma are filtered through the glomerulus. He states that as the glomerular filtrate proceeds through the tubules, only those substances are reabsorbed that are necessary to the plasma and tissues. Such substances are water, glucose, salts, amino acids, sodium bicarbonate, etc., and are termed "threshold" substances. There is no absorption of unnecessary constituents, such as urea and creatinine. These are termed "no-threshold" substances.

Wearn and Richards,[2a] have devised a method for the simultaneous collection of glomerular and bladder urine in frogs. They succeeded in showing that the bladder urine may be devoid of sugar and chloride at a time when these constituents are easily demonstrable in the glomerular fluid.

According to the view of Starling and Verney [3] there is some basis for the hypotheses of both Ludwig and Bowman. Starling and Verney were able to demonstrate that the glomeruli filter from the blood plasma its non-protein constituents. In addition, they observed that urea and sulfate are also secreted by the tubule cells, and pass into the glomerular fluid. Phenolsulfonphthalein, when injected into the dog, is likewise eliminated by the tubules. In turn, the tubule cells reabsorb water, chloride, bicarbonate, and glucose. According to Starling, the cells that reabsorb water occupy a lower position in the tubules than the cells concerned in the reabsorption of chloride.

That the secretory activity of the kidney may be under hormone control has also been suggested by Starling and Verney. Pituitrin was found to cause a marked increase in the percentage and absolute amount of chloride and a decrease in the amount of water elimination. This influence could not be explained on the basis of the vascular action of this hormone.

Blood pressure and the volume of blood flow through the kidney influence the amount of urine formation. Richards and Plant [4] perfused the rabbit's kidney with hirudinized blood in a manner which permitted of variations in the pressure within the renal circulation without alterations in the rate of blood flow. It was found that changes in renal blood pressure produced parallel changes in the rate of urine flow.

[2] A. R. Cushny, The Secretion of the Urine, New York, 1917.

[2a] Am. J. Physiol., **71**, 209 (1924); J. Biol. Chem., **66**, 247 (1925).

[3] Proc. Royal Soc., London, **97**, Ser. B., 321 (1925).

[4] Am. J. Physiol., **59**, 144 (1922).

The rate with which certain substances, such as urea and phosphates, are excreted by the kidneys seems to be regulated not only by their concentration in the blood but by a variety of other factors. For details the student is referred to the review of Marshall and the papers of Addis and his associates.[5]

It is instructive to compare the concentration of various constituents in the blood and urine. The values given here, while in no sense fixed, are nevertheless fairly representative. Urine contains 25 times as much uric acid, 40 times as much ammonia, 60 times as much urea, 100 times as much creatinine, 30 times as much PO_4, and 60 times as much SO_4, as is contained in an equivalent volume of blood plasma. On the other hand, there is little difference in the concentration of chloride, sodium, calcium, and magnesium; and, in the case of glucose, much less is present in the urine than in the blood. On the basis of Cushny's theory, this would mean the filtration of large quantities of plasma per day. Basing his computations on the difference in the concentration of urea, Cushny has calculated that in the formation of 1 liter of urine, 67 liters of plasma would have to filter through the glomeruli.

Volume.—The volume of urine secreted per day (twenty-four hours) may vary within wide limits. In the United States, a normal adult usually excretes 1200–1500 cc. In Germany, where beer is consumed freely, 2000 cc. is nearer the normal value. The most important factor determining the output of urine is obviously the water intake, in the form of water, milk, soup, or beverages. Temperature is another important modifying factor. During the summer months or in warm climate, less urine is formed, because of the loss of water in perspiration. Urination is, as a rule, more frequent in winter than in summer. A high-protein diet, by giving rise to nitrogenous end-products having a diuretic effect, causes an increased elimination of urine. Muscular exercise, on the contrary, results in a diminished volume of urine. Nervousness and excitement may cause abnormally frequent and abundant micturition. It is generally believed that mentally deranged people excrete more urine than normal individuals. The diuretic effect of coffee, tea, and chocolate is due largely to the presence of caffeine and other purine derivatives.

Normally, the amount of urine secreted varies with the time of day. For an hour or two after a meal, there is usually an increase in urine formation. During the night much less urine is formed than

[5] E. K. Marshall, Physiol. Reviews, **6**, 440 (1926); Addis, Barnett and Shevky, Am. J. Physiol., **46**, 1, 39, 52 (1918); Addis and Drury, J. Biol. Chem., **55**, 105, 629, 639 (1923); Addis, Meyers and Bayer, Am. J. Physiol., **72**, 125 (1925).

during the day. If the total urine for twenty-four hours is 1500 cc., that formed during the night is usually about 400 or 500 cc. Simpson has observed that these relations hold even whan a definite amount of water is given at hourly intervals during the day and night. Apparently there is a retention of water during the night, whereas during part of the day there is a negative water balance. These variations seem to be dependent, at least in part, on body temperature. Coincident with the rise in body temperature which occurs at about 6 A.M., there is an increased secretion of urine. Both the temperature and the urine volume continue to increase until late in the afternoon or evening, after which both begin to fall until the following morning when the cycle begins again.

The chloride and phosphate elimination and *p*H of the urine are likewise decreased during sleep, but it is interesting to note that these changes may occur irrespective of significant changes in the urine volume (Simpson [6]).

In nephritis, the urine collected during short intervals of the day and night shows less than the normal variation in volume and composition. As a rule, the night urine of nephritics is more abundant than that of normal individuals. The condition in which an excessive amount of urine is secreted at night is called nocturia.

The determination of urine volume may be of value in diagnosing kidney disease. In acute nephritis due to mercuric chloride, there may be complete suppression of urine, or anuria. Oliguria is the condition of low urine output and is observed in eclampsia, cardiac derangements, fever, and diarrhea. Polyuria, or excessive secretion of urine, occurs especially in diabetes insipidus and after injury to the pituitary gland. In diabetes insipidus, the daily elimination of urine may exceed 20 liters, and there is at least one case on record in which 50 liters were excreted during a period of twenty-four hours.

Color.—Normal urine is pale yellow in color, but may vary from a slight yellow tinge to deep amber-yellow, depending on its concentration. In fever, the urine is usually dark yellow or brown-red in color. In jaundice, the presence of bile pigments gives the urine a greenish-yellow or greenish-brown color. The presence of blood or hemoglobin would obviously cause a reddish tinge. Brown and black urine may be due to the presence of methemoglobin, melanin, and phenol derivatives such as are excreted in carbolic-acid poisoning. Drugs excreted in the urine may likewise give rise to peculiar colors.

[6] J. Biol. Chem., **59**, 107 (1924); **67**, 505 (1926); **84**, 393 (1929); compare N. Kleitman, Am. J. Physiol., **74**, 225 (1925).

Drabkin [7] has made the important observation that normally the output of urinary pigment is constant from day to day and is independent of the diet. It is accordingly a product of endogenous metabolism, being eliminated in quantities which are proportional to its intensity. Experimentally the output of pigment was increased by fasting, the administration of acids, or the administration of calorigenic agents such as epinephrine or thyroxin. A diminished urinary pigment output was observed after the administration of alkali, or following the surgical removal of the thyroid gland. In exophthalmic goiter, the quantity of pigment is abnormally high. In one case which was followed daily, the amount of urinary pigment paralleled the patient's metabolic rate.

Transparency.—Freshly voided urine is clear and transparent, except after a hearty meal, when the precipitation of calcium phosphate, due to the alkalinity of the urine (alkaline tide), may render it turbid. Strict vegetarians, as well as herbivorous animals, normally excrete an alkaline and turbid urine. Clear urine may become turbid on standing, owing to the precipitation of mucin derived from the urinary tract. The conversion of urea into ammonia by bacteria may cause an acid transparent urine to change into an alkaline turbid urine, the turbidity here being due, likewise, to the precipitation of calcium phosphate.

In the abnormal condition known as chyluria, the urine has a milky appearance, which is due to the presence of fat globules. In inflammations of the urinary tract, large amounts of pus may be excreted with the urine, causing it to acquire a similar turbid appearance.

Odor and Taste.—Urine has a faint aromatic odor which has been attributed to a substance of unknown chemical composition, called urinod. The odor of urine may be influenced by the ingestion of drugs and vegetables. Asparagus causes a peculiar odor, due to methyl mercapatan. Abnormal constituents, such as acetone bodies, may modify the odor of urine. Putrefactive changes cause urine to acquire an ammoniacal odor. Normal urine is salty to the taste; diabetic urine has a sweetish taste.

Specific Gravity.—The specific gravity of urine depends on its concentration. The greater the volume, the lower is the concentration, and hence, the specific gravity. Accordingly, the specific gravity of normal urine is not fixed but may vary within a wide range of values. The normal range is usually given as 1.008–1.030. A rough estimate of the total solids of the urine, in grams per liter, may be obtained by multiplying the last two figures of the specific gravity (i.e., the second and third decimal places) by the factor 2.66 (Long's coefficient).

[7] J. Biol. Chem., **75**, 443, 481 (1927).

Reaction.—Whereas the blood is faintly alkaline in reaction (*p*H 7.35–7.43), the urine is normally acid. Indeed, on an ordinary diet, about 250–350 cc. of N/10 acid is excreted daily. Henderson and Palmer [8] have calculated that the kidneys may normally remove from the body 600–700 cc. of N/10 acid, and in diabetes the excretion of acid may be ten times as great. It is because of this that the kidneys enable the blood to maintain its reaction within certain narrow limits. When the kidneys fail to function properly, retention of urinary constituents occurs in the blood and is followed by the well-known symptoms of intoxication, which have been incorrectly classed under the term of uremia or uremic poisoning. The coma that characterizes the terminal stages of nephritis is not due so much to the retention of urea and other nitrogenous constituents as to the accumulation of acid. Cushny [9] makes the statement that acid is probably the most poisonous of all the waste products of metabolism known at present.

The kidney exerts its regulatory effect by eliminating acid and at the same time retaining, for the use of the organism, as much alkali as possible. In the blood the ratio $Na_2HPO_4 : NaH_2PO_4$ is in favor of the basic phosphate, whereas in the urine there is a preponderance of the acid phosphate. The change which brings about this altered relation, and which is believed to take place in the tubule, may be represented as follows:

$$Na_2HPO_4 + H_2O \rightarrow NaH_2PO_4 + Na^+ + OH^-$$

The sodium is retained in combination as the bicarbonate. The important point to be emphasized here is this: The glomerular filtrate resembles in composition the blood plasma. Both, therefore, contain Na_2HPO_4 and NaH_2PO_4 in approximately the same proportion. It may be supposed that the acid phosphate is excreted unchanged, although we should not disregard the possibility of a certain amount of conversion into $NH_4H_2PO_4$. The Na_2HPO_4, on the other hand, as it proceeds through the tubule, gives up a part of its Na,which is reabsorbed by the tubular epithelium.

The shift from basic to acid phosphate and the replacement of fixed base by ammonia in the kidney has been demonstrated by a number of workers. Hendrix and Sanders [10] observed that the injection of dibasic phosphate caused a marked rise in the titratable acidity and ammonia in the urine. A similar effect was produced when sodium hippurate was injected. In fact, the rise in total acidity plus ammonia was

[8] J. Biol. Chem., **13**, 393 (1913).
[9] A. R. Cushny, The Secretion of the Urine, p. 165.
[10] J. Biol. Chem., **58**, 503 (1923–24).

very nearly equivalent to the total phosphate and hippurate injected. From these observations it may be surmised that when the rate of flow of glomerular fluid through the tubules is markedly increased, as in diuresis, there is an incomplete absorption of no-threshold substances. In later experiments, Hendrix and Calvin [11] have shown that, in diuresis produced by the injection of certain neutral salts (sodium chloride, sodium nitrate, and sodium sulfate) and urea, there is a loss of base through the kidney over and above that lost normally. The excretion of base is reflected in a marked fall in the alkali reserve of the blood, which occurs simultaneously and which is obviously due to the removal of fixed base from the body. These changes are apparently due to a failure in reabsorption from the tubules, since these are flooded and overtaxed in diuresis, and cannot be supposed to function with their normal efficiency in the retention of basic ions.

Titratable Acidity.—A measure of urinary acidity may be obtained by titration. The method in general use is that of Folin, according to which a certain amount of urine (25 cc.), to which 15–20 grams of finely pulverized potassium oxalate has been added, is titrated with standard sodium or potassium hydroxide (N/10), phenolphthalein being used as the indicator. The end-point is not sharp, because of the presence of ammonium salts. The oxalate is added to precipitate the calcium as oxalate, for otherwise it would interfere with the end-point by prematurely forming insoluble calcium phosphate as the point of neutrality was approached. The acidity is usually expressed in terms of cubic centimeters of N/10 alkali required to neutralize the twenty-four-hour output of urine.

The reaction of urine is dependent upon the character of the diet. Accordingly, the titratable acidity may fall normally within a wide range of values (150–500 cc.). The average is probably about 300–350 cc. Even an alkaline urine (pH > 7) has a titratable acidity, for the point of neutrality of phenolphthalein is well over on the alkaline side (pH = 8.5). On diets rich in acid-forming foods (meat, fish, oatmeal, rice, wheat, egg yolk, prunes, etc.), a very acid urine is produced. Values of 600–900 for titratable acidity may be obtained easily if sufficient quantities of such food are eaten. Most vegetables and fruits (oranges, potatoes, beans, raisins, apples, bananas, carrots, beets, etc.) are base-forming and yield an alkaline urine. This accounts for the fact that the urine of herbivorous animals is normally alkaline, whereas carnivorous animals ordinarily secrete an acid urine.

Urinary acidity bears a relationship to the excretion of ammonia, low acidity being associated with low values for ammonia, whereas

[11] *Ibid.*, **65**, 197 (1925).

urines having high acidities contain much larger quantities of ammonia. An exception to this is often observed in nephritis, where high acidity values are not always associated with correspondingly high values for ammonia.

Van Slyke and Palmer [12] have shown that men normally excrete the equivalent of about 6.0 cc. of N/10 organic acid per kilo of body weight in twenty-four hours. In starvation and in diabetes, the organic-acid elimination is markedly increased. A similar alteration, commensurate with the changes in titratable acidity, is observed in the ammonia output in the urine. Increase in ammonia elimination may be produced experimentally by feeding acid (Fiske and Sokhey [13]). In a recent experiment, Fiske [14] observed that when exceedingly large doses of acid were given there was relatively more loss of fixed base, and less of ammonia production, than when the dose was smaller; but for several days after the acid administration, the output of fixed base was lower than normal, whereas the ammonia elimination continued to be high, showing that there was a retention of fixed base at the expense of ammonia.

Blatherwick and Long [15] have observed that drinking large amounts of orange juice resulted in the production of alkaline urines, an increased organic acid excretion and a decreased output of ammonia. The explanation of the increased organic acidity is that a certain amount of the citric acid escapes oxidation and is eliminated in the urine as citrate and thus increases the titration value for organic acids. However, this affects only a small part of the citric acid even when large amounts are taken; in fact the authors state: "It was impossible to overreach the organism's ability to oxidize the contained citric acid even though the amounts (of orange juice) drunk in one day were the equivalent of 48 grams of acid." Accordingly, the excess of base in the orange juice in Blatherwick's experiments was sufficient to balance the organic acidity which escaped oxidation and to cause an alkaline urine, as well as a marked depression in the ammonia excretion.

The increased urinary acidity produced by eating prunes and cranberries is due to hippuric acid.

Hydrogen-ion Concentration.—The hydrogen-ion concentration, or the reciprocal of its logarithm, may be taken as a truer index of urinary acidity than is afforded by titration. Determinations of *p*H of urine are usually made by indicator methods previously mentioned. For

[12] J. Biol. Chem., **41**, 567 (1920).
[13] J. Biol. Chem., **63**, 309 (1925).
[14] *Ibid.*, **67**, 385 (1926).
[15] *Ibid.*, **53**, 103 (1922); **57**, 815 (1923).

details of these procedures the student is referred to laboratory manuals of biochemistry. The extreme range of urinary acidity is usually given as *p*H 4.80–7.50, the average normal value being about 6.0. Deviations from this mean value are dependent on the character of the diet, a high-acid diet yielding urine of low *p*H, whereas on a low-acid diet the urine obtained has a high *p*H. Low *p*H values are found in pathological conditions, especially in diabetes and cardio-renal disorders.

Perhaps the most striking change in the reaction of the urine is that which occurs after meals, when the urine becomes less acid and may even acquire a neutral or alkaline reaction. This is referred to as the "alkaline tide," and has been attributed to the withdrawal of hydrogen ions, attending the formation of the hydrochloric acid of the gastric juice. Inasmuch as in conditions of anacidity (lack of HCl formation) Hubbard, Munford and Allen [16] did not observe an alkaline tide, it may be inferred that the secretion of gastric juice is an important factor in causing it. Possibly the moderate diuresis which occurs after meals is another factor. The increased acidity of the urine secreted during sleep has been referred to in another connection.

Composition of Urine.—In view of all that has been written in the preceding chapters, it would be superfluous to explain why the composition of urine is so variable. It will therefore be sufficient, at this point, to review briefly the better-known constituents of normal urine. These may be grouped under three heads:

1. Nitrogen-containing waste products:—urea, uric acid, creatinine, creatine (in children), hippuric acid, indican (indoxyl-potassium sulfate), skatoxyl-sulfuric acid (as salt in traces), allantoin (absent or in traces), traces of purine bases other than uric acid (adenine, guanine, xanthine, epiguanine, paraxanthine, heteroxanthine, *l*-methylxanthine), amino acids.
2. Nitrogen-free organic constituents:—glucose, glucuronic acid or similar reducing substances in traces, aromatic oxyacids, oxalates, traces of acetone bodies, volatile fatty acids (acetic, formic and butyric in traces).
3. Inorganic constituents:—chlorides, sulfates, phosphates, carbonates, of calcium, sodium and potassium, traces of nitrates, silicates, fluorides, iron, copper, and zinc.

In addition to these there are the pigments, chief of which is urochrome, a number of neutral sulfur compounds, some of which contain

[16] Am. J. Physiol., **68**, 207 (1924); for the factors which cause the change in the reaction in morning urine see J. Biol. Chem., **84**, 191, 199 (1929).

nitrogen as well (cystine, chondroitin-sulfuric acid, thiocyanates, taurine derivatives, oxyproteic acid), ethereal sulfates other than those mentioned (phenol and para-cresol-sulfuric acids and pyro-catechol-sulfuric acid), traces of oxaluric acid, benzoic acid, peptides, phenaceturic acid, certain phosphorized compounds (glycerophosphoric acid, phosphocarnic acid), etc.[17]

An idea of the quantitative relations of the chief urinary constituents may be obtained from the data in Table XL, taken from Mitchell.[18]

TABLE XL

REPRESENTATIVE COMPOSITION OF NORMAL HUMAN URINE

Volume for 24 hours, 1250 cc.
Specific gravity, 1.019

	Weight, Grams	Approximate Per Cent
Water	1212.0	95.1
Solids	61.7	4.9
Nitrogen-containing constituents:		
Urea	28.5	2.28
Creatinine	1.7	0.13
Ammonia, computed as NH_3	0.7	0.05
Uric acid	0.65	0.05
Hippuric acid	0.6	0.04
Indican, indoxyl potassium sulfate	0.01	0.0008
Allantoin (not always present)	0.005	0.0004
Creatine (not usually present in the urine of healthy adults but may occur in traces).		
Nitrogen-free organic constituents:		
Glucose or similar carbohydrate	0.7	0.05
Aromatic oxyacids	0.05	0.004
Oxalates, as oxalic acid	0.015	0.001
Acetone, acetoacetic acid	0.01	0.0008
Inorganic constituents:		
Chlorides, as NaCl	11.0	0.90
Phosphates, as P_2O_5	2.2	0.18
Sulfates, as SO_3	1.7	0.14
Potassium	1.6	0.12
Calcium	0.2	0.01
Magnesium	0.2	0.01
Iron	0.005	0.0004

[17] Based on table given in P. B. Hawk's "Practical Physiological Chemistry," 9th edition, 1926, p. 592.

[18] P. H. Mitchell, General Physiology, McGraw-Hill Book Co., New York, 1923, p. 635.

The values given are not supposed to show the average composition of human urine. They are based upon a limited number of analyses but are nevertheless fairly representative results.

Composition of Urine in Relation to Diet.—At this stage of the discussion, it may be profitable to consider how the composition of the diet influences that of the urine. By this time, the student has no doubt become aware that the study of certain phases of metabolism requires a knowledge of the end-products appearing in the urine. The type of information which may be obtained from the quantitative analysis of urine will be illustrated by a few simple examples.

Let us suppose that the student wishes to study the effect of changing the amount of protein of the diet on the composition of the urine. He may proceed to do so by first examining the urine on a diet containing the amount of protein to which he is normally accustomed. The composition of the food should be known. This information may be obtained by analyzing the food, or it may be calculated from known data, since the composition of the common foodstuffs has been determined. On a given day the urine should be collected over a period of exactly twenty-four hours. This urine is then analyzed for the constituents to be given presently, as well as for any others which the student may wish to determine.

Having performed this preliminary experiment, the student may now vary his protein intake. It is desirable that he be on the experimental diet (high-protein intake, low-protein intake, etc.) not only on the day when the urine is collected, but for two or three days preceding this. The reason for going on the experimental diet several days before collecting the urine is that an alteration in the diet does not produce an immediate effect on the composition of the urine. There is usually a lag in the elimination of the nitrogenous end-products of metabolism. This is referred to as the " nitrogen lag."

In the table on p. 388 are recorded the results of representative analyses of twenty-four-hour specimens of urine collected:

(*a*) On a diet containing an ordinary amount of protein (equivalent to about 15 grams of nitrogen per day),

(*b*) On a diet containing more than the usual amount of protein (equivalent to about 25 grams of nitrogen, given in the form of meat and eggs, etc.),

(*c*) On a diet containing very little protein (cream, starch, butter, potatoes) but adequate as regards caloric requirements.

The subject was male and weighed 67 kg.[19]

[19] Total nitrogen is determined by the Kjeldahl method. For purposes of com-

TABLE XLI

INFLUENCE OF PROTEIN INTAKE ON THE COMPOSITION OF URINE

(Daily Output)

	Usual Protein Intake	Protein-rich Diet	Protein-poor Diet
Total nitrogen (g.)	13.20	23.28	4.20
Urea nitrogen (g.)	11.36	20.45	2.90
Ammonia nitrogen (g.)	0.40	0.82	0.17
Creatinine nitrogen (g.)	0.61	0.64	0.60
Uric acid nitrogen (g.)	0.21	0.30	0.11
Undetermined nitrogen (g.)	0.62	1.07	0.52
Titratable acidity (cc. 0.1 N)	284.0	655.0	160.0
Total sulfur as SO_3 (g.)	2.65	3.55	0.86
Inorganic sulfate as SO_3 (g.)	2.16	2.82	0.64
Ethereal sulfate as SO_3 (g.)	0.18	0.36	0.11
Neutral sulfur as SO_3 (g.)	0.31	0.37	0.11
Total phosphates as P_2O_5 (g.)	2.59	4.07	1.06
Chlorides as NaCl (g.)	12.10	15.10	9.80
Volume (cc.)	1260	1550	960

Urea nitrogen is usually 80–90 per cent of the total nitrogen, but when the total nitrogen is very low the urea nitrogen may be only 60–70 per cent of the total. On a high-protein diet, particularly on one containing meat, the output of total sulfur and phosphorus is increased, as well as the titratable acidity. The change in acidity influences the ammonia output, as indicated above. The increase in uric acid on the protein-rich diet and the decrease on the protein-poor diet are to be attributed to the presence of nucleic acid in the protein fed (part of it was meat). The undetermined nitrogen represents the nitrogenous constituents, other than those given, which are present in urine. The most important of these are probably hippuric acid and purine bases. Like ammonia, hippuric acid is believed to be synthesized in the kidney. Upon this fact is based a method for testing renal function, which consists in determining the rate of excretion of hippuric acid after the administration of sodium benzoate (Kingsbury and Swanson).[20] On a high-protein diet, there is usually a greater amount of intestinal putrefaction than otherwise occurs. This may account for the increase in

parison, the concentrations of the nitrogenous constituents are expressed usually in terms of nitrogen. The analytical procedures are described in laboratory manuals of biochemistry.

[20] J. Biol. Chem., **46**, iv (1921).

ethereal sulfates on the protein-rich diet. No special significance need be attached to the changes in the elimination of chlorides and water. When large quantities of food are consumed, an increased intake of chlorides and water is usually incidental.

The influence of purine-free and purine-rich diets is indicated in the following table. In the experiments represented by the data contained therein, an attempt was made to maintain the total nitrogen intake at approximately the same level as on the day of the normal protein diet described above. The purine-rich diet consisted largely of glandular tissues (thymus, pancreas, and liver). On the low-purine diet, the most important changes were those involving the titratable acidity and the output of uric acid and phosphates. All three were increased on the purine-rich diet and diminished on the low-purine diet. These changes were due to the relative abundance, in the first case, of uric acid and phosphate precursors, and the relative lack of these in the second case. With the increased acidity on the high-purine diet, there was a corresponding rise in ammonia excretion. The high value for undetermined nitrogen on this diet was due, no doubt, to an increased elimination of purine bases other than uric acid.

It will be observed that there was very little, if any, change in the

TABLE XLII

INFLUENCE OF HIGH- AND LOW-PURINE DIETS ON THE COMPOSITION OF URINE

(Daily Output)

	High-Purine Diet	Low-Purine Diet
Total nitrogen (g.)	15.75	13.54
Urea nitrogen (g.)	12.97	11.88
Ammonia nitrogen (g.)	0.90	0.51
Creatinine nitrogen (g.)	0.61	0.60
Uric acid nitrogen (g.)	0.43	0.11
Undetermined nitrogen (g.)	0.84	0.44
Titratable acidity (cc. 0.1 N)	638	182
Total sulfur as SO_3 (g.)	3.64	2.00
Inorganic sulfate as SO_3 (g.)	2.81	1.53
Ethereal sulfate as SO_3 (g.)	0.46	0.22
Neutral sulfur as SO_3 (g.)	0.39	0.25
Total phosphates as P_2O_5 (g.)	3.94	1.40
Chlorides as NaCl (g.)	13.20	12.80
Volume (cc.)	1620	1410

creatinine values in this and in the preceding series of experiments, as might be expected from the fact that creatinine is derived from endogenous and not exogenous sources.

Equally illuminating are the changes that occur during starvation. The following results are based upon analyses of urine collected during the first and fourth days of a fasting period.

TABLE XLIII

INFLUENCE OF FASTING ON THE COMPOSITION OF URINE

(Daily Output)

	First Day of Fast	Fourth Day of Fast
Total nitrogen (g.)	7.08	14.40
Urea nitrogen (g.)	5.80	11.82
Ammonia nitrogen (g.)	0.21	1.32
Creatinine nitrogen (g.)	0.59	0.44
Creatine nitrogen (g.)	.....	0.16
Uric acid nitrogen (g.)	0.15	0.08
Undetermined nitrogen (g.)	0.33	0.58
Titratable acidity (cc. 0.1 N)	176	720
Total sulfur as SO_3 (g.)	1.22	2.01
Total phosphates as P_2O_5 (g.)	1.71	1.14
Chlorides as NaCl (g.)	5.20	1.26
Acetone bodies (g.)	(trace)	3.86
Volume (cc.)	860	880

It is obvious that, during the first day of the fasting period, sufficient glycogen was available to supply most of the energy requirements. Consequently, relatively little protein was broken down for this purpose, as shown by the data. Since glucose metabolism was taking place, there was complete conversion of creatine to creatinine. There was likewise, complete oxidation of fatty acids, as evidenced by the fact that the urine did not contain abnormal quantities of acetone bodies. The excretion of ammonia, uric acid, sulfate and phosphate, and the titratable acidity were less than on the normal diet, owing to the diminished metabolism of amino acids and purines.

By the fourth day, the glycogen stores had been fairly well depleted, for there was on that day an increase in tissue break-down (rise in total N), as well as incomplete conversion of creatine into creatinine and failure in fat combustion (appearance of large amounts of acetone

bodies). The continued decrease in uric-acid elimination may be explained on the ground that there was diminished nuclear metabolism and probably a retention of uric acid in the blood, in accordance with the suggestion of Lennox.[21] Commensurate with this change, there was a marked decrease in the excretion of phosphates. Nevertheless, the titratable acidity was higher than normal, owing to the acetone bodies. Accordingly, there was a corresponding increase in the formation and excretion of ammonia. Creatine appeared in the urine at the expense of creatinine. Chloride elimination decreased to a low level.

Pathological Constituents of Urine.—Except in cases of alimentary glycosuria, the presence of more than traces of glucose in the urine is of pathological significance. This has been discussed in other connections. Fructose occurs frequently, together with glucose, in diabetic urine, but has been likewise observed in non-diabetic individuals. This is a rare anomaly, called fructosuria. Pentosuria is another rare anomaly. Failure to remove milk from the mammary glands of lactating animals may lead to the appearance of lactose in the urine. This condition, called lactosuria, is occasionally observed during pregnancy and lactation and is somewhat more frequent during the weaning period.

Albuminuria, or the presence of albumin or globulin in the urine, is usually indicative of renal injury, or nephritis. In this condition the kidneys become abnormally permeable to protein, with the result that a certain amount of it is excreted. However, protein may enter the urine below the kidneys, in which case the condition is usually called post-renal or "false" albuminuria in contradistinction to "true" albuminuria. Certain individuals, on standing for a variable length of time, develop albuminuria. This condition may be the result of stasis of the blood in the kidney, due to low blood pressure. It is called orthostatic or postural albuminuria.

In pneumonia, diphtheria, osteomalacia, atrophy of the kidneys, carcinoma, and other conditions, proteoses and peptones are frequently found in the urine. Perhaps the most important of the proteoses is "Bence-Jones protein," which has the property of precipitating at low temperatures and of redissolving as the temperature is raised. The presence of this substance in the urine is of diagnostic value in multiple myeloma.

Among the other proteins that are occasionally found in the urine are oxyhemoglobin and nucleoprotein. The former usually appears as a result of hemolysis, whereas the presence of the latter may be due to nephritis, pyelitis, or inflammation of the bladder.

[21] J. Biol. Chem., **66**, 521 (1925).

The formation of a communicating channel between the lymph vessels and the urinary tract, due to a lesion, may result in the appearance of fat in the urine. This condition is called chyluria. The appearance of blood in urine (hematuria) may be due to a lesion in the kidney or in the urinary passages. Inflammation of the genito-urinary tract leads to the presence of pus in urine (pyuria). In icterus or jaundice, bile pigments and bile salts appear in the urine.

The urine of persons with melanotic tumors, on exposure to air, gradually turns dark brown or black, owing to the presence of an oxidizable substance (melanogen) which on oxidation yields a black pigment (melanin). Another pigment found in certain pathological conditions (pulmonary tuberculosis, typhoid fever, nephritis, etc.) is urorosein, present as a chromogen which Herter[22] found to be indole-acetic acid. Homogentisic acid is present in the urine of alcaptonurics, and cystine in that of cystinurics.

Creatine and the acetone bodies have been discussed elsewhere.

Sediment.—Urine sediments may be collected readily by centrifuging the urine. Among the constituents that settle out are calcium phosphate, uric acid, and urates. Calcium carbonate occurs in the urine of herbivorous animals, but very rarely in human urine. Calcium oxalate crystals are frequently observed, especially after apples or sweet potatoes have been eaten. Crystals of leucine, tyrosine, and cystine are present occasionally, even in normal urine.

Among the cellular elements are epithelial cells and cell débris derived from the lining epithelium of the urinary tract. An occasional pus cell may be found, even in normal urine, on examining the sediment. These constituents are markedly increased in inflammatory conditions of the pelvis, kidney, ureters, bladder, or urethra. Spermatozoa may also be present in urine.

Microscopic examination of urinary sediment is often made with the object of determining the presence or absence of casts. These are derived from the renal-tubular epithelium and are usually cylindrical in shape, having parallel sides and rounded ends. Casts are classified according to their morphological characteristics. There are the so-called hyaline, granular, epithelial, and fatty casts. These are described in detail in textbooks devoted to clinical pathology. The presence of casts in the urine, together with a positive test for albumin, is diagnostic of renal disorder.

[22] J. Biol. Chem., **4**, 253 (1908).

CHAPTER XV

INTERNAL SECRETIONS

The glands of internal secretion, or endocrine organs, will be considered in this chapter mainly from the standpoint of their effect in chemically correlating the various activities of the animal organism, particularly with reference to metabolism. Certain glandular secretions, not those with which we are concerned here, are transported by means of ducts. The gastric and pancreatic juices and the saliva are familiar examples of such secretions. In contrast to these it has been discovered that certain glands pour their products directly into the blood stream. These ductless glands and their secretions are the subject of the present chapter. The internal secretions are of the utmost importance to the animal body because of their effect in correlating the activities of its many organs. In fact, certain secretions are indispensable to life.

Although the subject of endocrinology may be said to have an earlier history, nevertheless, the work done by Bayliss and Starling [1] on secretion, in 1902, is usually regarded as marking the beginning of the modern development of this important branch of physiology and biochemistry. Bayliss and Starling recognized that substances of the type of secretin were probably chemical in nature, and, since such substances appeared to stimulate or arouse organs and tissues to activity, they suggested the term "hormone," from the Greek, meaning "I rouse to activity." Schafer has suggested the general name "autocoid" for these substances.

Since a hormone is defined as a substance formed in one organ and carried to another organ where it sets up definite physiological activity, even such compounds as urea and carbon dioxide might possibly be regarded as hormones. Urea has its origin in the liver and stimulates the kidney; carbon dioxide exerts an effect on the respiratory center. While it is difficult at present to make a hard-and-fast rule as to what to include and what to exclude under the definition, nevertheless, the substances mentioned are not classed with the hormones. If all the by-products of metabolism that incidentally have a regulatory effect

[1] J. Physiol., **28**, 325 (1902).

on bodily functions were included under the definition of hormone, then, as Taylor [2] states the case, the number of hormones would be illimitable. The internal secretions with which we are more directly concerned at present are those that have a controlling effect on the metabolic functions of the body. From this standpoint, the most important organs of internal secretion are the thyroid, parathyroids, pituitary, suprarenals or adrenals, pancreas, and sex glands. Of these, the pancreas and the generative glands have both internal (ductless) and external (duct) secretions. The liver should perhaps be included, in view of the recent discovery in this organ of a substance which is a specific curative agent for pernicious anemia. As yet, however, it has not been determined that this substance is in the nature of a hormone. The intestinal mucosa, it will be recalled contains secretin, concerned with pancreatic secretion, and possibly a second hormone, " cholecystokinin," which is said to stimulate contraction of the gall bladder.

The Thyroid Gland.—In man, the thyroid gland is a bi-lobed, reddish-yellow, highly vascular organ, surrounded by a capsule of connective tissue; it weighs about 20 or 25 grams and is situated at the sides of the larynx and treachea. Histologically, the organ appears to be composed of numerous closed alveoli or vesicles containing a single layer of cuboidal epithelium and filled with translucent material known as " *colloid.*"

Baumann,[3] in 1895, made the important discovery that the thyroid gland of mammals contains iodine in firm organic combination. On acid hydrolysis of the thyroid, he obtained an iodine compound which was named " iodothyrine." Oswald [4] studied the colloid material of the thyroid gland and found it to be mainly globulin. He found, moreover, that in general the amount of iodine varied with the amount of visible colloid, although hyperplastic thyroids could be rich in globulin and yet be iodine-free. It was Oswald who introduced the term " iodothyreoglobuline " for the globulin-iodothyrine complex. This question has been carefully studied by Marine,[5] many of whose observations give support to those of Oswald. According to Marine, the iodine store in the thyroid varies, in general, with the amount of stainable colloid, and inversely with the degree of active hyperplasia; and, in the extreme degrees of active hyperplasia seen in cretinoid states in man and ani-

[2] N. B. Taylor in Macleod's " Physiology and Biochemistry in Modern Medicine," 1920 edition, p. 766.

[3] Z. physiol. Chem., **21**, 319, 481 (1896).

[4] *Ibid.*, **23**, 265 (1897); **32**, 121 (1901).

[5] Arch. Int. Med., **1**, 349 (1908); **3**, 66 (1909). David Marine, Functions of the Thyroid Gland, Physiol. Reviews, **2**, 521 (1922).

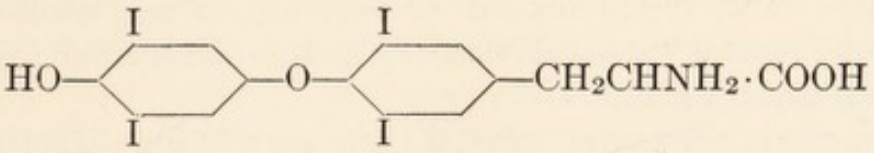
mals, the iodine store may be entirely exhausted. The relation of iodine to histological structure is brought out by the following data (Marine and Lenhart [5]):

	Normal	Early Hyperplastic Stage	Moderate Hyperplastic Stage	Marked Hyperplastic Stage	Colloid or Resting Stage
Man.....	2.17*	0.88	0.71	0.32	2.00
Dog.....	3.32	0.62	0.37	0.11	1.99

* Iodine in milligrams per gram of dried gland.

Thyroxin.—The isolation of the active principle of the thyroid gland was reported by Kendall [6] in 1916. From 3 tons of the fresh organ he obtained 33 grams of a substance which had the same pharmacological properties as whole thyroid gland. About 10 years later, Harington [7] improved the method of isolation and obtained yields as high as 0.027 per cent from fresh gland and 0.12 per cent from dried thyroid gland.

The relation of thyroxin to tyrosine and its chemical constitution was determined by Harington (and independently by Dakin [8]). Later Harington and Barger [9] accomplished its synthesis. The synthetic product has the same chemical and physiological properties as the hormone isolated from the gland.



Thyroxin
($C_{15}H_{11}O_4NI_4$)

β-[3 : 5-*Diiodo*-4-(3′ : 5′-*diiodo*-4′-*hydroxyphenoxy*) *phenyl*]-*α*-*amino*-*propionic acid*

Harington [10] has resolved the racemic form, *dl*-thyroxin into its two optically active isomers. It has been found that *l*-thyroxin is physiologically more active (about three times) than *d*-thyroxin.

In addition to thyroxin, the thyroid has a second iodine derivative

[6] Collected papers of the Mayo Clinic, **8**, 513 (1916): J. Biol. Chem., **40**, 265 (1919); see also Ann. Clin. Med., **1**, 256 (1923).

[7] Biochem. J., **20**, 293, 300 (1926).

[8] See footnote in paper by Harington and Barger.

[9] *Ibid.*, **21**, 169 (1927).

[10] *Ibid.*, **22**, 1429 (1928); Gaddum, J. H., **22**, 1434 (1928).

of tyrosine, 3, 5-diiodotyrosine, as recently shown by Harington and Randall [11] and by Foster.[12] From a sample of 100 grams of thyroglobulin, containing 760 mg. of iodine, Foster isolated 0.44 gram of diiodotyrosine, containing 248 mg. of iodine. This accounted for 33 per cent of the total iodine. The thyroxin which was isolated accounted for an additional 16 per cent of iodine. These observations indicate that thyroglobulin may contain still other iodine compounds and also that 3, 5-diiodotyrosine may be the precursor of thyroxin. It is to be noted, however, that this derivative of tyrosine is without the physiological properties possessed by thyroxin.[13]

3, 5-Diiodotyrosine is widely distributed in certain marine organisms. It was first discovered by Drechsel [14] among the hydrolytic products of the axial skeleton of the Gorgonian coral. Mörner [15] found it in the skeleton of certain anthozoa and Wheeler and Mendel [16] in sponges.

The oxidative processes of the organism are under the control of thyroxin. Excessive secretion of the thyroid hormone stimulates cellular oxidation and hence elevates the rate of metabolism. On the contrary cellular oxidation and the metabolic rate are depressed when there is an insufficiency of the thyroid hormone.

Diseases of the Thyroid Gland.—There are many classifications of thyroid disease in medical literature, largely because clinicians have been forced to base their distinctions on symptomatology alone. The manifestations of deranged thyroid function are exceedingly variable; nevertheless, most symptoms can be regarded as the result either of diminished function (hypofunction) or increased function (hyperfunction) of the thyroid gland.

Myxedema is a manifestation of hypothyroidism. This condition results from atrophy of the thyroid gland and the consequent reduction in the secretion of the hormone thyroxin. It may be produced also by partial or complete extirpation of the thyroid. Among the more important symptoms are the following:

(*a*) The face and hands become puffed and swollen. This is not due to edema, but to thickening of the subcutaneous connective tissue.

[11] Biochem. J., **23**, 373 (1929).

[12] J. Biol. Chem., **83**, 345 (1929).

[13] Compare with the observations of Abderhalden on tadpoles, Arch. ges. Physiol., **206**, 467 (1924).

[14] Z. Biol., **33**, 85 (1896).

[15] Z. Physiol. Chem., **51**, 33 (1907); **55**, 77, 223 (1908).

[16] J. Biol. Chem., **7**, 1 (1909).

(*b*) There is a marked reduction in the basal metabolic rate (see next chapter). The temperature of the body and the pulse rate tend to be subnormal.
(*c*) There is a tendency to obesity.
(*d*) The skin has an unhealthy appearance and the hair tends to fall out.
(*e*) Myxedematous individuals are sluggish both mentally and physically.
(*f*) Myxedematous individuals are often anemic.

The symptoms are relieved on feeding thyroid gland or extracts of it, or upon the administration of thyroxin. The effects of the administered thyroid hormone may last for several weeks. However, when treatment is discontinued for longer periods there is a relapse, showing that the active principle of the thyroid gland is not readily stored.

Myxedema is more frequent among women than among men.

Cretinism.—Failure in the embryonic development of the thyroid or its atrophy during fetal life or childhood results in the condition called cretinism. There are two forms of this disease. The endemic form is due presumably to goitrous degeneration of the gland and is found in districts where goiter is endemic. The failure in thyroid development in this form may be due to a lack of iodine in the organism of the mother. The sporadic form of cretinism may occur anywhere. As to its etiology, nothing is known except that it may have the same underlying causes as myxedema.

The most noticeable symptom is the practically complete cessation of physical and mental development, resulting in dwarfism and idiocy. Cretins are typically pot-bellied, ugly, and somewhat obese. The hair is thick and coarse; and the skin dry and pale. As in myxedema, the basal metabolic rate is low.

Concerning the deficiency of the thyroid hormone in cretinism, there can be little question. Cretins fed on whole thyroid gland or treated with thyroxin begin to develop normally. There is almost immediate improvement both mentally and physically; an ugly, idiotic child may be converted into an almost normal one. The cures which have been accomplished in this way are most remarkable. Improvement in the case of cretins of long standing is not always possible.

Endemic or Colloid Goiter.—Hyperplasia or enlargement of the thyroid does not necessarily indicate hyperfunction. Being easily recognized by the marked swelling which develops in the region of the neck, this disease was known to the ancients. The symptoms are due apparently to an increase in the colloid material of the gland, hence the name " colloid

goiter." This condition may or may not be accompanied by a decrease in hormone secretion. Accordingly, patients with endemic goiter may have either normal or subnormal basal metabolic rates.

Goiter is especially prevalent in certain parts of Switzerland and in the region of the Great Lakes in the United States. That it is due to a lack of iodine was shown by Marine and Kimball.[17] Accordingly, the method of treatment is to give small doses of iodides. Prophylactic measures have been taken in goiter regions by adding simple inorganic iodides to the drinking water supply, and to the table salt, and by periodically administering therapeutic doses of iodide to school children. This procedure is to be especially recommended in the case of adolescent girls and during pregnancy. McClendon[18] has reported the results of analyses of drinking water obtained from regions where goiter is endemic and from other regions. These show higher values for iodine in the water from non-goitrous regions than in that from regions where goiter is prevalent.

Simple goiter is so prevalent in the Great Lakes basin that a large proportion of the dogs and other animals of the region are afflicted with it. The practice of administering small amounts of iodine to stock animals for the purpose of preventing goiter has shown beneficial results.

Van Dyke[19] has determined the relative absorption of iodine and various iodine compounds, potassium iodide, potassium iodate and thyroxin, when injected intravenously, by the hyperplastic thyroid gland of the dog. He found that iodide iodine was most readily absorbed and that iodate iodine was also taken up in considerable amount. Free iodine was absorbed in small amount, probably because a considerable proportion must have combined with the unsaturated lipids of the blood. The least effect in increasing the iodine content of the thyroid was obtained on administering thyroxin which was apparently taken up by the gland very slowly and in only minute amounts.

Exophthalmic Goiter.—Exophthalmic goiter (also known as Graves' and as Basedow's disease) is the most important example of hyperthyroidism. Among the symptoms are the following:

(*a*) There is usually marked hyperplasia of the thyroid gland.

(*b*) There is an increase in the basal metabolic rate. The temperature of the body is usually above normal. The heart rate is faster than normal and is irregular (tachycardia).

[17] J. Am. Med. Assoc., **77**, 1068 (1921).

[18] *Ibid.*, **82**, 1669 (1924); see also the review "The Distribution of Iodine with Special Reference to Goiter," Physiological Reviews, **7**, 189 (1927).

[19] Arch. Int. Med., **41**, 615 (1928).

(*c*) There is a marked tendency to emaciation.
(*d*) Usually, though not always, there is protrusion of the eye-ball ("exophthalmos").
(*e*) Extreme restlessness and hyperexcitability.
(*f*) Gastro-intestinal disturbances.

Most physiologists believe exophthalmic goiter to be due to hypersecretion of the thyroid hormone, in view of the fact that some of the symptoms of this disease may be produced by the administration of sufficient doses of thyroxin. Another point in support of this general idea is that many of the symptoms of exophthalmic goiter are exactly the reverse of those noted in myxedema. Since the disease is presumably due to excessive secretion, the methods of treatment consist in diminishing the amount of active thyroid, by partial extirpation of the gland, by ligation of the thyroid arteries, or by exposure to Roentgen rays or radium. Most cases usually yield to these methods of treatment, although it is not always possible to clear up the exophthalmos and the cardiac symptoms.

Certain clinicians believe that simple goiter, which is essentially a condition of hypothyroidism, may pass over into Graves' disease. In the opinion of Marine and Lenhart, expressed in an early paper, there is no adequate evidence for the view that exophthalmic goiter is due to hypersecretion.

Biological Significance of the Thyroid.—A few additional words may be said concerning the thyroid in relation to development. The metamorphosis of tadpoles into frogs is dependent on thyroid secretion; if the gland is removed, this change does not take place, although the tadpole may continue to grow, as such. If at any time thyroid is given, prompt metamorphosis occurs. Gudernatsch [20] discovered that the feeding of thyroid to young tadpoles results in premature metamorphosis with the formation of exceedingly small frogs, often no larger than a fly. Abderhalden [13] reported similar results with 3, 5-diiodotyrosine. Traces of iodine may exert a similar effect, according to Swingle.[20a] There is a species of salamander, found in Mexico, which never undergoes metamorphosis, apparently because of the absence of the thyroid. Metamorphosis can be induced artificially, however, by thyroid feeding.

It may be pointed out here that thyroid function is apparently closely related to the activity of other organs of internal secretion (adrenals, pituitary, thymus, pancreas, etc.). For example, sexual development is depressed in conditions of hypothyroidism. That a

[20] Zentr. Physiol., **26**, 323 (1912).
[20a] J. Gen. Physiol., **1**, 593 (1919).

relationship exists between the thyroid and the generative glands of the female is also indicated by the fact that enlargement of the thyroid occurs at puberty, during menstruation, and during pregnancy. Other relationships will be mentioned as we proceed in our discussion of the endocrine organs.

It has been estimated that in man approximately 1 mg. of thyroxin is secreted per day. The administration to a normal individual of an additional milligram is sufficient to produce a definite calorigenic effect, and 2 mg. will raise the basal metabolic rate by about 20 per cent.[21]

The Parathyroid Glands.—The earlier physiologists and surgeons had observed that thyroidectomy frequently led to the development of tetany, followed by death. That this outcome was actually due to the accidental removal of an independent set of glands was not generally appreciated until the beginning of the present century despite the fact that the parathyroid glands had been discovered twenty years previously (1880) by the Danish anatomist Sandstrom.[22] There are usually two pairs of parathyroid glands, one pair lying on each side of the neck, close to the thyroid or embedded in it. At least in some animals (cat, rabbit, etc.) there are probably additional or accessory parathyroid structures scattered along the trachea near-by. The parathyroids are small glands, yellowish-brown to brown-red in color, usually bean-shaped in structure and about the size of a hemp-seed or somewhat larger; in man they are variable in length (3–15 mm.), about 2–3 mm. in breadth and 2 mm. in thickness.

In 1925, Collip [23] succeeded in isolating the active principle from the parathyroid gland. The hormone preparation exhibited a marked effect in raising the calcium concentration of the blood both in normal and parathyroidectomized animals. As yet, the chemical nature of the parathyroid hormone has not been determined; Collip's purest preparations give positive tests for protein and are said to contain sulfur and iron. The work of this investigator has contributed much toward establishing the theory that the parathyroid glands secrete a hormone which is concerned with the regulation of calcium metabolism and with controlling, in some way, the concentration of calcium in the blood.

Tetania parathyreopriva is the condition which follows the extirpation of the parathyroids in man and most animals, particularly the carnivora. The dog is especially susceptible. Usually in from one to four days

[21] Plummer, H. S., J. Am. Med. Assoc., **77**, 243 (1921).

[22] Upsala läkerför. forh., **15**, 44 (1880).

[23] J. Biol. Chem., **63**, 395 (1925); Am. J. Physiol., **72**, 182 (1925); Collip and Clark, Trans. Roy. Soc. Canada, **19**, Sect. V, 25 (1925); J. Biol. Chem., **64**, 485; **66**, 133 (1925); **67**, 679 (1926); Collip, Harvey Lectures, Series **21**, 1927.

after the operation, symptoms of intoxication become manifest. There is loss of appetite, the motor nerves become hyper-excitable to electrical but not to mechanical stimuli, and there is marked restlessness. Diarrhea, often bloody, is a frequent symptom. Soon fine tremors set in, and the animal gradually becomes stiff. The tremors become more and more violent, the temperature, respiration and heart action increase. After nine or ten days the animal dies in spasm or convulsions, or from exhaustion.[24] The effects of loss of parathyroid function are especially severe where the calcium requirement is increased as in pregnant or lactating animals, or in animals with active rickets. In parathyroid tetany, the outstanding change in the composition of the blood is a marked decrease in the concentration of calcium. Normally human and dog serum contains 10 to 11 mg. of calcium per 100 cc. Following the removal of the parathyroid, the concentration may fall to 5 to 6 mg., and even lower. The symptoms may be relieved at this time by the administration of calcium salts, such as calcium lactate, the disappearance of the symptoms being associated with an increased concentration of calcium in the blood. However, the administration of parathyroid extract is much more effective, especially if administered together with calcium lactate. The use of parathyroid hormone clinically has, on the whole, yielded satisfactory results.[25]

Tetany may occur spontaneously (idiopathic tetany), particularly in children and infants. It is frequently accompanied by severe gastro-intestinal disturbances. The spasms resemble those observed in tetania parathyropriva. The condition is believed to be due to deficient parathyroid function. This is likewise regarded by many clinicians as the cause of certain obscure nervous disorders, including chorea and epilepsy. Deficient parathyroid function interferes with calcium metabolism to such an extent that the teeth and bones fail to calcify properly.

In an interesting series of experiments, Greenwald [26] observed that the administration of calcium salts to thyro-parathyroidectomized dogs led, at first, to a deposition of a large part of the calcium in the tissues, chiefly as calcium phosphate. Later, calcium equilibrium was attained. Greenwald suggests that under normal conditions the calcium content of the plasma may be maintained at a constant level by

[24] For a more detailed description of the symptoms of parathyroid tetany and for a general review of the physiology of the parathyroid glands, consult L. R. Dragstedt, Physiol. Reviews, **7**, 499 (1927).

[25] Lisser and Shepardson's paper (Endocrinology, **13**, 427 (1929)) contains a review of several cases of parathyroid tetany, treated with parathyroid hormone, including their own case.

[26] J. Biol. Chem., **67**, 1 (1926).

an equilibrium between inorganic calcium and an organic compound of calcium which has some points of resemblance to calcium citrate but is not identical with it. The formation of this organic compound, it is suggested, may be dependent on the presence of the parathyroid hormone.

Greenwald and Gross [27] have observed that if cod-liver oil is administered for some time before thyroparathyroidectomy, in dogs, the fall in plasma calcium after the operation is more gradual and the onset of tetany is delayed and in some cases even avoided. The explanation offered is that cod-liver oil has a stimulating effect on the parathyroids and that dogs previously fed cod-liver oil are left after the operation with a larger store of the parathyroid hormone and with more active accessory parathyroid tissue than animals that have not been so treated. In this way, the authors state " the organism is given a longer time in which to adjust itself to the low concentration of plasma calcium or the remaining parathyroid tissue has a better opportunity to hypertrophy sufficiently to supply the animal's needs. Possibly, both processes are involved."

Sheard and Higgins [28] have made the interesting observation that chicks maintained on a diet low in vitamin D (p. 477), growing under amber, blue, or ordinary window glass, which cuts out the ultraviolet rays, develop rickets and associated with this there is a very marked hypertrophy of the parathyroid glands. The hyperplasia is believed to be a compensatory effect for the deficiency in vitamin D, which as we shall see later likewise exerts a regulating effect on calcium metabolism. The hyperplasia may be prevented, according to Sheard and Higgins, by feeding the chicks small amounts of cod-liver oil. From these observations it follows that cod-liver oil has the opposite effect from the one suggested by Greenwald and Gross.

Hyperparathyroidism.—The various forms of tetany to which we have referred are essentially due to a deficiency of parathyroid function, or *hypoparathyroidism*, the effects of which have been studied for about a generation. However, it is very recently that an interest and more thorough appreciation have been awakened in the effects of hypersecretion of the parathyroid gland. Collip has shown that when the parathyroid hormone is given to normal animals the blood calcium is increased above normal (hypercalcemia). Hypercalcemia may be produced in parathyroidectomized animals by overdosage. The symptoms associated with a high calcium content of the blood (above 15 mg. per 100 cc. is definitely dangerous) are anorexia, vomiting, apathy,

[27] *Ibid.*, **82**, 505 (1929); see also **82**, 531, 717 (1929).

[28] Am. J., Physiol., **85**, 299 (1928).

drowsiness verging into coma, and failing circulation. Collip has shown that hypercalcemia is fatal if allowed to persist. It may be relieved by the administration of sodium bicarbonate.

Enlarged parathyroid glands have been observed *post mortem* in certain diseases of the skeleton characterized by the depletion of the inorganic constituents of the bones, such as osteomalacia, osteitis fibrosa and rickets. The hyperplastic condition of the parathyroids was regarded as being a compensatory phenomenon until it was shown by Mandl [29] that the removal of a parathyroid adenoma in a case of osteitis fibrosa resulted in recovery. Several similar cases have since been reported in which osteitis fibrosa was found to be associated with a tumor of the parathyroid gland. In all cases the patients improved clinically after removal of the tumor. One case of osteitis fibrosa has been reported in which there was no evidence of a parathyroid tumor; however, excision of two normal glands led to recovery. In the case studied by Wilder, bone was removed for analysis. It contained a larger proportion of organic matter and less calcium and phosphorus than normal bone. The blood calcium was above normal (12.2 mg. per 100 cc.). X-ray plates showed extensive areas of decalcification in the bones. The surgical removal of the parathyroid tumor was followed by a marked improvement in strength and muscle tone, relief of pain in the bones, and increased calcification of the bones. The serum calcium concentration fell to about 8 mg. per 100 cc.

It is thus seen that hypersecretion of the parathyroid causes a depletion of calcium from bone and possibly from other tissues. Such changes as may occur in the composition of the inorganic constituents of the tissues are likely to have a profound effect on their normal physiology. The nature of the physiological derangements that occur in osteitis fibrosa, for example, is not fully understood, although it seems likely that the extreme weakness and loss of muscle tone observed may be due to the loss of calcium from the muscle. Hypercalcemia, as has been pointed out, is a characteristic feature of hyperparathyroidism.

The Hypophysis.—The pituitary body, or hypophysis cerebri, is a small organ weighing about 0.6 gram and located in the hypophyseal fossa of the sphenoid bone. Its two main parts are the anterior and posterior lobes. Lying between these is the pars intermedia. There are, in addition, smaller structures related to the hypophysis. The pars tuberalis extends toward the nose from the junction of the pars

[29] Mandl, F., Z. f. Chir., **53**, 260 (1926); Gold, H., Wien. med. Wochenschr., **77**, 1734 (1927); Barr, D. P., Bulger, H. A., and Dixon, H. H., J. Am. Med. Assoc., **92**, 951 (1929); Boyd, J. D., Milgram, J. E., and Stearns, G., *ibid.*, **93**, 684 (1929); Wilder, R. M., Endocrinology, **13**, 231 (1929).

intermedia and the anterior lobe. The stalk of the infundibulum connects the gland with the floor of the third ventricle. The anterior and posterior lobes differ in origin (consult textbooks on embryology). They also differ histologically and functionally. The former is glandular, whereas the latter is made up largely of neurogliar tissue. Indeed, it is believed by some that the active principle of the posterior lobe is merely stored there, being actually formed by the cells of the pars intermedia.

The Anterior Lobe.—The extirpation of the anterior lobe or of the entire gland, in young animals, has a marked inhibitory effect upon growth. In a celebrated experiment, Aschner [30] selected two puppies from the same litter, removed the hypophysis of one and used the other as a control. The dog without the hypophysis remained stunted, wheras the control animal grew to normal size.

Similar retardation of growth, as well as atrophy of the sex organs, occurs in rats following hypophysectomy, as shown by P. E. Smith.[30a] If such dwarfed rats are fed or are given injections of anterior lobe substance, growth and the development of the sex organs are resumed. Almost normal growth was obtained by Smith in a group of hypophysectomized rats in which a daily homeotransplant of anterior lobe tissue was made. The rôle of the anterior lobe is believed to be due to the presence of two hormones, one of which promotes sexual development and the other growth, particularly the growth of bone and connective tissue.[31]

In man, as in animals, the most important effects of hypopituitarism are incomplete growth, which if severe enough may amount to dwarfism, and sexual immaturity.

Obesity and polyuria have also been regarded as symptoms of hypophyseal hypofunction, but these may be due, not so much to disease of the anterior lobe as to involvement of adjacent structures. It has been demonstrated by Smith that if ablation of the hypophysis in rats is performed with sufficient care so as not to injure the adjacent midbrain structures, obesity and polyuria do not result. On the contrary, even though the hypophysis remains intact, injury to the hypothalamus results in the development of very marked obesity. Some evidence has also been presented (Borquin) that polyuria may be due to involvement of the mammillary bodies.

[30] Arch. f. d. ges. Physiol., **146**, 1 (1912).

[30a] Anat. Rec., **33**, 289 (1926); Am. J. Physiol., **80**, 114; **81**, 20 (1927); J. Am. Med. Assoc., **88**, 158 (1927). See also G. L. Foster and P. E. Smith, J. Am. Med. Assoc., **87**, 2151 (1926); Smith and Engle, E. T. Am. J. Anat., **40**, 159 (1927–28); H. M. Evans, Harvey Lecture, 1923–24, p. 212.

[31] Cushing, H., and Teel, H. M., Am. J. Physiol., **90**, 323 (1929).

Gigantism and *acromegaly* result from hyperactivity of the anterior lobe of the hypophysis (hyperpituitarism). The former condition occurs in young, growing individuals and consists in the growth of the long bones to gigantic proportions. The giants of the circus are usually victims of this disease. In older people, the more prominent manifestations are the growth of the hands and feet and the bones of the face. The jaw protrudes, the nose becomes widened and the lips very thick. This condition is called acromegaly. Among the symptoms frequently observed are severe headaches and progressive loss of vision, both being due usually to the pressure exerted by adenomatous growths of the gland. As the condition progresses and the posterior lobe becomes involved, carbohydrate tolerance is markedly diminished.

Operative methods of treatment, for which we are largely indebted to Harvey Cushing, have been found most effective in the treatment of diseases of the pituitary gland.

The Posterior Lobe.—To the posterior lobe have been attributed a multiplicity of functions, knowledge of which has been based largely on the effects obtained by the administration of extracts of this portion of the hypophysis. The more important physiological effects of extracts of the posterior lobe are as follows: (1) stimulation of uterine contractions (oxytocic effect), (2) rise in blood pressure (pressor effect), (3) diuretic effect in anesthetized animals and an antidiuretic effect produced clinically in diabetes insipidus (diuretic-antidiuretic effects). In addition extracts of the posterior lobe produce expansion of the melanophores or pigment cells of frogs, stimulate milk secretion (galactagogue effect), the action probably being exerted on the plain muscle of the milk sinuses.

Recognition of these many functions has naturally led to the supposition that the posterior lobe contains more than one hormone. Thus far, however, the presence of only two active principles has been demonstrated (Kamm, Aldrich, Grote, Rowe and Bugbee [32]). One of these stimulates contraction of the uterine muscle and the other raises the blood pressure. To the latter is also ascribed the diuretic-antidiuretic effect. These hormones are exceedingly active physiologically. As yet their chemical nature has not been determined, but it is suggested that they are basic nitrogenous substances, presumably amines.

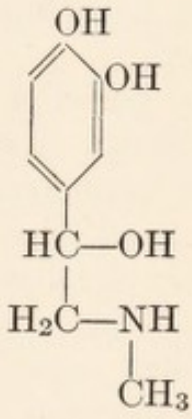
The Adrenals.—The adrenal or suprarenal glands are two small, highly vascular organs, usually situated at the upper poles of the kidneys and each weighing, in man, about 6 or 7 grams. Two parts are distinguishable, the *cortex* and the *medulla*. These differ from each other embryologically, histologically, and functionally. The medulla

[32] J. Am. Chem. Soc., **50**, 573 (1928).

is stained by potassium dichromate, hence it is frequently referred to as the chromaffin tissue. The importance of the adrenals is indicated by the fact that their extirpation results fatally. It is now generally held that death in adrenalectomized animals is due primarily to the absence of the cortex.

However, it was from the adrenal medula that the first hormone was isolated. It is variously called adrenaline, epinephrine, or suprarenine.

Epinephrine was first obtained as the benzoyl derivative by Abel [33] and subsequently as the free base by Aldrich [34] and Takamine.[35] It has since been prepared in the laboratory by synthetic methods. Epinephrine is closely related to tyrosine, as shown by the formula:



Epinephrine or Adrenaline

Before the discovery of adrenaline Oliver and Schäfer [36] had shown that extracts of the adrenals exert a powerful effect in raising blood pressure. It has since been shown that 0.001 mg. of adrenaline, when injected into a cat, is sufficient to cause constriction of the arterioles and, hence, a rise in blood pressure.

From the standpoint of metabolism may be mentioned the effect of adrenaline in causing increased glycogenolysis. The immediate effect is hyperglycemia. There is also an increase in the metabolic rate as well as in the respiratory quotient, the latter change showing an increased utilization of carbohydrate.[37] Associated with these changes is an increase in muscular power and an apparent resistance to fatigue.

[33] Abel, Johns Hopkins Hospital Bull., **9**, 215 (1898); **12**, 80 (1901).

[34] Aldrich, Am. J. Physiol., **5**, 457 (1901).

[35] Takamine, Am. J. Pharmacy, **73**, 523 (1901).

[36] J. Physiol., **18**, 230 (1895).

[37] For a review of the relation of epinephrine to insulin and carbohydrate metabolism see Cori, C. F., Harvey Lectures (1927–28), p. 76.

To explain the significance of adrenaline, Cannon [38] has postulated the theory that this substance enables the organism to cope with emergencies. Cannon believes that fear, rage, and other emotions stimulate the adrenals to increased production of adrenaline, which, on entering the circulation, produces prompt mobilization of carbohydrate. This provides ready fuel for the muscles. Among the other manifestations of hypersecretion or injection of adrenaline are an increase in blood pressure and increased efficiency of muscular contraction, including that of the heart muscle. These changes Cannon believes to be adaptations which enable the organism to work at its maximum capacity in the face of danger. The effect of adrenaline in diminishing the clotting time of blood is regarded by Cannon as another adaptation, useful to the organism in preventing excessive hemorrhage in the case of wounds.

In a well-known experiment, Cannon compared the concentration of adrenaline in the blood of normal cats with the concentration observed in the blood when the cats were frightened by the barking of a dog. Whereas, in the normal state, no evidence of adrenaline was found, the blood of the frightened animals was found to contain demonstrable amounts of this substance. These observations were not confirmed by Stewart and Rogoff.[39] Stewart [40] states that the best evidence for the view that the epinephrine output exerts no important or indispensable function is that, after its suppression, the animals do not differ notably from normal animals in their blood-sugar content, in their capacity to meet the emergencies of life, or in a variety of other ways in which Cannon observed significant differences.

In 1855, an English physician named Addison pointed out that the peculiar and fatal disease often associated with bronzing of the skin was in some way connected with degeneration of the adrenals. This condition, now known as Addison's disease, though often associated with tuberculosis of the adrenal glands as in the cases studied by Addison, may, however, be due to destruction of these glands by any process. The more important symptoms are pigmentation or bronzing of the skin and hair, excessive muscular weakness leading to prostration, mental depression and other nervous symptoms, gastro-intestinal disturbance including vomiting, atrophy of the sex organs, and hypoglycemia. Addison's disease is believed to be due to hypofunction of the

[38] W. B. Cannon, Bodily Changes in Pain, Hunger, Fear and Rage, New York and London, 1915.

[39] J. Exp. Med., **26**, 637 (1917); J. Pharmacol. Exp. Therap., **10**, 49 (1917); Am. J. Physiol., **44**, 543 (1917); *ibid.*, **51**, 366 (1920).

[40] Physiol. Reviews, **4**, 163 (1924).

adrenals, and it is probable that the more severe symptoms are due to involvement of the adrenal cortex (Kovács [41]).

Koehler and Eichelberger [42] have reported the isolation of an epinephrine-free substance from the suprarenal gland that is capable of elevating the basal metabolic rate and producing symptomatic improvement in various types of clinical asthenias, such as progressive muscular dystrophy. Using this extract, Koehler and Hastings [43] have found that in addition to the calorigenic effect there is an improvement in muscular efficiency, particularly in individuals with low metabolic rate and deficient capacity for muscular work. It is indicated that the suprarenal extracts promote the utilization of carbohydrate and the sparing of protein.

A condition has been described which is believed to be due to hyperfunction of the adrenal cortex. Hypertrophy in this region is frequently associated with tumors (hypernephromata) and has been observed in cases of sexual precocity. Sexually precocious children exhibit very remarkable changes. A boy of six or seven will rapidly acquire the sexual development of a much older individual. There will be enlargement of the testes, together with the appearance of hair in the pubic region. There may also be the beginning of the growth of a beard or mustache. Girls similarly afflicted show evidence of hypertrophy of the breasts and enlargement of the uterus; in some cases menstruation may occur. In a general way, these children may be said to resemble small men and women.

The Pancreas.—This organ produces an external as well as an internal secretion, the latter being formed in the so-called β-cells of the *islets* of Langerhans. The pancreatic hormone insulin has already been discussed in the chapter on carbohydrate metabolism, but it may not be out of place at this point to consider briefly certain features of pancreatic secretion which have not been mentioned heretofore.

The more familiar manifestation of abnormal pancreatic function is deficient secretion of insulin, which as we have seen is the underlying factor of the symptoms of diabetes, such as hyperglycemia, impaired utilization of carbohydrate, etc. Since the discovery of insulin, the results of experimental and therapeutic overdosage with this hormone have also received a considerable amount of attention. The outstanding effect of the presence of an excessive amount of insulin is hypoglycemia, which is accompanied by a train of symptoms, showing in man a considerable amount of variation. The initial symptom is

[41] Beitr. z. path. Anat. u. z. allg. Path., **79**, 213 (1928).
[42] Am. J. Physiol., **90**, 417 (1929).
[43] *Ibid.*, **90**, 418 (1929).

usually a feeling of nervousness or tremulousness; sometimes there is a feeling of hunger. This is followed by weakness and a sense of depression and later by perspiration; frequently there is an increase in the pulse rate. Extreme anxiety, sometimes excitement and emotional instability, confusion and delirium become evident. Convulsions do not occur in man according to the description given by Fletcher and Campbell.[44] A blood sugar concentration of 0.035 per cent is usually accompanied by coma.

Through a better understanding of the action of insulin clinical cases of "*hyperinsulinism*" have been more readily recognized and more diligently studied. Most important of the conditions associated with spontaneous hypoglycemia, due apparently to an increased production of insulin, is malignancy of the pancreas with proliferation of the islet tissue. The first case of this type to be thoroughly studied is one of carcinoma of the islands of Langerhans with metastases in the liver and lymph nodes, described by Wilder, Allan, Power and Robertson.[45] Their patient experienced frequent attacks of extreme weakness, faintness and paresthesia, accompanied by hypoglycemia. These symptoms could be relieved by eating between meals or taking sweet drinks. When food was withheld for 3 hours and 20 minutes after the noon meal, the blood sugar concentration fell to 0.055 per cent. At this time the patient appeared apprehensive and depressed. Fifteen minutes later perspiration and tremor were noted; at 4 hours the blood sugar had fallen to 0.036 per cent and 15 minutes later to 0.027 per cent, at which time the patient was stuporous and no longer able to speak and was jerking about convulsively. At this point 15 gm. of glucose were given by mouth; the blood sugar rose to 0.065 per cent and the patient became rational and able to converse. Hourly doses of glucose were required to prevent the patient from developing too severe an hypoglycemia.

A similar case has been described by Howland, Campbell, Maltby and Robinson.[46] This patient showed no metastases and surgical removal of the tumor was followed by recovery from the hypoglycemic tendency and associated symptoms.

The Thymus.—The thymus is a lymphoid organ situated at the lower end of the trachea. As to its function little is known, and even the belief that it is a gland of internal secretion is based on indirect

[44] The first complete study of the clinical effects of an overdose of insulin was made by Fletcher, A. A., and Campbell, W. R., J. Metab. Research, **2**, 637 (1922).

[45] J. Am. Med. Assoc., **89**, 348 (1927).

[46] *Ibid.*, **93**, 674 (1929); see also Thalheimer, W., and Murphy, F. D., *ibid.*, **91**, 890 (1928); Warren, S., Am. J. Path., **2**, 335 (1926); Wagner, R., and Parnas, J. K., Z. ges. exp. Med., **25**, 31 (1921).

evidence. One well-established fact concerning it is that it undergoes involution after puberty and may even disappear in the adult. It has likewise been observed that involution of the gland may be inhibited by castration. Another interesting observation, which has not, however, been fully substantiated, is that of Gudernatsch,[47] who reported that feeding tadpoles on thymus delayed their metamorphosis into frogs. Thus the thymus may be concerned in some way in delaying the development of the sex organs. However, attempts to prove this by experimental methods have not yielded consistent results.

There is apparently an interrelationship between the suprarenals and the thymus, it having been shown that hypoplasia of the former is often associated with hyperplasia of the latter. Jaffe [48] has demonstrated that double suprarenalectomy in the rat, performed after involution had normally begun, brings about regeneration of the thymus. In younger animals extirpation of the adrenals results in a marked enlargement of the thymus, as well as of lymphoid structures in general.

The Pineal Gland.—Another organ, the significance of which is obscure, is the pineal body, a small structure situated between the anterior corpora quadrigemina, in contact with the roof of the third ventricle, from which it develops embryologically. It grows until about the seventh year, but after puberty it undergoes marked involution. Extirpation of the pineal body of young roosters has been found to cause hypertrophy of the testes (Foâ). Similar results were obtained by Horrax [49] with guinea pigs. There are on record a number of cases of pineal tumor in boys, associated with precocious development in stature as well as of the sex organs. Hence this condition may be assumed to be one of hypopinealism. So marked are the changes brought about by the presence of these tumors that a boy of eight may actually have the appearance of a fourteen- or sixteen-year-old boy.

From these observations it is probably logical to assume that the pineal gland contains a hormone which exerts a regulatory effect on growth, particularly in relation to the generative organs. Direct evidence for this assumption is lacking, however. Feeding experiments with pineal gland have yielded conflicting results.

The Internal Secretions of the Reproductive Organs.[50]—The interstitial cells (cells of Leydig) of the testes are believed to be concerned

[47] Anat. Record, **11**, 357 (1917).

[48] J. Exp. Med., **40**, 325, 619, 753 (1924).

[49] Arch. Internal Med., **17**, 607 (1916).

[50] A detailed discussion of the older literature of the subject will be found in Marshall's Physiology of Sex Reproduction, London, 1922, as well as in a review by the same author in Physiol. Reviews, **3**, 335 (1923).

with the production of a hormone, the influence of which on the development of the sexual characteristics of the male is very profound. The view that the interstitial cells are solely concerned in the formation of the hormone is not conceded universally. Other cells may be concerned, in conjunction with the interstitial cells, or by themselves, in the production of the testicular hormone. The extreme condition of deficient testicular function is represented by the complete removal of the gland (castration). Castration has been practiced since ancient times, particularly in oriental countries, where there has always been a considerable demand for eunuchs as household servants. Both in man and animals, if the operation is performed at an early age, the secondary sex characteristics fail to develop (growth of beard and development of larynx in man, growth of antlers in stags, and of comb, spurs, wattles, etc., in the cock). On the other hand, female characteristics may become more or less prominent. Thus a eunuch will develop large breasts and hips, and his general contour, partly because of excessive fat deposition, will resemble that of a female. Profound changes are likewise observed in related organs of internal secretion, including atrophy of the thyroid and hypophysis and hypertrophy of the thymus and suprarenal cortex. The testicular hormone apparently stimulates metabolism, but it is not known with certainty whether this effect is a direct one or whether it is due to the influence of this hormone on the thyroid gland. The excessive deposition of fat may represent a derangement in metabolism. There is also evidence of low carbohydrate tolerance and of creatinuria in eunuchs.

Extracts of the lipid fraction of bull testicles, prepared in F. C. Koch's laboratory,[51] have been shown to produce a striking effect on the seconday sex characters of the capon. A substance acting similarly has been extracted from the urine of young men by Funk, Harrow and Lejwa.[51a]

Testicular hyperfunction is alleged to occur as a result of tumors of the testes in boys. The condition known as *pubertas precox* manifests itself in the premature development of secondary sex characteristics, such as the growth of hair on the face and deepening of the voice. A case of this type has been described in which a boy less than ten years old had actually grown a black beard. A marked tendency to return to normal occurs after removal of the tumor.

One of the results of ovariectomy is a cessation of the œstrus cycle.

[51] McGee, L. C., Juhn, M., and Domm, L. V., Am. J. Physiol., **87**, 406 (1928); Moore, C. R., and McGee, *ibid.*, **87**, 436 (1928); Gallagher, T. F., and Koch, J. Biol. Chem., **84**, 495 (1929).

[51a] Am. J. Physiol., **92**, 440 (1930).

If the ovaries are removed early in life, the uterus remains infantile and the mammary glands fail to develop normally. If this operation is performed in the adult, after these organs have reached a mature state of development, retrogressive changes will become apparent. However, if ovarian tissue is grafted in a region of the body where it will establish connection with the blood, and ovariectomy performed, these changes will not be observed.

After extrusion of the ovum, a lipid-rich yellowish body is deposited in the follicle during pregnancy. This is the *corpus luteum.* The injection of extracts of this yellow substance stimulates the secretion of milk and causes the contraction of the uterus. The development of the mammary glands during pregnancy is believed to be under the control of this secretion; and, even in virgin mammals, the continuous administration of luteal extracts has been shown to cause hypertrophy of the mammary glands. There also appears to be some relationship between the corpus luteum and the fixation of the embryo during the early stages of pregnancy, for if the corpus luteum is destroyed, by cautery or otherwise, the embryo becomes detached. Another effect of the removal of the corpus luteum is the arrested development of the mammary glands and uterus.

Menstruation ceases in adults upon the removal of the ovaries, and after the menopause when the organs begin to undergo retrogressive changes. Associated with absence or deficiency in ovarian function is an increased tendency to obesity. Hyperfunction resulting from tumors of the ovary has been observed in girls and women. In the former, symptoms of sexual precocity become very prominent.

It therefore seems certain that the ovary is an organ of internal secretion producing one or more hormones. Active preparations have been obtained from whole ovaries, as well as from the isolated contents of the ovarian follicles, the corpus luteum, urine of pregnant women, and placenta. These induce maximum œstrus phenomena in rats, stimulating the tissues of the genital tract to growth and secretion. The isolation of the ovarian hormone in a crystalline form has been accomplished by Doisy, Veler and Thayer.[52]

Various names have been given to the female sex hormone: œstrin (Parkes and Bellerby), menformon Laqueur, etc.

The composition of this hormone as well as its chemical properties have not been fully determined.[52a]

[52] Am. J. Physiol., **90**, 329 (1929); J. Biol. Chem., **86,** 499 (1930).

[52a] For a review of the literature, see Allen, E., and Doisy, E. A., Physiol. Reviews, **7**, 600 (1927); Frank, R. T., Gustavson, R. G., and collaborators, Endocrinology, **10**, 260 (1926); R. T. Frank, The Female Sex Hormone, Springfield (1929).

Internal Secretions in Other Organs.—The liver, spleen and kidneys have also been studied from the standpoint of endocrinology, but the available information is on the whole too fragmentary to be included here. However, reference may be made to the important observations of Minot and Murphy [53] that in cases of pernicious anemia, the feeding of liver produces an increased concentration of reticulocytes and mature erythrocytes in the peripheral blood. The active principle has been prepared in a relatively pure state; it is soluble in water and is believed to be a nitrogenous base.[54] The chemical constitution has not been determined and it has not been definitely classified as a hormone. Clinical experience thus far seems to show that liver extract is a specific cure for pernicious anemia. It has been recently reported that equally good results have been obtained in the treatment of pernicious anemia by the use of desiccated hog's stomach, indicating that here also an anti-anemic factor is present, which may or may not be identical with the substance occurring in the liver.[55]

Summary.—From the standpoint of function, the glands of internal secretion are very closely related to each other. In respect to a given physiological phenomenon, they may be antagonistic or indifferent to each other, or they may reinforce each other. Certain of the glands exert a profound effect on metabolism. Thus, the utilization of glucose in the body is largely under the control of insulin. The parathyroid hormone is concerned with calcium utilization. Glycogenolysis is produced by the internal secretions of the adrenals and thyroid. Metabolism is depressed in conditions where there is a lack of thyroid hormone; it is increased when there is an excess of the hormone. The value of hormones to the organism is best appreciated when they are absent or when they are present in excess. Thyroid deficiency results in cretinism in the child and myxedema in the adult. Loss of the parathyroids leads to tetany and death. Diabetes follows upon degenerative changes in the pancreas, whereas destructive changes in the adrenals may become manifest as Addison's disease.

[53] Minot, G. R., and Murphy, W. P., J. Am. Med. Assoc., **87**, 470 (1926); **89**, 759 (1927); Harvey Lectures (1927–1928), pp. 151–207.

[54] Cohn, E. J., and collaborators, J. Biol. Chem., **77**, 325 (1928); Am. J. Physiol., **90**, 316 (1929).

[55] Sturgis, C. C., and Isaacs, R., J. Am. Med. Assoc., **93**, 747 (1929); E. A. Sharp, *ibid.*, **93**, 749 (1929).

CHAPTER XVI

ANIMAL CALORIMETRY

THE unit of measurement of heat in animal calorimetry is the large or kilogram Calorie. It is the amount of heat required to raise the temperature of 1 kg. of water from 15° to 16° C. When 41,860,000 ergs (work units) are changed or dissipated into heat, 1 Calorie is formed.

The calorific value of organic compounds is usually determined by means of the bomb calorimeter. The essential part of this apparatus is a combustion bomb in which is supported a platinum capsule. The latter is used as the container for the material to be analyzed. A wire having electrical connections with the outside dips into it. Before an analysis, the bomb is closed tightly, filled with oxygen under a pressure of 20 to 25 atmospheres, and placed in a vessel containing water. Passing an electric current through the wire causes it to glow, thereby igniting the material in the platinum capsule. The heat evolved is calculated from the observed change in the temperature of the water.

Calorific Value of Foodstuffs.—The combustion calorimeter has been used extensively in determining the heat values of foodstuffs. Somewhat more elaborate methods are required for the determination of the calorific value of foods when burned in the body. These will be discussed presently. The combustion of one gram of a monosaccharide yields about 3.75 calories.[1] One gram of a disaccharide yields 3.95 calories; and a gram of starch, 4.23 calories. Hence, 4.0 or 4.1 is usually taken as the average calorific value of one gram of carbohydrate. This amount of heat is evolved whether the combustion occurs in the air or inside the body. The heat value of fats is considerably higher. Approximately 9.3 calories are obtained on combustion of one gram of fat. Here also, the amount of heat produced is the same whether the fat is burned in a bomb calorimeter or in the body. The situation is somewhat different in the case of the proteins. When burned in the bomb calorimeter, one gram of protein yields on an average about 5.7 calories, but in the body, the heat of combustion is found to be only about 4.1 calories. Proteins may differ somewhat in calorific value.

[1] In this book, unless specified otherwise, the term "calorie" will be used in referring to the large, or kilogram calorie.

Thus, casein produces 4.4 calories, whereas the vegetable proteins yield about 4.0 calories. The divergence in the calorific values of protein when burned outside and inside the body is due to the fact that protein combustion in the tissues is never complete. The end-products of protein metabolism (urea, etc.), while of no value as energy producers in the body, are capable of undergoing further combustion in the bomb calorimeter. One gram of urea, for example, on oxidation, yields 2.52 calories. It has been determined that on a mixed diet the ratio of carbon to nitrogen in the urine is about 0.75, and that a gram of urinary nitrogen is equivalent to 8.09 calories. This value is not constant, being influenced by a variety of factors. Following the ingestion of large amounts of carbohydrate, the urine, though practically free from sugar, may contain sufficient amounts of intermediary products of glucose metabolism to increase the calorific equivalent of a gram of urinary nitrogen to as much as 13 calories. After meat has been eaten, the calorific value of 1 gram of urinary nitrogen is 7.46 (Rubner, cited by Lusk [2]); during starvation, it is 8.49 calories.

Heat Production and Respiratory Exchange.—Total metabolism in the body may be determined by direct or indirect methods of calorimetry. The direct method consists in placing the individual in a suitably constructed chamber and measuring the amount of heat evolved. In principle, animal and bomb calorimeters are similar. By the indirect method, the heat given off is computed from the respiratory exchange. By determining the consumption of oxygen, the elimination of carbon dioxide, and the excretion of nitrogen in the urine, the necessary data are obtained for calculating, not only total heat production, but the nature and amount of each of the substances metabolized, as well.

Various forms of apparatus have been designed for the measurement of heat production in man and animals either by the direct or indirect method. For use in experiments on human beings, an exceedingly accurate calorimeter was invented by Atwater and Rosa [3] and improved by Benedict.[4] This apparatus measures heat production and respiratory exchange simultaneously.

Principle of the Atwater-Rosa-Benedict Respiration Calorimeter.—The respiration calorimeter is shown in Fig. 42, and is diagrammatically represented in Fig. 43. There are three walls, an inner and an outer copper wall and an insulating wall. The two copper walls are separated from each other by a dead-air space. A similar space separates the

[2] G. Lusk, Science of Nutrition, 1928 edition, p. 39.

[3] Atwater and Rosa, Report of the Storrs Agr. Exp. Sta., p. 212 (1897).

[4] Atwater and Benedict, Carnegie Inst. of Washington Pub., No. 42 (1905); Benedict and Carpenter, *ibid.*, No. 123 (1910).

outer copper wall from the insulating wall. The latter is constructed of two layers of compo-board separated by a layer of cork. Between the insulating wall and the outer copper wall are water pipes, along which run resistance wires, carrying an electric current. Thus the temperature of the interspace, as well as that of the outer copper wall, may be kept under control, either by the passage of cold water through the pipes or by warming. It is essential that the temperature of the two copper walls be maintained the same, for otherwise there would be an

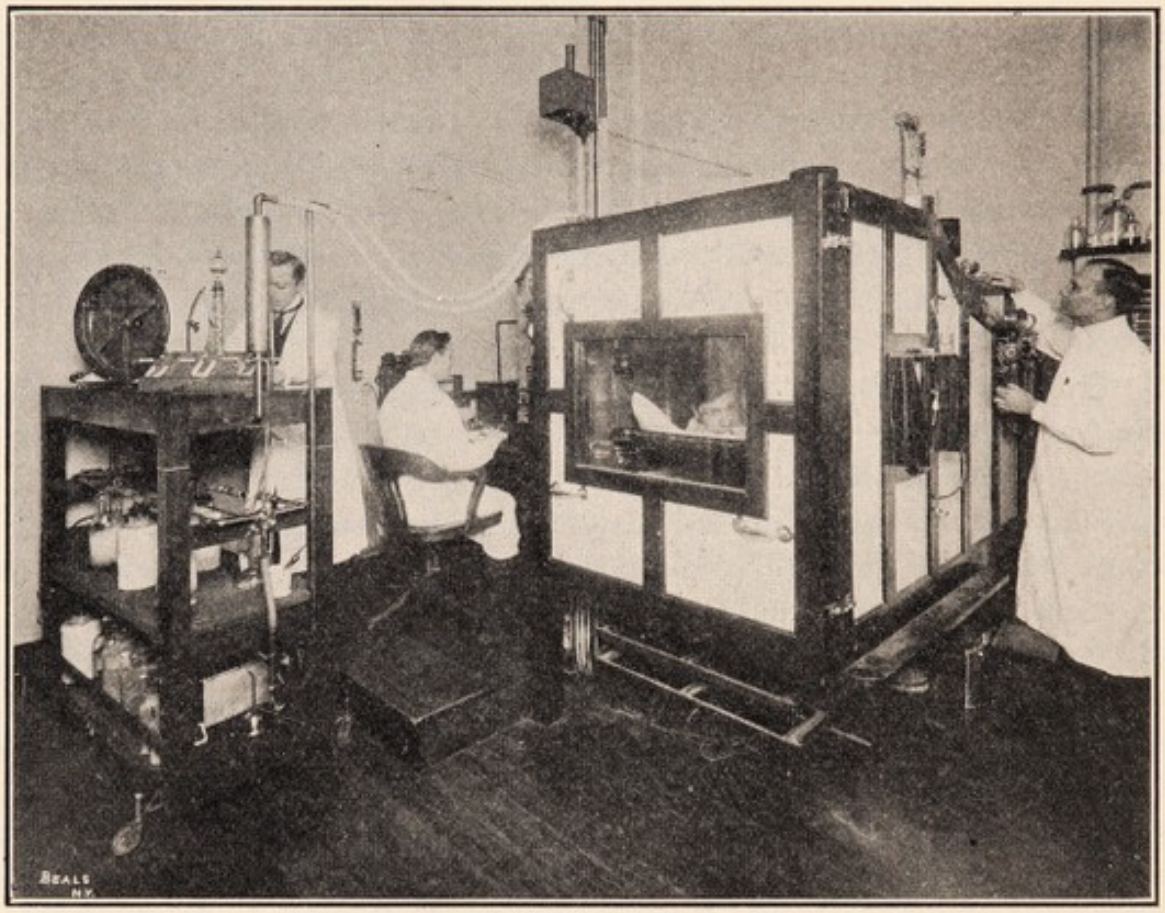


FIG. 42.—The respiration calorimeter of the Russell Sage Institute of Pathology. (After a photograph loaned by Dr. Eugene F. Du Bois)

exchange of heat between them and, hence, either a gain or loss of heat by the inner wall. Thermocouples are arranged between the two walls to determine their temperature. During the course of an experiment, this is done at intervals of about four minutes.

Inside the calorimeter, the temperature is maintained practically constant by passing a current of cool water through a series of pipes. The heat lost by an individual in the calorimeter through radiation and conduction is thus removed by the water. The total volume of water passing through the calorimeter is measured. Likewise, the tempera-

ture of the ingoing and outgoing stream of water is recorded at short intervals during the experiment. A considerable amount of heat is

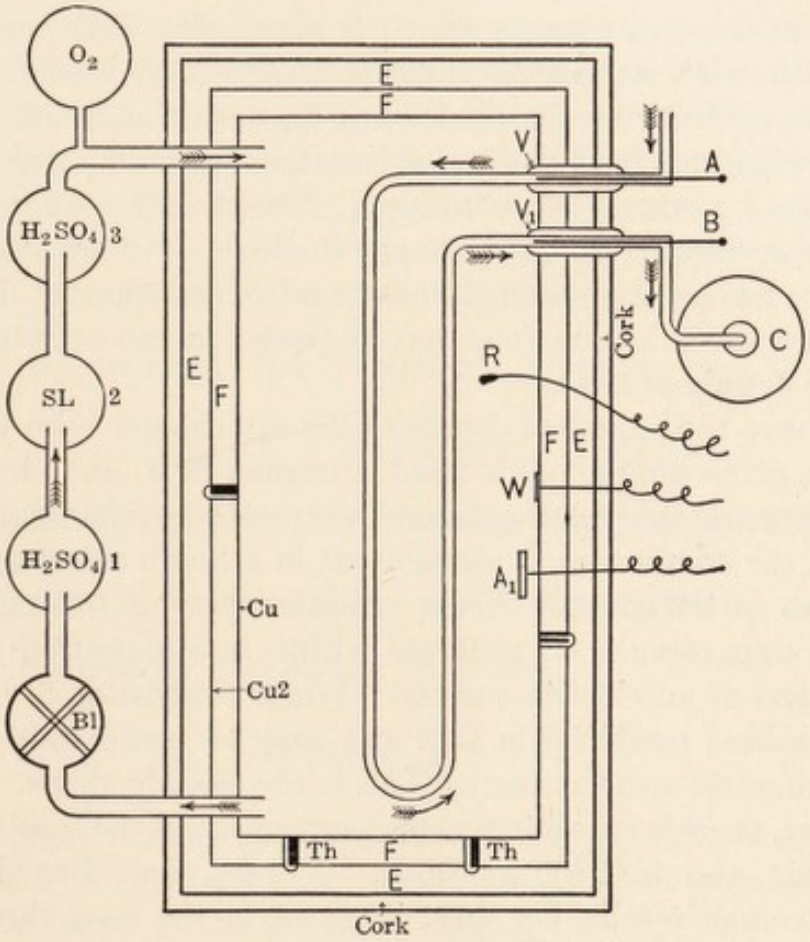


FIG. 43.—Schematic diagram of the Atwater-Rosa-Benedict respiration calorimeter. (After Graham Lusk, Elements of the Science of Nutrition, Saunders & Co., 1928 edition, p. 70.)

Ventilating System:
- O_2, Oxygen introduced as consumed by subject.
- 3, H_2SO_4 to catch moisture given off by soda lime.
- 2, Soda lime to remove CO_2.
- 1, H_2SO_4 to remove moisture given off by patient.
- Bl, Blower to keep air in circulation.

Indirect Calorimetry:
- Increase in weight of H_2SO_4 (1) = water elimination of subject.
- Increase in weight of soda lime (2) + increase in weight of H_2SO_4 (3) = CO_2 elimination.
- Decrease in weight of oxygen tank = oxygen consumption of subject.

Heat-absorbing System:
- A, Thermometer to record temperature of ingoing water.
- B, Thermometer to record temperature of outgoing water.
- V, Vacuum jacket.
- C, Tank for weighing water which has passed through calorimeter each hour.
- W, Thermometer for measuring temperature of wall.
- A_1, Thermometer for measuring temperature of the air.
- R, Rectal thermometer for measuring temperature of subject.

Direct Calorimetry:
- Average difference of A and B × liters of water + (gm. water eliminated × 0.586) ± (change in temperature of wall × hydrothermal equivalent of box) ± (change of temperature of body × hydrothermal equivalent of body) = total calories produced.

Th, thermocouple; Cu, inner copper wall; Cu_2, outer copper wall; E. F. dead air-spaces.

used in the evaporation of water. The water evaporated from the skin and the water vapor in the expired air are taken up by sulfuric acid

absorbers outside the chamber. From the amount of water thus collected, the latent heat is calculated. It is estimated that about one-quarter of the total heat produced by the human body is present as latent heat in the water vapor which is given off. Although the temperature of the air entering the calorimeter is always heated to exactly the same temperature as the air leaving it, nevertheless, the temperature of the calorimeter is determined at the beginning and end of an experiment and a correction introduced, if necessary. Another correction may be necessary, should a change in the body temperature occur. The chamber has a port-hole with inner and outer airtight doors. By opening these, one at a time, food may be passed in and excreta removed, without loss or gain of heat.

So well have the technical details of the calorimeter been worked out that when a given amount of alcohol is burned in it, and the heat production measured, the value obtained is practically identical with that found when the combustion is carried out in a bomb calorimeter. This is referred to as the alcohol check. Another way of testing the accuracy of the apparatus is to generate within it a measured amount of heat by means of an electric current. When everything is functioning properly the heat produced in this way may be completely accounted for by calorimetric measurement. This is the electric check.

Moreover, there is exceedingly close agreement in the results obtained by the direct and indirect methods. Atwater and Benedict [5] compared the average results per day, obtained in the case three individuals who were experimented upon for forty days each, and found an average difference of only 0.2 per cent.

	Average Calories per Day
Indirect calorimetry	2717
Direct calorimetry	2723
Difference	0.2 per cent

Murlin and Lusk [6] performed a series of twenty-two experiments on a dog and found the average difference in the results obtained by direct and indirect calorimetry to be only 0.6 per cent.

For the determination of respiratory exchange alone, less elaborate equipment is required than for the measurement of heat output by direct calorimetry. The fact that the indirect method yields results which are both reliable and valuable in the study of metabolic disorders

[5] Cited by Lusk, Science of Nutrition, 4th edition, p. 62. The student is referred to this book for a detailed description of the Atwater-Rosa-Benedict calorimeter.

[6] J. Biol. Chem., **22**, 15 (1915).

has been the stimulus for the invention of a large variety of appliances to be used in the determination of respiratory exchange.[7]

Respiratory Quotient, Influence of Metabolism.—The ratio between the carbon-dioxide output and the oxygen intake is termed the " respiratory quotient (R. Q.)." Early in the history of the science of nutrition, it was realized that this ratio was profoundly affected by the character of the material metabolized and that, therefore, the determination of the respiratory quotient would yield information concerning the nature of the substances which were being utilized. In the combustion of carbohydrate, the volume of carbon dioxide produced is equal to the volume of oxygen used. The respiratory quotient, $\frac{CO_2}{O_2}$, is therefore 1.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

The combustion of a fat (triolein) may be represented by the equation:

$$C_{57}H_{104}O_6 + 80O_2 = 57CO_2 + 52H_2O$$

Hence, the respiratory quotient of triolein is

$$\text{R. Q.} = \frac{57}{80} = 0.71$$

There are slight variations in the respiratory quotients of different fats, owing to the differences in molecular weight. For tripalmitin, the quotient is 0.703; for human fat, 0.713, etc.

It is somewhat more difficult to represent the oxidation of protein. The respiratory quotient may be computed as follows (Loewy [8]):

100 grams of meat protein contain:

52.38 g. C
7.27 g. H
22.68 g. O
16.65 g. N
1.02 g. S

[7] Some of the methods employed in the measurement of respiratory exchange in man and animals are to be found in the monograph by A. Krogh, The Respiratory Exchange of Animals and Man, Chapter II, London and New York, 1916. See also Du Bois, Basal Metabolism in Health and Disease, Philadelphia, 1924, and W. M. Boothby and I. Sandiford, Laboratory Manual of the Technique of Basal Metabolism Determinations, Philadelphia, 1920. The student is also referred to H. B. Richardson's review, "The Respiratory Quotient," in Physiol. Reviews, **9**, 61 (1929).

[8] Oppenheimer's "Handbuch der Biochemie," **4**, 1, 279 (1911); cited by Lusk, 4th edition, p. 64.

Of these amounts, the following portions are excreted in the urine and feces:

Urine	Feces
9.406 g. C	1.471 g. C
2.663 g. H	0.212 g. H
14.099 g. O	0.889 g. O
16.28 g. N	0.37 g. N
1.02 g. S	

This leaves a residuum for the respiratory process of:

41.50 g. C
4.40 g. H
7.69 g. O

The amount of oxygen here is sufficient to oxidize 0.961 g. of hydrogen, leaving for further oxidation

41.50 g. C
3.439 g. H

To oxidize these would require 138.18 grams of oxygen. The carbon-dioxide production would be 152.17 grams. These values may be converted into their volume equivalents, since 1 gram of oxygen occupies 0.699 liter and 1 gram of carbon dioxide 0.5087 liter. Computing for the value of the respiratory quotient:

$$\frac{77.39 \text{ l. } CO_2}{96.63 \text{ l. } O_2} = 0.801$$

If the combustion of carbohydrate alone were possible, the respiratory quotient would be 1; if only protein were being burned, it would be about 0.80–0.82; if fat, about 0.71. Under certain conditions, values outside the range 0.7 to 1.0 have been observed. Indeed, a quotient as high as 1.38 was obtained by Bleibtreu [9] on geese which were being stuffed and which were presumably converting carbohydrate (an oxygen-rich substance) into fat (an oxygen-poor substance). This process obviously involves the liberation of oxygen. Before hibernating, animals also show high respiratory quotients. Values lower then 0.70 have been observed in hibernating animals, the calorific requirements of which are obviously met by the stored fat. The glycerol arising in fat metabolism in these animals is probably converted, at least in part, into sugar. In diabetes, the respiratory quotient is low, since carbohydrate metabolism

[9] Pflüger's Arch., **85**, 345 (1901).

is deficient; and when the condition is especially severe, it may fall somewhat below 0.7 as a result of the conversion of amino acids and glycerol into glucose. In phlorhizin diabetes, Chambers and Deuel [10] recently observed a reduction in the respiratory quotient after the administration of glycerol, for, in the conversion of the latter into glucose, oxygen was required. Under ordinary conditions, the respiratory quotient is about 0.85, but it may vary within rather wide limits. The Hindoos and Chinese, who live largely on rice, are said to have a high respiratory quotient (above 0.9).

The amount of protein represented by 1 gram of urinary nitrogen requires for oxidation 5.923 liters (8.471 grams) of oxygen and produces 4.754 liters (9.347 grams) of carbon dioxide (Zuntz and Schumberg[11]). The calorific equivalent of 1 gram of urinary nitrogen (about 6.25 grams protein) is 26.51 calories (Lusk [12]).

Given the total oxygen consumption, carbon-dioxide production, and urinary-nitrogen elimination for a certain period, it is possible to calculate the amounts of protein, fat, and carbohydrate metabolized during that period. This may be illustrated by a simple example.

Suppose Mr. A during a period of twenty-four hours consumed 400 liters of oxygen and eliminated 340 liters of carbon dioxide and 12 grams of nitrogen. For the combustion of the protein represented by the urinary nitrogen,

$12 \times 5.923 = 71.076$ liters of oxygen were used, and

$12 \times 4.754 = 57.048$ liters of carbon dioxide produced.

Subtracting these values from the total volumes:

Total O_2 used	400 liters
O_2 used for protein	71 liters
O_2 used by carbohydrate and fat	329 liters
Total CO_2 produced	340 liters
CO_2 produced by protein	57 liters
CO_2 produced by carbohydrate and fat	283 liters

The ratio $\frac{283}{329} = 0.86$ is the non-protein respiratory quotient. From this figure may be computed the relative amounts of carbohydrate and fat used, or these may be determined more simply from the table

[10] J. Biol. Chem., **65**, 21 (1925).

[11] Studien zu einer Physiologie des Marsches, Berlin (1901).

[12] For further details and other methods of calculation, consult the books by Lusk and Du Bois, previously cited.

of Zuntz and Schumberg, modified by Lusk [13] and by McClendon [14] (page 423). It is to be noted that when the non-protein R. Q. is 0.86, 1 liter of oxygen is equivalent to 0.622 gram of carbohydrate and 0.249 gram of fat. Hence, 329 liters of oxygen are equivalent to $329 \times 0.622 = 204.6$ grams of carbohydrate and $329 \times 0.249 = 81.92$ grams of fat. Accordingly, during the period of experimentation, the following amounts were utilized:

Protein (12×6.25)	75	grams
Fat	81.92	grams
Carbohydrate	204.6	grams

From these figures may be calculated the total calorific output:

$$[75 \times 4.1] + [81.92 \times 9.3] + [204.6 \times 4.1] = 1908.3 \text{ calories}$$

According to Loewy:

1 liter of O_2 from protein corresponds to	4.485 calories
1 liter of O_2 from fat corresponds to	4.686 calories
1 liter of O_2 from carbohydrate corresponds to	5.047 calories

Referring to Table XLIV, it will be seen that when the non-protein respiratory quotient is 0.86, 1 liter of oxygen corresponds to 4.875 calories. From this value and the figure given by Loewy for protein, the calorific output may be computed readily as follows:

$$[71 \times 4.485] + [329 \times 4.875] = 1922.3 \text{ calories}$$ [15]

These calculations illustrate the method for computing heat production by indirect calorimetry.

Basal Metabolism.—The respiratory exchange and, hence, the heat production of an individual may vary within wide limits, being influenced by such factors as muscular activity, food intake, and external temperature. The influence of these factors is reduced to a minimum when the individual is lying perfectly still, sufficiently long after the last meal, so that no digestion is going on, and at a temperature ranging between 30° and 35° C. This condition of minimum metabolism and heat production is called basal metabolism (*Grundumsatz*—Magnus Levy). It is also referred to as maintenance metabolism (*Erhaltungs-*

[13] Lusk, Science of Nutrition, 1928 edition, p. 65.

[14] McClendon and Medes, Physical Chemistry in Biology and Medicine, p. 158.

[15] The slight discrepancy between this value and 1908.3 calories (less than 1 per cent) is due to the somewhat divergent factors introduced by different authorities and to dropping, in the calculations, of the last decimal places by the present author.

TABLE XLIV

THE SIGNIFICANCE OF THE NON-PROTEIN RESPIRATORY QUOTIENT AS REGARDS THE HEAT VALUE OF 1 LITER OF OXYGEN, AND THE RELATIVE QUANTITY IN CALORIES OF CARBOHYDRATE AND FAT CONSUMED (ZUNTZ AND SCHUMBERG, MODIFIED BY LUSK, MODIFIED BY MCCLENDON).

One Liter of Oxygen is Equivalent to

Non-protein Respiratory Quotient	Grams		Calories
	Carbohydrate	Fat	
0.707	0.000	0.502	4.686
0.71	0.016	0.497	4.690
0.72	0.055	0.482	4.702
0.73	0.094	0.465	4.714
0.74	0.134	0.450	4.727
0.75	0.173	0.433	4.739
0.76	0.213	0.417	4.751
0.77	0.254	0.400	4.764
0.78	0.294	0.384	4.776
0.79	0.334	0.368	4.788
0.80	0.375	0.350	4.801
0.81	0.415	0.334	4.813
0.82	0.456	0.317	4.825
0.83	0.498	0.301	4.838
0.84	0.539	0.284	4.850
0.85	0.580	0.267	4.862
0.86	0.622	0.249	4.875
0.87	0.666	0.232	4.887
0.88	0.708	0.215	4.899
0.89	0.741	0.197	4.911
0.90	0.793	0.180	4.924
0.91	0.836	0.162	4.936
0.92	0.878	0.145	4.948
0.93	0.922	0.127	4.961
0.94	0.966	0.109	4.973
0.95	1.010	0.091	4.985
0.96	1.053	0.073	4.998
0.97	1.098	0.055	5.010
0.98	1.142	0.036	5.022
0.99	1.185	0.018	5.035
1.00	1.232	0.000	5.047

umsatz—Loewy), post-absorptive metabolism (F. G. Benedict), basal metabolic rate (Plummer and Boothby), and standard metabolism (Krogh).

Influence of Surface Area.—It was pointed out by Voit [16] that the heat production, during rest, of such animals as the mouse, rabbit, fowl, dog, and horse, was dependent on the surface area. Calculated on this basis, the heat output of these animals, as well as of man, would amount to about 1000 calories per square meter per day. In proportion to its weight, the mouse has a greater surface and a greater heat output than the horse. Rubner [17] postulated the law that the metabolism is proportional to the superficial area of an animal. In the normal male, between the ages of twenty and forty years, the heat output per square meter per hour is, on an average, 39.5 calories; in females between these ages, it is somewhat lower, namely, 36.5–37 calories.

The surface area of human subjects may be calculated by means of the following formula, proposed by D. Du Bois and E. F. Du Bois: [18]

$$A = W^{0.425} \times H^{0.725} \times 71.84$$

where A = area in square meters, W = weight in kilograms, and H = height in centimeters.

Influence of Age and Sex.—New-born infants have a low basal metabolism, as was clearly demonstrated by Hasselbach,[19] who found that the heat production per kilogram of body weight was about the same during the first twenty-four hours of life as in the adult, despite the relatively larger surface of the infant. These findings were confirmed by Benedict and Talbot,[20] who observed a caloric output per square meter per twenty-four hours of 612 calories or about 25 calories per square meter per hour; and by Murlin, Conklin and Marsh,[21] who obtained a value of 700 calories in the new-born. It is interesting to note that during the first hours of life the respiratory quotient is high, often approximating 1, which means that the infant is burning carbohydrate, for the most part. The quotient falls rapidly, so that at the end of the first day it may approximate 0.7 to 0.72. It then increases gradually until the fifth or sixth day, when it reaches 0.81, or the respiratory quotient of the adult. Carpenter and Murlin [22]

[16] Z. f. Biologie, **41**, 120 (1901).

[17] Rubner, Energiegesetze, 1902, p. 282.

[18] The surface area of human subjects may be determined more directly from a chart prepared by D. and E. F. Du Bois. For the methods employed in deriving the equation, consult the original papers by Du Bois [Arch. Int. Med., **15**, 868 (1915); *ibid.*, **17**, 863, 887 (1916)], and the book previously cited.

[19] Trans. Pub. No. 233, Carnegie Inst., Wash., 1915.

[20] Carnegie Inst., Wash., 1921 Pub. No. 302; see also, Talbot, Basal Metabolism of Children, Physiol. Reviews, **5**, 477 (1925).

[21] Am. J. Dis. Child., **29**, 1 (1925).

[22] Arch. Int. Med., **7**, 184 (1911).

made the interesting observation that the energy metabolism of the pregnant mother, immediately before delivery, is equal to the sum of the metabolism of the mother and infant taken 3–10 days after childbirth.

The basal metabolism of premature infants is lower than that of full-term infants, both at birth and for several months thereafter. The basal metabolism of the child increases very rapidly during the first year of life and continues to be high for three or four years thereafter (15–20 per cent per square meter of surface above adult). A second rise has been reported by Du Bois and his associates immediately preceding puberty (twelfth to thirteenth year), which is followed by a gradual decline after puberty is reached. This point has not been fully substantiated, however. According to Boothby and Sandiford,[23] there is a decrease in the basal metabolism of male children between the ages of five and twenty-one and a more rapid decrease in female children between the ages of five and seventeen, followed in both sexes by a gradual and nearly parallel decline to old age. The influence on metabolism of age and sex is shown in the following table:

TABLE XLV

THE DU BOIS NORMAL STANDARDS AS MODIFIED BY BOOTHBY AND SANDIFORD

Calories per square meter per hour

Age	Males	Females	Age	Males	Females
5	(53.0)	(51.6)	20–24	41.0	36.9
6	52.7	50.7	25–29	40.3	36.6
7	52.0	49.3			
8	51.2	48.1	30–34	39.8	36.2
9	50.4	46.9	35–39	39.2	35.8
10	49.5	45.8	40–44	38.3	35.3
11	48.6	44.6	45–49	37.8	35.0
12	47.8	43.4	50–54	37.2	34.5
13	47.1	42.0	55–59	36.6	34.1
14	46.2	41.0			
15	45.3	39.6	60–64	36.0	33.8
16	44.7	38.5	65–69	35.3	33.4
17	43.7	37.4			
18	42.9	37.3	70–74	(34.8)	(32.8)
19	42.1	37.2	75–79	(34.2)	(32.3)

[23] Am. J. Physiol., **90**, 290 (1929).

Seasonal and Other Variations.—According to Gustafson and Benedict,[24] metabolism tends to be at a low level in winter and to rise to a higher level during the spring and summer.

Benedict and Finn [25] have observed that menstruation is a real factor in lowering the metabolism. Oxygen consumption was found to be lowest and most uniform during the menstrual period and highest about one week after menstruation ceased.

There are racial differences. Thus the Chinese are said to have a lower metabolism than Europeans or Americans and the Eskimos a higher metabolism than individuals living in the temperate zones. Necheles [25a] has attributed the lower basal metabolic rate of Orientals partly to a greater degree of constant relaxation. He also found that, unlike Caucasians, the Chinese do not show a marked drop in basal metabolism during sleep.

Starvation.—Turning to the abnormal variations of the basal metabolic rate, we may consider first the effect of undernutrition and starvation. In a celebrated experiment, Benedict [26] studied the changes in basal metabolism of a man subjected to a fast of thirty-one days. At the beginning of the experiment the subject weighed 60.64 kg.; on the thirty-first day the weight was 47.39 kg. On the first day of the fast the heat output was 904 calories per square meter of body surface. By the twenty-first day this had diminished to 664 calories. Then followed an increase to 737 calories on the last day of the experiment.

The effect of undernutrition has been studied in individuals who, voluntarily or otherwise, were victims of chronic inanition.[27] During the period of the Great War, Zuntz and Loewy [28] followed their own basal metabolism and observed reductions of 15 and 12 per cent respectively. Benedict [29] placed a squad of athletic men, who had been accustomed to a daily caloric intake of 3200–3600 calories, on a diet containing 1400 calories for a period of three weeks. As a result, the men lost, on an average, 12 per cent of their weight. The basal metabolism was reduced 18 per cent. After this the men were able to maintain themselves, without further loss of weight, on 1950 calories, although on this reduced intake they were not as active or energetic as previously,

[24] Am. J. Physiol., **86**, 43 (1928).

[25] *Ibid.*, **86**, 59 (1928).

[25a] Am. J. Physiol., **91**, 661 (1930).

[26] Carnegie Inst. Pub., No. 203, 1915; Am. J. Physiol., **41**, 292 (1916).

[27] The subject of Fasting and Undernutrition is discussed in a book of that name by S. Morgulis, New York, Dutton and Co., 1923.

[28] Biochem. Zeit., **90**, 244 (1918).

[29] Benedict, Miles, Roth and Smith, Carnegie Inst. Pub., No. 280 (1919).

nor were they able to withstand the cold as well. Clearly, undernourishment and starvation are effective in lowering basal metabolism.

Influence of Disease.—An increase of 1 degree Centigrade in the body temperature causes a rise in metabolism of about 13 per cent. Thus, in typhoid fever there may be an increased heat production of as much as 40 or 50 cent above the normal level. Similar changes are observed in pneumonia and malaria, but not in tuberculosis where alterations in metabolism with changes in body temperature are not so marked. It is believed that the toxic destruction of protein, which is characteristic of certain febrile conditions, is responsible for the increased metabolism. It is not definitely known whether the rise in temperature in fever is the cause or the result of increased catabolism.

The determination of basal metabolism has found wide application in the study of pathological conditions, particularly those associated with the thyroid gland. Boothby and Sandiford [30] have summarized their observations on 1689 patients with thyroid disorders, and find that 92 per cent of their cases of exophthalmic goiter show a basal metabolism which is 20 per cent or more above normal. Fifty-two per cent of their 452 cases of exophthalmic goiter show a basal metabolism of 50 per cent and more above normal. On the other hand, the basal metabolic rate of 83 per cent of their myxedema cases are subnormal by 20 per cent or more, the remaining 17 per cent being 11 to 15 per cent below normal.[31] Following operative treatment in cases of hyperthyroidism, the basal metabolic rate tends to approach normal levels. In myxedema, the administration of thyroxin is followed by an increase in metabolism.

Boothby and Sandiford have likewise summarized their results on 1642 individuals with different diseases other than those of the thyroid gland. These show that 89 per cent are within 15 per cent of the Du Bois normal standards. Of the pathological conditions listed in this group, leukemia shows the only marked variations from the normal. Of 15 cases of lymphatic and myelogenous leukemia cited, 10 have a basal metabolism which is 20 per cent or more above normal.

Subnormal metabolism is indicated in many of the cases diagnosed as hypopituitarism. On the experimental side, Benedict and Homans [32] have shown a decrease in the basal metabolism of dogs following hypo-

[30] Physiol. Reviews, **4**, 69 (1924).

[31] These calculations are based on the Du Bois normal standards. Thus, if the normal basal metabolism of a woman for a given age is 37 calories per square meter, per hour, and the actual metabolism is 44.4 calories, the metabolism is $44.4 - 37 = 7.4$; $7.4/37 \times 100 = +20$ per cent.

[32] J. Med. Research, **25**, 409 (1912).

physectomy. Low metabolic rates are also frequent in obesity, whether due to castration, to hypopituitarism, or to some other cause.[33]

Returning to derangements of the thyroid, Talbot [34] observed a minus 20 per cent metabolism in a $3\frac{2}{3}$-year-old cretin. In a 36-year-old cretin, Du Bois [35] found the basal metabolism to be low by 18 to 25 per cent. A minus metabolism of 48 per cent was observed in a case of cretinism studied by Janet.[36] The administration of thyroid extract or thyroxin in this condition causes a rise in metabolism, whereas discontinuance of the treatment is followed by a marked fall of the basal metabolic rate.

Regulation of Body Temperature.—Animals may be divided into two groups according to their ability to maintain a constant body temperature. The temperature of cold-blooded animals varies with the environment, and they are therefore called heterothermic or poikilothermic. Reptiles, batrachians, fish, molluscs, and insects belong to this group. At low temperatures (1–20° C.), these animals are usually warmer than their environment by about 1° C. (Burns [37]). When the temperature of the environment of a frog is increased to about 40° C., its own temperature remains somewhat lower.

Birds and mammals belong to the group of homoiothermic animals, which are able to resist environmental temperature changes. It is not to be supposed, however, that the mechanism for the maintenance of body temperature in these animals is never broken down. Reference has already been made to the body-temperature changes which occur during fever. During hibernation, the homoiothermic animal is essentially heterothermic. Curare produces a similar effect. This drug inhibits the transmission of motor impulses to voluntary muscles and causes, in addition, the breakdown of the temperature-regulating mechanism (Roehrig and Zuntz [38]). Its administration is also followed, at ordinary temperatures, by a marked diminution in metabolism. It is a matter of general knowledge that the regulation of body temperature is deficient in infants as well as in other new-born warm-blooded animals. These are usually unable to withstand long exposure to temperatures below 20° C.

The temperature of birds varies with the size and is usually between 40° and 43° C. Small mammals have a higher body temperature (39°–

[33] Consult Lusk, Science of Nutrition.
[34] Am. J. Dis. Child., **12**, 145 (1916).
[35] J. Am. Med. Assoc., **63**, 827 (1914).
[36] Le Journ. Médic. Français, **12**, 1, No. 6 (1923); cited by Talbot.
[37] D. Burns, An Introduction to Biophysics, London, 1921, Chapter XXXI.
[38] Pflüger's Arch., **4**, 57 (1871).

40° C.) than larger mammals. In man, the temperature is normally about 37.5° C., but may fall somewhat below 37° during sleep.

Loss of Heat from the Body.—Heat is lost from the body through the following channels:

1. Conduction and convection;
2. In warming the inspired air (conduction);
3. In warming the ingested food;
4. In excreta (CO_2, urine and feces are warm);
5. Radiation;
6. Evaporation of water from lungs and skin.

Among the factors that influence the dissipation of heat from the body are the area and moistness of the surface, time of exposure, temperature gradient between the surface and outside atmosphere, humidity of the atmosphere, and the force of the wind, if any. The loss of heat by radiation is affected by the color of the surface. A black surface has a higher absorptive and emissive power than a white surface. Accordingly, there should be a greater amount of heat lost by radiation from the body of a negro than from that of a white person. On the whole, however, the loss of heat by radiation is comparatively small. In the following table (taken from Burns [39]) is given a rough estimate of the amount of heat lost per day through the more important channels:

TABLE XLVI

LOSS OF HEAT FROM THE BODY

	Per Cent	Calories per Day
1. Radiation and conduction	73.0	1792
2. Evaporation:		
(*a*) Lungs, etc	7.2	182
(*b*) Skin	14.5	364
3. Excreta:		
(*a*) CO_2	3.5	84
(*b*) Urine and feces	1.8	48
Total heat loss per day		2470

[39] For further discussion of the factors, consult Burns' "An Introduction to Biophysics," Churchill, London, 1921 edition, p. 348; see also Lusk's "Science of Nutrition," Chapter IV.

Having considered the way in which the accumulation of heat is prevented, we may now turn our attention to the factors which prevent the excessive loss of heat from the body when the temperature of the environment is reduced. These are usually discussed under two heads, namely:

1. Physical regulation;
2. Chemical regulation.

Regulation of the temperature by physical forces is believed to predominate above 20° C. Below this temperature, chemical regulation enters into play to a greater degree than physical regulation.

Physical Regulation.—Man adjusts the temperature of his environment by living in houses, by heating his dwelling during the winter, and by wearing clothes. Animals, likewise, provide themselves with shelter and have fur and feathers to enable them to diminish the loss of heat by conduction. The thickness of the skin and the amount of subcutaneous fat are additional factors which reduce loss of heat from the body. It is well known that lean people suffer more from cold and less from heat than obese individuals.

Heat and cold exert an important effect on the cutaneous nerve endings, causing a reflex vasomotor stimulation. When the temperature is high, the blood vessels of the skin and respiratory passages become dilated; there is an increased flow of blood to these areas, and hence a greater effective cooling surface, together with increased evaporation of water from sweat glands and mucous surfaces. On the other hand, cold, by causing vaso-constriction, decreases the flow of blood to the skin and respiratory surfaces and therefore diminishes the cooling area as well as the amount of perspiration.

In sleeping, an animal will curl up when it is cold and stretch out when hot, thereby diminishing or increasing the area of the exposed surface.

Chemical Regulation.—The increased heat production incident to exposure to cold is referred to as the heat of chemical regulation. Opinion is divided as to its cause. Voit [40] suggested the view that exposure of the skin to cold brought about a reflex stimulation of metabolism in muscle cells without necessarily causing muscular activity such as shivering. From the work of Loewy,[41] Johansson,[42] Lusk [43] and others, however, it appears that the increased heat output is to be

[40] Z. f. Biologie, **14**, 80 (1878).
[41] Pflüger's Arch., **46**, 189 (1890).
[42] Skand. Arch. Physiol., **7**, 123 (1896); cited by Krogh.
[43] Am. J. Physiol., **27**, 427 (1910).

attributed to involuntary muscular activity. During the period of shivering which follows immersion in a cold bath, the heat production may increase to 180 per cent above the normal (Lusk). The energy required for involuntary muscular activity is derived from the metabolism in the tissues. It is well known that exposure to cold is an effective method of depleting the glycogen supply of the body. Shivering may be avoided, however, by voluntary muscular exercise, for in either case the effect on combustion in the tissues is the same. When the difference in temperature between the body and the surrounding medium becomes so great that the dissipation of heat from the former is markedly increased, the " fires of metabolism " are caused to burn more briskly in order to make up for this loss. The chemical regulation of body temperature is essentially the result of increased metabolism.

Influence of Food on Metabolism; Specific Dynamic Action.— For a variable period (usually twelve to eighteen hours) after the ingestion of food, the calorific output is greater than that determined under basal conditions. To illustrate what is meant, let us suppose that the basal metabolism of a given individual is found to be 1800 calories per day. If exactly 1800 calories were now supplied to this individual in the form of a mixed diet, over a period of twenty-four hours, his heat production for that period would be, not 1800 calories, but more nearly 2000 calories. The problem we have to consider is the cause of this increased heat production.

It is conceivable that the processes of digestion and absorption may constitute a factor; but when this supposition is subjected to experimental study, it is found that the energy requirement due to increased activity of the muscles and glands of the alimentary tract accounts for but a fraction of the increased heat production. Meat extract, though stimulating the digestive glands to secretion, nevertheless produces no appreciable effect on metabolism; nor does the presence of agar in the alimentary canal influence the rate of metabolism, although it probably increases the muscular activity of the intestinal wall. Moreover, it has been shown that the intravenous injection of glucose and amino acids leads to an increase in metabolism which is almost comparable to that observed following the administration of these substances by mouth (Benedict and Carpenter [44]).

The effect of foods in stimulating metabolism is called the specific dynamic action. In the study of this phenomenon, Rubner was the pioneer. Among other discoveries, he made the important observation that the heat due to specific dynamic action can be used by the

[44] Carnegie Inst. Wash., Pub. No. 261, 1918.

animal organism in the regulation of body temperature. Our knowledge of the effect of foodstuffs in stimulating metabolism has been greatly extended by the work of Lusk and his students in this country. The effect is not the same for all foods. Williams, Riche, and Lusk [45] showed an increase of 30 calories in heat production for every 100 calories contained in the protein of 1220 grams of meat given to a dog. Numerous similar observations place the value for the specific dynamic action of protein at about 30 per cent. In the case of fat, it is about 12 per cent; and of carbohydrate, 5 to 6 per cent.

From the standpoint of nutrition, the significance of specific dynamic action is of considerable importance. Let us imagine a hypothetical organism which has a basal metabolism of 100 calories. Suppose this animal were given exactly 100 calories in the form of protein. As a result, the actual heat production would be about 130 calories. The 30 additional calories must obviously come from the combustion of the organism's own tissues. If this process were continued, the organism would eventually diminish in size until its basal metabolism, plus the specific dynamic action, would be 100 calories. From that point on, it would remain in caloric equilibrium. But, if we persisted in giving the animal its basal requirements from day to day, it would ultimately vanish. The same would be true if fat were given, except that the initial 100 calories given in the form of fat would result in the production of 112 calories, the extra 12 calories representing the specific dynamic effect of the fat. If 100 calories were given in the form of carbohydrate, it would stimulate sufficient additional metabolism to yield 6 more calories. In arranging a dietary, about 15 per cent should be added to the basal caloric requirement to allow for the specific dynamic action (or S. D. A.) of the foodstuffs.

As to the fundamental cause of this phenomenon, there is much uncertainty. In attempting to elucidate the significance of the specific dynamic action of foodstuffs, attention has been directed particularly toward the behavior of the amino acids. Lusk [46] has shown that both glycine and alanine exert a very marked effect, whereas this is not so in the case of aspartic acid and other amino acids. Phenylalanine has a greater specific dynamic action than any other amino acid (Rapport and Beard [47]). The products of the intermediary metabolism of amino acids do not exhibit this effect, nor is there an obvious relationship

[45] Cited by Lusk, Science of Nutrition, 1917 edition, p. 238.

[46] Lusk, J. Biol. Chem., **12**, 349 (1912); **20**, viii (1915); Atkinson and Lusk, **36**, 415 (1918); Lusk, **49**, 453 (1921); also Lusk, Science of Nutrition, 4th edition, Chapter XII.

[47] J. Biol. Chem., **73**, 299 (1927).

between the specific dynamic action and the rate or extent of deamination. Seth and Luck [48] studied the effect of administering various amino acids on the amino-nitrogen concentration of the blood, and reached the conclusion that the specific dynamic action of an amino acid is proportional to its power of increasing the amino nitrogen content of the blood. This may be accepted as evidence in support of the idea that the velocity of oxidative reactions in the tissues is modified by the concentration of the metabolites, as would be expected from the law of mass action.

Wilhelmj and Bollman [49] have observed that the injection of alanine, glycine or phenylalanine causes an immediate rise in heat production, which usually reaches its height during the injection, and with glycine and alanine the return to the basal value may take from four and a half to nine hours. Accompanying the increased heat production there is a definite elevation of the respiratory quotient, indicating the probable utilization of carbohydrate in the process. An equally interesting observation is that of Rapport and Katz [50] who have shown that when glycine is added to perfused muscle, the oxygen absorption is 40 per cent higher than otherwise, indicating that the presence of the amino acid stimulates the combustion of other tissue constituents. According to Rapport and Beard, the specific dynamic action of meat and gelatin may be accounted for by the summated effect of five of their amino acids, namely glycine, alanine, leucine, phenylalanine and tyrosine.

It has been reported that specific dynamic action is abolished in experimental, as well as in clinical hypopituitarism.[50a]

Influence of Work on Metabolism.—Work is accomplished by the body at the expense of increased metabolism, whether food is eaten or not. In a series of experiments, Rubner [51] was able to show that heat production incident to mechanical work is independent of the heat produced because of specific dynamic action, especially in the case of protein food. This is indicated by the data in Table XLVII.

Although, fundamentally, there is little similarity between the animal body and the steam or petrol engine, it is nevertheless of interest

[48] Biochem. J., **19**, 366 (1925).

[49] J. Biol. Chem., **77**, 127 (1928).

[50] Am. J. Physiol., **80**, 185 (1927).

[50a] Foster, G. L., and Smith, P. E., J. Am. Med. Assoc., **87**, 2151 (1926); Plaut, R., Deut. Arch. klin. Med., **139**, 285 (1922); **142**, 266 (1923); Kestner, O., and collaborators, Klin. Wochnschr., **5**, 1646 (1926); see also Lusk, Science of Nutrition, 4th edition, pp. 613, 614.

[51] Sitzungsberichte der preussischen Akademie der Wissenschaften, **16**, 316 (1910); cited by Lusk, p. 311.

TABLE XLVII

INFLUENCE OF DIET AND MECHANICAL WORK ON METABOLISM

Diet and Conditions	Calories Produced		
	24 Hours, Calories	Increase, Per Cent	Increase Due to Work, Calories
No food, rest	1976		
Cane sugar 600 g. + rest	2023	2.4	
Same + work (100,000 kg.-meters)	2868	45.2	845
Protein (meat) + rest	2515	27.2	
Protein + work (100,000 kg.-meters)	3370	70.5	855

to compare the efficiency of external muscular work with the thermal efficiencies of mechanical devices. The efficiency of the steam engine is 8–10 per cent. The usual type of gasoline motors have an efficiency of about 20 per cent; i.e., of every 100 gallons of gasoline which are burned to completion, about 20 are converted into mechanical energy. It has been estimated that the efficiency of the human body is between 25 and 33 per cent. In this regard, it is excelled only by special types of engines, such as the Still-Diesel combination which has an efficiency of about 44 per cent.

Training is apparently a factor determining the amount of energy required in the performance of a given task. An individual unaccustomed to a certain type of muscular exertion, such as mountain climbing, uses proportionately more energy than one who has been trained for this work. It has also been determined that the speed with which muscular work is done influences the degree of energy expenditure. Less energy is used in covering a given distance by slow walking than in covering it by fast walking or running.

Provided an adequate amount of fat and carbohydrate is available, muscular exercise does not influence materially the amount of protein metabolism. In fact, in the well-nourished individual, violent exertion is associated with a high respiratory quotient without marked alteration in nitrogen excretion, showing that carbohydrate is the chief fuel under these circumstances.

Heat produced in doing mechanical work can take the place of heat of chemical regulation. In other words, there is not a summation of these factors when an individual performing muscular exercise is exposed to cold.

Caloric Requirements.—Rubner developed the conception that, under certain conditions, the foodstuffs may replace each other in accordance with their heat-producing value. This is known as the "isodynamic law." According to this view, 100 grams of fat, 232 grams of starch, and 243 grams of protein (as dried meat, etc.) are equally effective in providing the animal body with the energy required for muscular work as well as with heat. Rubner's hypothesis has been questioned by Cathcart,[52] who believes that glucose and fat are not interchangeable in providing energy demands, since carbohydrate is a more effective protein sparer than fat. Evidence has been presented, likewise, by Krogh,[53] which shows that carbohydrate is superior to other foodstuffs in supplying energy for muscular contraction.[54] Accordingly, the isodynamic law is not to be interpreted too strictly. As pointed out by Cathcart, the calorific value is simply a very convenient physical standard for the assessment of diets; but the mere fact that such a standard has proved of great utilitarian value is not real justification for adopting this standard as the foundation stone of hypotheses framed to offer an explanation for cellular activity. The calorific value of a given amount of food is therefore not necessarily a criterion of its nutritional or tissue-sparing effect. With this in mind, we may continue our discussion of the caloric requirements of the animal body.

The caloric needs of man and animals are determined by the total heat production due to the various factors which have been discussed in the preceding paragraphs. When the caloric intake is equivalent to the output, the condition of calorific balance or equilibrium is said to exist. This is the normal state in the adult individual; but in the growing animal the intake should exceed the outgo. The energy of maintenance, including that of the vital functions (circulation, respiration, secretion, excretion, maintenance of muscle tone, etc.), is represented by the basal heat production. The basal metabolism of an adult, weighing 70 kg., is approximately 1750 calories for twenty-four hours. The heat production is increased above the basal level even by slight activity, such as sitting or standing. Depending on the character of the diet, allowance should be made for the specific dynamic action of the food ingested. An allowance of 10–12 per cent above the

[52] Biochem., J., **16**, 747 (1922).

[53] J. Physiol., **52**, p. lxxiv (1919).

[54] The energy transformations which occur in muscle have been studied recently by Meyerhof (Chemical Dynamics of Life Phenomena, Phila., 1924, and a series of papers in Arch. ges. Physiol., 1920–1922), and Hill (The Mechanism of Muscular Contraction, Physiol. Reviews, **2**, 310, 1922). As this work does not permit of a brief formulation, the student is urged to refer to the original literature.

basal metabolic requirement is ordinarily sufficient when the individual is maintained on a mixed diet.

The most variable factor to be reckoned with is the food required for the performance of physical work. The relation of occupation to energy requirement has been the subject of numerous investigations both in this country and abroad. Individuals engaged in sedentary occupations have a total daily metabolism of 2500–2800 calories. These figures allow 550–900 calories for the performance of mechanical work (walking to and from work, etc.).

In Finland, Becker and Hämäläinen [55] made a study of the energy requirements of men and women engaged in various occupation. A portion of their results is summarized in Table XLVIII.

The food consumption of the average American farmer is about 3500–4000 calories. Lusk states that a bicyclist riding for sixteen hours may have a metabolism amounting to 9000 calories daily. The food ration of a Maine lumberman may rise to 7000 and even 8000 calories per day.[56]

TABLE XLVIII

Sex	Occupation	Calorific Requirement for 24 Hours
Men	Tailors (2)	2400 to 2500
	Bookbinder	2700
	Shoemaker	2800
	Metal workers (2)	3100 and 3200
	Painters (2)	3200 and 3300
	Carpenters (2)	3200 and 3300
	Stonemasons (2)	4300 and 4700
	Men sawing wood (2)	5000 and 5400
Women	Seamstress (needle)	1800
	Seamstress (machine)	1900 and 2100
	Household servants	2300 to 2900
	Washerwomen (2)	2600 and 3400

Thus far, we have not considered the allowance to be made for "chemical regulation." This factor would become operative only

[55] Skan. Archiv. f. Physiol., **31**, 198 (1914). Lusk, Science of Nutrition, p. 463; see also numerous papers from the Nutrition Laboratory of the Carnegie Institution of Washington.

[56] For a review of the food requirements of soldiers, see Lusk, Science of Nutrition, 4th edition, pp. 469–473

when an individual doing little or no work was exposed to extreme cold, a combination of circumstances not ordinarily met with. In considering the quantitative relation between work and total metabolism, it is important to bear in mind the interplay of all the factors. The heat due to mechanical work can replace the heat of chemical regulation. Demands for chemical regulation of body temperature may be met, likewise, by the specific dynamic action of foodstuffs. Accordingly, protein and fat would be more effective in this regard than carbohydrate. On the other hand, the specific dynamic action of foodstuffs cannot be converted into muscular work. These two factors are totally independent.

It is a mistaken idea that a child is a fraction of an adult as far as its food requirements are concerned. There are three points to be considered in this connection, namely, the relatively high basal metabolic rate during childhood, the unusual physical activity of boys and girls, and the necessity of maintaining the caloric intake well above the expenditure in order to allow for growth. Between the ages of 1 and 2 years, infants require approximately 1000–1200 calories per day. It has been estimated that between the ages of 10 and 13 years, the requirements of a boy are 2300–3000 calories, and even more in the case of a very active child. This explains the apparent voraciousness of boys and girls, particularly the former. That there is actually a physiological need for large quantities of food is shown in the work of several authorities,[57] all of whom recommend a most liberal food allowance for the growing child.

[57] The problem of energy requirements of children is discussed in the contribution of F. G. Benedict and Talbot (Carnegie Inst. of Washington, Publication No. 302 (1921), as well as in the monograph of Mendel, Nutrition—The Chemistry of Life, Yale University Press, 1923).

CHAPTER XVII

NUTRITION

Sir Michael Foster likened the growth of knowledge to the ascent of a spiral stair from which the observer periodically surveys the same landscape, but each time from a higher level than the last.—Joseph Barcroft.

The chemistry of nutrition is customarily treated from the standpoints of (*a*) caloric, (*b*) mineral, (*c*) protein, and (*d*) vitamin requirements. These are generally regarded as the most important factors to be considered. Evidence is accumulating, however, that although the body is capable of synthesizing fat from carbohydrate and indirectly from protein, nevertheless, a certain amount of it, present in the food as such, is indispensable to proper nutrition. The water balance of the organism, as has been pointed out in other connections, is another factor to be considered, being of special importance in the young. It may be emphasized at the outset that this manner of presentation has not been in vogue very long, for much of our knowledge of the subject is of comparatively recent origin. Very probably, as further progress is made in this important field, new facts may come to light—facts which may modify or even alter our present conceptions.

To be adequate, a diet must provide for all the needs of the organism, particularly for maintenance, growth, and reproduction. The minimum requirements for proper nutrition are fulfilled only (*a*) when the organism is maintained in caloric equilibrium; (*b*) when it is maintained in nitrogen equilibrium; (*c*) when there is an adequate supply of inorganic elements; and (*d*) when there is an adequate supply of vitamins. As can be readily appreciated, the needs of the young and growing animal are far in excess of the minimal requirements.

In addition to these factors, consideration may also be given to certain others which are presumably of secondary importance. There is some reason for believing that variety in the selection of food is beneficial. The Eskimo is limited by his environment to a few staples and subsists in certain localities largely on fish and meat. The everyday food of the Oriental of the poorer classes is rice with variable additions of fish. Europeans and Americans seek a more varied diet. The

benefits of this may be purely psychological; but it is reasonable to suppose that the larger the variety of foods, the less would be the likelihood of missing some essential ingredient.[1]

There is also the question of cooking, seasoning, and flavoring. Aside from the effect of cooking in increasing the digestibility of many substances, the beneficial effect of these treatments is due largely to the increased palatability, and, hence, to increased consumption of food. Moreover, seasoning and flavoring materials are not without influence in stimulating the secretion of digestive juices (both chemical and psychic stimulation). While these substances can hardly be regarded as essential to nutrition, they may nevertheless be included here as constituting a factor of secondary importance. It may also be pointed out, in this connection, that diet is too often a matter of habit. Certain individuals and peoples relish foods that others find distasteful.

Caloric Requirements.—A large portion of the preceding chapter was devoted to the energy factor in nutrition. The subject may therefore be dismissed here with a few words. The normal adult requires just sufficient calories to balance the total loss from his body. It is obvious that the manual laborer needs more calories than the individual who is engaged in light work. The caloric demands are also influenced by external temperature and, hence, by climate. When more calories are given off by an individual than are taken in the form of food, he is no longer in a state of caloric equilibrium. Calories are produced, under these circumstances, at the expense of the tissues, and there is a loss of weight. It is important to bear in mind that the growing child should be provided with more food than is sufficient for the maintenance of the energy balance. The normal state for the growing animal is a condition of positive caloric balance.

Mineral Requirements.—The extraordinary influence of the mineral elements in nutrition, although it has been appreciated to some degree for many years, was not clearly understood or quantitatively studied until quite recently. In a long series of experiments which began not quite twenty years ago, Hart, McCollum and Steenbock [2] studied the growth and reproduction of cattle upon restricted diets of various grains. These investigators divided their experimental animals into four groups. All the animals received approximately the same amount of sodium

[1] The dietary habits of man in different parts of the world are described by E. V. McCollum and N. Simmonds in "The Newer Knowledge of Nutrition," Macmillan and Co., New York, 4th edition (1929), Chapter XXVII.

[2] For details of these studies the student is referred to McCollum and Simmonds, Newer Knowledge of Nutrition, New York, 1929, as well as to Mendel's monograph on Nutrition, Chapter II.

chloride. The ration which was fed to one group was derived solely from the wheat plant and consisted of wheat straw, wheat gluten, and the entire wheat grain. The second group received a ration derived from the corn plant. The third group was fed on the products of the oat plant. The fourth group of animals received a ration consisting of a mixture of wheat, corn, and oats in about equal proportions. It was discovered that the nutritive condition of the corn-fed animals was much better than that of any of the remaining groups. The wheat-fed cattle fared worst. The corn-fed animals gave birth to normal young and reared them. The offspring of the wheat-fed cows were not carried to full term, and those that were not born dead usually died several days after birth. The behavior of the animals in the remaining two groups was intermediate between the two extremes observed in the corn-rationed and wheat-rationed groups. The untoward manifestations noted in the wheat-fed animals, as well as in those maintained exclusively on oats, were shown to be due largely to a deficiency in inorganic constituents, chiefly calcium.

Much of the progress attained in the field of nutrition has been made possible by using small animals, particularly albino rats. These reach maturity and begin to breed at about ninety days of age and rarely live to be more than three years old. Thus it is possible to study, in a comparatively short time, the complete life cycle of the animal. The use of synthetic or artificial diets in feeding experiments may likewise be mentioned in this connection. It is possible to maintain rats and other animals in excellent nutritive condition upon a diet consisting of purified protein, fat, carbohydrate, and salts, provided the necessary vitamins are added to the food. These may be derived from yeasts and other sources. The value of employing suitable artificial mixtures in nutrition studies is obvious, for it becomes possible by this method to exclude, at will, more or less completely, one or more ingredients. It then remains only to compare the progress of the experimental animals in respect to growth, reproduction, etc., with control animals maintained on an adequate diet. Mendel and others have done much to establish the concept that a deficiency of any factor essential for growth is followed by a failure in growth of the body as a whole, and not by the production of abnormal tissues due to the lack of some element. When the minimum requirements are not met, even with regard to a single constituent, such as the growth-promoting vitamin, an essential amino acid, or an inorganic element like calcium, failure in growth results.

Using artificial food preparations, Osborne and Mendel [3] investi-

[3] J. Biol. Chem., **34**, 131 (1918).

gated the influence of inorganic elements in nutrition, by excluding (except for traces), from the diets of different groups of rats, one or more of the following constituents: potassium, magnesium, calcium, sodium, chlorine, and phosphorus. They found that the rats fed on diets low in magnesium, sodium, and chlorine grew with vigor in so far as could be judged by gains in body weight, and that even when these elements were present only in traces good growth was obtained. Apparently less than 0.04 per cent of either sodium or chlorine and 0.01 per cent of magnesium (per cent of total food) was sufficient to permit these rats to complete their growth. The growth of the animals was fairly satisfactory on a potassium intake of about 0.03 per cent, provided the sodium intake was adequate. When both sodium and potassium were reduced in amount, growth ceased. Under these circumstances, the addition of sodium alone, at an early stage of growth, did not result in gain; but when potassium was added, normal growth was resumed. At a later stage in development, Osborne and Mendel found it possible to replace sodium for potassium.

With regard to the potassium requirement, the observations of Miller [4] differ somewhat from the findings of Osborne and Mendel. Miller noted that the growth of rats could be greatly retarded by reducing the potassium content of the ration below a certain level, approximately 0.1 per cent. The minimal requirement, according to this author, is therefore at least three times as much as that given by Osborne and Mendel. Moreover, Miller observed that potassium deficiency during the early development of the organism may not only prevent the growth of the body but also cause abnormal physiological disturbances which make themselves apparent later. In fact, rats deprived of potassium early in life usually die despite an adequate supply of potassium at a later stage (fourth to eleventh week) of development. Miller did not obtain normal growth by substituting sodium for potassium. Nor is it possible to substitute potassium for sodium in a sodium-deficient diet (St. John [5]).

According to Mitchell and Carman [6] the lack of sodium and chlorine limits the food value of rations composed largely of corn. Miller,[7] on the other hand, found corn rations to contain sufficient chlorine for growth and maintenance.

An oft-repeated observation, first made by Bunge,[8] is that the admin-

[4] J. Biol. Chem., **55**, 61 (1923); **62**, 259 (1924); **70**, 587 (1926).
[5] J. Agr. Res., **37**, 55 (1928).
[6] J. Biol. Chem., **68**, 165 (1926).
[7] *Ibid.*, **70**, 759 (1926).
[8] Zeit. Biol., **9**, 104 (1873).

istration of potassium increases the excretion of sodium and chlorine in the urine. According to Whelan,[9] the increased elimination of these elements may be due to the diuretic effect of potassium. It has been supposed that the tissues retain potassium at the expense of sodium and other elements because of its relatively greater physiological importance (regulatory effect upon the heart, muscular contraction, presence in red corpuscles, etc.). However, in view of the recent observations of Miller,[10] it is probably incorrect to assume that a high level of potassium intake continues indefinitely to modify the excretion of other elements which may be needed for normal physiological development. In Miller's experiments, the introduction of potassium salts into the diet caused an immediate increase in the total output of sodium and chlorine, after which the loss of these elements in the urine was only slightly greater than on a basal ration. As to calcium and phosphorus, the levels of excretion on a high-potassium intake were but slightly increased over the low-potassium period.

The magnesium requirement is extremely low, as shown by the experiments of Osborne and Mendel in which diets containing only about 0.01 per cent magnesium were found adequate for growth. In her recent study of magnesium metabolism, Medes [11] found little variation in the concentration of magnesium in rats. Analyses of a series of whole animals, at 29, 60 and 90 days of age, revealed the interesting fact that the magnesium content remained constant during growth, the amount determined in all cases being 0.045 per cent. These observations are similar to those of Buckner and Peter,[12] who had shown previously that, whereas the percentages of phosphorus and calcium increased with age, the magnesium content remained about the same percentage of the body weight. It is of interest to note, at this point, that the contents of calcium, potassium, and magnesium are higher in female than in male rats. Another interesting conclusion reached by Medes is that the composition of the rat with respect to magnesium is more constant under varying conditions (as influenced by diet, etc.) than with respect to calcium. Of importance in relation to the metabolism of magnesium are the earlier observations of Mendel and Benedict,[13] who noted that an increased excretion of magnesium could be induced by the administration of calcium, and that an increased elimination of calcium could be brought about by the administration of

[9] J. Biol. Chem., **63**, 585 (1925).
[10] J. Biol. Chem., **55**, 45 (1923); *ibid.*, **67**, 71 (1926); **70**, 593 (1926).
[11] J. Biol. Chem., **68**, 295 (1926).
[12] *Ibid.*, **54**, 5 (1922).
[13] Am. J. Physiol., **25**, 1, 23 (1909–10).

magnesium. Similar relations have been shown to hold for man by Bogert and McKittrick.[14]

Approximately two-thirds of the magnesium in the body is present in bone. The analyses of Hammett[15] show that the ash of the femur and humerus of rats contains slightly less than 1 per cent of magnesium and that this value decreases somewhat with age. More magnesium than calcium is present in muscle tissue. Katz[16] analyzed fresh human muscle and found it to contain 0.212 parts per thousand of magnesium as compared with 0.075 parts per thousand of calcium. Muscle tissue likewise contains more potassium than sodium. The values given by Katz are 3.20 and 0.80 parts per thousand of the fresh tissue, respectively. In human blood, Kramer and Tisdall[17] obtained the following values per 100 cc.: sodium, 170–225 mg.; potassium, 153–201 mg.; calcium, 5.3–6.8 mg.; magnesium, 2.3–4.0 mg. In the serum the normal concentration of calcium varies between 9 and 11 mg., and the magnesium concentration between 1.8 and 2.0 mg. per 100 cc.

From the standpoint of adequacy in nutrition, no difficulties present themselves in the selection of diets containing sufficient amounts of sodium, chlorine, potassium, and magnesium. The last two elements are especially abundant in both plant and animal tissues, and the quantities derived from these sources are far in excess of the normal requirements for proper nutrition. Sodium and chlorine are likewise widely distributed in nature. An adequate supply of these elements, particularly in the nutrition of man, is not difficult to secure, since the quantities of common salt used in seasoning are greater than the natural requirements. In herbivorous animals, however, particularly during lactation, there is occasional evidence of salt deficiency. It is well known that buffalo and deer frequently travel long distances and brave many dangers in search of rock-salt deposits, or salt licks. Observations reported by Babcock[18] show that the milk of cows deprived of salt may become very low with respect to the sodium chloride content and that continued deprivation may result even in the death of these animals. The practice of supplying common salt to cattle therefore has a scientific basis.

Calcium Requirement.—To return to the experiments of Osborne and Mendel,[2] these investigators found that there was a striking con-

[14] J. Biol. Chem., **54**, 363 (1922).

[15] *Ibid.*, **64**, 693 (1925).

[16] Pflüger's Arch., **63**, 1 (1896).

[17] J. Biol. Chem., **48**, 223 (1921).

[18] Wisconsin Agr. Exp. Sta. Ann. Report, 129 (1905); cited by McCollum and Simmonds, The Newer Knowledge of Nutrition, 4th edition, 1929, p. 411.

trast between the influence of sodium, potassium, magnesium, and chlorine and that of calcium and phosphorus. While growth occurred in rats despite low levels of intake of sodium, potassium, magnesium, or chlorine, inhibition of growth was noted in rats maintained on diets low in either calcium or phosphorus. The importance of calcium in human and animal nutrition has been emphasized, especially by Sherman [19] and by McCollum, Simmonds, Parsons and their associates. In a critical study of a large number of American dietaries, Sherman was led to the conclusion that the intake of calcium in this country is frequently below the level of requirement and that the adequate supply of this element in a "mixed diet" constitutes a real problem in human as well as in animal nutrition. From a study of calcium excretion in adults, Sherman has determined that the minimum requirement of calcium is, on an average, about 0.45 g. per day (equivalent to 0.63 g. when expressed in terms of CaO). If a margin of safety of 50 per cent is allowed—a practice which has been found valuable in computing the needs for protein and other essential components of the diet,—the so-called "standard requirement" of a normal adult of about 70 kg. weight would be 0.68 g. per day. The data given by Sherman show that 52 per cent of the dietaries studied were below this level, and that as many as 16 per cent were below even the minimal requirement of 0.45 g.

The effects of a low calcium intake during pregnancy and lactation when the needs of both the mother and child are involved may be readily imagined. Calcium deficiency under these circumstances results in a depletion of calcium from the bones which thereby become soft, a condition termed osteomalacia. The victims of this disease become badly deformed, owing to the flexibility of the bones. Osteomalacia is very prevalent among women in certain parts of India and China.

The effects of calcium deficiency are especially serious in children. In a study of calcium and phosphorus metabolism, Sherman and Hawley [20] have shown that children from three to thirteen years old require an intake of one gram of calcium per day, an amount which is necessary to induce optimum storage of this element and to insure the proper development of bones and teeth. Milk is the best and most available source of calcium, particularly for children, who do not seem to utilize the calcium of vegetables very efficiently. McClugage and Mendel [21] have reported that the calcium supply of the organism is normally derived from milk in greater abundance than from any other dietary

[19] J. Biol. Chem., **44**, 21 (1920); see also "The Harvey Lectures," 1917–1919, p.114.
[20] J. Biol. Chem., **53**, 375 (1922).
[21] *Ibid.*, **35**, 353 (1918).

source and that the calcium in spinach and carrots is poorly assimilated. It is concluded, therefore, that vegetables should not be used extensively as a substitute for milk with the idea of providing the requisite amount of calcium. However, it is not impossible, in the case of adults, to meet the maintenance needs of calcium, as well as phosphorus, from exclusively vegetable sources, such as carrots, as has been shown by M. S. Rose,[22] Blatherwick and Long,[23] and others.

Sherman and Hawley [20] recommend 750 to 1000 cc. of milk per day for the growing child. The calcium derived from this amount of milk, together with that obtained from other dietary sources, would provide about one gram of the element.

Calcium Metabolism in Relation to Rickets.—The disease known as rickets is very prevalent among children, particularly those of the poor, and is associated with faulty bone formation. Depending upon the degree of its severity, the disease leads to various types of malformation, such as bow legs, deformed chest and skull, knock knees, etc. Concerning the etiology, it is now believed that any one or more than one of the following factors may be operative: (*a*) calcium deficiency, (*b*) phosphorus deficiency, (*c*) improper balance between calcium and phosphorus, (*d*) lack or deficiency of the anti-rachitic vitamin or vitamin D, (*e*) lack of direct sunlight which includes ultraviolet radiations. These factors are closely interrelated, and it even appears that the anti-rachitic vitamin may be completely replaced by ultraviolet light. Rickets is essentially a condition of deranged calcium and phosphorus metabolism.

Mitchell and Johnson [23a] have studied the nutrition of rats on a diet deficient in calcium, phosphorus, and vitamin D. This diet is known to produce rickets in these animals. One group of the rats was exposed to ultraviolet light, and it was found that this not only aided in the healing of rickets but that it caused a higher degree of retention of the available calcium and phosphorus in the diet than would otherwise have been the case. Presumably, the metabolism of calcium and phosphorus is in some way controlled by radiant energy. That sunshine is an important factor in the prevention and treatment of rickets has been suspected for a number of years. From this standpoint, it is instructive to compare the living conditions of the poor, as well as those of the rich, in various parts of the world with the incidence of rickets. When one considers the dark and dingy quarters of the laboring classes in many of the industrial sections of Europe and America and the fact

[22] *Ibid.*, **41**, 349 (1920).
[23] *Ibid.*, **52**, 125 (1922).
[23a] Am. J. Physiol., **72**, 143 (1924).

that during the first years of life the children of the poor are kept indoors much of the time, it is little wonder that the incidence of rickets is so high. The more leisurely classes in Europe and America receive more liberal provisions of sunshine during infancy and the incidence of rickets is therefore much lower than among the poor. The situation is exactly reversed in India, as has been admirably shown by Hutchison and Shah.[24] The female Hindoo farmhand, for convenience, keeps her infant near her while at work. The child thus plays in the sunshine for a considerable part of the day. On the other hand, children belonging to the higher castes remain indoors during much of their infancy. As a result, despite a more liberal diet, the wealthier Hindoo children are more prone to develop rickets than are those of the lower social strata. The situation is comparable in other Oriental countries. The relative non-prevalence of rickets among the Eskimos may be partly explained by the fact that, after weaning, which as with the Chinese occurs as late as 4 or 5 years of age, the diet of Eskimo children is especially rich in fats which contain the anti-rachitic vitamin. In another connection, the influence of this vitamin in nutrition will be considered in greater detail.

It has been emphasized by several groups of investigators[25] that a proper ratio between the concentrations of calcium and phosphorus in the diet is very important and may, within certain limits, be of greater significance to the welfare of the animal than the absolute amounts of these substances. Indeed, it has been shown that pathological changes in bone may be produced on a diet excessively high in calcium. A very severe form of rickets was observed in rats maintained on a diet high in calcium but low in phosphorus.

The addition of phosphate to a high-calcium, low-phosphorus, rickets-producing diet, so that the calcium-phosphate ratio is equal to 1, prevents rickets or causes a rapid healing of rickets in rats. Karelitz and Shohl[26] state that under these conditions the metaphyses show more rapid calcium phosphate deposition than is observed when any other method of curing rickets in rats is employed.

Rickets may result either from a deficiency of calcium (low-calcium rickets), or phosphorus (low-phosphorus rickets), or both calcium and phosphorus (low-calcium, low-phosphorus rickets). The form of rickets which is most frequently seen clinically and which is easily pro-

[24] Quart. J. Med., **15**, 167 (1921–22).

[25] McCollum and Simmonds, The Newer Knowledge of Nutrition, 4th edition, pp. 298, 323, *et seq.*

[26] J. Biol. Chem., **73**, 665 (1927); see also Shohl *et al.*, *ibid.*, **74**, 247 (1927); **78**, 181 (1928); **84**, 501 (1929).

duced experimentally is the low-phosphorus rickets. In this condition the concentration of calcium in the serum is normal, whereas the phosphate content is reduced. This form of rickets is associated with skeletal abnormalities, bowed legs, enlarged joints, "rachitic rosary," etc. In the low-calcium form of rickets, the phosphorus content is normal, whereas the calcium content is low, this being often accompanied by tetany.[27]

Experience in the rearing of lions and other carnivorous animals in several zoological gardens has brought out very strikingly the importance of calcium and phosphorus in nutrition. It is now fully understood that the difficulty formerly encountered in bringing up lions in captivity was due largely to the fact that the diet, after weaning, was inadequate, consisting as it did almost entirely of raw meat. Even lions, when they are young, find it difficult to chew large bones, and this was formerly the chief source of calcium and phosphorus provided to them. As a result, the young animals kept on this diet frequently developed a severe form of rickets and succumbed. However, when the diet was supplemented by the addition of calcium- and phosphorus-rich food, such as milk and crushed bones, and by the addition of cod-liver oil (the latter contains the anti-rachitic vitamin), the animals grew normally and those which had previously developed rickets were frequently cured.

The Phosphorus Requirement.—The intimate relationship between calcium and phosphorus metabolism has been referred to in the preceding paragraphs. Phosphorus is essential to growth and is one of the limiting factors in the formation of bone. The requirement of the growing animal for this element is therefore fairly high. In the adult, where phosphorus is required merely to replace the loss from the body, the minimum requirement averages about 0.88 g. per day. In his statistical studies, Sherman found that only 4 per cent of the American dietaries examined fell below this level. As a rule, there is therefore in the case of the adult less danger of phosphorus deficiency than of calcium deficiency. Phosphorus deficiency is also an important factor in the nutrition of cattle, particularly in regions where the soil and vegetation are poor in this element. Cattle may develop an intense craving for phosphorus, which manifests itself in bone-eating. This condition is known as osteophagia. An outbreak of this abnormality in South Africa has been described by Green,[28] who states that the craving may be produced experimentally upon phosphorus-low rations

[27] For details consult the review of E. A. Park on the etiology of rickets, Physiol. Rev., **3**, 106 (1923), as well as the papers of Howland and Kramer.

[28] J. Biol. Chem., **46**, p. xix (1921).

and removed by administration of phosphorus compounds and by phosphatic manuring of the soil.

Attention has been drawn to the so-called " anti-calcifying " action of cereals. It is not entirely clear whether this effect is due to a low concentration of calcium or phosphorus, as suggested by the work of Steenbock [29] and his associates, or to an unsuitable ratio of the two elements, or as suggested by E. Mellanby,[30] to some specific anti-calcifying agent such as fatty acids. In her recent review on the influence of diet on the structure of teeth, May Mellanby [31] states that oatmeal and wheat germ, which experimentally produce the worst calcified teeth, have far more calcium and phosphorus than other cereals which do not exert as marked an effect. Nor does it appear that the ratio of calcium to phosphorus in these cereals is the significant factor. The Ca : P ratio of oatmeal is 1 : 5.7 and of wheat germ 1 : 14.8. Rye flour has a Ca : P ratio of 1 : 16.1, rice 1 : 10.7, and barley 1 : 9.0. None of these is nearly so effective as oatmeal or wheat germ in preventing good tooth calcification.

Iron.—Sherman [32] places the actual requirement for iron in the case of adults at about 0.010 gram per day, and therefore believes that there is comparatively little danger of iron deficiency in freely chosen diets. Iron is used by the organism in the production of the hemoglobin of the red corpuscles and in the formation of other heme compounds which, as we have seen, are widely distributed in tissues where they play an important part in cellular oxidation. The problem of iron metabolism has only recently been studied as energetically as is warranted by its importance. Whipple and his associates [33] have contributed much to our knowledge of the relation of diet to blood regeneration in anemic conditions. They have shown that meat, particularly that of organs (heart, liver), is especially beneficial in hemoglobin and red-cell formation. That this is at least partly due to the relatively high concentration of iron in these substances is borne out by the analyses of Forbes and Swift.[34] These workers have found that beef contains twice as much iron as do potatoes; two and a half times as

[29] Steenbock, H., Black, A., and Thomas, B. H., J. Ind. Eng. Chem., **19**, 906 (1927).

[30] Brit. Med. J., **1**, 831 (1922); **2**, 849 (1922); **1**, 895 (1924); **1**, 515 (1926); Green, H. N., and Mellanby, Biochem. J., **22**, 102 (1928).

[31] Physiol. Rev., **8**, 545 (1928).

[32] H. C. Sherman, Harvey Lectures (1917–1919), p. 117.

[33] Am. J. Physiol., **53**, 151 (1920); **72**, 408, 419, 431 (1925); for a review of recent work on the regeneration of hemoglobin and erythrocytes, see F. S. Robscheit-Robbins, Physiol. Rev., **9**, 666 (1929).

[34] J. Biol. Chem., **67**, 517 (1926); Miller, R. C., Forbes, E. B., and Smythe, C. V., J. Nutrition, **1**, 217 (1929).

much as white flour and corn meal; and eight times as much as apples. Certain vegetables (peas, beans, lentils, spinach, graham flour, oatmeal, and shredded wheat), contain more iron than does beef. The richest source of iron is to be found, however, in organ meats or " extra carcass parts." Beef heart and brain contain about twice as much iron as do beef and veal. Beef liver is twice as rich in iron as is beef heart. The iron content of beef spleen exceeds that of the liver by 50 per cent. These observations are of the utmost importance and suggest that the utilization of these parts in human nutrition should be given serious consideration.

The low concentration of iron in milk (0.0002–0.0003 per cent) is an important factor to consider from the standpoint of infant nutrition. Fortunately, most animals are born with an extra supply of iron (the guinea pig is an exception) which is utilized in the formation of hemoglobin during the early part of life. However, if an animal's diet is restricted to milk for much longer than its normal lactation period, anemia may result, as has been shown by Abderhalden [35] and others. In the artificial feeding of babies on diluted cow's milk, the supply of iron may be reduced to such an extent as to cause a nutritional form of anemia. In this connection, the recent observations of Hart and his collaborators [36] are of much significance. These workers produced anemia in rats and rabbits by restricting them to a diet of cow's milk containing sodium citrate, the latter being added to prevent the formation of large curds in the stomach and to reduce the possibility of gastritis. Inorganic iron added to this basal ration did not correct the anemia, but when the iron was given in conjunction with fresh cabbage, lettuce, an alcoholic extract of desiccated cabbage, an alcoholic extract of yellow corn meal, or chlorophyll, the anemia was either cured or prevented.

Later work [37] showed that the ash of these substances, as well as the ash of beef liver, were equally effective in stimulating the utilization of iron and the production of red blood corpuscles. The active constituent of the ash proved to be copper. Indeed, the addition of pure copper salts, such as copper sulfate, were found to exert a favorable effect in preventing or curing this type of nutritional anemia.[38]

[35] Zeit. Biol., **39**, 193 (1900).

[36] Hart, Steenbock, Elvehjem and Waddell, J. Biol. Chem., **65**, 67 (1925); **72**, 299 (1927); **77**, 777 (1928)

[37] Hart, Steenbock, Waddell, and Elvehjem, J. Biol. Chem., **77**, 797 (1928); **83**, 243, 251 (1929); **84**, 115 (1929).

[38] The discovery by Hart and his co-workers that copper has a specific rôle in nutrition has stimulated considerable research interest not only in this element but in other elements, aluminum, zinc and manganese, known to be widely distributed

THE PROTEIN REQUIREMENT IN NUTRITION

Amount of Protein Needed.—The statistical studies of Voit in Germany showed, for adults, an average daily consumption of 118 grams of protein. Atwater in this country, and other workers both in America and in Europe, have made similar estimates of the average protein intake. This quantity has therefore been accepted by many students of nutrition as representing an adequate supply. As this amount of protein provides less than 500 calories, the energy needs of the body must obviously be met largely from carbohydrate and fat. From this standpoint, a well-balanced diet for an individual engaged in moderate physical work may include 50–60 grams of fat (465 to 560 calories) and about 500 grams of carbohydrate (approximately 2,000 calories).

Chittenden [39] studied the protein requirement in human nutrition very exhaustively and reached the conclusion that the Voit standard of 118 grams was far in excess of the actual needs of the body. In his investigations were included individuals engaged in various occupations (soldiers, professors, students, athletes, etc.) Chittenden determined that the nitrogen requirement per day per kilogram of body weight was fairly uniform for different individuals and amounted to 0.10 to 0.12 gram. A man weighing 70 kg. would therefore require 7–8.4 grams daily, or 44–53 grams of protein. Accordingly, an allowance of 60

in plant and animal tissues. The indications are that in addition to copper, at least zinc and manganese may be of importance from the standpoint of nutrition and metabolism. It would be premature, however, to give here an account of the results that have been obtained so far. M. S. Rose has ably reviewed a large portion of the recent literature dealing with the place of aluminum, copper, manganese and zinc in animal nutrition (J. Nutrition, **1**, 541 (1929). This review includes an extensive bibliography. Reference may also be made to the following papers which have appeared recently: Studies in the Metabolism of Aluminum, Underhill, F. P., Peterman, F. I., *et al.*, Am. J. Physiol., **90**, 1–82 (1929). These studies deal with the occurrence of aluminum in foods and tissues, its absorption, storage, toxic effect and excretion. V. C. Myers, J. Killian, Mull, J. W., *et al.*, have studied the occurrence of aluminum in tissues, including autopsy material, as well as the effect of aluminum on growth in rats; J. Biol., Chem., **78**, 591–626 (1928). Robscheit-Robbins, Elden, Sperry and Whipple have observed that apricots, which are high in copper, are especially effective in stimulating blood regeneration. J. Biol. Chem., **79**, 563, 577 (1928). Titus and Hughes, J. Biol. Chem., **83**, 463 (1929) state that manganese as well as copper stimulates the utilization of iron and the production of hemoglobin. In experiments on mice maintained on low zinc diets, Hubbell and Mendel, J. Biol. Chem., **75**, 567 (1927), observed retardation of growth and a loss of zinc from the tissues. Hart, Steenbock and their co-workers at the University of Wisconsin have continued their studies on copper; their results are to be found in a series of papers which have appeared during the last two years in the Journal of Biological Chemistry.

[39] R. H. Chittenden, Nutrition of Man, New York, 1904.

grams of protein per day should be entirely adequate. This calls for two assumptions, namely, that there is adequate provision, through other food elements, to meet the energy requirements, and that the protein ingested provides a complete and adequate assortment of all the amino acids essential to the formation of tissue protein. These assumptions cannot always be made, for as we shall see presently, all proteins are not of equivalent nutritive value. It is obvious that fixed standards are of limited value. What the optimum proportions of the various foodstuffs will be in any particular case will depend on many circumstances. Thus, in cold climates a high level of protein intake is dictated by sound physiological reasoning. In warm climates a lower protein level would seen more suitable. Fortunately, in some particulars, the dietary habits of people frequently tend in the proper direction.

Denis and Borgstrom [40] in a large group of students in a Southern medical school found an average daily urinary excretion of 10.63 grams of nitrogen. This figure, plus 10 per cent added to account for the nitrogen lost through the feces, indicates an average consumption of 73.8 grams of protein, an amount not much higher than Chittenden's standard and distinctly below the average protein intake (121 grams) recorded for inhabitants of the United States. During the winter months the same group of students showed higher values for nitrogen excretion than in April or July. Youngburg and Finch [41] observed essentially the same level of protein intake in a group of medical students in the North and were unable to demonstrate seasonal variations in nitrogen excretion. Similar results reported by others show that the protein intake of students in the North and South, per 70 kg. body weight is approximately the same (Brooks,[42] Beard,[43] Lusk [44]).

Hindhede [45] has also been among those advocating low protein dietaries and has reported that it is possible for the body to remain in nitrogen equilibrium indefinitely on diets consisting of bread, potatoes, fruit, and small amounts of milk. The chief protein of the potato, tuberin, a globulin, is according to Kon [46] apparently a good, complete protein, i.e., it contains all the essential amino acids. Kon and Klein have described an experiment in which two adults, a man and a woman, lived over a period of 167 days in nitrogen equilibrium and in good

[40] J. Biol. Chem., **61**, 109 (1924).

[41] *Ibid.*, **68**, 335 (1926).

[42] Am. J. Physiol., **89**, 403 (1929).

[43] *Ibid.*, **82**, 577 (1927).

[44] Science of Nutrition, 4th edition, 1928, p. 455.

[45] Skand. Arch. Physiol., **30**, 97 (1913); "Protein and Nutrition," London, 1913.

[46] Biochem. J., **22**, 258, 261 (1928).

health on a diet in which the nitrogen was almost entirely derived from the potato, the nitrogen intake averaging 5.7 grams daily.

The absolute minimum probably falls considerably below the standard set by Chittenden. Numerous investigators have attempted to determine the minimum protein intake sufficient to maintain nitrogen balance. Folin [47] obtained a minimum excretion of 2.6 grams of nitrogen on the twelfth day of an experiment on a low-protein diet. The subject of this experiment weighed 64.0 kg. In a similar experiment Thomas [48] (body weight 76.2 kg.) obtained a minimum excretion of 2.98 grams on the nineteenth day. Deuel [49] was able to reduce his nitrogen elimination to a minimum of 2.1 grams. The injection of thyroxin was followed after an interval of 7 days by an increased output of nitrogen, due obviously to an increased "wear and tear" of the tissues. Smith,[50] by insuring for himself an abundant caloric supply in the form of carbohydrate and fat, was able to reduce his endogenous protein metabolism to an extremely low level, the lowest recorded in the literature. During the course of the last 24 days of a 28-day experiment, he excreted but 80.08 grams of nitrogen, or an average of 3.34 grams per day. The lowest point was reached on the twenty-fourth day of the experiment when the total nitrogen excreted in the urine was only 1.58 grams. These figures represent an amount of protein metabolism of 20 grams or less. From this discussion, the inference is not to be drawn, however, that a low protein intake is at all desirable. It is true that if the protein fed were one containing all the essential amino acids in suitable proportions for tissue synthesis, a daily allowance of 50 or 60 grams would be adequate. Proteins of animal origin are usually complete or adequate in this sense, but an adequate supply of the required amino acids can hardly be expected from 50 or 60 grams of many of the vegetable proteins, such as zein of corn, gliadin of wheat, hordein of barley, and phaseolin of kidney beans. It is therefore of the utmost importance to allow a liberal margin of safety, particularly when part of the protein is derived from plant sources.

Few problems in nutrition have aroused more discussion than the question whether kidney damage may be related to a high protein dietary. Newburgh [51] and later Newburgh and Clarkson [52] reported that rabbits fed a diet high in protein (meat) over a period of months develop definite lesions in the kidney. Evidence of renal involvement (hema-

[47] Am. J. Physiol., **13**, 66 (1905).

[48] Archiv. f. Anat. u. Physiol., Physiol. Abt., 219 (1909); cited by Smith.

[49] Deuel, Sandiford, Sandiford, and Boothby, J. Biol. Chem., **76**, 391 (1928)

[50] J. Biol. Chem., **68**, 15 (1926).

[51] Arch. Int. Med., **24**, 359 (1919).

[52] *Ibid.*, **32**, 850 (1923).

turia and albuminuria) was also noted by Squire and Newburgh [53] in human subjects after taking excessive amounts of protein. On the contrary, other workers [54] have reported that rats may be raised successfully and without any determinable physiologically deleterious effect when protein is fed in great excess (70 to 90 per cent of the total food). However, even those investigators who were unable to produce nephritis by feeding protein, recognized considerable hypertrophy of the kidneys, a change attributable to the increased activity of the kidneys in excreting the end products of protein metabolism.

Moise and A. H. Smith [55] working with unilaterally nephrectomized rats were able to demonstrate definite lesions in the remaining kidney (fibrosis of the glomerular tuft, dilation of the tubules, albuminuria, casts, etc.), particularly in the maturer animals. These results show that when the functional burden of kidney tissue is intensified, it may lead to pathological change.

The fact that most workers who obtained negative results fed casein led Newburgh and Marsh [56] and more recently Newburgh and Curtis [57] to consider the possibility that the nephropathic action of protein may depend on the constituent amino acids. They were able to show that whereas certain amino acids, glycine, alanine, leucine, phenylalanine produce no renal injury, others, tyrosine, tryptophane and particularly cystine [58] are apparently nephrotoxic.

While this introduces for serious consideration the possibility that chronic interstitial nephritis in man may be related to the protein intake, proof of this is lacking. In fact those [59] who have studied the dietary habits of Eskimos, whose daily consumption of protein may exceed 500 grams, have not observed any unusual prevalence of cardiac or renal disease among them. Nor has a detailed metabolic study of two distinguished Arctic explorers, maintained on an exclusive meat diet for one year, given any evidence suggesting that renal damage had occurred.[60]

Considerable light has been thrown on this perplexing problem by

[53] *Ibid.*, **28**, 1 (1921).

[54] Osborne and Mendel, Proc. Nat. Acad. Sci., **7**, 157 (1921); Osborne, Mendel, Park and Winternitz, J. Biol. Chem., **71**, 317 (1927); McCollum, Simmonds and Parsons, *ibid.*, **47**, 111 (1921); Addis, MacKay and MacKay, *ibid.*, **71**, 139, 157 (1926); Drummond, Crowden and Hill, J. Physiol., **56**, 413 (1922); Reader and Drummond, Biochem. J., **20**, 1256 (1926).

[55] J. Exp. Med., **46**, 27 (1927).

[56] Arch. Int. Med., **36**, 682 (1925).

[57] *Ibid.*, **42**, 801 (1928).

[58] Lewis, H. B., J. Biol. Chem., **63**, p. xx (1925).

[59] Thomas, W. A., J. Am. Med. Assoc., **88**, 1559 (1927).

[60] Tolstoi, E., J. Biol. Chem., **83**, 753 (1929).

Cox [61] and his co-workers who find that only very young rats are susceptible to cystine nephrosis. It is suggested that this susceptibility may be hereditary. Furthermore, even in young susceptible rats, injury to the kidney may be prevented by supplementing the diet with sufficient vitamin B concentrate (prepared according to Osborne and Wakeman). Cox and Hudson state that " the active substance of yeast extract which protects rats from cystine nephrosis is probably distinct from any of the known accessory food factors."

To a considerable extent modern discussions of the protein factor in nutrition center about the relative efficiency of different proteins in allowing for maintenance and growth. That this depends on the amino-acid make-up of the proteins will be shown in the following paragraphs.

Essential Amino Acids.—That the value of a protein in nutrition depends largely on the nature and amounts of the amino acids which it yields on hydrolysis has been definitely established. As indicated on p. 116, there are notable variations in the composition of different proteins as regards the constituent amino acids. It will be observed that not only are there differences in the proportions in which the amino acids are present, but that one or more of these are totally lacking in certain proteins. The question therefore arises as to whether a protein that does not have a complete assortment of amino acids can be adequate in nutrition when it is the sole protein provided in the diet. Much depends on the nature of the deficiency. Many proteins lack glycocoll, but this is not a limiting factor in nutrition because the animal organism is capable of synthesizing this amino acid from other substances that are available. On the contrary, a deficiency of tyrosine, tryptophane, cystine, arginine, histidine, or lysine and possibly proline materially reduces the biological value of a protein. Maintenance and growth do not occur in the absence of these amino acids, for their synthesis in the animal body does not take place. This statement may be qualified to some extent. Mendel [62] states that sheep have been observed to gain many pounds over considerable periods of time on a diet of starch, denitrogenized straw, inorganic salts, and urea, an exceedingly simple nitrogenous mixture that readily disintegrates to form ammonia and carbon dioxide. In the rumen, or paunch, of the sheep, as well as in that of other ruminants, there is ample opportunity for the synthesis of amino acids by bacteria. These amino acids may even be synthesized into protein, which is incorporated into the protoplasm of the bacteria. When the bacteria pass into the acid-secreting stomach and die, this protein is presumably digested and utilized in the usual manner. How-

[61] Cox, Smythe and Fishback, J. Biol. Chem., **82**, 95 (1929); Cox and Hudson, Nutrition, **2**, 271 (1930).

[62] L. B. Mendel, Nutrition, p. 124. See also J. Franklin Institute, July, 1921.

ever, amino-acid synthesis by bacteria is not a factor in the nutrition of man and most of the higher animals. These depend on an exogenous supply of the essential amino acids.

One method of studying the nutritive value of different proteins has been to provide rats with basal diets adequate in all other respects but containing no protein or amino acids. By this method, the protein element in the diet may be made the only variable factor. As knowledge of the remaining factors in nutrition has increased, the composition of the basal non-protein rations has been subjected to numerous modifications, but the principle has remained the same. By adding to such rations suitable amounts of a single protein, it has been possible to determine which are and which are not adequate for maintenance and growth.

Although several investigators had previously attempted to compare the nutritive value of different proteins, the first important contributions to the subject were the classical studies of Osborne and Mendel.[63] Osborne and Mendel have done much to establish the value of feeding isolated food substances in nutritional studies. As milk was known to be an adequate diet for young rats, these workers thought it desirable to include in their experimental rations a protein-free milk preparation for the purpose of supplying the necessary mineral constituents and other possibly essential ingredients. The dried " protein-free milk " constituted 28.2 per cent of the ration. The other constituents were starch 20.8, agar-agar 5.0, and fat 28.0 per cent. The proteins used in the experiments were highly purified and were supplied in liberal amount (18 per cent of the total food).

The observations of Osborne and Mendel showed that growth in rats could be secured with certain proteins but not with others. The proteins which, when fed singly in suitable concentration, proved adequate for growth included:

Proteins of Animal Origin	Proteins of Vegetable Origin
Casein (milk)	Edestin (hemp-seed)
Lactalbumin (milk)	Globulin (squash-seed)
Ovalbumin (hen's egg)	Excelsin (Brazil-nut)
Ovovitellin (hen's egg)	Glutelin (maize)
	Globulin (cotton-seed)
	Glutenin (wheat)
	Glycinin (soy bean)
	Cannabin (hemp-seed)

[63] T. B. Osborne and L. B. Mendel, Feeding Experiments with Isolated Food Substances, Carnegie Inst. Pub., 156, Parts I and II, 1911. Osborne and Mendel, J. Biol. Chem., **12**, 81 (1912); **15**, 311 (1913); **16**, 423 (1913); **17**, 325 (1914); L. B. Mendel, The Harvey Lectures, 1914–1915, 101.

The following proteins, when fed alone, failed to induce growth:

Legumelin (soy bean)	Hordein (barley)
Vignin (vetch)	Conglutin (blue or yellow lupin)
Gliadin (wheat or rye)	Gelatin (horn)
Legumin (pea)	Zein (maize)
Legumin (vetch)	Phaseolin (white kidney bean)

The inadequacy of certain proteins to promote growth cannot be attributed to any toxic effect which they may possess; nor is the effect

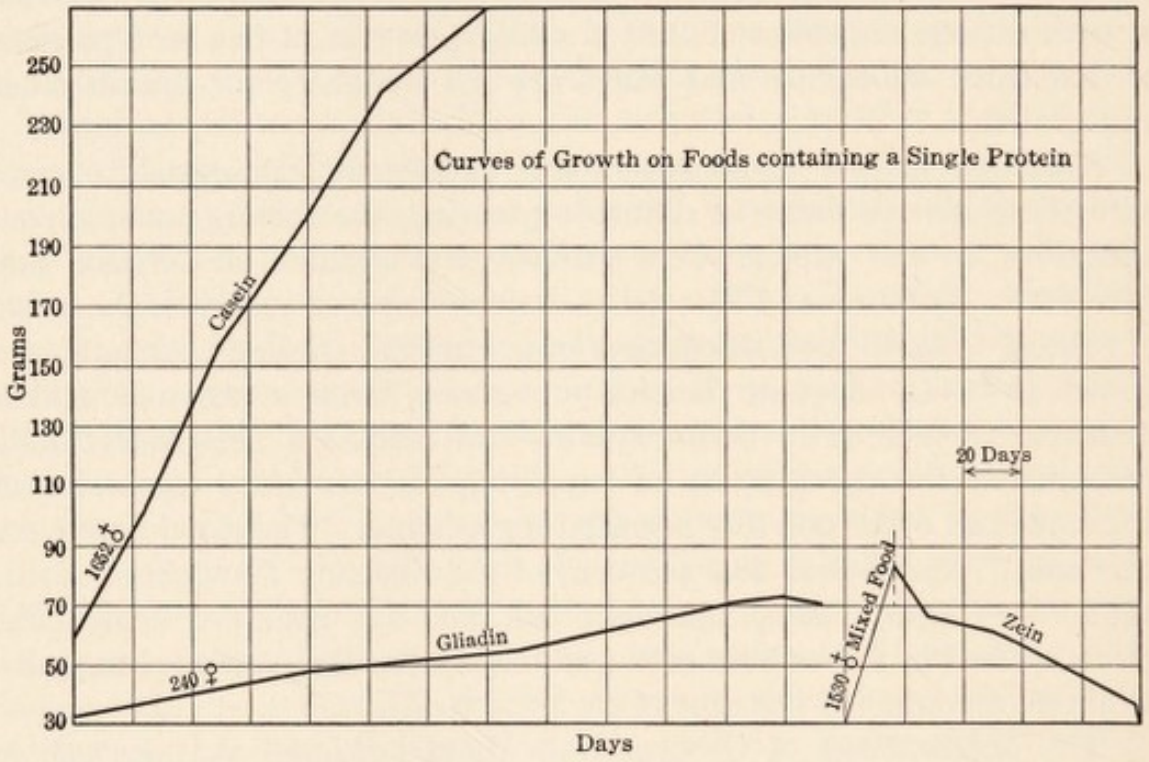


FIG. 44.—Showing typical curves of growth of rats maintained on diets containing a single protein. On the casein food (deficient in glycocoll) satisfactory growth is obtained; on the gliadin food (deficient in lysine) little more than maintenance of body weight is possible; on the zein food (devoid of glycocoll, lysine and tryptophane) even maintenance of body weight is impossible. (After L. B. Mendel, J. Am. Med. Assoc., **64**, 1539 (1915); Nutrition: The Chemistry of Life, New Haven, 1923, p. 117.)

referable to a diminished utilization due to incomplete digestion. The evidence all points in one direction, namely, that it is a question of amino-acid deficiency. A comparison may be made of the results obtained with foods containing either casein or gliadin as the sole protein. Osborne and Mendel have shown that when the former protein is fed to rats, normal growth occurs, but when gliadin is the sole protein of the diet, growth occurs very slowly or not at all. Their results are represented in Fig. 44. When a comparison is made of the amino acids present in the two proteins, it is seen that casein contains all the

amino acids, although the content of cystine is low. On the contrary, gliadin lacks glycocoll, and, as compared with casein, is poor in lysine. The absence of glycocoll does not constitute an actual deficiency, for this amino acid is readily synthesized in the body. The content of lysine in gliadin is apparently sufficient for maintenance and apparently also for a slight amount of growth, but insufficient to permit normal growth. That lysine is the factor limiting the nutritional value of gliadin may be shown by supplementing such diets with this amino acid.

An even more striking illustration is to be found in the experiments (Osborne and Mendel) in which zein was fed as the sole protein. One of the curves included in Fig. 44 shows that Rat No. 1530 gained weight

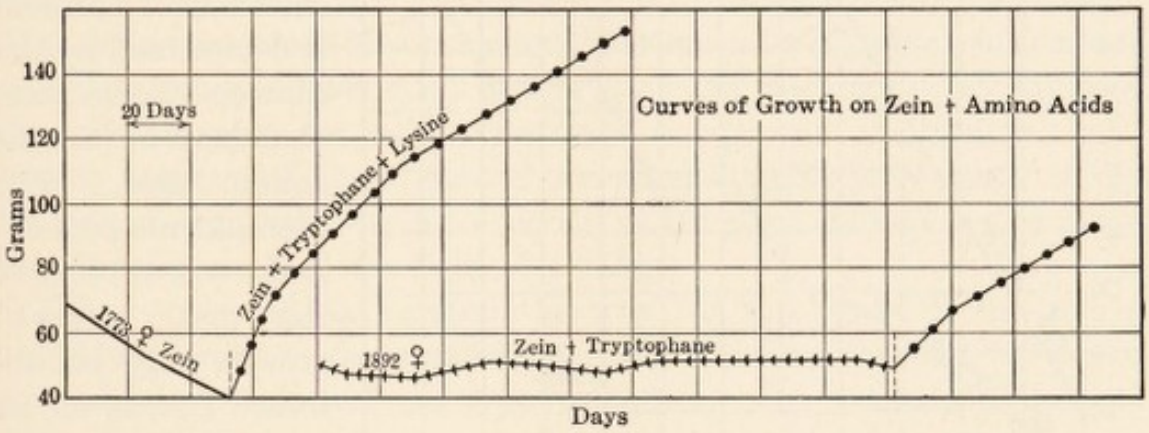


FIG. 45.—Showing the effect of the addition of the amino acids tryptophane and lysine to zein which fails to yield them. With zein alone (rat 1773) there is nutritive decline. The addition of tryptophane (rat 1892) permits maintenance without growth on foods containing zein as the sole protein. The addition of tryptophane and lysine to zein enables the animals to make considerable growth. It is interesting to note, in relation to rat 1892, that the growth of this animal was inhibited for six months without material change in its body weight. That the capacity to grow is not lost by prolonged dwarfing on imperfect food is shown by the subsequent growth of the animal when lysine was added to the food containing zein and tryptophane. (After L. B. Mendel, J. Am. Med. Assoc., **64**, 1539 (1915); Nutrition: The Chemistry of Life, p. 118.)

on a mixed diet, but that with the restriction of the protein element to zein there resulted at once a loss of weight. Zein is deficient in glycocoll, lysine, and tryptophane, the last two of which are indispensable to proper nutrition. The addition of tryptophane to the deficient diet prevented further loss of weight but did not induce any growth, whereas the addition of both tryptophane and lysine caused prompt gain in weight (Fig. 45). The dietary deficiency caused by zein may be removed in still another way, as by the addition of small amounts of lactalbumin, a protein rich in both tryptophane and lysine. Concerning the

nutritional value of lactalbumin, there is a difference of opinion.[64] According to Osborne and Mendel [65] lactalbumin is a "complete" protein. It is possible to select suitable combinations of two or more incomplete proteins that contain jointly a complete assortment of amino acids and are, hence, adequate for nutrition.

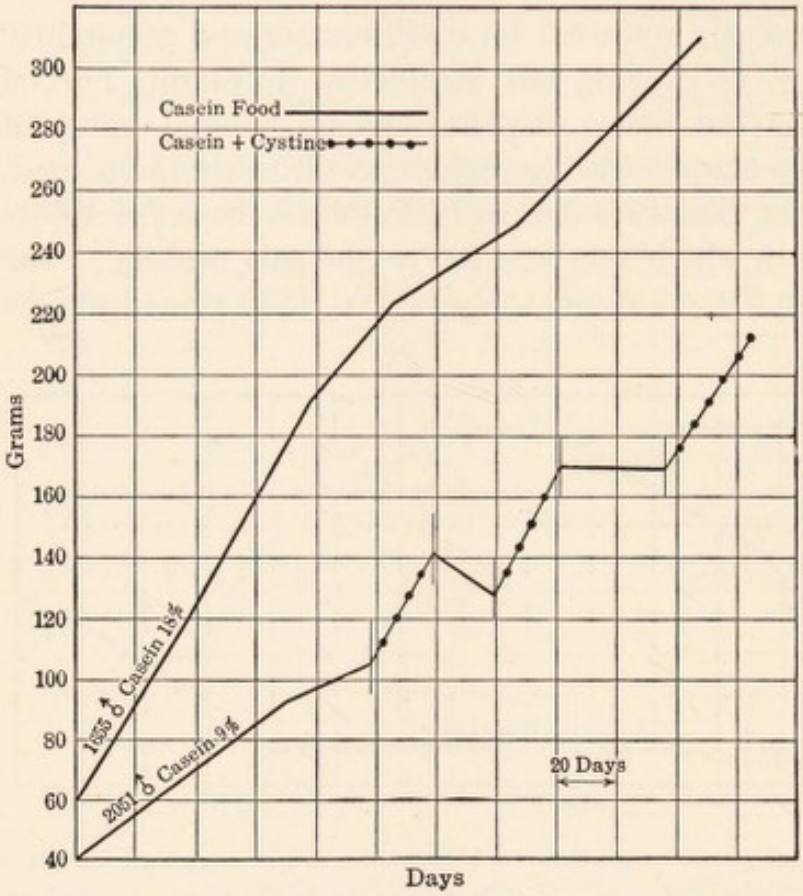


FIG. 46.—The curve for rat 1655 shows the satisfactory growth obtained when 18 per cent of casein was present in the diet as the sole protein. With a smaller amount of casein (rat 2051)—9 per cent—much less rapid growth ensued. That the insufficiency of the smaller amount of casein is essentially due to its relative deficiency in cystine-yielding groups is shown by the marked accelerating influence upon growth brought about by the addition of the amino acid, cystine, to the food containing 9 per cent of casein, and the prompt contrary effect when the cystine was withdrawn from the diet. (After L. B. Mendel, J. Am. Med. Assoc., **64**, 1539 (1915).)

There is another aspect to the problem. The nutritive efficiency of a protein is determined by the content of the least abundant essential amino acid. For example, when casein is the sole protein of the diet, normal growth may be obtained in rats on an intake of 18 per cent, but when this is reduced to a lower level (9 per cent of total food intake), growth is greatly retarded. The retarding effect is not due to a lack of sufficient protein *per se*, but rather to a deficiency of cystine. The addition of cystine raises the nutritive efficiency of the ration as shown strikingly by Osborne and Mendel. Their observations are represented in Fig. 46.

Similar results have been obtained with edestin, which supports growth when fed in liberal amounts (18 per cent), but which cannot support normal growth when fed in moderate amounts (9 per cent).

[64] McCollum and Simmonds, The Newer Knowledge of Nutrition, 1929 edition, p. 54.

[65] J. Biol. Chem., **59**, 339 (1924).

A large number of proteins have been investigated with the object of determining their biological value. Johns and Finks [66] and others have shown that beans of the genus *Phaseolus* are deficient in cystine. The mixed proteins of corn, according to Hogan,[67] have tryptophane as the first limiting factor and lysine as the second. Lack of space does not permit even a résumé of these and similar investigations. In his recent review, Mitchell [68] has dealt with the literature pertaining to the subject.

Feeding Experiments with Amino-acid Mixtures.—The most direct approach to the study of the part played by amino acids in nutrition would obviously be one involving the complete replacement of the protein fraction of the diet by mixtures of amino acids. The first important contribution of this type was made by Abderhalden,[69] who fed to dogs amino-acid mixtures prepared from meat by digestion with appropriate enzymes. With such mixtures as the sole source of nitrogen, the animals were not only maintained in nitrogen equilibrium, but a certain number of them showed remarkable gains in weight. A dog that was fed in this way for a period of 100 days showed at the end of that time an increase in weight of 9.35 kg.

These observations opened the way for the study of the rôle of individual amino acids in nutrition. Abderhalden removed both tyrosine and tryptophane from protein digests. The resulting amino-acid mixture was inadequate for maintenance or growth unless supplemented by both of these amino acids. Henriques and Hansen [70] may be regarded as having been pioneers in this type of work, although their results have been proved to be incorrect. These workers removed arginine, histidine, and lysine from pre-digested protein by precipitation with phosphotungstic acid. They reported that the resulting mixture of amino acids was adequate for the maintenance of positive nitrogen balances. Ackroyd and Hopkins [71] hydrolyzed casein with acid and removed histidine and arginine from the digest. The remaining material, added to non-protein synthetic rations, was inadequate for the growth and maintenance of rats, but when either arginine or histidine was added, further loss of weight was avoided and growth was often resumed. The interpretation given to these observations

[66] Am. J. Physiol., **57**, 61 (1921); J. Biol. Chem., **41**, 379 (1920).

[67] J. Biol. Chem., **29**, 485 (1917).

[68] H. H. Mitchell, The Nutritive Value of Proteins, Physiol. Reviews, **4**, 424 (1924); see also Mitchell and Hamilton, The Biochemistry of Amino Acids, New York, 1929, Chap. X.

[69] Z. physiol. Chem., **77**, 22 (1912); Abderhalden and P. Hirsch, *ibid.*, **81**, 323 (1912); *ibid.*, **83**, 444 (1913).

[70] Z. physiol. Chem., **43**, 417 (1904–05).

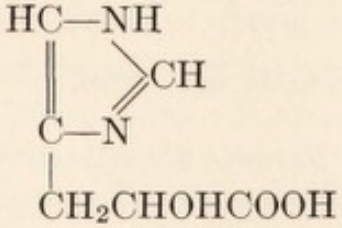
[71] Biochem. J., **10**, 551 (1916).

was that histidine and arginine were interchangeable in metabolism, but that one or the other must be present in the diet.

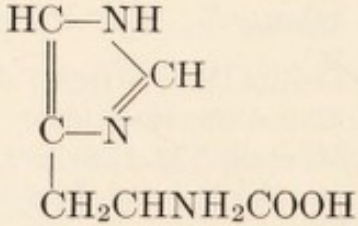
The question of the transmutability of histidine and arginine into each other has been subjected to critical study by Rose and Cox.[72] These investigators compared the growth of rats upon diets in which the nitrogen was supplied (*a*) by casein, (*b*) by completely hydrolyzed casein, and (*c*) by hydrolyzed casein from which arginine and histidine had been removed. Rose and Cox were able to show that the rats fed upon completely hydrolyzed casein grew to maturity, but at a slower rate than animals of the same age fed upon whole casein. On the contrary the rats that were given the arginine-histidine-free rations were neither able to grow nor to maintain body weight. Instead, there was a prompt and continuous loss of weight which could be remedied or avoided only by the addition of histidine. This part of the work, therefore, showed that histidine is an essential component of the diet.

As to arginine, it was found that its addition to the deficient diet exerted no perceptible influence upon growth. The animals continued to lose weight as rapidly as before the addition of this amino acid. Moreover, rations containing the minimum maintenance requirement of histidine and supplemented by large amounts of arginine were shown to be inadequate for growth. The work of Rose and Cox therefore furnishes conclusive evidence that arginine and histidine are not mutually interchangeable in metabolism.

Cox and Rose [73] first demonstrated the replacement of an indispensable amino acid by a non-amino compound. These investigators showed that the addition of *dl*-β-4-imidazole lactic acid to a histidine-deficient diet caused an immediate resumption of growth (in rats) at a rate slightly slower than that induced by the equivalent quantity of histidine. Cox and Rose state, " It is evident that under the conditions of the experiments the synthetic product in question is capable of serving in place of histidine, probably through being transformed by the cells into the amino acid." The results of Cox and Rose have been confirmed by Harrow and Sherwin.[74] The close relationship between the two compounds is brought out by the following formulas:



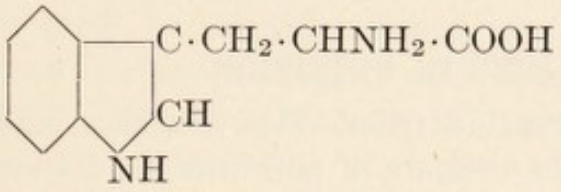
β-4-Imidazole lactic acid



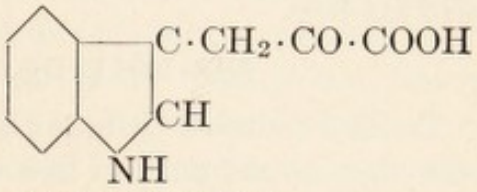
Histidine

[72] J. Biol. Chem., **61**, 747 (1924).
[73] J. Biol. Chem., **68**, 781 (1926).
[74] J. Biol. Chem., **70**, 683 (1926).

Tryptophane, another essential amino acid, may be replaced by synthetic, 3-indole-pruvic acid, but not by 3-indole-propionic acid or other closely related derivatives (Berg, Rose and Marvel,[75] Jackson).[76]



Tryptophane



3-Indole-pyruvic acid

On the contrary, α-dihydroxy-β-dithiodipropionic acid can not be used as a substitute for cystine (α-diamino-β-dithiodipropionic acid) in the diet of rats (Westerman and Rose).[77] Nor can α-hydroxy-ϵ-amino caproic acid be used in place of lysine (α, ϵ-diamino caproic acid) according to the observations of McGinty, Lewis and Marvel.[78]

THE INDISPENSABILITY OF FAT

Two papers have appeared recently which suggest that a certain amount of fat is indispensable to proper nutrition. McAmis, Anderson and Mendel [79] fed rats a diet of very low fat content, but otherwise adequate (consisting of extracted casein, sucrose, Osborne and Mendel salt mixture, yeast concentrate, cod-liver oil concentrate and a hot-water extract of liver). These animals grew moderately well, but not as well as rats fed the same diet supplemented by a small amount of fat. Burr and Burr [80] in a very carefully executed series of experiments fed rats a diet containing all the known ingredients necessary to rear healthy animals. Practically all the fat was removed from these substances, however, by repeated extraction with fat solvents. The animals grew for a time, but soon developed symptoms indicative of a dietary deficiency disease; the skin became scaly, the tip of the tail appeared inflamed and swollen, later becoming heavily scaled and ridged and often followed by necrosis. The hair on the back of the body became filled with dandruff and the hair about the face and neck tended to fall out. Hemorrhagic spots and sores appeared on the skin. Growth stopped at about the time these symptoms became prominent and death soon followed. The disease could be readily prevented or cured by the addition of a very small amount of fatty acid to the fat-free diet.

[75] J. Biol. Chem., **85**, 207, 219 (1929).
[76] *Ibid.*, **84**, 1 (1929).
[77] *Ibid.*, **79**, 413, 423 (1928).
[78] *Ibid.*, **62**, 75 (1923–24).
[79] J. Biol. Chem., **82**, 247 (1929).
[80] *Ibid.*, **82**, 345 (1929); in a second paper recently published (*ibid.*, **86**, 587 (1930)), Burr and Burr state that linoleic acid is an essential fatty acid.

This newly described deficiency disease is due either to the exclusion of the fatty acids, or possibly to the lack of some ether-soluble substance removed from the food ingredients in the process of extraction to render them fat-free.

THE RÔLE OF VITAMINS IN NUTRITION

To Sir Frederick Gowland Hopkins is attributed the first clear statement that no animal can live upon a mixture of pure protein, fat, and carbohydrate, and that, "even when the necessary inorganic material is carefully supplied in order to supplement this diet, the animal cannot flourish." He recognized that, in diseases such as rickets and scurvy, dietary factors were involved that were as yet obscure, and he predicted in 1906 that the later development of the science of dietetics would deal with these complex unknown factors. This was indeed a remarkable prophecy. The history of the vitamin problem is of extraordinary interest, but it is possible here only to outline a few of the steps which have led to its remarkable development. More adequate discussions are to be found in a number of excellent books [81] that have appeared within the past few years.[82]

Deficiency Diseases.—The disease beri-beri is known to have existed since ancient times in India, Japan, the Malay Peninsula, Southern China, the Philippine Islands, the Dutch East Indies, and to a lesser degree in other portions of the globe. The symptoms of the disease may vary somewhat. "Wet" beri-beri is associated with edema or swelling of the tissues, whereas "dry" beri-beri is accompanied by rapid atrophy of the muscles, resulting in complete helplessness. In the early stages of both forms of the disease, there is fatigue, mental depression, loss of appetite, and gastro-intestinal disturbances. As the disease progresses, breathing becomes difficult; there is paralysis of the extremities, and other symptoms of multiple neuritis become manifest.

In 1897, Eijkman,[83] a Dutch physician and chemist working in Java, reached the conclusion that beri-beri was caused by long-continued

[81] H. C. Sherman, Physiol. Reviews, **1**, 598 (1921). H. C. Sherman and S. L. Smith, Vitamins, Am. Chem. Soc. Monographs, Chem. Catalog Co., 1922. C. Funk and H. E. Dubin, Vitamines, Baltimore, 1922. W. H. Eddy, The Vitamine Manual, Baltimore, 1921. C. Ellis and A. L. Macleod, Vital Factors of Foods, New York, 1922. B. Harrow, Vitamines, New York, 1921; E. V. McCollum and N. Simmonds, The Newer Knowledge of Nutrition, New York, 1929; H. D. Kruse and E. V. McCollum, Physiol. Rev., **9**, 126 (1929).

[82] Lunin, a student of Bunge, is said to have recognized, as early as 1881, that substances other than protein, fat, carbohydrate, and salts were indispensable for proper nutrition.

[83] Eijkman, C., Virch. Arch., **148**, 523 (1897); **149**, 187 (1897); Arch. f. Hyg., **58**, 150 (1906).

consumption of polished rice. He obtained evidence that the deficiency could be removed by the addition of the rice polishings to the diet. Moreover, he succeeded in producing experimentally a similar condition in birds by feeding polished rice, having previously observed (1896) that chickens fed largely upon the remains of the food used in the hospital developed a form of polyneuritis (polyneuritis gallinarum). Later, it was shown by Fraser and Stanton [84] that alcohol extracts of rice polishings exerted a curative effect on a beri-beri patient. The problem was studied experimentally by Casimir Funk,[85] who fractionated extracts of rice polishings and obtained a crystalline product which was very active in curing and preventing polyneuritis in pigeons, and which he believed to be the physiologically active principle in preventing beri-beri in man. Soon after, Funk reported the isolation of the same substance from brewer's yeast. His analyses showed it to contain nitrogen in basic combination, and he therefore considered that it might be in the nature of an amine. As this principle seemed to be essential to life, he suggested the term " vitamine " for substances of this character. The same year, Suzuki, Shimamura and Odake [86] obtained results identical with those of Funk, but designated the active substance by the name " oryzanin." It has been definitely shown, however, that neither Funk nor the other investigators actually isolated the vitamin; the crystalline substance which they obtained was really a complex of the active principle with other substances. Moreover, it does not appear to be an amine. Therefore, in the original sense, the term " vitamine " is regarded by some as a misnomer. The more accepted spelling of the term at present is " vitamin." Hopkins [87] has suggested the term " accessory food factors " for the substances which Funk called " vitamines," but this designation has likewise met with criticism since the word " accessory " does not imply that these components are indispensable.

The discovery of the importance of the water and alcohol-soluble constituent of rice polishings as the etiological factor in beri-beri stimulated many investigations of other diseases which appeared to be due to dietary deficiencies, such as scurvy, rickets and pellagra. Since 1911, additional vitamins have been discovered. The vitamins are usually designated by the letters A, B, C, D, and E, in accordance with the original suggestion of McCollum.

[84] Lancet, i, 733 (1910); ii, 1755 (1910).

[85] J. Physiol., **43**, 395 (1911); *ibid.*, **45**, 75, 489 (1912–13); *ibid.*, **46**, 173 (1913); Z. physiol. Chem., **88**, 352 (1913).

[86] Biochem. Z., **43**, 89 (1912).

[87] J. Physiol., **44**, 425 (1912).

Vitamin A.—In 1912, Hopkins [88] drew attention to certain observations showing that normal growth does not occur on rations consisting of purified foodstuffs, but that it does take place upon the addition to such rations of small amounts of milk. In the following year, Osborne and Mendel [89] reported similar observations; they showed, moreover, that rats restricted to synthetic diets with lard as the source of fat frequently developed a peculiar infection of the eyes (ophthalmia or xerophthalmia), but that this condition could be remedied by the introduction of butter fat into the food. Somewhat later, Osborne and Mendel found that cod-liver oil likewise exerted an anti-xerophthalmic effect. McCollum and Davis [90] were also actively engaged in the study of the problem at this time, and in 1915 definitely attributed the growth-promoting effect induced by feeding butter, egg yolk, cod-liver oil, etc., to the presence of an accessory factor differing from the water-soluble vitamin obtained by Funk from yeast and rice polishings. Thus, by the work of Osborne and Mendel, McCollum, and others, the second vitamin was discovered. Because of its solubility in fats, it became known as fat-soluble A, in contradistinction to Funk's vitamin, which received the designation water-soluble B. In 1917, McCollum and Simmonds [91] were able to show that xerophthalmia was due specifically to a lack of the fat-soluble A vitamin. Hence the term " anti-xerophthalmic vitamin " also came to be used in designating this vitamin. At this point, it may be emphasized that as early as 1913 Osborne and Mendel fully appreciated the nature of xerophthalmia as a deficiency disease, for they referred to it as a nutritive deficiency prevalent in animals inappropriately fed, and speedily alleviated by the introduction of butter fat into the diet. It is to be noted also that the protein-free milk used by Osborne and Mendel in their early nutritional studies, as a component of their experimental rations, contained vitamin B.

Occurrence of Vitamin A.—Milk, butter, egg yolk, and the green leaves of plants are the most abundant sources of vitamin A. The common vegetables that are especially rich in it are cabbage, carrots, lettuce, and spinach. It occurs in cod-liver oil and in certain organs, such as liver and kidney. It is present in beef fat and is said to be present in very small amounts in lard. Vitamin A does not occur in oleomargarine nor in vegetable oils (cottonseed, olive, almond, etc.)

The capacity of the animal body to store vitamin A was suspected by Osborne and Mendel, McCollum and Davis, and others. The most

[88] J. Physiol., **44**, 425 (1912).
[89] J. Biol. Chem., **15**, 311 (1913); **16**, 423 (1913); **17**, 401 (1914).
[90] *Ibid.*, **15**, 167 (1913); **19**, 245 (1914); **20**, 641 (1915); **23**, 231 (1915).
[91] *Ibid.*, **32**, 181 (1917).

recent study (Sherman and Cammack) [92] of the storage of vitamin A shows that on a liberal intake, animals continue to increase their bodily store throughout the entire period of growth. At first the process is relatively rapid, but as the maximum quantity which the body is capable of storing is approached, the accumulation becomes more gradual.

Chemical Properties of Vitamin A.—Vitamin A is soluble in fats and is a constituent of the non-saponifiable residue. It may be separated from cholesterol by precipitating the latter with digitonin. If the cholesterol-free fraction of the unsaponifiable matter of cod-liver oil is distilled under reduced pressure (3 mm.) vitamin A comes over mainly between 180–220° C.[93] Vitamin A is readily oxidized, being thereby destroyed. In an oxygen-free atmosphere it is heat-stable. With arsenic trichloride vitamin A gives a characteristic ultramarine blue color which changes to purple and then gradually fades. This color reaction is considered to be specific (Rosenheim and Drummond).[94] According to Morton and Heilbron,[95] the presence of vitamin A in oils and other preparations may be detected by its absorption band which is said to be in the ultraviolet with a maximum at 328$\mu\mu$.

The chemical identity of vitamin A has not been established. It is believed to be an unsaturated sterol with a molecular weight of about 300 and an iodine number of about 100.

Nutritional Significance of Vitamin A.—The *growth-promoting* properties of vitamin A were the first to be recognized and studied. Soon, however, it was appreciated that deficiency with respect to this vitamin may result in a well-defined infection of the eyes, which begins in the lids and later involves the cornea. This becomes dry and opaque owing to the formation of a horny layer, a process called keratinization or cornification. The disease is usually termed *xerophthalmia.*[96]

Medical literature contains many references to the prevalence of eye disease in communities in which malnutrition may be supposed to exist. Among Japanese children, Mori [97] found the incidence of xerophthalmia to be very high, especially in times of famine. It is interesting to note in this connection how even empirical methods often prove to be of

[92] J. Biol. Chem., **68**, 69 (1926).

[93] Drummond, J. C., Channon, H. J., and Coward, K. H., Biochem. J., **19**, 1047 (1925).

[94] Biochem. J., **19**, 753 (1925).

[95] *Ibid.*, **22**, 987 (1928).

[96] Yudkin (J. Am. Med. Assoc., **79**, 2206 (1922)) and Yudkin and Lambert (Proc. Soc. Exp. Biol. and Med., **19**, 375 (1922)) have made a study of the pathology of xerophthalmia.

[97] Jahrb. Kinderheilk., **59**, 175 (1904); J. Am. Med. Assoc., **79**, 197 (1922).

lasting value, for Mori's treatment consisted in feeding his patients cod-liver oil and chicken livers. A similar condition prevailed in Denmark several years ago. Owing to the demands of the butter industry, the use of skim milk in infant and child feeding was so general among the poor that xerophthalmia became very prevalent. Bloch [98] studied the condition exhaustively and ascribed it to fat deficiency. Butter fat and cod-liver oil proved effective in treating the disease. In the light of our present knowledge, the conclusion is justified that both in Mori's and Bloch's clinical studies, the conditions were largely due to a deficiency of vitamin A. To remedy the situation in Denmark, the government found it necessary to limit the exportation of butter, thereby increasing its consumption within the country. This measure is said to have decreased, to a very marked extent, the incidence of xerophthalmia among the poorer classes.

Night blindness is very common among the inhabitants of Newfoundland, Labrador, Russia, Japan, certain parts of India, and other places. McCollum [99] is of the opinion that this condition, as well as other eye disturbances, may be the result of a deficiency of vitamin A.

Lowered resistance to infection in vitamin A deficiency is not limited to the eyes alone. There is good reason to believe that an increased susceptibility to upper respiratory tract infections, including those of the throat, nose, ears and sinuses, may be a manifestation of an inadequate supply of this essential vitamin in the diet. Indeed, the recent observations of Sherman and Burtis [100] even suggest the possibility that vitamin A deficiency early in life may render the organism more susceptible to infectious diseases at a later age.

Keratinization of epithelial tissues in vitamin A deficiency is more generalized than was formerly supposed. It occurs extensively in the respiratory tract, including the lungs (Mori),[101] the alimentary tract, the urino-genital tract, salivary glands and ductless glands (Wolbach and Howe[102]). Evans and Bishop [103] made the interesting observation that in experimental animals deprived of vitamin A, there is a constant appearance of cornified cells in the vaginal smears. Evans states that this is even more constant in appearance than xerophthalmia and occurs earlier. The injury to the female reproductive tract thus produced

[98] J. Hyg. (Cambridge), **19**, 283 (1921).

[99] E. V. McCollum and N. Simmonds, Newer Knowledge of Nutrition, 4th edition, p. 167.

[100] Proc. Soc. Exp. Biol. and Med., **25**, 649 (1928).

[101] Bull. Johns Hopkins Hosp., **33**, 357 (1922).

[102] J. Exp. Med., **42**, 753 (1925).

[103] Anal. Rec., **23**, 17 (1922); Evans, J. Biol. Chem., **77**, 651 (1928).

may be sufficient to prevent the fertilization and implantation of the ovum.

Another manifestation of vitamin A deficiency is the tendency to form calcium phosphate concretions in the urinary tract. This was first described by Osborne and Mendel [104] and has since been confirmed by van Leersum.[105] This condition of urolithiasis occurs extensively in the tropics and the Far East which suggests the possibility of it being associated with a dietary low in fat and fat-soluble vitamins (Mendel).

In short, our present knowledge of the subject leads to the conclusion that vitamin A is growth-promoting and that it increases resistance to infections. In its absence, growth ceases; resistance to infection is greatly diminished, resulting in such conditions as xerophthalmia; epithelial tissues, particularly mucous membranes become cornified; and there is a marked tendency for the formation of urinary calculi.

The Water-soluble (B) Vitamins.—What was formerly taken to be the water-soluble, growth-promoting, anti-neuritic vitamin is not an entity but is composed of at least two different factors. Of these, the anti-neuritic vitamin is readily destroyed by heat, whereas the growth-promoting and, as shown by Goldberger and associates,[106] the pellagra-preventing vitamin is relatively stable to heat. As yet, a uniform terminology for the water-soluble vitamins has not been decided upon. British biochemists designate the heat-labile factor by " B_1" and the heat-stable factor by " B_2," reserving " B " to identify the complex of the two vitamins. In America, it has been tentatively suggested that the term " B " be restricted to designate the more heat-labile (anti-neuritic) factor and that the term " G " be used to denote the more heat-stable, water-soluble dietary factor, called the P-P (pellagra-preventive) factor by Goldberger and associates, and which also has to do with maintenance and growth.[107]

Occurrence.—It is to be appreciated that while the earlier studies on " vitamin B " have taught us important lessons in nutrition, nevertheless, in view of our present knowledge of the existence of two separate factors, much of the older work will require repetition or critical reinterpretation. Of particular value would be a reinvestigation of the foodstuffs as sources of the two water-soluble vitamins. As formerly taught, the principal sources of " vitamin B " are: yeast, eggs of birds, plant seeds, milk and a large variety of fruits and vegetables.

[104] J. Am. Med. Assoc., **69**, 32 (1917).

[105] J. Biol. Chem., **76**, 137 (1928).

[106] U. S. P. H. Reports, **40**, 927 (1925); **41**, 297, 1025 (1926); **42**, 2383 (1927); **43**, 172 (1928).

[107] Science. **69**. 276 (1929).

Nitrous acid destroys the heat stable factor (vitamin G), presumably as a result of deaminization, but has no effect on the antineuritic vitamin.

Ultraviolet rays are said to destroy the growth-promoting vitamin, without changing the antineuritic potency of the vitamin B complex.

Although considerable progress has been made [108] in obtaining relatively pure and highly active preparations of vitamins B and G (or B_1 and B_2), their chemical constitution is as yet unknown.

Yeast contains an abundance of both factors. When heated in an autoclave, at 120° C., under pressure, the antineuritic potency is rapidly destroyed, whereas the growth-promoting, anti-pellagra (G or B_2) vitamin remains active. Wheat and corn contain more of the antineuritic fraction, whereas milk, bananas, spinach are proportionately richer in vitamin G.

In the cereal grains the vitamin is present largely in the embryo; in other seeds it is more evenly distributed. Vitamin B occurs in timothy hay and alfalfa. Meat is a poor source, but glandular tissues (liver, brain, kidney, thymus, thyroid, etc.) contain the vitamin in greater abundance. Although large amounts of vitamin A may be stored in the tissues for further use, the storage of vitamin B is not very pronounced. Osborne and Mendel [109] have reported that "when an adequate supply of vitamin B is lacking in the diet the store of this factor in the liver tissue, where it is ordinarily found in abundance, becomes largely depleted." The synthesis of vitamin B is not believed to occur in the mammary gland. Accordingly, it must be supplied in the food of the mother to insure its presence in adequate amount in the milk.

Chemical Properties.—The antineuritic vitamin is soluble in water, acetone, benzene, ethyl acetate, and glacial acetic acid, 70–80 per cent alcohol, but not in 95 per cent alcohol. It is insoluble in ether. The solubilities of the growth-promoting factor are about the same, but it is stated that benzene and acetone do not extract the vitamin directly from original food sources. However, after the vitamin has been extracted by alcohol, it is soluble in these solvents.

Heat destroys the antineuritic vitamin, the temperature required being 115 to 120° C., for 1 hour. Under these conditions no effect is produced on vitamin G.

Both components are remarkably stable toward acids. On the contrary, long exposure to alkali destroys the antineuritic fraction, leaving the growth-promoting factor unaffected.

[108] Kinnersley and Peters, Biochem., J., **22,** 419 (1928); Levene, J. Biol. Chem., **79,** 465 (1929).

[109] J. Biol. Chem., **58,** 363 (1923–24).

Nutritional Significance of the Water-soluble Vitamins (B and G).— Our present knowledge of the effects of water-soluble vitamin deficiency falls into three groups. First, most of what we know is based on studies performed before the dual nature of "vitamin B" was appreciated and therefore such physiological and pathological changes as have been described are to be regarded, at least for the present, as having been due either to one or the other, or both factors. Under this head may be considered the effect of "vitamin B" on appetite. Osborne and Mendel had observed in their earlier nutritional studies that deprivation of what are now known as the water-soluble vitamins was frequently accompanied by loss of appetite (anorexia). Karr [110] examined the problem more closely, using dogs as the experimental animals. He found that "some relation exists between the desire to partake of food and the amount of the so-called water-soluble B vitamin ingested." Animals that had lost their appetite through avitaminosis regained it upon receiving substances rich in vitamin B (brewery yeast, baker's yeast, tomatoes, and milk). These findings have been confirmed by Cowgill,[111] who has demonstrated, moreover, that alcoholic extracts of wheat embryo, rice polishing, and navy beans exert an appetite-provoking effect.

Gastric motility is markedly depressed in dogs maintained on water-soluble vitamin deficient diets. In fact, in some cases complete gastric atony has been observed. Nevertheless, this is not believed to be the direct cause of the loss of appetite for the vitamin deficient food (Rose, Stucky and Cowgill [112]).

The appetite-provoking effect of vitamin B is not associated with increased secretion of the digestive glands, according to the observations of Cowgill and Mendel.[113] In a more recent statement, Cowgill [114] ascribes the loss of appetite to a general systemic disturbance rather than to an abnormal condition localized in the alimentary tract. The symptoms of vitamin-B deficiency are difficult to interpret. There is, nevertheless, a strong suggestion that avitaminosis with respect to this food factor results in some form of severe intoxication.

Owing to the loss of appetite, the effects of water-soluble vitamin deprivation should be regarded at least partly in the light of what may happen in starvation. Thus, the anhydremia (diminished water content of the blood) and other changes in the composition of the blood

[110] J. Biol. Chem., **44,** 255 (1920).
[111] Am. J. Physiol., **57,** 420 (1921).
[112] *Ibid.*, **91,** 531 (1929–30).
[113] *Ibid.*, **58,** 131 (1921).
[114] *Ibid.*, **77,** 389 (1926).

which appear to be characteristic of this type of avitaminosis is probably fundamentally due to the diminished food and water consumption (Rose, Stucky and Mendel[115]).

To the combined effects of vitamins B and G deficiency and the associated anorexia may perhaps be attributed the generalized atrophy of various tissues and organs of the body, including the ductless glands, with the exception of the adrenals.

Having disposed of what may well be the combined effects of vitamins B and G deficiency, we may consider secondly the effects of avitaminosis with respect to the factor B (B_1 according to the present British terminology, " F," according to a suggestion made by Professor Sherman). As described elsewhere, the absence of this essential substance was early recognized as the etiological factor in the disease *beri-beri*, affecting human individuals, and the similar disease seen in birds, called polyneuritis, the characteristic symptoms of which are loss of muscular coordination, progressive weakness, convulsive seizures, and involvement of the central nervous system. Rats maintained on a diet deficient in this vitamin develop within three or four weeks paralysis and symptoms of general collapse. Polyneuritis may also be produced in monkeys, cats and other animals.

The more important symptoms of polyneuritis in the dog may be reviewed briefly. The first symptom is usually vomiting. There soon follows a loss of control of the hind limbs, presumably because of the production of lesions in the brain. Later, convulsions set in, together with severe gastro-intestinal disturbances. The animal becomes extremely sensitive to pain. Upon the administration of substances containing the anti-neuritic vitamin, these symptoms are alleviated. Two of Cowgill's dogs that showed paralytic symptoms and incoordination of gait, on attempting to leave their cages, were seized with spasms and suddenly died. Cowgill believes that heart failure was the cause of death of these animals.

Vitamin B (the anti-neuritic vitamin) is the specific preventive or curative agent of human beri-beri and the similar deficiency diseases observed in birds, dogs and other animals.

Finally remains to be stated the significance of the heat-stable vitamin G (B_2 according to the British terminology). Yeast concentrates have been prepared containing only the anti-neuritic factor. When used as the source of the water-soluble vitamins, polyneuritis is prevented, but growth is subnormal. When to such a deficient diet, autoclaved yeast (which is devoid of the anti-neuritic vitamin) is also added, growth is normal. This is good evidence that the active principle

[115] *Ibid.*, **91**, 520 (1929–30).

present in autoclaved yeast and which has been designated vitamin G, is *growth-promoting*. To what extent the increase in weight of experimental animals is due indirectly to a primary effect of the vitamin on appetite and on the condition of the gastro-intestinal tract is something that needs to be further elucidated.

Vitamin G is of further significance in relation to pellagra, a disease associated with characteristic skin lesions, gastro-intestinal disturbances and symptoms of involvement of the central nervous system. Pellagra has been described as a disease entity for nearly two hundred years and has been frequently associated with faulty diet. This relationship, however, was not widely accepted until very recently, when it became more or less generally recognized that pellagra may be a deficiency disease belonging to the same category as beri-beri, scurvy and rickets. The absence of fresh meat and milk from the dietary of a large proportion of the victims of pellagra first made it appear probable that it was due primarily to protein deficiency.

A condition resembling pellagra has been produced in rats,[116] monkeys,[117] and dogs; in the latter by Chittenden and Underhill,[118] who were the first to produce pellagra (or an analogous condition) experimentally. The disease in dogs is called "black tongue," the symptoms of which are remarkably similar to those of human pellagra. Smith and Hendrick [119] found that they could prevent or cure the pellagra-like condition in rats by feeding autoclaved yeast. Goldberger and Wheeler [120] demonstrated that experimental "black tongue" in dogs could be cured in the same way. Finally it was shown that autoclaved yeast was also curative of human pellagra. The active principle was designated by Goldberger as the P–P (pellagra-preventive) vitamin. As the sole source of the water soluble vitamins, autoclaved yeast does not prevent the development of polyneuritis in dogs.

The evidence seems to be fairly conclusive that factor G is a growth-promoting and pellagra-preventing vitamin. However, in conclusion it should be stated that there is also good evidence for the view that autoclaved yeast may not be specific as regards its anti-pellagra effect. Underhill and Mendel [121] several years ago reported that "black tongue" in dogs is due to a deficiency of a fat-soluble substance present in butter which differs from vitamin A. More recently, the effective

[116] McCollum, Simmonds and Parsons, cited by McCollum and Simmonds, "Newer Knowledge of Nutrition," 4th edition, p. 388.

[117] Chick, H., and Hume, E. M., Biochem. J., **14**, 135 (1920).

[118] Am. J. Physiol., **44**, 13 (1917).

[119] U. S. Pub. Health Reports, **41**, 201 (1926).

[120] *Ibid.*, **43**, 172 (1928).

[121] *Ibid.*, **40**, 1087 (1925); Am. J. Physiol., **83**, 589 (1928).

substance has been isolated in a crystalline form from carrots and is reported to be the familiar unsaturated hydrocarbon, carotin ($C_{40}H_{56}$), the pigment to which carrots, butter and other plant and animal substances owe some of their yellow color.

Bios.—As early as 1901, Wildiers[122] emphasized the fact that yeast contains a substance essential for its growth and without which yeast cells do not multiply in artificial media. He assigned the term " bios " to the growth-promoting factor of yeast. Interest in Wildiers' bios was revived after the discovery of the anti-neuritic principle in yeast, and certain investigators even adopted the view that bios and " vitamin B " were identical. Based on this supposition, methods were introduced for determining the potency of anti-neuritic preparations by measuring their effect in promoting the growth of yeast. Sufficient evidence is now available to show that bios and vitamin B are not identical. Eddy, Kerr, and R. R. Williams [123] have reported the isolation of a pure crystalline compound possessing the functions of Wildiers' bios. The physiological effect of this preparation is very remarkable, for extremely small doses (0.005 mg. per cc.) are sufficient to stimulate the growth of yeast. According to Eddy and his associates, bios has a melting-point of 223° C., and a molecular weight of approximately 133. The compound has the formula $C_5H_{11}NO_3$ and is believed to be heterocyclic.

Deas [124] has reported the isolation of two substances from malt rootlets, Bios I which is not adsorbed by charcoal and Bios II which is adsorbed. Growth of yeast is said to depend on the presence of both in certain proportions, an excess of either, particularly of Bios II exerting an inhibitory effect.

Vitamin C; Anti-scorbutic Vitamin; Water-soluble C.—Authoritative accounts of scurvy may be found in the literature of the fifteenth century. Lind in 1752 wrote a treatise on scurvy which is still a source of valuable information. This is indeed remarkable, for we rarely go back so far to past generations for lucid accounts of nutritional disorders. The history of scurvy has been traced by Hess [125] in his excellent monograph on the subject.

As to the prevalence of scurvy, it is stated that between 1556 and 1887 there are known to have occurred 143 epidemics of this disease on land. In most cases the outbreaks were among troops, prisoners, and inmates of institutions. In times of famine and war, scurvy has been very prevalent. During the siege of Paris, which lasted from

[122] Wildiers, E., La Cellule, **18**, 314 (1901).
[123] J. Am. Chem. Soc., **46**, 2846 (1924).
[124] J. Biol. Chem., **61**, 5 (1924).
[125] A. F. Hess, Scurvy—Past and Present, Philadelphia, 1920.

September 17, 1870, to January 27, 1871, scurvy broke out among the inmates of the prisons, patients in the military hospitals as well as among the civilian population. It is stated by Hess that in the Russo-Japanese War, after the siege of Port Arthur, it was found that one-half of the garrison of 17,000 men had scurvy. Scurvy was likewise prevalent during the Great War, the highest incidence having been reported from Austria. Both the civilian population and troops suffered from this deficiency disease in Russia.

Scurvy has been more frequently associated with life at sea. On long voyages, such as those made by the early explorers, serious outbreaks of the disease occurred. An early account of such an outbreak is that of Vasco da Gama, who, about 1497, reached the East Indies by way of the Cape of Good Hope. In our own day, explorers have suffered most from scurvy, particularly in Arctic and Antarctic expeditions. This does not apply, of course, to the extremely well-organized expeditions of the last few years, such as those of Rear Admiral Byrd. The relation of diet to scurvy has been known to sailors and explorers since the eighteenth century. Sprouted barley, wheat, beans and lentils were used as a protection against the disease. The high incidence of scurvy at sea is readily comprehended when one considers that for many months the diet of the sailors lacked fresh animal and vegetable food. The efficacy of the juice of limes, lemons, and oranges was likewise known at an early date, and, in 1795, lime and lemon juice were introduced into the rations of sailors in the British Navy, with the result that the incidence of scurvy decreased to a remarkable extent. Occasionally outbreaks occurred on what appeared to be liberal allowances of lime juice and consequently many lost faith in its value as an anti-scorbutic. As we shall see presently, the anti-scorbutic substance is readily destroyed, and this may have occurred in the process of preparation of the juice. Moreover, not all limes are equally efficacious; those grown in the Mediterranean region apparently contain more of the anti-scorbutic factor than those grown in the West Indies. From his own experience, the Arctic explorer Stefánsson has been led to believe that the large amounts of salt which sailors formerly consumed in salted meat may have been a factor in the development of the disease.

The more prominent symptoms of scurvy in man are loss of weight, pallor (due often to anemia), weakness, breathlessness, palpitation of the heart, swelling of the gums, loosening of the teeth, hemorrhage into the skin and mucous membranes, pains in the bones and joints, edema, nervousness, and hypersensitivity to pain. Scorbutic patients frequently die in delirium, but death may also occur suddenly, suggesting the possibility of cardiac involvement. The pathology and symptoma-

tology of scurvy, both in adults and infants, are adequately discussed by Hess (Chapters IV and VII).

In 1895, Theobald Smith [126] reported a peculiar hemorrhagic condition in guinea pigs restricted to a diet containing cereal but no grass, clover, or succulent vegetable like cabbage. The importance of this observation was not appreciated until Holst and Frölich,[127] in 1912, pointed out the similarity between this disease in guinea pigs and human scurvy. The resemblance is indeed very striking, for a diet that causes scurvy in man likewise produces it in the guinea pig, and those substances that exert a curative effect in man are equally efficacious in experimental scurvy in the guinea pig. However, despite the acceptance of the vitamin theory in relation to beri-beri and xerophthalmia, the idea that scurvy may also be a deficiency disease, brought about by some form of avitaminosis, did not gain much headway at first. Several important papers led ultimately to the adoption of the present view.

In 1917, Chick and Hume [128] emphasized certain differences in the distribution of the anti-neuritic vitamin and the factor which prevented scurvy, and discussed the independent need of both factors in nutrition. In a critical study, Cohen and Mendel,[129] in 1918, produced scurvy in guinea pigs maintained on rations adequate in all respects and containing liberal amounts of vitamins A and B. Similar results were obtained by Hess and Unger,[130] who found, moreover, that orange juice which had been allowed to stand in a refrigerator for several months lost its anti-scorbutic power.

Rats seem to be immune to scurvy, as they do not develop the disease on diets that produce it in man, guinea pigs and monkeys. Why this should be is not clear, although it has been suggested that the rat is capable of synthesizing the anti-scorbutic substance. Harden and Zilva [131] in 1918, expressed the view that, " rats existing on a scorbutic diet, although capable of gaining in weight and reproducing themselves without any apparent manifestation of pathological symptoms for months, do not thrive as well as animals which have their diets supplemented with an anti-scorbutic." Drummond [132] reached similar conclusions, and in 1919 proposed the admission into the family of vitamins of the anti-scorbutic vitamin, or vitamin C. The view that this

[126] U. S. Dept. Agr. Bureau Animal Industry Ann. Rept., 1895–96, 172.

[127] Zeit. f. Hyg. u. Infect-Krank., **72**, 1 (1912).

[128] J. Roy. Army Med. Corps, **29**, 121 (1917); Proc. Roy. Soc., London, B, **90**, 44 (1917).

[129] J. Biol. Chem., **35**, 425 (1918).

[130] *Ibid.*, **35**, 479 (1918).

[131] Biochem. J., **12**, 408 (1918).

[132] *Ibid.*, **13**, 77 (1919).

vitamin is synthesized in the body of the rat is rendered plausible by the observations of Parsons,[133] who found that scorbutic guinea pigs could be cured by feeding them livers of rats that had been kept on rations deficient in anti-scorbutic vitamin. These results could not have been obtained unless synthesis of the vitamin had taken place in the rats, and Parsons therefore concluded that these observations indicated the need for the anti-scorbutic factor in the normal metabolism of the rat. Even after long intervals on diets lacking vitamin C, this factor persists in the liver of the rat. In the guinea pig the situation is different, for when these animals are fed rations low in vitamin C, its reserve supply in the liver is rapidly depleted (Parsons and Reynolds[134]). The question of vitamin-C synthesis has been pursued further by Hart, Steenbock, Lepkovsky, and Halpin,[135] who found that chicks subsisted on scorbutic rations without developing scurvy. What is even more striking is that the livers of these birds proved to be potent sources of vitamin C and effected rapid cures when fed to guinea pigs suffering from this disease. It therefore appears that birds do not require vitamin C.

Vitamin C deficiency is regarded by Hanke[136] to be the principal etiological factor in dental caries and other diseases of the .gums and teeth. He has stated, " It is possible, by means of a diet containing an abundance of vitamin C, to produce solid gum tissue, to arrest caries and, with the aid of prophylactic measures, to cure pyorrhea and induce bone regeneration in the alveolar tissue."

Occurrence of Vitamin C.—Grain products, sugars, starches, fats, oils, and yeast do not contain the anti-scorbutic vitamin. Variable amounts are present in fresh meats and fish, but canned meat and meat extracts are deficient in this respect. Fruits and vegetables are, as a rule, excellent sources of vitamin C, being present in greatest abundance in lemons and oranges, cloudberries, raspberries, tomatoes, cabbage, lettuce, rutabaga, swedes (a root vegetable grown in England), and bananas. Milk is not a very satisfactory source of vitamin C; in its raw state it is more effective as an anti-scorbutic than after pasteurization. The anti-scorbutic potency of evaporated milk is negligible. The presence of vitamin C in dried milk depends on the process employed in drying. It has been estimated that 500 cc. of cow's milk (16 ounces), per day, is the minimum required to protect an infant from scurvy. Hess's experience has been[137] that 12 ounces of the best grade

[133] J. Biol. Chem., **44**, 587 (1920).
[134] J. Biol. Chem., **59**, 731 (1924).
[135] *Ibid.*, **66**, 813 (1925).
[136] Am. J. Physiol., **90**, 376 (1929).
[137] A. F. Hess, Scurvy, Past and Present, p. 152.

of raw milk, per day, is at times insufficient to effect a cure. Breast-fed babies and those given raw cow's milk are less likely to develop scurvy than are infants maintained on pasteurized, condensed, or evaporated milk. The greatest incidence is observed in cases where proprietary infant foods are permitted to replace a part of the daily milk allowance. Human milk possesses approximately the same anti-scorbutic power as cow's milk. Clearly, the diet of the mother is an important factor to consider. It should contain an abundance of anti-scorbutic foods.

Chemical Properties of Vitamin C.—The method of assaying vitamin C consists essentially in determining the minimum amount of the anti-scorbutic food (or vitamin concentrate) required to prevent scurvy in guinea pigs.

Vitamin C is readily destroyed by heat. Cabbage when boiled for one hour at a temperature of about 100° C., may lose as much as 90 per cent of its anti-scorbutic property. The destructive effect is especially marked in alkaline solution and in the presence of oxygen, destruction of the vitamin being essentially an oxidative process. Decitrated lemon juice (of about *p*H 6.8) loses its anti-scorbutic potency much more rapidly than the original lemon juice, which has a considerable acidity, owing to its citric acid content.

Heating at high temperatures for short periods is less destructive than heating at lower temperatures for longer intervals. Vitamin C is likewise destroyed in the process of drying and aging of foods, especially when exposed to oxygen. When moisture is present, foods may lose the anti-scorbutic factor even during storage. It is apparently stable to ultraviolet light.

Vitamin C is soluble in water and alcohol, thus resembling the anti-neuritic vitamin. It is diffusible through parchment and porcelain filters and is not adsorbed by fuller's earth, differing in the last particular from vitamins B and G.

A highly active concentrate of vitamin C has been prepared by Zilva [138] from lemon juice. Briefly stated the method of preparation consists in removing the citric acid by precipitation and the sugar by fermentation. The juice is then concentrated and treated with alcohol; a precipitate forms. Vitamin C is contained in the supernatant liquid, from which it is precipitated with basic lead acetate. The precipitate is dissolved in the smallest possible volume of acetic acid and the extraneous material precipitated by neutral lead acetate. Vitamin C is contained in the supernatant liquid which may be further concentrated. The purest preparations of vitamin C thus far obtained contain iron, phosphorus and sulfur and exhibit marked reducing proper-

[138] Biochem. J., **19**, 589 (1925); **21**, 354, 689 (1927); **22**, 779 (1928).

ties. It is believed that the vitamin is a relatively simple organic compound.

Vitamin D; the Anti-rachitic Factor in Nutrition.—Mention has been made elsewhere of the prevalence of rickets and of the factors that are believed to contribute toward its production. Rickets, according to Park's definition, is a condition in which the mineral metabolism is disturbed in such a way that calcification of the bones does not take place normally. Even before there was any direct evidence of vitamin deficiency in this disease, it was suspected by Hopkins, Funk, and other pioneers. In 1919, Mellanby [139] published a paper in which he described the experimental production of rickets in puppies and in which he set forth the view that rickets is due probably to a lack of the fat-soluble A vitamin. In 1922, McCollum, Simmonds, Becker and Shipley [140] subjected cod-liver oil to oxidation, thereby destroying vitamin A, as demonstrated by the fact that the product failed to cure xerophthalmia. It was now a question of determining whether the power to cure rickets had likewise been destroyed in the process. A year earlier, Shipley, Park, McCollum, Simmonds and Parsons [141] were able to show that the sudden introduction of cod-liver oil into the diet of a rachitic rat is followed by a beautiful deposition of lime salts in bone, in a transverse line across the cartilage, at right angles to the long axis of the shaft. This phenomenon was employed by this group of investigators at Johns Hopkins University in developing a delicate biological test, the so-called "line test," by which curative effects in rickets may be determined. Using this method, McCollum and his associates found that the oxidized cod-liver oil, though lacking vitamin A, has nevertheless retained its power of curing rickets. It was determined, moreover, in this investigation, that cocoanut oil, though deficient in fat-soluble A, possessed the power of stimulating the deposition of calcium in a manner similar to that of cod-liver oil. It was therefore concluded "that the power of certain fats to initiate the healing of rickets depends on the presence in them of a substance which is distinct from fat-soluble A." The existence of a fourth vitamin was thus postulated.

Relation of the Anti-rachitic Factor to Radiant Energy.—In his excellent review, Park [142] summarizes the earlier literature dealing with the influence of radiant energy in the prevention and cure of rickets. The value of sunlight in the treatment of this disease has been frequently emphasized by clinicians for nearly forty years, but clear-cut demon-

[139] Lancet, i, 407 (1919).
[140] J. Biol. Chem., **53**, 293 (1922).
[141] *Ibid.*, **45**, 343 (1921).
[142] Physiol. Reviews, **3**, 106 (1923).

strations of curative effects were naturally lacking, as X-ray methods of diagnosis, making possible periodic examination of the condition of the bones, had not yet been developed sufficiently. The effect of ultraviolet radiations in causing the deposition of calcium salts in bone and curing rickets in children was clearly shown by Huldschinsky [143] in 1919, with the aid of X-ray photography. Hess and Unger [144] obtained similar results by exposure to sunlight. These studies were soon extended to experimental animals (Hess, Unger and Pappenheimer,[145] Powers, Park, Shipley, McCollum and Simmonds [146]). It was found that rachitic lesions could be prevented in rats by short exposures to direct sunlight. It is important to bear in mind that window glass filters out of sunlight the ultraviolet rays. Accordingly, light received by a child behind windows does not protect it against rickets. It has been determined that light of wave length 300$\mu\mu$ or shorter is most effective.

The problem of rickets acquired a novel aspect upon the appearance, in 1924 and 1925, of a number of papers in which was described the remarkable effect of ultraviolet irradiation in endowing foods, otherwise ineffective, with anti-rachitic potency. Attention was called to this phenomenon at approximately the same time by two groups of workers in this country, Hess and his associates, and Steenbock and his students. It was observed by Steenbock and Nelson [147] that a ration which ordinarily induced rickets in rats could be made definitely anti-rachitic by the simple expedient of exposing it to ultraviolet light. Hess [148] reported that, by means of irradiation, vegetable oils could be endowed with anti-rachitic properties. In a more extensive study, Hess and Weinstock [149] investigated the effect of irradiating various inert fluids, and determined that cottonseed oil and linseed oil could be made very effective as anti-rachitic agents by this method. The daily addition to a ricket-producing diet of 0.1 cc. of these irradiated oils was sufficient to protect rats from rickets. It was observed, moreover, that wheat which had been grown in the dark (etiolated) possessed no anti-rachitic potency, whereas wheat grown in the light and irradiated with the mercury vapor lamp exercised this power. Irradiation of green vegetables produced a similar effect. In a subsequent paper,

[143] Deutsche med. Wochenschr., **45**, 712 (1919).
[144] J. Am. Med. Assoc., **77**, 39 (1921).
[145] J. Biol. Chem., **50**, 77 (1922).
[146] J. Am. Med. Assoc., **78**, 159 (1922).
[147] J. Biol. Chem., **62**, 209 (1924–25).
[148] Am. J. Dis. Child., **28**, 517 (1924).
[149] J. Biol. Chem., **62**, 301 (1924–25).

Hess and Weinstock [150] showed that dry milk, flour, and spinach can be rendered anti-rachitic by radiations from the quartz mercury lamp. On the contrary, oleic acid and egg phosphatide could not be activated by this means.

That the anti-rachitic properties of hays are related to their exposure to sunlight has been emphasized by Steenbock and his associates.[151] It appears that clover hay, made with exposure to sunlight, exercises considerable calcifying power, whereas when made in the dark, it is inactive. When excessively weathered, clover hay loses its potency, as compared with hay less exposed to dew and rain. Another interesting observation [152] from the same laboratory is that, by irradiation, the anti-rachitic potency of cow's milk may be increased eight or more times. Goat's milk, similarly treated, may increase its activity as much as twenty-four times. Furthermore, direct irradiation of the animals produces the same effect, though to a lesser degree. The importance of these observations to the dairy industry is self-evident.

Cow's milk contains an appreciably greater amount of vitamin D than human milk.[153]

Bills [154] has determined the relative antirachitic potency of various fats and oils. Puffer fish oil contains about fifteen times as much vitamin D as cod-liver oil, the latter having about the same potency as goosefish liver, herring and sardine oils. Vitamin D is absent from seal blubber, whale blubber, hydrogenated cod-liver oil, veal fat, linseed oil, corn oil and olive oil. As stated previously some of these may acquire anti-rachitic properties upon irradiation.

Chemistry of Vitamin D.—Evidence rapidly accumulated to show that the anti-rachitic factor was confined to the unsaponifiable fraction of fats and oils. Simultaneously two groups of workers [155] reported that on irradiation (by ultraviolet rays of wave length approximating 300$\mu\mu$) cholesterol acquired antirachitic properties, but it soon became clear that highly purified preparations of cholesterol did not behave in this way and that the actual substance involved was ergosterol ($C_{27}H_{41}OH$).[156] This is an unsaturated sterol (3 double bonds) discovered by Tanret in the fungus ergot. Yeast is its principal source,

[150] *Ibid.*, **64**, 181 (1925).

[151] Steenbock, Hart, Elvehjem, and Kletzien, J. Biol. Chem., **66**, 425 (1925).

[152] Steenbock, Hart, Hoppert and Black, *ibid.*, **66**, 441 (1925).

[153] Outhouse, Macy and Brekke, J. Biol. Chem., **78**, 129 (1928).

[154] *Ibid.*, **72**, 751 (1927).

[155] Hess and Weinstock, J. Biol. Chem., **64**, 193 (1925); Steenbock and Black, *ibid.*, **64**, 263 (1925).

[156] Rosenheim and Webster, Biochem. J., **21**, 127, 389 (1927); Windaus and Hess, Nachr. ges. Wiss., Göttingen, **175**, 84 (1927).

although it has a wide distribution in plant and animal tissues. Ergosterol, which is itself inactive, upon being exposed to ultraviolet light of about 300$\mu\mu$ wave lengths acquires the properties of vitamin D, the maximum potency being attained after 30 minutes' irradiation. Prolonged exposure tends to destroy the antirachitic property of the reaction products of ergosterol. The chemical reactions underlying the conversion of ergosterol into vitamin D are not definitely known. Whereas ergosterol, the parent substance of vitamin D, has been prepared in crystalline form, this has not yet been accomplished in the case of the vitamin itself.

Irradiated Ergosterol and Rickets.—It is stated that a daily dose of 0.0001–0.0002 mg. of irradiated ergosterol is sufficient to cure rickets in rats. Clinical experience[157] so far has definitely established the therapeutic value of irradiated ergosterol in human rickets, 2–4 mg. per day being the curative dose.

Reports have appeared [158] showing that the administration of massive doses of irradiated ergosterol produces a condition of "hypervitaminosis" which is associated with hypercalcemia, the widespread deposition of calcium in various organs and tissues, other pathological changes and death. This in no way, however, reflects against the therapeutic usefulness of irradiated ergosterol, for the quantities required to produce experimental hypervitaminosis are many thousand times as great as the amounts needed to produce the desired effect in experimental rickets.

Vitamin E; the Reproductive Factor in Nutrition.—Mason,[159] in experiments on rats, found that vitamin E was a specific requirement of the germinal epithelium of the testis. In the absence of this vitamin, testicular degeneration occurred and sterility resulted. Evans and Burr,[160] referring to the occurrence of sterility in rats maintained on otherwise adequate "synthetic" food mixtures, make the following statement:

> The sterility is a dietary deficiency disease, for it can be cured or prevented by a change in dietary régime, a change involving the addition of certain single natural foodstuffs high in a new factor, vitamin E, or the addition of very much smaller amounts of extracts of these foods.

In another place Evans[160a] asserts:

> It has already been possible to show [161] that in the female, sterility may be produced by dietary régimes which nevertheless contain the

[157] Hess, Lewis and Rivkin, J. Am. Med. Assoc., **93**, 661 (1929).

[158] Moll, München. med. Wochenschr., **75**, 637 (1928); Klein, J. Am. Med. Assoc., **92**, 621 (1929); Harris and Moore, Biochem. J., **22**, 1461 (1928).

[159] Mason, K. E., Proc. Nat. Acad. Sci., **11**, 377 (1925); J. Nutrition, **1**, 311 (1928–29).

[160] Proc. Nat. Acad. Sci., **11**, 334 (1925).

[160a] *Ibid.*, **11**, 373 (1925).

[161] Evans and Bishop, Science, **60**, 20 (1924); J. Metabolic Res., **3**, 233 (1923); see also, Evans, Burr and Althausen, Memoir, Univ. California, **8**, 1 (1927).

known vitamins and are adequate for growth. We have reported the cure of such sterility by a variety of natural foods and by small doses of alcoholic and ethereal extracts of those foods. The evidence at hand is thus conclusively in favor of the existence of a new vitamin or food accessory to which the designation of fat-soluble E may be given.

When male rats from mothers on natural foods are weaned on the twenty-first day of life and reared upon a basal or pure food ration, they are usually at first fertile but when from ninety to one hundred and fifty days of age (usually at the close of the fourth month) become sterile.

The theory postulating the existence of a vitamin that is essential for reproduction is shared by Sure,[162] who has been an active worker in this field of investigation. Vitamin E is said to be present in green leaves, such as alfalfa, lettuce, peas, and tea. It occurs in grains and cereals, such as wheat, oats, and corn. Wheat germ is especially rich in this respect. Milk fat is a poor source of this vitamin. The reproductive factor seems to be lacking altogether in cod-liver oil. It is to be pointed out that vitamin E is fat-soluble, and, accordingly, should be found in abundance in oils. It appears, however, that certain oils do not favor fertility. Commercial linseed oil, cocoanut oil and sesame oil are examples belonging to this group. Then there are those that favor fertility but fail to produce lactation. Commercial olive oil, peach-kernel oil and directly expressed soy-bean and peanut oils may be included in this group. In the same general group are included oils that produce fertility but are only partially successful in producing lactation. Commercial cottonseed oil behaves in this manner. The third group consists of those oils that are potent in producing both fertility and lactation. Examples of this group are wheat-germ oil, hempseed oil, yellow-corn oil (when prepared by extraction with ether, acetone, or benzene).

It is not clear whether in reproduction and lactation one factor or more than one is involved. The demands of the lactating mammal may be far in excess of the requirements for reproduction. Whether this is the case with regard to vitamin E has not been determined, but there is some evidence to show that the requirement of water-soluble B vitamin for normal mammary gland function is much greater than that for growth (Sure).

The sterility disease affects males and females differently, the difference being that in the male there is destruction of the germ cells, whereas in the female the ovary and ovulation remain unimpaired throughout life. A characteristic disturbance develops, however, in

[162] J. Biol. Chem., **58**, 693 (1923–24); **62**, 371 (1924–25); **63**, lxxiv (1925); **69**, 29, 41, 53 (1926); **74**, 651 (1927), etc.

the female, which consists in the death and resorption of the embryos. Evans holds that the existence of typical "resorption gestations" is a clear sign of deficiency in the specific substance designated as vitamin E.

There are doubtless gradations in the degree of deficiency. Thus, if it is marked, there will be no fertility, but if less marked there may be fertility with inadequate lactation. The disinclination of the mother rat to care for her offspring is associated with failure in lactation. The situation is vividly described by Sure:[163]

When a mother has normal mammary function her maternal instinct guides her to be very solicitous toward the welfare of her litter. She stays in the box provided for her, keeps the young warm, and nurses them most of the time except during intervals when she must go to drink and eat her food. When, however, there is lack of milk flow and she feels she is unable to supply nourishment, the mother becomes very irritable and makes no attempt to save her baby rats. She either devours them or scatters them on the screen, so that the young are later found cold in the shavings of the galvanized pan below the screen. After the young have been separated from the mother any length of time, they naturally die. Sometimes the mother scatters the young on the platform of the feeding pan and completely ignores them.

Mattill and his associates[164] have studied the question with reference to the sterility produced in rats on rations consisting of milk and fat. Their results are of interest in that they show the effect to be related to the fat intake. On a milk diet that is high in fat (added lard) there is marked failure in reproduction, whereas on a milk diet that is low in fat (i.e., without the addition of lard) reproductive failure is not as pronounced. Mattill, Carman and Clayton do not regard this as evidence for the non-existence of vitamin E, but on the contrary believe that the amount of vitamin required for the normal reproductive functions depends upon the nature of the diet. They were able to prevent sterility by supplementing the deficient rations with 5 or 10 per cent of wheat embryo.

As regards the reproductive process, it is probable that not only vitamin E, but each of the other known vitamins exerts its own special influence.

Vitamin E is remarkably resistant to the effects of heat, light, and air. It is not destroyed when wheat germ is heated to 170° C., or

[163] J. Biol. Chem., **62**, 379 (1924–25).

[164] Mattill and Stone, N. C., J. Biol. Chem., **55**, 443 (1923).
Mattill, H. A., Carman, J. S., and Clayton, M. M., *ibid.*, **61**, 729 (1924).
Mattill, H. A., and Clayton, M. M., *ibid.*, **68**, 665 (1926).

when wheat-germ oil is distilled in superheated steam at 180°, or by distillation *in vacuo* at 233° C. At ordinary temperatures, acid and alkali exercise no injurious action. Even saponification with 20 per cent alcoholic KOH at 30° C. results in no great loss of the vitamin.

Summary.—In this chapter the requirements of proper nutrition have been considered. It has been pointed out that the organism should receive an adequate amount of food to supply its calorific needs. A sufficient supply of inorganic elements is likewise imperative. Fortunately, our ordinary food contains many of these in amounts that are in excess of the natural requirements, but frequently the supply of such elements as calcium, phosphorus, iron, and iodine may be deficient. The protein of the diet should be adequate, both from the standpoint of quantity and from that of quality. The biological value of proteins depends upon the presence of certain essential amino acids, without which the organism is unable to restore its worn-out tissue or to maintain nitrogen equilibrium. The vitamins are equally indispensable. In the absence of vitamin A, growth ceases, the normal integrity of epithelial tissues is not maintained and there is a lowered resistance of cells to injury and infection resulting in such disease manifestations as xerophthalmia. Moreover, it seems likely that vitamin A deficiency early in life may produce lasting effects on the ability of the organism to resist infection and disease later in life. Without the water-soluble vitamins B and G, there is marked loss of appetite. In man, vitamin B prevents beri-beri and vitamin G is believed to promote growth and prevent pellagra. In the absence of vitamin C, scurvy is produced. Vitamin D, which may be derived from ergosterol by ultraviolet light irradiation, is an important factor in controlling calcium and phosphorus metabolism and exerts a beneficial effect in curing rickets and osteomalacia. There is likewise evidence for the existence of a sixth vitamin or accessory food factor, vitamin E, which is believed to be essential to reproduction.

There are other aspects to the problem of nutrition that have not been considered and which in fact have been observed so recently that their significance is not yet fully appreciated. Thus, a new phase has been brought out by the studies of Sherman and Campbell [165] which indicate that a diet that is more than adequate, in the accepted sense, may have a direct influence in prolonging the normal life-span of experimental animals. In addition to this possible relationship of diet to longevity which would affect the individual, there is perhaps another relationship of even greater biological significance. It has been the experi-

[165] Proc. Nat. Acad. Sci., **14**, 852 (1928); J. Nutrition, **2**, 415 (1930).

ence of those [166] who have been engaged for many years in the study of nutrition and who have had an opportunity to observe colonies of white rats through many generations that under favorable dietary and environmental conditions the individuals of successive generations grow more rapidly, are larger and more flourishing. While the elements of selection and heredity are not to be excluded, there is obviously a nutritional factor which is responsible for the improvement of the stock. Those who are interested in individual and racial physical betterment may well be guided by scientific observations such as these.

[166] Osborne, T. B., and Mendel, L. B., J. Biol. Chem., **69**, 661 (1926); Mendel and Cannon, **75**, 779 (1927); see also Smith, A. H., and Bing, F. C., J. Nutrition, **1**, 179 (1929).

CHAPTER XVIII

THE COMPOSITION OF MILK AND CERTAIN TISSUES

Milk.—The young mammal depends for its nourishment almost entirely upon milk, which is probably the most complete single food found in nature. It contains protein, fat, in a finely emulsified state, the sugar lactose, inorganic salts, organic acids, certain non-protein nitrogenous constituents, and vitamins.

Milk is normally slightly acid in reaction, having a *p*H of approxi- 6.6 to 6.9.

Of the proteins in cow's milk, all but about 15 per cent is casein. The remainder is lactalbumin together with a small amount of lactoglobulin and traces of other proteins. The available data of the proportion of lactalbumin and casein in human milk lacks uniformity and further information on this point is needed. It is usually stated that the two proteins are present in about equal amounts. The protein of milk is derived from the amino acids of the blood, the synthesis occurring in the mammary glands.[1]

About 90 per cent of milk fat is composed of the glycerides of the higher fatty acids, including myristic, palmitic, stearic and oleic. The remainder consists of the glycerides of the lower fatty acids, butyric, caproic, caprylic, capric and lauric. Small amounts of other lipids are present, including lecithin, cephalin, cholesterol and free fatty acids. Milk fat is believed to have its origin in the phospholipids of the blood.[2]

The lactose of the milk (milk sugar) is derived from the glucose of the blood.[3]

The inorganic salts and other constituents are likewise derived from the blood, some by a process of simple filtration. The ash content of milk varies in different mammals, being, for example, much higher in cow's than in human milk. The elements contained in the ash are: Ca, P, K, Na, Mg, S, Cl, and traces of Fe, I, Cu, Zn, etc.[4] The amounts in

[1] Cary, C. A., J. Biol. Chem., **43**, 477 (1920).

[2] Meigs, E. B., Blatherwick, N. R., and Cary, C. A., *ibid.*, **37**, 1 (1919).

[3] Kaufman, M., and Magne, H., Compt. rend. de l'Acad. des Sciences, **143**, 779 (1906).

[4] In addition to these elements Wright and Papish (Science **69**, 78 (1929)), have reported the detection, spectroscopically, of traces of the following elements: Al, Mn, Si, B, Ti, Vd, Rb, Li and Sr.

which the more important of these are present is indicated by the following data:[5]

TABLE XLIX

	Cow's Milk, Per Cent	Human Milk, Per Cent
Phosphorus (inorganic)	0.087	0.0148
Calcium	0.144	0.0354
Magnesium	0.013	0.0030
Potassium	0.120	0.0711
Sodium	0.055	0.0147
Chlorine	0.076	0.0711
Total ash	0.725	.....

The factors which influence the yield and composition of cow's milk are: breed, age, stage of lactation, frequency of milkings, diet, pain, anxiety, fatigue, etc.[6] Human milk is similarly influenced by a variety of factors. The data in Table L show the limits of variation, as well as

TABLE L

	No. of Analyses	Specific Gravity	Water	Fat	Lactose	Total Protein	Casein	Albumin	Ash	Fuel Value per Lb., Calories
Cow's milk..	800									
Maximum.		1.0370	90.32	6.47	6.12	6.40	6.29	1.44	1.21	
Minimum..		1.0264	80.32	1.67	2.11	2.07	1.79	0.25	0.35	
Average...		1.0315	87.27	3.64	4.88	3.55	3.02	0.53	0.71	310
Human milk.	94									
Maximum.		1.0426	.....	9.05	8.89	5.56	.....	.....	0.50	
Minimum..		1.0240	.....	0.47	4.22	0.85	.....	.....	0.09	
Average...		1.0313	88.20	3.30	6.80	1.50	.....	.....	0.20	295
Goat's milk..	200									
Maximum.		1.0360	90.16	7.55	5.77	.....	3.94	2.01	1.06	
Minimum..		1.0298	74.47	2.81	2.76	.....	3.59	0.83	0.13	
Average...		1.0305	85.71	4.78	4.46	4.29	3.20	1.09	0.76	364

[5] According to Bosworth (J. Biol. Chem., **20**, 707 (1915)), the probable condition of these constituents in human milk is as follows: Calcium, in combination with protein, 0.014 per cent; calcium chloride, 0.059 per cent; mono-potassium phosphate, 0.069 per cent; sodium citrate, 0.055 per cent; potassium citrate, 0.0103 per cent; mono-magnesium phosphate, 0.027 per cent.

[6] Consult, E. B. Meigs, Milk Secretion as Related to Diet, Physiol. Rev., **2**, 204 (1922).

the average values, for the composition of cow's, human and goat's milk.[7]

Human milk differs from cow's milk in having less casein and ash and more albumin and lactose.

The secretion produced by the mammary glands for two to four days after the birth of the young is termed *colostrum*. It is a yellowish, alkaline fluid of greater viscosity and specific gravity than milk and has a much higher content of total solids, which in cow's colostrum may exceed 25 per cent. Albumin is the chief constituent, frequently forming more than 15 per cent of the colostrum. Colostrum exerts a purging effect on the new-born mammal.

Human colostrum contains 8 to 10 per cent protein and more inorganic constituents and less lactose and fat than milk. From the fifth day *post partum* until the end of the first month the milk shows a gradual change in composition, the protein and ash contents diminishing, whereas the amounts of lactose and fat tend to increase. The milk secreted during this period is often termed "transition" milk. "Mature" milk is secreted after the first few weeks. The limits of variation as well as the average composition of human milk at different periods is shown by the data in the following table (after Bell[8]):

TABLE LI

AVERAGE COMPOSITION OF HUMAN MILK AT DIFFERENT PERIODS

Time	No. of Cases	Protein			Sugar			Fat		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
		Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
5 days	88	1.45	2.83	2.00	4.62	7.37	6.42	0.9	8.2	3.2
9 "	88	1.12	2.65	1.73	4.76	7.65	6.73	1.6	7.1	3.7
3–4 wks.	35	1.03	1.79	1.37	6.17	7.89	7.11	1.4	6.1	3.6
5–6 "	32	0.98	1.57	1.30	5.97	8.33	7.11	1.3	7.6	4.0
7–8 "	14	1.04	1.40	1.21	6.25	7.83	7.11	1.1	7.0	4.0

[7] Leach, A. E., Food Inspection and Analysis, revised by A. L. Winton, 4th ed., John Wiley & Sons, Inc., 1920, p. 113.

[8] Bell, M., J. Biol. Chem., **80**, 239 (1928); see also Kleiner, I. S., Tritsch, J. E., and Graves, L. G., Am. J. Obst. and Gynec., **15**, 172 (1928).

Connective Tissue and Cartilage.—Connective tissue contains approximately 60 per cent water and 40 per cent solids. Of the latter, about 0.5 per cent consists of inorganic matter. The principal organic constituent of white fibrous connective tissue is the albuminoid collagen, which composes about 32 per cent of the tissue, the remaining 6–7 per cent being made up of elastin, mucoid, ether-soluble lipids, coagulable protein and non-protein nitrogenous constituents, or extractives.

The composition of connective tissue varies somewhat with age, the tissue of younger animals containing more water and mucoid and less collagen than that of older animals. On hydrolysis collagen is changed to gelatin.

The principal constituent of yellow elastic tissue is the albuminoid elastin, which forms about 30–32 per cent of the tissue. About 7 per cent of collagen is also present. The remaining constituents are the same as those found in white fibrous tissue.

Collagen is likewise a constituent of cartilage, which contains in addition chondromucoid, chondroitin-sulfuric acid, and another albuminoid. The following data are typical of the composition of cartilage:

	Per Cent
Water	68–74
Solids	26–32
Organic matter	25–30
Inorganic matter	1.5–2

Considerable variation in composition may be shown by cartilage from different parts of the body.

Bone.—Bone which is free from marrow contains 20 to 25 per cent of water. The organic matrix resembles the matrix of cartilage.[9] It consists principally of *ossein* which is probably identical with collagen, a mucoid, *osseomucoid*, and an albuminoid. These constitute about 40 per cent of normal, dried, marrowless bone, the remaining 60 per cent consisting almost entirely of calcium in combination with phosphate and carbonate. Magnesium (0.2–0.3 per cent), fluorine and traces of iron are present.

In chemical composition the *cement* and *dentine* of the teeth resemble bone, though the dentine contains less water. The *enamel* which is a derivative of epithelium, contains still less water, only about 5 per cent, and is the hardest structure in the body. It differs from bone in having a higher phosphorus content and a somewhat different organic matrix,

[9] For a brief though comprehensive account consult P. G. Shipley, "Cartilage and Bone," in Cowdry's "Special Cytology," vol. II, pp. 705–733.

for on boiling with water, enamel does not yield gelatin as is the case with bone.

According to recent studies of Taylor and Sheard [10] the solid inorganic phase of bone consists essentially of small crystals of mineral of the *apatite* group and may therefore be designated by the general formula $3Ca_3(PO_4)_2 \cdot CaX_2$, where X_2 ordinarily represents CO_3, F_2, $(OH)_2$, O, SO_4, or Ca. To some extent the last may be replaced by Mg. Taylor and Sheard's conclusions are based on the chemical analysis of the inorganic phase of bone and of apatite, the resemblance of their X-ray diffraction patterns and refractive indices.

Several theories have been advanced to explain the deposition of calcium in bone. One concept, associated with the names of Freudenberg and György,[11] is that calcium reacts with protein to form a calcium-protein compound which in turn combines with phosphate and carbonate to yield a calcium-protein-phosphate-carbonate complex. Much of the carbonate is supposed to react gradually with tissue acids, carbon dioxide being thus liberated. Eventually the calcium phosphate is also split off, presumably leaving the protein free again to combine with calcium and repeating the cycle.

As another explanation, it has been pointed out by Robison [12] that ossifying bone and teeth, as well as certain other tissues (kidney, liver, spleen, pancreas) contain an enzyme (*phosphatase*), capable of hydrolyzing organic phosphoric acid esters, e.g., the esters of hexose-monophosphoric acid and glycero-monophosphoric acid. The view has been advanced that this enzyme, by liberating inorganic phosphate from organic combination, plays an important part in the formation of bone.

It is clear that the mechanism of ossification is not fully understood as yet. Our knowledge of the subject is nevertheless far in advance of what it was only a decade ago. For example, it has been shown that calcification depends, not so much on the individual concentrations of calcium and phosphorus in the serum (practically all of the blood calcium is in the plasma or serum) as on the product of these concentrations.[13] Thus, if the calcium content is 9 mg. per 100 cc. of serum and the phosphorus concentration is 2.8 mg., the product is only 25.2, under which conditions bone formation is not to be expected. Low Ca × P products are characteristic of active rickets and osteomalacia, but just as soon as this product is increased to 40 or above, either as a

[10] J. Biol. Chem., **81**, 479 (1929).

[11] Biochem. Z., **142**, 1, 407 (1923).

[12] Biochem. J., **17**, 286 (1923); **18**, 740, 755, 1139 (1924); **19**, 153 (1925); **21**, 665 (1927).

[13] Tr. Am. Ped. Soc., **34**, 204 (1922); Monatschr. Kinderheilk., **25**, 279 (1923).

result of ultra-violet ray treatment, exposure to sunshine, administration of vitamin D, or improvement in diet, the process of healing of the bones begins immediately.

It was first observed by Shipley [14] that if an isolated piece of bone of a rachitic rat is placed in serum or plasma taken from normal animals, or from those in which rickets is in the process of healing, calcification begins in the bone in 48 hours. The new deposits of calcium are similar to those which are found in the bones of rats recovering from rickets. This simple demonstration of the *in vitro* healing of rickety bones is indeed remarkable.

A further step in this direction was made by Shipley, Kramer and Howland,[15] who obtained calcification *in vitro* by using solutions containing only inorganic constituents. Calcium phosphate was deposited in rachitic bone in a narrow zone across the epiphyses in a manner similar to that occurring in the body of an animal which is being cured from rickets. Calcification was obtained only when the Ca × P concentration exceeded 40; no calcification occurred when the product was below 40. It is to be noted that calcification may occur even though the calcium concentration is only 5 mg. per 100 cc., provided that the phosphorus concentration is at least 8 mg. Shipley, Kramer and Howland state that the process of calcification is not one of simple precipitation of an insoluble calcium salt, but that it depends on the activity of living tissue, protoplasmic poisons exerting a pronounced inhibitory effect.

Bone may be prepared for analysis by extracting the lipids with suitable solvents, drying and crushing the bone to a fine powder, at the same time avoiding the liberation of carbon dioxide. By determining the amount of carbonate in such bone, it is possible to calculate the amount of calcium present as carbonate. The difference between the total calcium and the carbonate calcium is called the " residual " calcium. This bears a definite relation to the phosphorus content, the ratio $\frac{\text{Residual Ca}}{\text{P}}$ being equal to approximately 1.94. This value would be exactly 1.94 if all the calcium of bone were combined only as carbonate and phosphate. Actually, of course, some magnesium is present which is also combined with phosphate and carbonate, and, on the other hand, a small portion of the calcium is combined with fluorine and possibly with other elements and radicals. Accordingly, a small deviation from the value 1.94 is to be expected. Yet it is remarkable how closely the analyses of bone are in agreement with this ratio.

[14] Johns Hopkins Hosp. Bull., **35**, 304 (1924).

[15] Biochem. J., **20**, 379 (1926).

The composition of the inorganic phase is essentially the same in pathological calcifications as it is in normal bone, as has been shown by Wells [16] and others. Recently Kramer and Shear [17] analyzed several specimens of pathological calcification (thyroid, tuberculous lymph node, cusp of the aortic valve, capsule of the spleen, lung and mesenteric lymph nodes) and found them to have the same composition as normal bone. The ratio between carbonate calcium to the total calcium was approximately 15 per cent, as in normal bone, and the ratio of the residual calcium to phosphorus ranged between 1.86 to 2.01, which is essentially within normal limits. Somewhat different results were obtained on analysis of a fibroid uterus. This specimen contained two kinds of calcification, one a chalky deposit, and the other a firm, tough deposit. The chalky deposit had the same composition as normal bone, the Ca : P ratio being 1.98, whereas the hard deposit had a ratio of 2.23. Similarly high ratios have been obtained by Kramer and Shear [18] in analyses of newly laid down calcium deposits, formed in healing rickets in rats, and of the bones of young rats. These authors state that the proportion of carbonate in normal rat bone increases with age and that the ratio of carbonate calcium to total calcium is about 8 to 10 per cent in the bones of young rats as compared with 15 to 16 per cent in the bones of adult rats. Kramer and Shear have attempted to explain the high Ca : P ratio in primary calcification by suggesting that $CaHPO_4$ is possibly laid down as such, admitting, however, that the actual presence of this substance in bone has not been demonstrated. On the basis of Taylor and Sheard's results, which seem fairly conclusive, the predominant constituent of bone would appear to be *podolite*, $3Ca_3(PO_4)_2 \cdot CaCO_3$, one of the apatite group of minerals. This, according to Shear, Washburn and Kramer [19] does not explain the normal carbonate calcium : total calcium ratio. There is thus still a good deal of uncertainty regarding the mechanism of ossification, although we are undoubtedly much nearer the solution of the problem than formerly.[20]

In considering the relation of bone to the organism it is to be clearly

[16] Arch. Int. Med., **7**, 721 (1911); Calcification and Ossification, Harvey Lectures, 1910–11, p. 102; Chemical Pathology, Philadelphia, 1925, p. 487.

[17] J. Biol. Chem., **79**, 121 (1929).

[18] *Ibid.*, **79**, 147 (1929).

[19] *Ibid.*, **83**, 697 (1929).

[20] Among the important papers, not previously referred to, dealing with the physico-chemical aspects of the subject are the following: Holt, L. E., La Mer, V. K., and Chown, H. B., J. Biol. Chem., **64**, 509, 567 (1925); Holt, *ibid.*, **64**, 579 (1925); Hastings, A. B., Murray, C. D., and Sendroy, J., *ibid.*, **71**, 723 (1926–27); Sendroy and Hastings, *ibid.*, p. 783.

understood that the mineral deposits of bone are by no means inert masses of material, but are, on the contrary, actively involved in the " swirl " of metabolism. There is a continual interchange of inorganic salts between bone, the circulation and other tissues and organs, this interchange being subject to certain equilibrium relations. Directly or indirectly, the deposition and depletion of the mineral constituents of bone are under the control of such diverse factors as the acid-base balance of the blood, the anti-rachitic vitamin and the hormone of the parathyroid gland. As an illustration of what may happen if the normal control of one of these factors is removed, reference may be made again to Wilder's interesting case of hyperparathyroidism (p. 402). Normally, dried bone contains 40 per cent organic and 60 per cent inorganic matter. The crest of the ileum of Wilder's patient contained 70 per cent organic and 30 per cent inorganic matter. Instead of a normal calcium content of about 22.8 per cent, the patient's bone contained only 11.2 per cent; instead of 14.6 per cent phosphorus, there was only 8.4 per cent; all of this impoverishment being due to hypersecretion of the hormone of the parathyroid glands.

The Skin.—Of the two principal layers which compose the skin, the lower layer, the *dermis*, or *corium*, is vascular and the upper layer, or *epidermis* is avascular. The epidermis, in turn, may be said to consist of four layers or strata, the deepest of which derives considerable nourishment from the blood vessels and lymphatics of the corium. This is the *stratum germinativum*, in which cells are continually formed and are displaced toward the surface of the skin, the cells forming successively the other three layers, the strata *granulosum*, *lucidum* and *corneum*. These cells, by the time they form the stratum corneum are essentially dead and are eventually lost by desquamation. In these transitions important chemical changes are involved, our knowledge of which is unfortunately far from being complete. The cells of the stratum germinativum are metabolically active, one index of this being the relatively high concentration of a substance, presumably glutathione, which gives the sulfhydryl (SH) group reaction. The water content which is greatest in the lowest layer of the epidermis diminishes in the upper layers, as the surface is approached. Granules of an albuminoid, called *keratohyalin*, are scattered irregularly in the stratum germinativum and are very abundant in the stratum granulosum. These granules fuse together in the stratum lucidum and undergo still more profound change in the stratum corneum, where the characteristic properties of the keratohyalin are lost and keratin is formed.[21]

[21] For a more detailed account, consult E. V. Cowdry, " The Skin and Its Derivatives," in Cowdry's Special Cytology, pp. 13–43.

Keratin is an albuminoid and is the chief constituent not only of the epidermis but of its derivatives, including the hair, nails, hoofs, horns, feathers, tortoise shells and the shell membrane of bird's eggs. Since the keratins from various sources differ somewhat in composition, it is to be assumed that there is not one keratin but a group of these albuminoids. Indeed, there is a strong probability that even in the same source there may be more than one keratin. A distinction has been made between the so-called keratin "A," which is so resistant that it is even insoluble in fuming nitric acid and in a mixture of sulfuric acid and hydrogen peroxide, and keratin "B," which is soluble in these reagents. The keratins are insoluble in the usual protein solvents and are not acted on by pepsin and trypsin. They give positive xanthoproteic and Millon's reactions. Cystine is the principal amino acid obtained on hydrolysis of keratin (when heated for a long time with strong acid).

A second important constituent of the skin, present especially in the deeper layers of the epidermis, is the pigment melanin, the chemistry of which has been discussed in an earlier chapter (p. 330). In small aggregates melanin appears yellowish-brown in color, but more dense masses appear black. It is present in larger amounts in negroes than in Caucasians. Melanin is deposited in the skin when one is sunburned. Besides its occurrence in the skin of man and animals, it is normally present as the pigment of the hair and the choroid of the eye. It is also found in many low forms of life, as in the black secretion of the squid.

The formation of melanin from its precursors is brought about by an enzyme, the existence of which in the epidermal melanoblasts of the skin has been demonstrated by Bloch.[22] This enzyme has been shown to produce melanin from 3 : 4 dioxyphenylalanine. The failure of melanin formation in albinos has been associated with the absence of this enzyme.[23]

In addition to melanin, the presence of a lipochrome has been described in skin and hair, to which is attributed the characteristic red coloration which is often seen in hair (Cowdry, p. 28).

[22] Z. physiol. Chem., **98**, 226 (1917). This enzyme was named *dopa* oxidase by Bloch, the term being derived from the initial letters of the name of its substrate, *di-oxy-phenyl-alanine*. The action of this enzyme is not limited, however, to the conversion of only this substrate into melanin. Compare with the work of Raper on tyrosinase, Physiol. Rev., **8**, 245 (1928); see also p. 331.

[23] Garrod describes albinism as an inborn error of metabolism. For a description of the nature of this condition, the student is referred to A. E. Garrod, "Inborn Errors of Metabolism," 2d edition, Oxford University Press, 1923.

The pathological occurrence of melanin is discussed by H. G. Wells in his "Chemical Pathology," Philadelphia (1925), Chapter XX.

All layers of the epidermis contain fatty substances, approximately one-fifth of which, according to Eckstein's analyses,[24] is free cholesterol. It, as well as the phospholipid fraction, is present in the skin in much greater proportion than in subcutaneous fat. Associated with cholesterol in the skin, as elsewhere, is ergosterol. When the body is exposed to direct sunshine, the ergosterol is converted into the antirachitic factor, as has been described in the preceding chapter. This may be the explanation for the beneficial effect of sunbaths.

The skin contains a wide variety of other organic and inorganic substances, including glycogen and mucin, sodium, potassium, calcium, magnesium, iron, silicon, arsenic, in traces, etc. The sebaceous glands produce a waxy secretion, called sebum. The perspiration is formed by the sweat glands and contains among its constituents many substances which are also found in the urine.[25]

Muscle.—Skeletal muscle contains approximately 75 per cent water and 25 per cent solids. Of the latter about 20 per cent is protein, the remaining 5 per cent consisting of lipids, carbohydrate, inorganic salts and the so-called extractives. Plain muscle has a somewhat higher water content (80 per cent) than striated muscle and contains more nucleoprotein and less creatine. The two kinds of muscle are also said to differ in their proportions of sodium to potassium. Other differences have been described, such as the relative amounts of the various soluble proteins, but these differences are not so well defined.

Concerning the nature of the muscle proteins there is still a great deal of confusion partly because the work of various investigators (Halliburton,[25a] von Fürth,[26] etc.) has not been adequately correlated. An attempt along this line has been made by Howe,[27] whose conclusions seem worthy of consideration.

The maximum extraction of muscle protein is obtained with a 0.225 to 0.525 molar solution of potassium phosphate. (The solution used by Howe consists of a 1 : 2 mixture of KH_2PO_4 and K_2HPO_4 suitably diluted; the *p*H of this solution is 7.0.) In a 1.125 molar solution less protein is extracted. The protein present in the 0.225 to 0.525 molar phosphate extracts, but not in the 1.125 extract corresponds to Halliburton's *paramyosinogen* (or von Fürth's *myosin*). It coagulates at

[24] J. Biol. Chem., **69**, 181 (1926).

[25] The composition of sweat under various conditions has been recently studied by Talbert and associates, Am. J. Physiol., **81**, 74, 81 (1927); **84**, 577 (1928); **85**, 224 (1928) and earlier papers in this journal.

[25a] J. Physiol., **8**, 133 (1887).

[26] Arch. exp. Path. u. Pharmakol., **36**, 231 (1895); **37**, 389 (1896); Ergebnisse der Physiol., **1**, Abt. I, 110 (1902); **2**, I, 574 (1903).

[27] J. Biol. Chem., **61**, 493 (1924).

47° C. The isoelectric point is said to be *p*H 5.15.[28] Actually, paramyosinogen probably consists of at least two separable protein fractions. In some of its properties, particularly in its solubility, paramyosinogen is analagous, at least in part, to the fibrinogen fraction of the blood.

On extracting muscle in 1.725 molar phosphate another fraction remains behind which is found in the 1.125 molar extract. This fraction corresponds to Halliburton's *myosinogen* (or von Fürth's *myogen*). It coagulates at 56° C. The isoelectric point is 6.3.[28] Myosinogen is analagous, in its properties, to two of the globulin fractions (euglobulin and pseudoglobulin I, see p. 216) of the blood plasma. On these grounds, Howe is of the opinion that " myosinogen " consists of at least two protein fractions.

Myoglobulin (Halliburton) corresponds, according to Howe, to the fraction precipitated between 1.725 and 2.025 molar potassium phosphate. This protein is apparently analogous to the pseudoglobulin II fraction of the plasma.

The protein present in the 2.025 molar phosphate extract has the same precipitation limits as egg or serum albumin. It is *myoalbumin.*

The proteins which are thus extracted from freshly minced muscle constitute the protein of what is often termed the " muscle plasma." On standing muscle plasma forms a clot due presumably to the conversion of " paramyosinogen " and " myosinogen " into " myosin," or muscle fibrin. The same process may occur in the muscle itself. Living muscle is regarded as a semi-fluid muscle plasma. As a result of death, the soluble proteins, at least certain of the fractions, undergo clotting, being converted in the process into insoluble protein. This change is an accompaniment of *rigor mortis.* Eventually, the rigor disappears due to enzymic (autolytic) action on the protein.

Muscle stroma consists of the proteins insoluble in water and neutral salts, including nucleoprotein. It also contains lipids, the phospholipids predominating. Howe's analyses of cattle and rabbit's muscle show that about one-half of the total protein is " soluble " protein, of which the major part (80 to 90 per cent consists of the globulin fractions (paramyosinogen, myosinogen and myoglobulin), the remainder consisting of albumin (or albumins). A pigment, *myochrom,* is present in muscle, especially in the red variety, a large part of which is probably related to the heme compounds.

Boiling water extracts from muscle both inorganic salts and a variety of organic compounds, the latter being termed " extractives." Of these, creatine, creatine-phosphate, creatinine, inosinic acid, adenylic acid,

[28] Weber, H. H., Biochem. Z., **158**, 443, 473 (1925).

glutathione, various purines, such as hypoxanthine, etc., have been described in other connections. The amount of creatine in human skeletal muscle is about 350 to 400 mg. per 100 grams, and in smooth muscle (such as that of the human uterus) about one-fifth as much. About 5 to 10 mg. of creatinine per 100 grams is present in striated muscle and somewhat smaller quantities in smooth muscle. The nitrogenous base carnosine, $C_9H_{14}N_4O_3$, has been isolated from meat extracts. On hydrolysis it yields histidine and β-alanine. Another base is carnitine, $C_7H_{15}NO_3$, which is a derivative of betaine. The non-nitrogenous organic extractives include glycogen, the hexahydric alcohol, inosite, or inositol, $C_6H_6(OH)_6$, the various hexose-phosphates described in the discussion of carbohydrate metabolism, a small amount of *l*-lactic acid (absent in resting muscle) and *d*-lactic, or sarcolactic acid.

The inorganic constituents (found in the ash) of striated muscle include potassium (0.25 to 0.4 per cent), sodium (0.06 to 0.16 per cent), magnesium (0.02 to 0.03 per cent), chloride (0.04 to 0.08 per cent), sulfur (0.19 to 0.23 per cent), and phosphorus (0.17 to 0.25 per cent).[29] The sulfur is practically all present in organic combination in protein. In striated muscle about 80 per cent of the phosphorus is inorganic and the remainder organic. The relation is very different in smooth muscle, where the inorganic phosphorus is frequently 40 per cent, or less (as in uterine muscle) and the organic phosphorus about 60 per cent. The buffering power of smooth muscle is said to be less than that of striated muscle.

More sodium chloride and less potassium chloride is present in plain muscle than in striated muscle. The ratio of sodium to potassium in the former is 1 : 1.5, whereas in the latter it is 1 : 5.[30]

Brain and Nerve Tissue.—An outstanding difference in the composition of the gray and white matter of the brain is the water content, which in the former varies, on an average, between 83 to 85 per cent and in the latter between 68 to 73 per cent. The white matter, accordingly, contains 27 to 32 per cent solids; these are distributed as follows:

(1) protein, 7 per cent, including globulin and nucleoprotein, the latter amounting to about 3.7 per cent.
(2) neurokeratin, an albuminoid, 3 per cent.
(3) lecithin, 5 per cent.
(4) cephalin, 3.5 per cent.
(5) cerebrosides, including phrenosin and kerasin, 5 per cent.

[29] For detailed analyses consult Meigs and Ryan, J. Biol. Chem., **11**, 401 (1912).

[30] The reader is referred to the following reviews: D. M. Needham, Red and White Muscle, Physiol. Rev., **6**, 1 (1926); C. L. Evans, Physiology of Plain Muscle, *ibid.*, p. 358.

(6) cholesterol, 5 per cent.

(7) inorganic matter, 0.8 per cent, as follows: potassium, 0.3 per cent; sodium, 0.02 per cent; chloride, 0.1 per cent; magnesium, 0.02 per cent; calcium, 0.01 per cent; iron, 0.006 per cent.[31]

The total solids of the gray matter approximate 15 to 17 per cent and are distributed as follows:

(1) protein, 8 per cent, of which the nucleoprotein is 3 per cent.
(2) neurokeratin, 0.4 per cent.
(3) lecithin, 3 per cent.
(4) cephalin, 0.7 per cent.
(5) cerebrosides (phrenosin and kerasin), 3 per cent.
(6) cholesterol, 0.7 per cent.
(7) inorganic matter, 0.8 per cent.

Other important constituents are: sphingomyelin, sulfolipids, aminolipids and various extractives, creatine, creatine-phosphate, inosite, amino acids and organic acids.

The spinal cord contains a greater proportion of unsaturated phospholipids than any other part of the central nervous system. The water content is 74 per cent and the total amount of lipids is about 18 per cent. The peripheral nerves, on the other hand, contain only about 60 per cent water. Medullated fibers have more cerebrosides than phospholipids, whereas the reverse relationship is present in the non-medullated fibers. Approximately the same amount of neurokeratin is contained in peripheral nerves as in the gray substance of the brain.

[31] These data are based chiefly on those compiled by P. Hari, in his "Kurzes Lehrbuch der physiologischen Chemie," Berlin, 1928, p. 223.

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