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A TEXTBOOK OF BIOCHEMISTRY

FOR STUDENTS OF MEDICINE AND SCIENCE

BY

A. T. CAMERON

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PREFACE TO THE FOURTH EDITION

A TEXTBOOK of biochemistry, even if fresh editions appear every two or three years, must inevitably suffer from one of two faults. If the author waits for certainty, the book will always be a step or two behind the advances; each year important advances are made, which should not be ignored. If the author attempts to keep the book up to date, he must incorporate accounts of recent work which still lacks complete confirmation, and some of which will possibly be refuted. If he attempts to present recent work so carefully that he can escape criticism if some part of it is disproved, the cautious and hesitating method of presentation necessitated will scarcely tend to that clarity needed by students who are being introduced to the subject.

I prefer to risk incorporating as much pertinent recent work as possible, and can only hope to be sufficiently fortunate to include but little that will need retraction.

In this edition the chapter on the endocrine secretions has been very largely revised. The many recent discoveries concerning the vitamins necessitated the almost complete rewriting of the chapter concerned with them. The new and important ideas concerning the constitution of the sterols, which now link up with these compounds the endocrine principles secreted by the organs of reproduction, have been briefly dealt with. Minor changes have been necessary in almost every other chapter.

I have carefully reconsidered the problem of to what extent references should be given in a text-book of this nature. Full references are an obvious necessity for the teacher. I cannot believe that it is desirable to refer the student, in his early years of medicine or science, to long lists

of references. The policy of the previous editions has therefore been maintained. A few selected references—reviews where possible—are given at the end of most of the chapters. Such reviews themselves usually provide a full bibliography. Considerable use has been made of the first (1932) volume of the "Annual Review of Biochemistry," in which all recent work has been adequately dealt with and completely documented.

A. T. CAMERON.

WINNIPEG.

PREFACE TO THE THIRD EDITION

BIOCHEMICAL progress is so rapid that in even the two years since the appearance of the last edition new work has necessitated a considerable degree of revision, affecting almost every chapter.

The treatment of biochemical catalysis, the chemistry of respiration, enzymic synthesis, and the vitamins has been extended. It has been considered desirable to deal with the endocrine secretions at greater length, and in a separate chapter, although this completer treatment necessitates the inclusion of material which is not strictly biochemical. A new chapter has been added on tissue respiration and muscular activity; it seemed most convenient, although not perfectly logical, to place this at the end of the section dealing with quantitative metabolism.

These additions have somewhat increased the size of the book, but it is hoped that their inclusion will render this edition still more useful, both as a text for medical students, and as a general introduction to the science.

I have again to express my thanks to Messrs. J. & A. Churchill for their kind and helpful co-operation.

A. T. CAMERON.

WINNIPEG, CANADA.

PREFACE TO THE FIRST EDITION

THIS little textbook is based on lectures that have been given for a number of years to students of medicine who have just previously completed a special course of organic chemistry. A sufficient knowledge of organic chemistry is assumed. The requisite physical chemistry is introduced throughout the book as it becomes necessary. It is assumed also that the student is simultaneously taking a course in practical work, for which many excellent practical textbooks are available.

I have endeavoured to compass in small volume such a knowledge of the subject as is required by the student of Medicine *before* proceeding to the study of Pathology and Internal Medicine. So much is now demanded of this student in so many different subjects that it seems reasonable to limit the treatment of biochemistry to the essentials which will be useful to him.

As far as possible the essential feature of a good course of lectures has been preserved—no knowledge of one part of the subject has been assumed before this has been dealt with. This, and the incompleteness of our present knowledge, perhaps have led to an order of arrangement which is not completely logical, but I have found it most suitable in teaching, and believe that it permits the subject to be read most easily.

I have not considered it desirable to puzzle the student in the early years of Medicine by emphasis of the many points on which there still exists lack of harmony amongst active biochemists. Of necessity, therefore, the treatment adopted is somewhat dogmatic ; equally of necessity I have endeavoured to present as accurate a series of statements as pos-

sible. Where there is grave conflict of opinion this has been indicated.

While undoubtedly the student should be introduced to the original literature of any subject at an early stage, I have considered it more desirable in this little book to give references to monographs where these are available than to series of scientific papers. Should the student read these he will undoubtedly find some contradictions, both between them and with this textbook. The subject is advancing rapidly.

Certain chapters, and other interspersed material, are printed in small type. These are cognate to the subject, but less essential to the student of Medicine. It is hoped that they may make the book more useful as an introduction to biochemistry for students of Science, and also more useful to students and graduates of Medicine who may wish for a wider knowledge of the subject than is frequently presented to them.

At the present time, fortunately, students of Medicine and of Science are wasting less and less time in the acquisition of a fragmentary knowledge of the dead languages, but, unfortunately, scientists still seize every opportunity to coin new words and hybrids from these dead tongues, and so to render a scientific treatise in our language as un-English as possible. I have endeavoured throughout to indicate the sources and meanings of such terms, to enable, at any rate, some understanding of the ideas underlying the coinage of them.

In spite of many efforts to break down the watertight compartments of the modern sciences, they continue to be impervious with remarkable tenacity. Every effort to destroy them should be of some use, and I have added several chapters on borderline subjects with this aim in view.

I wish to acknowledge my indebtedness to all those monographs and larger treatises from which I have consciously and unconsciously acquired assistance. To many of them

references are given for further reading. I wish especially to mention that mine of information, Hammarsten's "Lehrbuch der physiologischen Chemie," whose ninth German edition I have repeatedly consulted.

I hope to deal with the chemical phases of endocrinology and the most important pathological features of biochemistry in a later volume.

My colleagues, Professors H. P. Armes, V. H. K. Moorhouse, and F. D. White, have read through the whole manuscript, while Professors Cadham, O'Donoghue, and Shipley, and Dr. W. G. Mackersie have read through chapters related to special subjects, and my thanks are due to all of them for this kindness, and for the numerous valuable suggestions that they have made. Professor Wm. Boyd very kindly had made from my preparations the microphotographs shown in the two plates.

My thanks are also due to Dr. J. B. Sumner and the Editors of the *Journal of Biological Chemistry* for permission to reproduce Figure 1 from that Journal, to Dr. E. F. Du Bois and the American Medical Association Press for permission to reproduce Figure 11 from the *Archives of Internal Medicine*, and also to Dr. Du Bois for permission to reproduce Figure 12 from his book "Basal Metabolism in Health and Disease."

I wish also to express my appreciation of the constant co-operation of Messrs. J. & A. Churchill. This has materially diminished the difficulties due to the great distance between publishers and author.

A. T. CAMERON.

WINNIPEG, CANADA.

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SECTION I

INTRODUCTION, AND SOME PHYSICAL-CHEMICAL CONCEPTIONS

CHAPTER I

INTRODUCTION. A BIRD'S-EYE VIEW OF THE SUBJECT

THE term *biochemistry* means the chemistry of life (Gk. *bios*). In studying biochemistry we study living matter (and matter which once was living) from the point of view of a chemist. That is to say, we try to ascertain of what compounds living matter is composed, how these compounds are formed and how destroyed, and what functions they carry on—in other words, how life proceeds chemically (and physico-chemically).

To this study we have to bring all the resources of chemistry and of physical chemistry, both their pertinent facts and their varied technique, and along with these we must apply, whenever advantage can be obtained, the facts of physiology, of botany, of zoology, of bacteriology, of pharmacology, and of pathology.

Biochemistry is the mortar which is building together the bricks, the facts of other sciences, into a structure of life, of which, when we have built far enough, we shall recognise the design. But, however much we have to draw upon these other sciences, we must remember that this is essentially a

chemical structure, viewed with chemical eyes, and built upon firm chemical foundations. And unless a sure knowledge of these chemical foundations has been acquired, of the facts embodied in general and inorganic chemistry, and organic chemistry, then no adequate conception can be obtained of the superstructure.

Again, in order to decipher easily the inscriptions upon the superstructure, we must know the alphabet. Our alphabet is the alphabet of organic chemistry. We must be able to read, write, and comprehend constitutional formulæ, or else we can make no intelligent effort to understand the language of biochemistry.

What is to be gained from such a study, beyond acquiring a knowledge of some portion of the truth of life ? Such an acquisition, to the philosopher, is of itself a sufficient reward for much painful effort, but at the end of the first quarter of the twentieth century we have little time to be philosophers (the practice of philosophy is far different from study of the so-called philosophies).

In the first place, biochemistry is rapidly becoming the handmaiden of the new science of medicine, that science which is placing the practice of medicine on a firm basis which is no longer empirical. Primarily, for students of medicine, the facts of biochemistry must be applied to man, to a study of his normal living, and of his pathological living, to the cause of disease, the diagnosis of disease, and, whenever possible, to lend assistance in the cure of disease. Most of the facts that apply to man apply also to the lower animals, and biochemistry must equally be the handmaiden of veterinary medicine, when that becomes an exact science.

The subject is involved in, and in fact dominates, the study of diet in all its bearings, including such crude applications as are at present made in what is termed " home economics." We shall have to deal with food from every standpoint, composition, preparation, physiological value, economic value.

Biochemistry has a wide application in agriculture, in all that concerns both animals and plants ; it includes the study of the chemical facts of normal plant growth and composition, and may have much to contribute in the study of plant diseases and their causes.

In all probability the biochemist who has specialised in this subject itself will, in the future, be more extensively used in industry than is the ordinary chemist of to-day. For we are slowly acquiring command of a most powerful chemical engine, the enzymic actions of bacteria, of the fungi, and of moulds, and are beginning to realise what tremendous chemical changes can be brought about easily by these simple organisms. Whilst such a biological engine has been employed by man ever since he realised that beverages containing alcohol had an important beneficial pharmacodynamical action, it is only recently that this type of action has begun to have the attention that it merits.

Biochemistry is the newest of the sciences. It is true that the Iatro-chemists—the medical chemists of whom Paracelsus (1493–1541) is perhaps the most outstanding figure—believing that human illnesses result from abnormal chemical processes within the body, which could only be counteracted by appropriate chemical remedies, had a biochemical concept (however erroneous), but they started the study of pharmacology, rather than of biochemistry, although, of course, pharmacology can quite properly be regarded as applied biochemistry—the study of the action of specific chemical compounds on the living organism.

The ovum of biochemistry was fructified when Liebig and Woehler synthesised urea, and so commenced destruction of the belief that there existed a class of chemical compounds which require vital force, some unknown power within the living body, for their production. And biochemistry may be said to have been born during the late decades of last century. But, although the first chair of Physiological Chemistry was established in Strasbourg in 1883 we cannot regard the

subject as having outgrown its swaddling clothes and gained freedom from its foster-parent Physiology until in this century in America Folin, and Van Slyke, and others, by development of a refined technique, showed its immediate and important application in clinical medicine. Since which time its progress has been rapid. This lusty child has grown so fast in the last dozen years that no one can foretell its growth in the next.

A Bird's-eye View of Biochemistry. Any living organism, as simple as the amœba or as complex as ourselves, manifests in living two important and distinct types of activity, the first, the reproduction of its kind, the second, the transformation of food material received from without it into material for its own growth and repair, and into heat and external work. The first activity becomes relatively a less important factor in the whole life-period of the organism, the higher the species is in the scale of evolution; the second constitutes by far the greater part of our chemical study.

We have to study the nature of the chemical processes whereby the organism converts the potential energy of its food into the work and heat that it produces; since these chemical processes also involve the " wear and tear " of the organism itself, we must study also, as far as we can, the chemical degradations of the organism and its chemical repairs. All these processes are fundamentally comparable, whether carried out by the single-celled organism, or by man himself. In *all* organisms there occurs the following series of events: conversion of food material into such a form that it can be absorbed within the body wall, utilisation of the absorbed food products for repair, and for the production of energy (work and heat), and rejection from the body of the products formed from the cell degradations and during the chemical actions involved in the production of energy.

The formation of heat obviously involves oxidations. In

most organisms the necessary oxygen is derived from the atmosphere ; part of our study deals with the mechanism by which this oxygen is made available where it is required.

Man lives on a very varied diet. Chemical analysis resolves it into several important classes of compounds : carbohydrates and fats (which are built up from the three elements, carbon, hydrogen and oxygen), proteins (which contain, in addition, nitrogen, usually sulphur, and sometimes phosphorus and other elements), certain inorganic salts, vitamins (which are organic compounds with specific and important properties, though we do not yet know their chemical nature), and, by no means least, water and oxygen.

The first three of these classes are eaten in large part in forms in which they are insoluble and incapable of passing through an animal membrane. It is essential that we learn to regard the alimentary tract of an animal as a tube formed of animal membrane and passing from mouth to rectum, whose contents are not truly within the body, but outside it. The function of the tube is to enable certain chemical processes to be carried on which will break up the complex molecules of proteins, fats, and most of the carbohydrates, into simpler ones, soluble in watery fluids, and, in such solutions, capable of passing through an animal membrane. These changes are brought about by chemical compounds manufactured in different glands, such as the salivary glands and the pancreas, and poured out by ducts into the alimentary canal. These compounds are called *ferments* or *enzymes*, and their behaviour is comparable to that of inorganic catalysts, which, it will be remembered, cause various chemical actions to proceed more rapidly. By the action of a particular enzyme the complex molecule of starch, whose formula we may write

$$(C_6H_{10}O_5) \text{ multiplied by } n$$

where n is an unknown number, but large, is caused to unite with many molecules of water until most of it has been

changed to the sugar maltose, $C_{12}H_{22}O_{11}$; this can be broken down by a second enzyme to twice its number of molecules of the sugar glucose, $C_6H_{12}O_6$. A third enzyme will cause a molecule of cane sugar, $C_{12}H_{22}O_{11}$, to unite with a molecule of water and so break up into a molecule of glucose, and another of the similar sugar, fructose, also $C_6H_{12}O_6$. Various enzymes will cause molecules of protein to combine with water and break up into a mixture of much simpler compounds that we call amino-acids. Similarly, fat molecules are caused to react with water and break up to the compound glycerol, and fatty acids such as stearic acid.

As these processes go on the mixture of food and digestive juices in the alimentary canal gradually becomes more fluid, and absorption of a large part of the fluid (along with the substances in solution in it) takes place in the intestine. Then these absorbed food products are carried by the circulating fluid of the body—the blood—to all the body cells. At the same time oxygen is absorbed through the lungs and conveyed in chemical combination in the blood to all these body cells. These bring about a multitude of chemical reactions, a large proportion of which are oxidations. Some of the chemical actions are governed by nerve control of particular cells, others are influenced or caused to take place by the " internal secretions," which are a number of very powerful chemical compounds, manufactured by particular glands, such as the thyroid, the pancreas and the adrenal bodies, and poured out directly into the circulating blood. This multitude of different chemical actions constitutes what we call " metabolism " or " intermediate metabolism " (Gk. *metabole*, change). Those which involve oxidation necessarily involve heat production, and the temperature of the body results from a balance between the heat produced by the millions of individual oxidations going on in the millions of cells in the body, and the heat lost from its surface.

The different chemical reactions result in two types of

products, either more complex compounds retained in the cell as part of itself (its own protoplasm), or for its own processes (cell enzymes), or poured forth for specific purposes (enzymes and internal secretions), or stored (glycogen, fat), or, on the other hand, degradation and waste products, of which the most important are carbon dioxide, water, and urea. These pass from the tissue cells into the circulating blood, and the amounts of them which it carries are perpetually kept within bounds by the lungs (which remove carbon dioxide and water), the kidneys (removing water and urea), and the skin (water). The bulk of the soluble *excreta* are removed by the kidneys, though certain compounds are excreted through the intestines and form part of the fæces.

Since our study is primarily concerned with the chemical processes involved in the transformations of potential to kinetic energies, by however involved and devious routes, it is not surprising that it has been found that the law of conservation of energy holds just as truly in living as in non-living processes. The body neither creates nor destroys energy, and accurate chemical experiments studying the intake and output have demonstrated the truth of this very exactly. We shall have to take some note of these experiments. From the quantitative standpoint also we must take into consideration the energy needs and the total food needs of the individual. Certain parts of a dietary, such as vitamins and salts, are perhaps not directly involved in these energy transformations, but unless the body is furnished with them in correct amounts it cannot guide its energy transformations correctly.

There is a very close connection between the chemistry of normal and pathological living processes ; frequently study of the pathological processes has yielded clues to the normal. To some extent our outlook must therefore be extended to certain of the more essentially chemical diseases, amongst which diabetes mellitus stands out pre-eminently.

No attempt will be made in this volume to deal in any detail with the problems of plant chemistry. Generally speaking, plants have a much greater and more varied power of synthesis than have animals, and they alone, in virtue of their chlorophyll content, possess the power of storing the radiant energy of sunlight in the form of the potential energy of complex carbohydrates.

CHAPTER II

CATALYSIS AND BIOCHEMICAL CATALYSIS

" *It has been whimsically said that life is just one enzyme reaction after another.*" (From Walton's preface to Waldschmidt-Leitz's " Enzyme Actions and Properties.")

Catalysis. Ordinary reactions may be divided into two classes. The first class consists of the practically instantaneous reactions exemplified in ionised solutions, such as the formation of silver chloride when solutions of sodium chloride and silver nitrate are added together, or the formation of unionised water which occurs in the neutralisation of a strong acid by a strong base.

The second class of reaction requires a measurable time. We may take as an example the saponification of such an ester as ethyl acetate. This reaction with water never reaches completion, but after many days an " equilibrium " is attained.

A *catalyst* (Gk. *kata*, down ; *lysis*, loosing, setting free) alters the *rate* of reactions of this class, and usually accelerates it. It is necessary to bear in mind the essential factors in catalytic actions in discussing enzymes ; these will, therefore, be recapitulated briefly.

Examples of catalysis are the hydrolysis of cane-sugar to a mixture of glucose and fructose in the presence of mineral acids (that is, of a fairly high concentration of hydrogen ions), the rapid combination of hydrogen and oxygen gases in the presence of finely divided platinum, and the liberation of oxygen from hydrogen peroxide in the presence of ferrous and manganous salts.

One of the most important effects in catalytic action is

the shortening of the time of reaction. Thus a mixture of ethyl acetate and water changes *very slowly* into a mixture of ethyl alcohol and acetic acid, until after many days an *equilibrium* is reached :

$$C_2H_5.O.CO.CH_3 + HOH \rightleftharpoons C_2H_5OH + HO.CO.CH_3.$$

At the point of equilibrium the reaction proceeds at an equal rate in each direction, so that the concentration of each of the four substances remains constant. If the hydrogen ion concentration is considerably increased by the addition of a small quantity of hydrochloric acid, equilibrium is attained within a few hours, but *the point of equilibrium is practically unaltered.* Catalysts will accelerate such a reversible reaction from whichever end it is started ; the same equilibrium point is finally reached.

Another of the essential points about a catalytic reaction is that a small quantity of catalyst will produce an effect on very much larger amounts of the reacting compounds. Thus colloidal platinum will catalyse a million times its weight of hydrogen peroxide. In many instances it can be demonstrated that the concentration of catalyst has not been altered at the end of the reaction ; it must not necessarily be concluded from this that the catalyst has played no chemical part in the reaction. Again, the degree of acceleration depends to some extent on the concentration of the catalyst ; the more of the catalyst there is present the faster will be the reaction.

Whether or not any catalyst can actually initiate a reaction is still an unsettled question. When finely divided platinum brings about the rapid union of a mixture of hydrogen and oxygen, it is usually considered that, in the absence of the platinum, there is a very slow combination taking place. Such theoretical explanations can be stretched too far, and there is reasonable ground for belief that at least certain biochemical catalysts can cause reactions to proceed which would not do so in their absence.

Mechanical Parallel. Various mechanical parallels have been suggested by different writers. The following is one of the simplest :—

Many of the properties of catalysts are exemplified in the behaviour of a brass weight—of 500 gm., say—placed at the top of a sheet of flat glass, which is inclined at such a slope that the weight slowly moves downwards. This movement can be considered to represent a slow reaction. If to the bottom of the weight is applied a little oil (representing the catalyst) then the weight moves downwards much more swiftly. The oil has accelerated the action. But in either case, with or without the oil, the weight sooner or later reaches the bottom, its position of equilibrium. The more oil that is applied (up to a certain limit) the faster is the fall, but the work done (depending on the actual weight and the height fallen through) is independent of the oil.

Some of the oil sticks to the glass on the way down. In certain reactions, such as those which occur when the oxides of nitrogen catalyse the formation of sulphuric acid, the catalyst is slowly transformed into something which cannot catalyse.

We can select such an angle of the glass plate that the weight will not move *unless* oil is applied to it. This parallels the initiation of a reaction by a biochemical catalyst.

We may therefore define a catalyst as a substance which changes the rate of a reaction, and which, in addition, in some cases may remove inhibiting factors which normally prevent the reaction from proceeding. The meaning of the term—" unloosener "—is in harmony with this wider conception.

But if we admit the possibility that a catalyst can cause a reaction to commence, we must be careful to distinguish such action from the so-called " trigger-action." Thus we may have a weight held by a trigger release at the top of a steep incline. Immediately the trigger is pulled the weight falls, but the trigger plays no further part in the fall, and does not govern the rate of fall in any way, nor does the work done in pulling the trigger bear any relation to the work accomplished by the falling weight. An example of trigger-action is the addition of a crystal to a supersaturated solution of the same salt, when crystallisation proceeds almost instantaneously.

Two distinct types of mechanism seem to be possible with catalysts. They may take part in the chemical reaction, by

bringing about various intermediate stages (compare the action of the oxides of nitrogen in the formation of sulphuric acid), or they may act merely by physical means, through bringing molecules of the reacting substances into closer juxtaposition (as with the *adsorption* of hydrogen and oxygen on the *large* surface of spongy platinum).

While the great majority of catalysts accelerate reactions, some, termed *negative catalysts*, lengthen the time of reaction or suppress the action altogether. When a stick of phosphorus is exposed to the atmosphere it slowly oxidises. The presence of a trace of ether vapour stops this oxidation. While traces of hydrogen sulphide markedly increase the rapidity of oxidation of stannous chloride exposed to air, manganese and chromium salts and certain organic compounds such as mannitol and aniline greatly reduce the speed of oxidation. It has been suggested that a " negative catalyst " produces its results indirectly, by suppressing the action of some true catalyst.

Biochemical Catalysis

The preparation of alcohol and carbon dioxide from sugar by yeast is one of the oldest known chemical reactions brought about by a living organism. Similar long known reactions are the bacterial transformation of lactose to lactic acid which occurs in the souring of milk, and of wine (a solution of alcohol) into vinegar (a dilute solution of acetic acid), and the transformation of starch into glucose by germinating barley. The *rôle* of the living organism in producing these reactions was first definitely recognised by Pasteur.

In 1832 Payen and Persoz prepared an extract from barley malt which converted starch into sugar just as do strong acids, although no living cells were present. This extract they called *diastase* (whence, until quite recently, French writers have called all enzymes *diastases*). They made the extract by macerating the germinating barley with water, and adding to the clear solution alcohol, which precipitated the diastase as a white powder, soluble in water.

Later, a series of analogous preparations were made, such as that of *pepsin*, from gastric juice, which would digest meat in glass vessels in the same way that it is digested in the stomach, of *trypsin* from pancreatic juice, with a similar effect on meat, and of *emulsin* from bitter almonds, which hydrolyses glucosides into glucose and other constituents.

The original actions in which the living cell is present were called *fermentations*, from the liberation of carbon dioxide, in the action of yeast on sugar, producing a frothing resembling boiling (L. *fervimentum*, boiling). Pasteur showed that this fermentation was due to living organisms, and yeast and other living organisms that bring about such changes were spoken of as " organised ferments." Diastase, and similar preparations, were called for distinction " soluble " or " unorganised ferments," and later, to prevent confusion, *enzymes* (Gk. *en zyme*, in yeast).

The resulting controversy as to whether an essential difference existed between organised and unorganised ferments was settled by Buchner in 1897. He proved that by the application of great pressure to ground up yeast it was possible to express a liquid which contained no cells, but which possessed all the fermentative properties of the original yeast.

Since that time, by similar means, many of the ferments have been extracted from living cells, and it has been amply demonstrated that, although these ferments are produced by the living cells, once they have been produced life itself is unnecessary for their actions.

All these compounds are therefore of one class, and we may call them ferments or enzymes as we choose. Neither term is satisfactory. Most of them do not produce an effervescence resembling boiling, and the vast majority of them do not occur in yeast. As we shall see, they produce catalytic actions, and they may be more correctly termed *biochemical catalysts*, or *bio-catalysts*.

Not only do they produce catalytic actions, but frequently they are much more powerful than corresponding inorganic catalysts. Thus, E. F. Armstrong showed in 1904 that a preparation of *lactase*, the ferment that acts on milk-sugar (lactose), producing from it two simpler sugars, would hydrolyse one-quarter of the lactose contained in a certain volume of 5 per cent. solution in one hour at 35° C., while a twice normal solution of hydrochloric acid required at the same temperature five weeks to produce the same degree of change.

These compounds, for convenience, will henceforth be referred to as *enzymes*. Each enzyme is named after the compound (or class of compounds) it acts upon, by replacement of the final syllable by the termination *-ase*. Thus *lactase* acts on lactose, *amylase* on starch (L. *amylum*), *lipases* on fats (Gk. *lipos*), *proteases* on proteins, and so on. Some few of the older specific names, as *pepsin* of the gastric juice, and *trypsin* of the pancreatic juice, have been retained.

To determine whether enzymic action is truly catalytic in nature the following criteria of catalysis can usefully be applied : acceleration of a reaction ; catalysis of amounts of material much larger than that of the catalyst ; non-alteration of the equilibrium of the reaction ; reversibility of the reaction.

The examples already quoted show that enzymes undoubtedly accelerate a reaction. In some cases we may conceive that the reaction is capable of proceeding very very slowly in the absence of an enzyme. Thus a solution of cane-sugar, sucrose, appears to decompose to glucose and fructose very slowly at the temperature of boiling water, from which we may conclude that the reaction still proceeds, though even more slowly, at the temperature of the blood. If we add a little *sucrase* (an enzyme that occurs in intestinal juice) we get an immensely accelerated reaction.

On the other hand, if we take some starch suspended in water, and sterilised, such a solution remains unchanged for

very long periods. But if we add a little *amylase* (we can regard saliva as a dilute solution of amylase) the starch is almost completely transformed to maltose in less than an hour. It would seem that in this case the amylase actually initiates the reaction, though we cannot regard this as absolutely proved. A purified protein, such as the fibrin from a blood-clot, in contact with water, will remain unaltered for indefinite periods if kept sterile. Addition of a solution containing trypsin rapidly breaks it down and brings it into solution, and it is difficult to believe that trypsin does not initiate the reaction.

A given weight of enzyme can catalyse many times that weight of the substance it acts upon. It is impossible as yet to say how many times, since, at the present time, only very impure preparations of the vast majority of enzymes are available. But it has been shown that sucrase will act on *at least* two hundred thousand times its weight of sucrose, while rennin of the gastric juice will clot at least four hundred thousand times its weight of casein, the coagulable protein of milk.

Just as with an inorganic catalyst, the greater the concentration of the enzyme the faster the reaction proceeds (though if sufficient time be given the final result is the same).

An enzyme seems to disappear gradually during the reaction it is accelerating. There are various explanations possible (we may compare this effect with the sticking of the oil to the glass as the weight moves down it).

Enzymes do not alter the equilibrium-point of a balanced reaction, provided *the same intermediate products* are formed as during the action of some other catalyst. Such identity of intermediate products frequently does not occur when the actions are composed of, on the one hand, an enzyme, and, on the other, a marked concentration of hydrogen ions (such as is furnished by the presence of a mineral acid). Further, such equilibria can only be

compared when the reactions take place within glass vessels, since when they occur in animals or in plants they take place in contact with living membranes, and the products of the reactions can be removed as fast as they are formed, so that the reactions may proceed almost to completion.

Certain enzymes can induce reversible reactions to some extent. Sometimes the reversion is complete, qualitatively. Lipase accelerates the hydrolysis of ethyl butyrate into butyric acid and alcohol, and also accelerates its formation from these compounds.

Hence we may consider enzymes as true catalysts, and define them as follows : *An enzyme, or ferment, or biochemical catalyst, is a catalyst produced by a living cell, but whose action is independent of the living cell that produces it.*

Properties of Biochemical Catalysts

Enzymes are very widely distributed in living material, but only in the minutest quantities. Hence they are extremely difficult to prepare in a state even approximating to purity, and there is by no means general agreement as to whether any enzyme has been isolated in absolutely pure condition. With preparations that are, at best, mixtures of enzymes and impurities, it is, of course, uncertain whether the apparent chemical and physical properties are due to the enzyme or to the impurities. We can therefore only test for an enzyme by ascertaining if a solution supposed to contain it will bring about the catalytic reaction characteristic of that enzyme, and we can only study its properties by submitting its solutions to various treatments, and finding by subsequent tests whether the enzyme remains unaffected, or has been in whole or in part destroyed.

Such tests show that *enzymes are very unstable compounds;* they are very easily changed to substances that do not catalyse.

Enzymes are soluble in water, in dilute glycerol, in solutions of sodium chloride, and in *dilute* alcohol. They are precipitated from solution by saturation with ammonium sulphate, or by adding to the solution excess of alcohol. Such treatment effects a partial purification of the enzymes, but only a partial purification, since many other compounds will also be thrown down by the ammonium sulphate or the alcohol. They appear to be colloidal and relatively non-diffusible, properties which suggest, as we shall see later, that they are compounds with relatively large molecules. Studies of their actual rates of diffusion lead to the conclusion that their molecular sizes are comparable with those of such a protein as egg albumin.

Effect of Temperature. In solution most enzymes are decomposed at definite temperatures between 70° and 100° C., that is, below the temperature at which the solutions boil. Freezing their solutions, in most cases, has no permanent effect on the enzymes, though it slows their catalytic action, and may completely stop it until the temperature is again raised. A few enzymes still possess activity at 0° C.

Optimum Temperature. Enzymes differ entirely from inorganic catalysts in that there is for each a specific temperature at which its catalytic power is a maximum. These optimal temperatures usually lie between 35° and 45° C., so that they approximate to the blood temperature of mammals.

The existence of an optimum temperature depends upon two factors : increasing temperature increases the speed of the reaction that is being catalysed by a given enzyme ; decomposition of an enzyme by heat *commences* at relatively low temperatures, and increases rapidly with increasing temperature. At temperatures above the optimum the rate of decomposition of the enzyme is so great that the increased speed of the reaction it is producing is more than counterbalanced ; at the optimum temperature the effect is a balance between increasing speed of reaction and increasing rate of

decomposition. (The optimum temperature for an enzymic synthesis differs from that for the usual decomposition.)

Co-enzymes. Many, if not all, enzymes require the presence of specific *inorganic ions*, in order that they can produce their effect. Thus the amylases, the starch-splitting enzymes of saliva and of pancreatic juice, require the presence of slight traces of chloride ions. If we *dialyse* pancreatic juice, *i.e.*, if we enclose some of it in a collodion bag, or an animal membrane bag, which will permit small molecules and ions to pass through, but will hold back large molecules, and such a bag is then immersed in flowing water, and finally in distilled water, in time all the chloride ions will be completely dialysed away, leaving the enzyme behind. This is then without action on starch, until a trace of chloride has been added. (For this particular reaction bromide ions can replace chloride ions in activating the enzyme.)

Most enzymes require a definite degree of acidity or alkalinity, *i.e.*, a definite concentration of hydrogen ions, before they are able to produce their maximum effects. Pepsin requires for its optimum action an acid concentration corresponding to about 0·2 per cent. hydrochloric acid (we actually find this acidity in stomach contents). In neutral solutions pepsin is without action. At the relatively high degree of acidity of the stomach contents salivary amylase is completely decomposed. Its optimum action takes place in a medium which is only just acid, in which the concentration of hydrogen ions is only about one-ten thousandth of that in the gastric juice. In certain of these cases we can regard the hydrogen ions as acting as co-enzymes.

It is rather striking, and at first sight unexpected, that many of the *oxidases*, a class of enzymes which, as their name suggests, are concerned with oxidations, require as their co-enzyme manganese ions, and it is not at all improbable that a number of other elements which are present only as traces in living tissues may be necessary in the *rôle* of co-enzymes.

Instances are also known in which simple organic compounds act as co-enzymes. The co-enzyme is obviously of great importance.

Decomposition of Enzymes. Enzymes are changed into inactive substances not only by heat, and by a marked change in hydrogen ion concentration, but even by such factors as mechanical shaking and the intramolecular vibrations produced by ultra-violet light.

Zymogen is a term given to the supposed precursors of certain enzymes. These enzymes, or their zymogens, if the latter really exist, require treatment of a specific nature to produce the activity associated with such enzymes. It is usually considered that pepsin of the gastric juice is formed from the inactive zymogen *pepsinogen* produced by the cells of the stomach wall, by the action of dilute hydrochloric acid. The activator—in this case the acid—is termed a *zymo-excitor.*

Classification of Enzymes. Enzymes are classified in accordance with the type of action they cause and accelerate. *Hydrolytic enzymes* produce *hydrolysis*, which may be defined as the reaction of a large molecule with one or more molecules of water, with the production of two or more smaller molecules. *Oxidases* and *peroxidases* effect oxidations. (*Reductases* bring about reduction.) *Protein-coagulating* enzymes produce coagulation of proteins (*e.g.*, rennin coagulates the caseinogen of milk).

Hydrolytic enzymes are subdivided in accordance with the class of compound they act upon, using a terminology in which the ending *-lytic* or *-clastic* replaces the final syllable of the compound or class (Gk. *lysis*, loosing ; *klastos*, broken in pieces). *Proteolytic* or *proteoclastic* enzymes catalyse the hydrolysis of proteins, *amylolytic* or *amyloclastic* enzymes catalyse the hydrolysis of starches, and *lipolytic* or *lipoclastic* enzymes catalyse the hydrolysis of fats. We shall find that there are many individual enzymes which each produce a very specific type of hydrolysis.

The substance which is acted on by an enzyme is termed its substrate.

Specificity of Enzymes. The classification just given at once raises the question as to the degree to which the action of a particular enzyme is specific for a particular substrate. It is found that enzymes, unlike inorganic catalysts, exhibit a marked selective action with regard to the substances they react with. An enzyme that will hydrolyse a fat has no effect on a carbohydrate, and *vice versâ*, but both fats and carbohydrates can be hydrolysed by warming them with dilute acids. An enzyme that will hydrolyse starch has no effect on cane-sugar, but both starch and cane-sugar are hydrolysed by dilute acid.

The specificity of action is still further exemplified by the fact that sucrase, the enzyme of the intestinal juice which hydrolyses cane-sugar, has no effect on malt-sugar, nor on milk-sugar, although these three sugars have somewhat similar constitutional formulæ, and the common empirical formula $C_{12}H_{22}O_{11}$.

On the other hand, many enzymes will act on a whole class of similar compounds. Thus pepsin will hydrolyse many of the proteins. The enzyme emulsin will act on a whole series of glucose compounds that we call β-glucosides.

The Nature of Enzyme Action. It has been pointed out that catalysts appear to be capable of acting in virtue either of adsorptive properties, or of capacity for playing some intermediate chemical *rôle ;* the latter is predominant, and even when catalysis is associated with adsorption there is evidence that not infrequently the adsorbent acts through chemical as well as physical mechanisms.

Enzymes likewise are probably active through a combination of physical and chemical mechanisms. Their large molecular size gives to them some of the properties of adsorbents, while they undoubtedly play an intermediate chemical *rôle.* It seems not improbable that they unite with their substrates in chemical union, forming an unstable complex

which breaks down into one product of the decomposition of the substrate, and a compound of enzyme and a second product of this decomposition ; this decomposes less readily, and since the bound enzyme is inactive, such a theory accounts in part for the apparent loss of an enzyme during a reaction.

If the substrate S is broken down into A and B by the action of the enzyme E then the suggested changes may be represented :

$$E + S \rightleftharpoons E{-}S \rightleftharpoons A + E{-}B \rightleftharpoons A + B + E.$$

The marked specificity exhibited by the majority of enzymes is associated, not with individual compounds, but with special types of linkages present in a series of compounds. This association has led to the so-called *lock and key* conception of enzyme action.

Reversibility of Enzyme Action. It has already been stated that the reverse action induced by some enzymes leads to production of precisely those compounds that such an enzyme usually hydrolyses, as with lipase and ethyl butyrate. More frequently a reverse action will take place when an enzyme is added to a concentrated solution of the hydrolysed products of its own action, but the compound built up is not quite the same as the one originally hydrolysed. Thus if a protein is hydrolysed by pepsin, and then more pepsin added to the concentrated products, a protein, termed a *plastein*, is built up, very similar to the original, but less soluble.

Purification of Enzymes. The technique of purification varies for different enzymes. Except in cases in which the enzyme is contained in some special secretion (such as the gastric and pancreatic juices) the structure of the containing cells must be destroyed. To this end methods such as Buchner's can be used, and the expressed cell juice obtained, or the cells can be killed and allowed to decompose (by autolysis ; see Chapter XX.), the resulting products being dried at low temperature and the enzymes extracted by

suitable solvents. The usual chemical methods of separation, including precipitation by suitable agents, or adsorption on to suitable material such as alumina or kaolin, can be utilised.

Sherman has prepared a pancreatic amylase in amorphous form ; he regards it as essentially the pure enzyme, and a description of his method can be taken as illustrative of the general procedures. Commercial " pancreatin " is extracted with about ten times its weight of 87 per cent. glycerol, the solid residue centrifuged off, and excess of ice-cold water added, in which is dissolved sufficient acid phosphate mixture to produce a very slight degree of acidity. The succeeding operations are all carried out at 0° C. The insoluble material thrown down is centrifuged off, and the solution added to twice its volume of an alcohol-alumina suspension, with occasional shaking. The alumina is centrifuged off and carries down with it the enzyme. This precipitate is at once stirred with ice-cold dilute sodium hydroxide in such amount and concentration as to produce the faintest degree of alkalinity, then immediately centrifuged, and the solution added to twice its volume of a mixture of alcohol and ether in equal parts. A precipitate of the enzyme is thrown down, centrifuged off, and dried *in vacuo* over sulphuric acid. From 40 gm. of " pancreatin " Sherman obtains 0·7 gm. of precipitate. This gives all the reactions of a protein, and is so active that it will produce nearly 10,000 times its weight of maltose from a 2 per cent. solution of starch in thirty minutes at 40° C.

Isolation of Crystalline Enzymes. The preparation of a compound in crystalline form is usually accepted as evidence that it has been obtained in a condition of relative purity. Recrystallisation increases the degree of purity. Claims have been made that two enzymes have been obtained in crystalline form, *urease* (which decomposes urea into ammonia and carbon dioxide), and *pepsin* of the gastric juice.

J. B. Sumner, in 1926, published the results—at length successful—of nine years' attempts to prepare pure urease.

His final successful procedure consisted in the extraction of Jack-bean meal (a rich source of urease) with a mixture of acetone and water in definite proportions, the extract being then cooled and kept for twelve hours just above 0° C. Crystals, colourless octahedra, microscopic in size, separated in amounts minute, but sufficient to be centrifuged off and examined. Their appearance is shown in Fig. 1. The material so obtained is a protein of the globulin class,

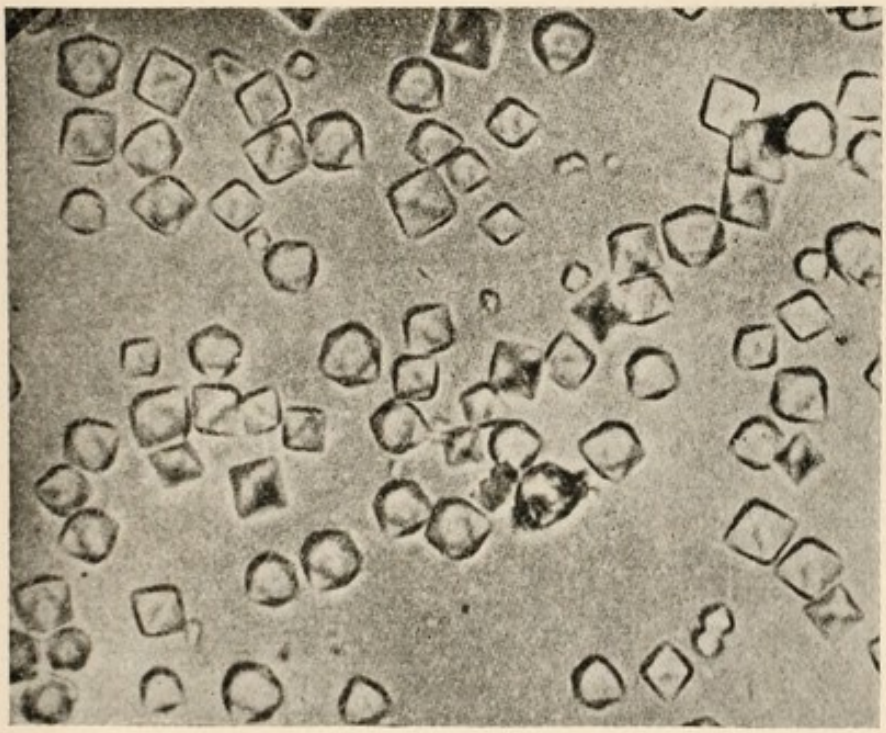

FIG. 1.—Pure crystals of the enzyme *urease*. Magnification 710. (From J. B. Sumner's photomicrograph, *J. Biol. Chem.*, 1926, lxix., 436.)

showing such marked activity that it seems a rational conclusion to regard it as urease itself. It can be recrystallised, and this treatment still further increases its activity, strengthening the argument in favour of the crystals being the pure enzyme. The crystals so obtained are 8,400 times more active than their weight of soy-bean meal (another potent source of the enzyme), and so powerful that they will liberate from a urea solution at 20° C. 120 times their own weight of ammonia in each five minutes.

Tauber (1930) confirmed Sumner's results, as has also Waldschmidt-Leitz (1931). The latter claims that when these crystals of protein material are hydrolysed by proteolytic enzymes, the hydrolysed products appear to retain the full original enzymic activity, from which he concludes that the crystalline globulin itself is not urease the enzyme, but a " carrier " of some complex radical which possesses urea-decomposing activity. Such a result would support Willstätter's view that the intrinsic part of an enzyme is some specifically active radical anchored to a carrier which is characterised by high molecular weight but is not necessarily markedly specific in character. However, Sumner states that proteolytic decomposition of urease is accompanied by a corresponding degree of inactivation, and he has apparently shown that this is true even when a solution of crystalline pepsin is allowed to act on a solution of crystalline urease.

Willstätter's view, just referred to, that the intrinsic part of an enzyme is some specifically active radical, is by no means wrong, even if it should prove that the radical, when isolated, is, without its " anchor," an inactive substance. Such a view draws attention to another point of importance. Many other bio-catalysts exist, of which glutathione (*cf.* p. 259) is a good example. Had these happened to be protein in nature, and therefore to be altered in behaviour by boiling their solutions, they would have been classified as enzymes.

Our present definition of enzymes is a little artificial. The simpler bio-catalysts are equally enzymic in their action, but they cannot be termed enzymes merely because of the simplicity of their chemical nature. Perhaps the only specificity of enzyme action which is associated with the large enzyme molecule is the possibility of surface adsorption.

Northrop (1930) has announced the isolation of crystalline pepsin. A commercial pepsin preparation was dissolved in dilute sulphuric acid and excess of a saturated solution of magnesium sulphate added. A precipitate, carrying the enzyme, was thrown down. This was treated with dilute sodium hydroxide until just slightly acid, the enzyme passing into solution. To the solution a little dilute sulphuric acid was added, and the heavy precipitate which formed was filtered off, stirred with water to a thick paste, dissolved by

addition of dilute sodium hydroxide, and the temperature raised to 45°. On slow cooling a heavy crystalline precipitate separated. These crystals showed all the properties of a protein, and their proteolytic activity remained constant through seven successive recrystallisations. They consisted of microscopic regular hexahedra, somewhat similar in general appearance to the urease crystals shown in Fig. 1. They exhibited marked enzymic activity. Crystalline pepsin is a protein of molecular weight between 33,000 and 38,000. When it is hydrolysed the split products show no activity.

Sherman (1931), from buffered alcohol-water solutions of his purified amylase preparations, has obtained crystals exhibiting marked activity.

Velocity of Enzyme Action. The velocity of a catalysed reaction depends upon a number of factors such as the concentration and nature of the substance or substances which are being catalysed, the concentration of the catalyst, the degree of acidity or alkalinity, and the temperature. When a single substance is decomposed into two or more substances, or when a single substance is hydrolysed in presence of a great excess of water, under conditions in which the amount of catalyst is fixed and the temperature remains constant, then the reaction is said to be *monomolecular*. In such a monomolecular reaction it follows from the law of mass action that at any instant the amount of material changed is a fixed proportion of the total amount present *at that instant*, and the relationship is expressed by the equation

$$\frac{1}{t} \log \frac{a}{(a - x)} = k$$

where a is the initial quantity of substance, x is the quantity which has been transformed at time t, and k is a constant.

The effect of a biochemical catalyst *approximates* to a monomolecular reaction. As example, Hudson's results can be quoted, for the action of saccharase (which hydrolyses cane-sugar to glucose and fructose) on a solution of cane-sugar.

The rate of reaction can be followed by the change in optical rotation of the sugar solution (see Chapter V.). It will be seen that the figures for k in Table I. are reasonably constant.

TABLE I. VELOCITY OF TRANSFORMATION OF CANE-SUGAR BY SACCHARASE AT 30° C.

Time.	Observed Rotation.	Velocity Constant.
Minutes.	Degrees.	$k \times 10^5$.
0	+ 27·50	—
30	+ 14·27	558
60	+ 7·90	530
90	+ 3·00	539
110	+ 0·80	534
130	− 1·49	559
150	− 2·40	533
∞	− 7·47	—

The effect of the concentration of enzyme upon the velocity of reaction varies with different enzymes. With saccharase, for example, the rule holds over a somewhat wide range of enzyme concentration, that the amount of sugar transformed is proportional to the product of (quantity of enzyme) multiplied by (time of action). This is shown by the figures in Table II., based upon Hudson's results.

TABLE II. RELATION BETWEEN CONCENTRATION OF ENZYME, TIME OF ACTION, AND AMOUNT OF TRANSFORMATION.

Relative Concentration of Saccharase.	Time in Minutes.	(Enzyme) × (Time).	Percentage Transformation of Sugar.	
			Initial Concentration of 4·55 per cent.	Initial concentration of 27·3 per cent.
			Per cent.	Per cent.
2·00	15	30·0	73·2	11·2
1·50	20	30·0	73·2	11·2
1·00	30	30·0	72·9	11·5
0·50	60	30·0	72·9	11·4
0·25	120	30·0	73·1	10·9

On the other hand, Schütz, studying the action of pepsin on protein, came to the conclusion that quantities of protein hydrolysed in equal periods of time by different quantities of pepsin are proportional to the square roots of the amounts of enzyme used. ($x = k\sqrt{\mathrm{E}}$, where x is the amount of protein transformed and E is the amount of enzyme present.) This relation, termed Schütz's law, only holds within a limited range. Various modifications of the law have been suggested.

Enzymes are the important chemical agents of life. They are involved in almost all the multitude of chemical processes which constitute life, and certain of them are largely responsible for the decay of an organism after death.

Anti-enzymes. Although the mucous membranes of the stomach and intestine consist largely of protein material, and are brought into contact for many hours at a time with powerful protein-splitting enzymes, these membranes are not affected by the enzymes. Further, certain parasitic organisms, the intestinal worms, can survive such enzymic action throughout their period of life. Evidently they possess some protective agency.

If intestinal worms are ground up with sand, and then subjected to high pressure, and the expressed juice filtered, addition of alcohol until its concentration in the mixture is 60 per cent. may result in a precipitate. If this is filtered off, and then more alcohol added to a concentration of 85 per cent., a precipitate forms. If this be filtered off, washed with alcohol and ether, and dried in a vacuum over concentrated sulphuric acid, a sticky powder is left which is soluble in water, and which, when added to a pepsin or trypsin solution, will stop its usual hydrolysing action on proteins.

Evidently this powder contains some compound which is antagonistic to proteolytic enzyme action. Such compounds have not been obtained in pure condition, and we know little of their properties except this antagonistic action, in virtue of which they are called *anti-enzymes*. We must suppose that it is due to the presence of such compounds in the external tissues of intestinal worms that the worms escape digestion in the intestine, and of similar compounds in the stomach and intestinal wall, that these also withstand digestion.

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CHAPTER III

pH

One of the factors which affects biochemical reactions very greatly is the degree of acidity or alkalinity of the medium in which the reaction takes place. Most of the reactions of the organism take place in a medium very nearly neutral in reaction—just on the acid, or just on the alkaline side of neutrality. Frequently, as for example with blood, an extremely slight change in the degree of alkalinity (or acidity) has marked and deleterious effect on the organism.

The degree of acidity or alkalinity of a solution bears a definite relation to the number of ions of the element hydrogen present in any particular volume of that solution. Neutral water has only 1 gm. of these ions in ten million litres. An average normal specimen of human arterial blood has less than half this number, actually 0·47 gm. in ten million litres of blood. It is difficult to grasp the meaning of such figures; they convey little to the mind beyond the tremendous dilution. This chapter is designed to explain how such figures are arrived at, and how a much simpler method of expressing the facts can be employed through the use of the shorthand expression *p*H.

We know that acids in solution in water ionise into *hydrogen ions* carrying a positive electric charge, and other ions (characteristic for each acid) carrying one or more negative electric charges (electrons). For example, when hydrochloric acid (gas) is passed into water its molecules ionise. After ceasing to pass the gas an equilibrium is set up

$$HCl \rightleftharpoons H^+ + Cl^-.$$

Alkalies, similarly, ionise into positively charged ions (characteristic for each alkali) and negatively charged *hydroxyl* (OH) *ions*. When a little solid sodium hydroxide is dissolved in water most of its molecules break up into ions, and again an equilibrium is set up :

$$NaOH \rightleftharpoons Na^+ + OH^-.$$

All biochemical reactions take place in a medium containing water, and even the purest water ionises, though to a very small extent, into hydrogen and hydroxyl ions, an equilibrium being established :

$$HOH \rightleftharpoons H^+ + OH^-.$$

The act of solution of acid or alkali in water brings about the type of ionisation just described. If we add an acid solution to water, *i.e.*, add many hydrogen ions to an equilibrised mixture of unionised HOH, and H^+ and OH^- ions, we cause a certain definite change in the equilibrium ; some hydrogen and hydroxyl ions combine :

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

so that the number of OH^- ions are diminished. The diminution is greater the larger the number of H^+ ions added, that is, the more strongly acid becomes the solution.

Similarly, if we make the water alkaline, *i.e.*, increase the number of OH^- ions, there is a corresponding shift in the equilibrium and again some hydroxyl and hydrogen ions combine :

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

so that the number of H^+ ions is diminished, and the more strongly alkaline the solution becomes the fewer H^+ ions remain.

The relationship is governed by a definite *mass law* (is related to the actual masses taking part in the reaction), and this law can be written for any equilibrium

$$\frac{(\text{Concentration of } X^+) \times (\text{Concentration of } Y^-)}{(\text{Concentration of unionised } XY)} = k(\text{a constant}).$$

Such a mass law governs all reactions, ionised or unionised, in which equilibria are established. A simple example is the reaction between ethyl acetate and water on the one hand and ethyl alcohol and acetic acid on the other (*cf.* previous Chapter, p. 10). At equilibrium it is found that two-thirds of the ethyl acetate remains unaltered. If its initial concentration be unity its final concentration will be 2/3, the molecular concentration of the water capable of reacting with it will be the same, and the corresponding concentrations of ethyl alcohol and acetic acid will both be 1/3. Hence :

$$\frac{\text{(Molecular concn. of ester)} \times \text{(Molecular concn. of water)}}{\text{(Molecular concn. of alcohol)} \times \text{(Molecular concn. of acid)}} = \frac{2/3 \times 2/3}{1/3 \times 1/3} = 4 = k.$$

In the particular case now under discussion, if we express the concentration of hydrogen ions as (H^+), that of hydroxyl ions as (OH^-), and that of unionised water as (HOH), then

$$\frac{(H^+) \times (OH^-)}{(HOH)} = k \text{ (some other constant)},$$

and since (HOH) is extremely large, when compared with (H^+) and (OH^-), (HOH) can be considered as constant, and we can write

$$(H^+) \times (OH^-) = k \times (HOH) = K,$$

where K is another constant.

If we add to neutral water, for which this relation holds, acid or base, or acid or basic salts, so that hydrogen ions or hydroxyl ions are increased, then hydroxyl or hydrogen ions will respectively be diminished, and always to such a degree that the equilibrium equation holds.

We express the concentration of a solution in terms of the number of gram-molecules of the *solute* (the substance dissolved) in one litre of the solution. We treat ions in precisely the same way, so that *the hydrogen-ion concentration in a solution is the number of gram-ions of hydrogen present in* 1 *litre of the solution.*

Accurate measurements made with the purest water show that for it

$$K = 10^{-14}.$$

Since pure water must contain an equal number of hydrogen and hydroxyl ions, their ionic concentration must also be equal, so that, in pure water,

$$(H^+) \times (OH^-) = 10^{-7} \times 10^{-7} = 10^{-14}$$

and pure water will contain one-ten millionth of a gram-ion of ionised hydrogen (and of hydroxyl ions) in one litre, *i.e.*, 0·0000001 gram of H^+ and 0·0000017 gram of OH^- in 1 litre. The relationship is grasped a little easier when expressed as 1 gram of hydrogen ions and 17 grams of hydroxyl ions in 10 million litres of water.

No matter how alkaline a solution may be made, the equilibrium equation still holds, so that strongly alkaline solutions of sodium hydroxide have a definite hydrogen-ion concentration expressed by

$$(H^+) = \frac{10^{-14}}{(OH^-)}.$$

Therefore *all aqueous solutions have a definite hydrogen-ion concentration*, which expresses their degree of acidity or alkalinity.

The *strength* of an acid solution depends not on the weight of acid present in a given volume of water, but on the extent of ionisation of that particular concentration of acid, *i.e.*, on the concentration of hydrogen ions present. The strength of an alkaline solution depends on the concentration of hydroxyl ions present, and therefore, from the last equation, it is equally definitely expressed by stating its concentration of hydrogen ions.

A dissolved salt, acid, or alkali, is only completely dissociated into ions at infinite dilution ; therefore, since the *effective strength* of an acid or alkaline solution depends on the concentration of hydrogen ions, it is necessary to know the *degree of dissociation* in order to know the strength.

The degree of dissociation can be measured in several ways. Thus we may measure the *conductivity of* a solution ; this is the inverse of the electrical resistance offered by the

solution to the passage of an electric current, and is proportional to the relative number of charged ions present in the solution. Again, pure water freezes at 0° C. If chemical compounds are dissolved in water, its point of solidification is lowered below 0°, and it has been found that the degree of lowering is proportional to the molecular concentration of the solution, each molecule producing the same degree of lowering. But if solutions of cane-sugar (which does not ionise) and sodium chloride of the same molecular concentrations are compared it is found that the depression of the freezing point (Δ) is for sodium chloride almost twice as much as for cane-sugar. The sodium chloride has been almost completely ionised, and the sodium and chloride ions have each the same quantitative effect as the unionised sodium chloride molecule. Obviously a comparison of Δ in the two cases gives the degree of ionisation of sodium chloride in the particular concentration measured.

An illustration of the degree to which ionisation may control a reaction is given in the following table (from Macleod's Handbook). In considering this table it must be remembered that the relative conductivity is proportional to the degree of ionisation, and therefore, for the acids dealt with, to the hydrogen-ion concentration. In this table the figures for hydrochloric acid are taken as standard, 100.

TABLE III. RELATION BETWEEN CATALYTIC POWER AND CONDUCTIVITY.

Acid.	Catalytic Power.	Relative Conductivity.
Hydrochloric acid, HCl . . .	100	100
Dichloracetic acid, $CHCl_2.COOH$.	27	25
Monochloracetic acid, $CH_2Cl.COOH$.	4·8	4·9
Formic acid, H.COOH . . .	1·5	1·7
Acetic acid, $CH_3.COOH$ . . .	0·40	0·42

Experiment shows that the degree of dissociation of 0·1 *N*

(one-tenth *normal*) hydrochloric acid, a solution containing one-tenth the molecular weight (3·65 gm.) per litre (just less than the 0·4 per cent. strength of this acid in gastric juice) is 91 per cent. Hence the hydrogen-ion concentration is not 0·1 gram-ions of hydrogen per litre, but

$0{\cdot}1 \times \frac{91}{100} = 0{\cdot}091$, which can also be written $0{\cdot}91 \times 10^{-1}$ or $9{\cdot}1 \times 10^{-2}$.

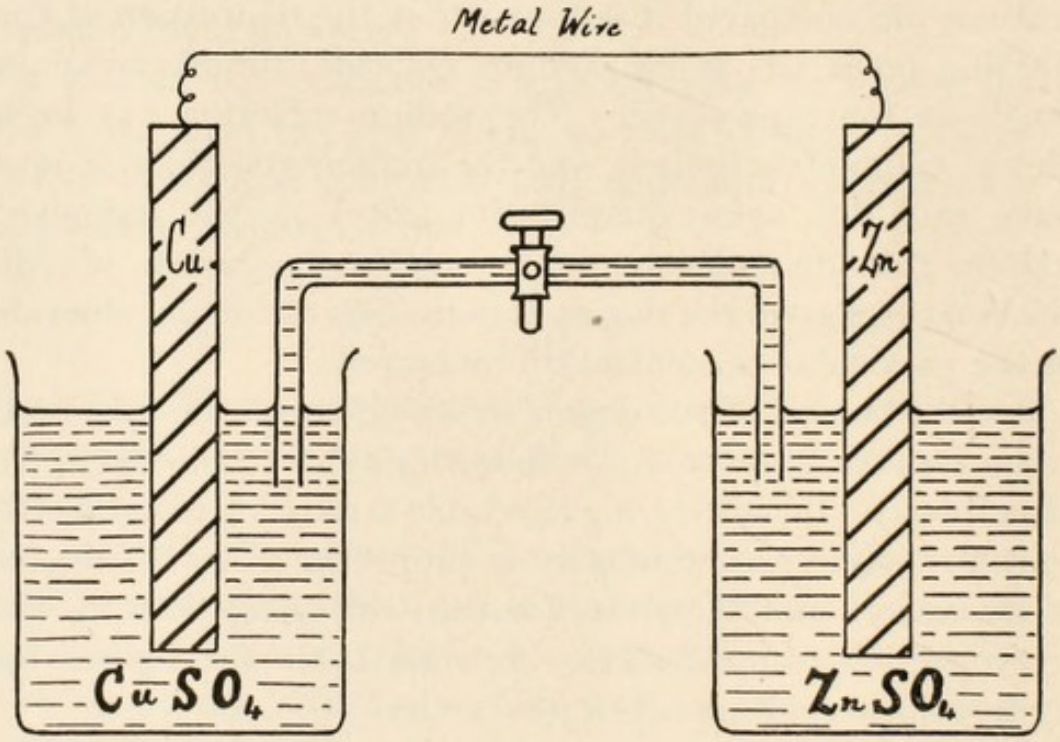


FIG. 2.—Schematic representation of two electrodes united to form a cell.

Again, 0·1 *N* acetic acid is only 1·3 per cent. dissociated, whence for this acid

$$(H^+) = 0{\cdot}1 \times \frac{1{\cdot}3}{100} = 0{\cdot}0013, \text{ or } 1{\cdot}3 \times 10^{-3}$$

Since biochemical reactions depend to such a great extent on hydrogen-ion concentration, it is obviously of great importance that we should be able to measure it.

We possess two methods, one, the electrical method, accurate, but requiring much special and costly apparatus ;

the other, the colorimetric, not quite so accurate, but in most cases much more easily applied.

The Electrical Method of determining Hydrogen-ion Concentration, and the Theory on which it is based. A simple chemical battery can be constructed by immersing two different metals in solutions of their respective salts, connecting the solutions through a third solution of an electrolyte, and the ends of the metals that are not immersed by a metal wire. As soon as the wire connection is made an electric current flows through the wire from metal to metal (and through the solution from metal to metal in the reverse direction). Such an arrangement is shown in Fig. 2.

If a voltameter be inserted in the wire part of the circuit the voltage can be measured.

The existence of such a potential depends essentially on the fact that a metal in contact with a solution tends to dissolve, of course in ionic condition. The degree of this tendency is called the *electrolytic solution pressure of the metal.* The salt is ionised, and the metallic ions from the salt also exert a solution pressure, the *osmotic pressure* of these ions. If this *ion-osmotic pressure* is less than the solution pressure of the metal there will be a slight passage of ions from the metal into the solution ; these will carry electric charges so that an *electric potential* will be set up. They will be held in the neighbourhood of the metal surface by the opposite electric charges set free simultaneously on that surface, thus constituting the Helmholtz electrical double layer. If the solution pressure of the metal is less than the ion-osmotic pressure of the solution, there will be a deposition of metal from the solution on the metal, and an electric potential (of opposite kind) will be created.

In the system that has been figured, as long as the two metal-solution units remain unconnected they constitute two isolated *electrodes*, and as soon as equilibrium between solution and metal is reached each will have a definite potential, a definite pressure (single electrode potential). As soon as these two electrodes are doubly connected (from solution to solution and from metal to metal) unless the two potentials are exactly equal an electric current will flow from one to the other, the direction of the current (of the flow of electrons) depending on the relative potentials, such a current always proceeding through the solution from the higher to the lower potential.

Various electrodes have been constructed of which the poten-

tials are known. Such an electrode is the so-called *calomel electrode*, in which metallic mercury is the metal, placed in contact with a saturated solution of calomel (mercurous chloride) in potassium chloride. Under certain exactly defined conditions this electrode will develop a potential of 0·56 volt. By constructing a cell with such an electrode, and a second of unknown potential, it is possible, by connecting the cell with a potentiometer and measuring the potential produced, to calculate the potential of the second electrode.

A *hydrogen electrode* has been constructed, in which platinum black, saturated with hydrogen gas, is immersed in a solution containing free hydrogen ions, and in contact with an atmosphere of hydrogen gas. It is found that the voltage of this electrode varies with the concentration of hydrogen ions around it. If the concentration of hydrogen ions in the solution is known, then we have a standard hydrogen electrode, which, linked up with a standard calomel electrode, gives us a standard cell for hydrogen-ion concentration measurements. Then the concentration of hydrogen ions in any other solution can be measured by converting this into a hydrogen electrode by immersing in it platinum black saturated with hydrogen gas, linking it up with a second calomel electrode, and comparing the potential developed from this cell with that developed from the standard cell.

For the mathematics of the calculation special treatises should be referred to.

The meaning of pH

The conception of a hydrogen potential developed in such a hydrogen electrode enables us to give at this point a shorthand method of expressing hydrogen-ion concentration. It is obvious that numbers such as (H^+) or $C_{H^+} = 1{\cdot}3 \times 10^{-3}$ (the value for the hydrogen-ion concentration of tenth-normal acetic acid) are extremely cumbrous, and difficult to visualise. Most of the reactions going on in the human body take place at hydrogen-ion concentrations between 1×10^{-6} and 1×10^{-8}, numbers still more difficult to visualise.

If, instead, the corresponding values

$$\log \frac{1}{(H^+)}$$

are taken, a series of simple numbers are obtained. From

the exponential (*power*) value of the hydrogen-ion concentration the expression *p*H has been coined :

$$(H^+) = 10^{-pH} \text{ and } pH = \log \frac{1}{(H^+)}.$$

The term also suggests the idea of a hydrogen potential, developed in a hydrogen electrode ; under specified conditions the actual values coincide.

The calculation of any *p*H value from the corresponding (H^+) value requires a knowledge of simple logarithmic procedures ; the following are examples :

The value of (H^+) for tenth-normal hydrochloric acid is, as we have seen, $9{\cdot}1 \times 10^{-2}$.

Hence

$$pH = \log \frac{1}{(9{\cdot}1 \times 10^{-2})} = 2 - \log 9{\cdot}1 = 2 - 0{\cdot}959 = 1{\cdot}041.$$

For N/10 acetic acid the value for (H^+) is $1{\cdot}3 \times 10^{-3}$. In this case

$$pH = \log \frac{1}{(1{\cdot}3 \times 10^{-3})} = 3 - \log 1{\cdot}3 = 3 - 0{\cdot}1139 = 2{\cdot}886.$$

If we are told that the *p*H of a solution is 7·33 (the mean normal value for arterial blood), then the hydrogen-ion value can be calculated as follows :

$$pH = 7{\cdot}33 = 8 - 0{\cdot}67 = 8 - \log 4{\cdot}68 = \log \frac{1}{(4{\cdot}68 \times 10^{-8})}$$

whence (H^+) is $4{\cdot}68 \times 10^{-8}$.

Based on this calculation, still another method of expressing the *p*H of a solution can be demonstrated. It happens to be, from its definition, the logarithm of the number of litres of solution which contain exactly 1 gram-ion of hydrogen. It has just been shown that the hydrogen-ion concentration of arterial blood is $4{\cdot}68 \times 10^{-8}$ gm. per litre of blood. Hence 1 gram is contained in $10^8/4{\cdot}68$, *i.e.*, 21,367,500 litres of blood, of which the logarithm is 7·33, the actual *p*H value.

The value of *p*H for the purest water, for which (H^+) is 10^{-7}, is obviously log $1/10^{-7}$, *i.e.*, 7.

It will be remembered that

$$(H^+) \times (OH^-) = K = 1 \times 10^{-14},$$

and from the above calculations, and from the nature of this equation, it is obvious that the higher the acidity, the higher the value of (H^+), the lower will be the value of *p*H, the extreme values being 0 and 14.

Extremely acid solutions will have *p*H values approximating to zero, neutral solutions the value of 7, and extremely alkaline solutions values approximating to 14.

A comparison between the hydrogen-ion concentration (H^+), and the corresponding *p*H figure for a few body fluids, will stress the value of the latter form of expression in recording the degree of acidity or alkalinity.

Gastric contents during digestion (adult), *p*H, 1·3 ;
(H^+), $5{\cdot}0 \times 10^{-2}$ or 0·05.

Normal urine (acid limit), *p*H, 4·8 ;
(H^+), $1{\cdot}6 \times 10^{-5}$ or 0·000016.

Gastric contents during digestion (infant), *p*H, 5·0 ;
(H^+), $1{\cdot}0 \times 10^{-5}$ or 0·000010.

Saliva (average figure), *p*H, 6·6 ;
(H^+), $2{\cdot}5 \times 10^{-7}$ or 0·00000025.

Purest water, *p*H, 7·0 ;
(H^+), $1{\cdot}0 \times 10^{-7}$ or 0·00000010.

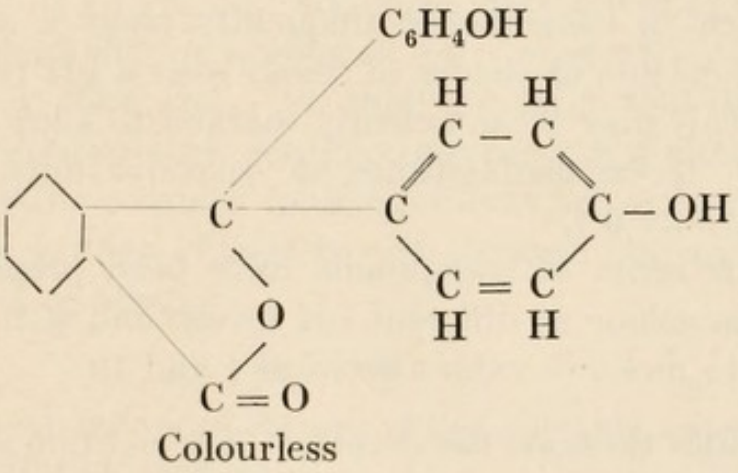
Arterial blood, *p*H, 7·3 ;
(H^+), $4{\cdot}7 \times 10^{-8}$ or 0·000000047.

Normal urine (alkaline limit), *p*H, 7·5 ;
(H^+), $3{\cdot}2 \times 10^{-8}$ or 0·000000032.

*The Colorimetric Method of Determining Values of p*H.

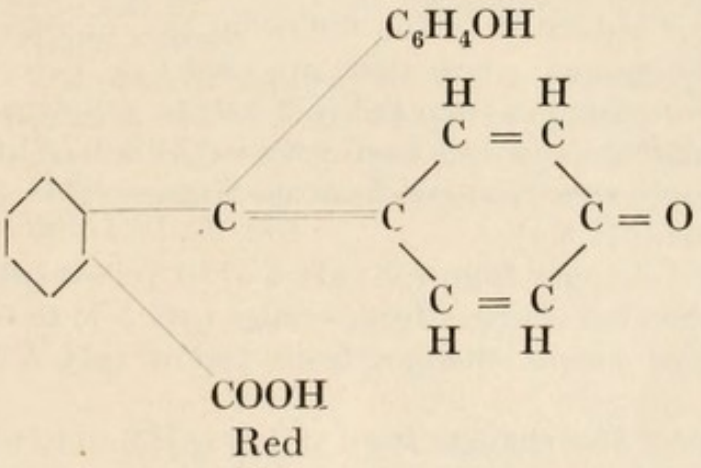
We are accustomed to determine the acidity or alkalinity of a solution by various indicators, such as litmus and phenolphthalein, which show definite colour changes. These changes of colour are traceable to different factors, for dif-

ferent indicators. The indicators, all organic compounds, may be bases, or acids, the undissociated molecules having one colour, the dissociated radicals another. Or in some cases there may be tautomeric changes (molecular rearrangements) in the molecules in solution, the different variations showing different colours. Thus there are two tautomeric forms of phenolphthalein :



Colourless

and



Red

and in the latter a hydrogen atom has migrated from one of the phenol radicals to form a carboxyl group, leaving a quinonoid structure which is responsible for the colour. It is found that these colour changes are all governed by the hydrogen-ion concentrations of the solutions. The indicators that were mentioned, litmus and phenolphthalein, and others like congo red, all change colour fairly sharply, *i.e.*, with only slight modification of (H^+), and so it is possible to use these in

acidimetric and alkalimetric volumetric measurements, in order to determine accurately the end-point of a reaction.

These indicators do not change colour at the same *p*H value. The colour-change for congo red occurs at about *p*H 4, that for litmus at *p*H 7, and that for phenolphthalein at *p*H 9.

But other compounds can be employed which only change colour gradually as the hydrogen-ion concentration changes, and which in consequence frequently show a sequence of different colours or shades of colour over a *p*H range of two units. This may be sufficiently marked to allow differences of colour to be distinguished in solutions differing in *p*H value by only 0·1.

Various series of compounds have been prepared which change in colour at different *p*H levels, and such series can be used to measure values between 1 and 10.

Typicalof these are the so-called Clark and Lub series, which used in concentrations of 0·04 per cent. or less, can be employed in the ranges indicated in the following list, in which the trade names of the organic compounds are used :

Thymol blue changes from red (*p*H 1·2) to yellow (*p*H 2·8).

Brom phenol blue changes from yellow (*p*H 3·0) to blue (*p*H 4·6).

Brom cresol green changes from olive green (*p*H 3·8) through green to blue (*p*H 5·4).

Methyl red changes from red (*p*H 4·4) to yellow (*p*H 6·0).

Chlor phenol red changes from orange (*p*H 5·1) to red (*p*H 6·7).

Brom cresol purple changes from yellow (*p*H 5·2) to purple (*p*H 6·8).

Brom thymol blue changes from yellow (*p*H 6·0) to blue (*p*H 7·6).

Phenol red changes from yellow (*p*H 6·8) to red (*p*H 8·4).

Cresol red changes from yellow (*p*H 7·2) to red (*p*H 8·8).

Thymol blue changes from yellow (*p*H 8·0) to blue (*p*H 9·6).

The measurement is simple. To 10 c.c. of the (preferably) colourless solution under examination a definite number of drops of the indicator is added, and the colour comparison is made either with a series of solutions of known *p*H value (using the same volumes in the same sized test-tubes, with the same number of drops of the indicator) or with a colour-chart, prepared by matching the colours developed in such solutions.

In body fluids and other solutions of biochemical interest there are always present a large number of different ions, so that many acids or bases, and salts, might be regarded as present in varying concentrations. It is vastly simpler to regard such solutions as mixtures of a large number of ions in equilibrium with small amounts of unionised compounds, and the conception of the hydrogen-ion concentration, especially in its shorthand, *p*H, form, determines at once the degree of alkalinity or acidity of such mixtures, no matter how complex they are. We shall see later that the main difference between such complex mixtures and simple solutions is that the complex ionic equilibria act as *buffers*, preventing any sudden change in *p*H through the addition of external acid or alkali.

References

For further details of the theory of hydrogen-ion concentration the student should consult :

Cole. "Practical Physiological Chemistry," 7th ed., Chap. I. (Cambridge, Heffer, 1926.)

Clark. "The determination of Hydrogen Ions," 3rd ed. (Baltimore, Williams and Wilkins Company, 1928.)

Steel. "Physical Chemistry and Biophysics," Chaps. VIII., IX., and X. (New York, John Wiley & Sons, Inc.; London, Chapman & Hall, Ltd., 1928.)

SECTION II

THE FOODSTUFFS, THEIR DERIVATIVES, AND RELATED COMPOUNDS

CHAPTER IV

THE SIMPLE CARBOHYDRATES AND THEIR IMPORTANT DERIVATIVES

Foreword. We may take starch and cane-sugar as typifying carbohydrates of our food, lean meat and egg-white as typifying proteins, and lard, suet and olive oil as typifying fats. As illustrated by the examples chosen, most of the food carbohydrates are of plant origin.

In vegetable tissues carbohydrates are of importance as foodstuffs for the plant (starches and sugars), and as structural material, since the walls of plant cells are largely composed of cellulose, a complex carbohydrate.

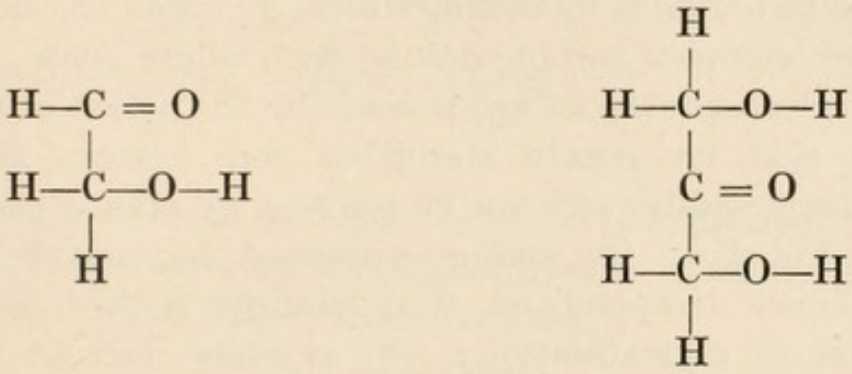
When carbohydrates are obtained in pure condition they are white compounds, amorphous or crystalline, according to the size of the molecule.

This statement holds true for all the classes of foodstuff compounds. The coloured compounds, to which are due all the colours of living tissues, belong to five groups, hæmoglobin and its derivatives (in animals), carotins and their derivatives (in plants and animals), melanins (in animals), anthocyans and their derivatives (in plants), and chlorophyll (in plants). The coloured compounds do not concern us at present.

The name *carbohydrate** suggests at once *carbon* and *water*, and most carbohydrates have the empirical formula

$$C_x(H_2O)_y.$$

However, this in no way suggests their composition. They may be regarded as aldehyde and ketone alcohols. The simplest of these derivatives are $C_2H_4O_2$ and $C_3H_6O_3$, represented graphically :



Carbohydrates may be classified as *monosaccharides* (simple sugars), *disaccharides* (more complex sugars), and *polysaccharides*. The simpler sugars are further classified according to the number of carbon atoms they contain, as *trioses* (three carbon atoms), *tetroses* (four carbon atoms), *pentoses* (five carbon atoms), *hexoses* (six), and *heptoses* (seven). A number of pentoses and hexoses occur in living organisms.

Disaccharides, which all have the empirical formula $C_{12}H_{22}O_{11}$, are to be regarded as built up from two hexoses with elimination of a molecule of water, and *polysaccharides* from a number of *monosaccharide* molecules through elimination of a number of molecules of water.

Let us study *glucose* as a typical *simple sugar*.

Glucose

Glucose, also named grape-sugar, and dextrose, occurs in plants and animals. In plants it is found in grapes (whence the name *grape-sugar*), in seeds, in various roots, and in

* The term *glucide* has been suggested by the International Committee on Biochemical Nomenclature to replace " carbohydrate." It has not been very widely adopted.

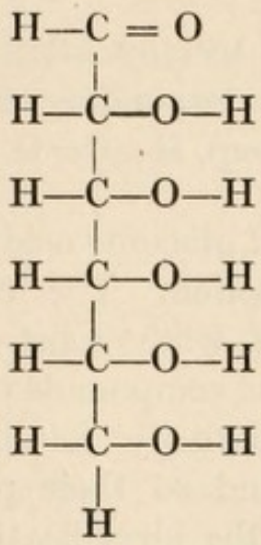
various juices, such as that of the sugar-cane. In such sources it is usually present mixed with other sugars.

In animals (and animal products) it is found in honey, in blood, in the intestine during digestion, perhaps in minute traces in normal human urine, and in large amounts in various types of pathological urines, especially those of patients suffering from diabetes mellitus.

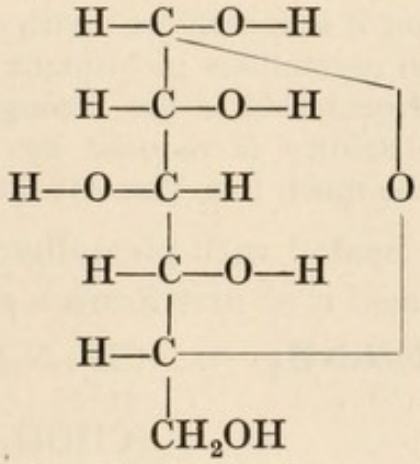
It can be obtained by boiling starch, glycogen and dextrins (all more complex carbohydrates) with dilute acids. Commercial glucose (corn syrup) is made by the action of hydrochloric acid on potato starch or corn starch. Glucose crystallises readily, and can be purified by adding excess of ethyl alcohol to its boiling saturated aqueous solution. At ordinary temperatures it crystallises with 1 molecule of water of crystallisation; the crystals melt at 86° C. From concentrated solutions at higher temperatures it crystallises in anhydrous condition, and these crystals melt at 146° C.

Crystals of anhydrous glucose are white, fine needles or prisms. It is fairly soluble in water; 100 parts of water at room temperature dissolve over eighty parts of glucose. It is sweet, but much less sweet than cane-sugar. Its sweetness is the sweetness of grapes.

Elementary analysis shows that glucose has the empirical formula CH_2O. Molecular weight determinations show that the formula is $C_6H_{12}O_6$. If it is treated with hydriodic acid it is converted into normal hexyl iodide, $CH_3.CH_2.CH_2.CH_2.CH_2.CH_2I$, showing that all the carbon atoms in glucose are united in one continuous chain. Treated with acetyl chloride, $CH_3.COCl$, it yields glucose pentacetate, $C_6H_7O(O.CO.CH_3)_5$, so behaving like a compound with five hydroxyl groups. That the remaining group may be an aldehyde group is shown by the fact that glucose gives certain aldehyde reactions, and that it is easily oxidised to gluconic acid. On these and similar grounds the formula for glucose may be written



This formula will be largely used, for convenience, though, as will be shown very shortly, glucose is more truly represented by such a ring or oxide formula as



Glucose possesses four important properties, each of which must be considered in some detail. It (*a*) reduces alkaline metallic solutions, (*b*) gives a characteristic *osazone*, (*c*) is fermented by yeast, and (*d*) is dextro-rotatory.

(*a*) **Reduction by Glucose.** Various alkaline solutions of copper are reduced by boiling with glucose, cuprous oxide separating as a yellow or red powder. The reaction consists essentially in the change

$$Cu^{++} \longrightarrow Cu^{+} \longrightarrow Cu_2O.$$

Similarly, silver salts in ammoniacal solution are reduced to metallic silver (which can be caused to deposit on a glass surface to form a mirror), and mercuric salts can be reduced to (grey, amorphous) mercury.

These *reductions* are usually attributed to the aldehyde group in glucose, the corresponding *oxidation* being regarded as the change of this group, H—C=O, into the corresponding acid group, —COOH, of gluconic acid.

(*b*) **The Osazone Reaction.** The importance of this reaction lies in the fact that, whilst most of the sugars are fairly or extremely soluble, the compounds they form with phenylhydrazine are only sparingly soluble, and crystallise in characteristic forms, and so their preparation is of considerable assistance in the identification of the sugars that give rise to them.

Phenylhydrazine, $C_6H_5.NH.NH_2$, which can be regarded as a derivative of hydrazine, $H_2N.NH_2$, forms plate-like crystals, melting at 23°, so that it is usually met with as an oil. It forms a hydrochloride, which crystallises in brilliant white leaflets. The solution of the hydrochloride is too strongly acid for osazone formation, and the acidity is reduced by addition of sodium acetate, producing the much less dissociated acetic acid.

When glucose is heated with phenylhydrazine in presence of excess of acetic acid it at first forms a *phenylhydrazone :*

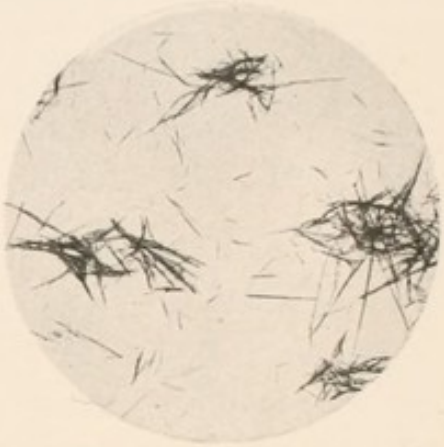
$$\begin{array}{l} CHO \\ | \\ (CHOH)_4 \\ | \\ CH_2OH \end{array} + C_6H_5.NH.NH_2 = \begin{array}{l} CH:N.NH.C_6H_5 \\ | \\ (CHOH)_4 \\ | \\ CH_2OH \end{array} + H_2O$$

Glucose phenylhydrazone is readily soluble. It immediately reacts with more of the phenylhydrazine, the reaction apparently taking place in two stages, a hypothetical oxidation product being assumed to be first formed :

$$\begin{array}{l} CH:N.NH.C_6H_5 \\ | \\ CHOH \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array} + C_6H_5.NH.NH_2 = \begin{array}{l} CH:N.NH.C_6H_5 \\ | \\ CO \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array} + \begin{array}{l} C_6H_5.NH_2 + NH_3 \\ \textit{Aniline} \end{array}$$

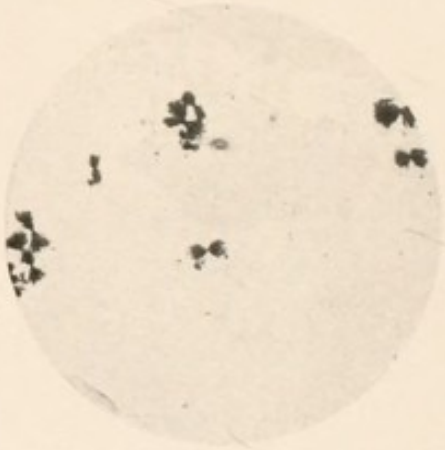
PLATE I

(*a*) Glucosazone from 1 per cent. glucose.

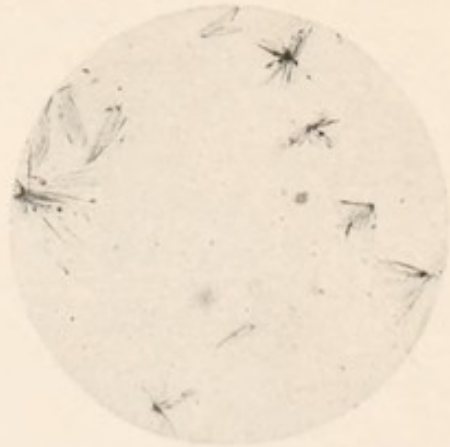


(*d*) Glucosazone from 0·2 per cent. glucose.

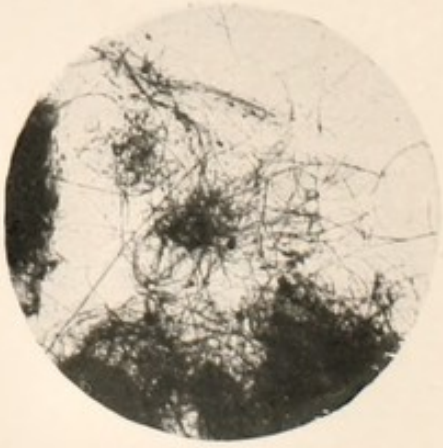
(*b*) Lactosazone from 1 per cent. lactose.



(*e*) Glucosazone from 0·2 per cent. glucose.



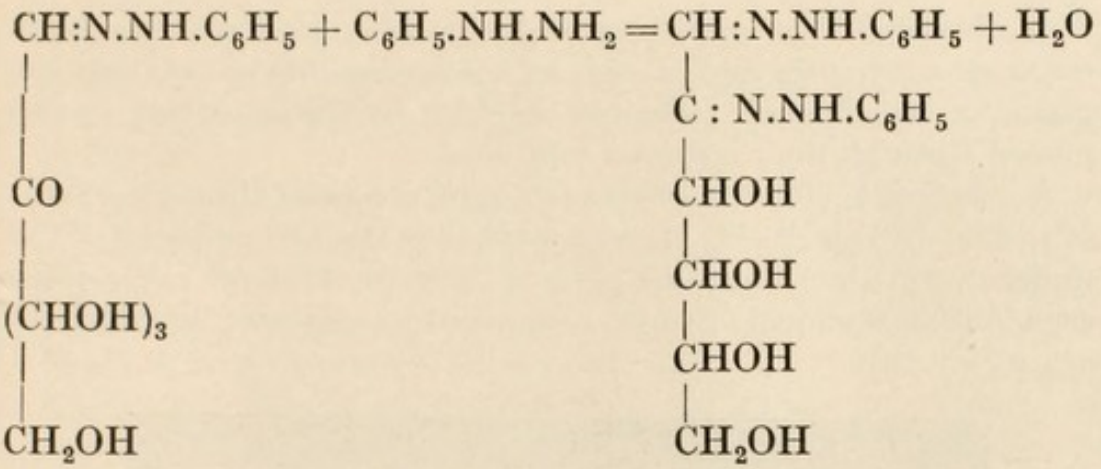
(*c*) Maltosazone from 1 per cent. maltose.



(*f*) Arabinosazone from 1 per cent. arabinose.

Black and white micro-photographs of the yellow osazones prepared by heating for thirty minutes on the water-bath the corresponding sugar solutions with 0·3 gm. of a mixture of 2 parts pure phenylhydrazine hydrochloride and 4 parts sodium acetate (for (*e*) commercial phenylhydrazine hydrochloride and a 2 : 3 ratio were employed). In each case 5 c.c. of solution were used. *Magnification* × 45.

[*To face p.* 47.



Glucose-osazone, or *glucosazone*, separates from the hot solution in yellow needles. All the osazones form yellow, well-defined crystals, which are relatively only slightly soluble in water. The way in which they are grouped together is characteristic for each sugar (see Plate I.). They have definite melting points, which serve further to identify the sugars.

(*c*) **Fermentation of Glucose.** When a solution containing glucose is rubbed up with fresh yeast, some of the enzymes in the yeast decompose the glucose with the formation of alcohol and carbon dioxide, the gas coming off from the solution in bubbles (giving it the appearance of boiling).

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

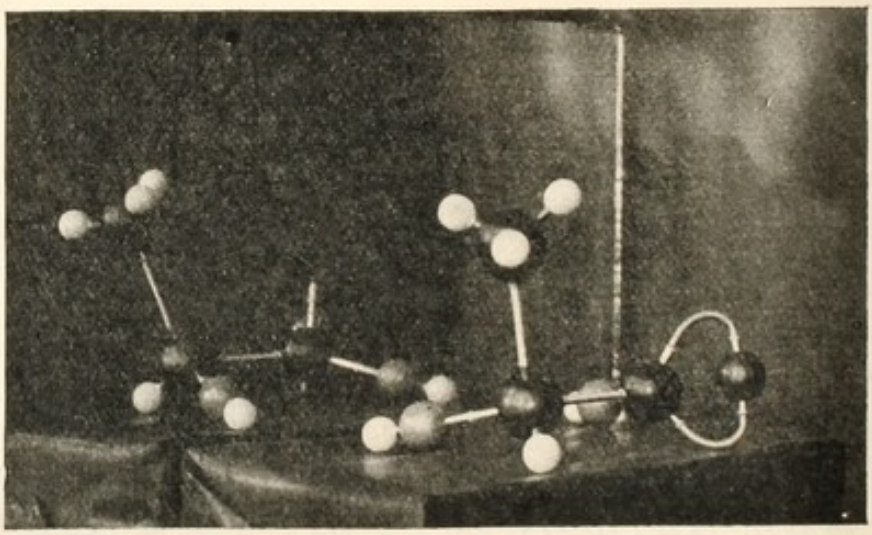
Only certain sugars are fermented by yeast, so that the liberation of carbon dioxide can be used as a test for these sugars.

(*d*) The Optical Activity of Glucose

When a ray of ordinary white light, vibrating in space in all three directions, is passed through certain crystals, such as Iceland Spar (calcium carbonate in one of its crystalline forms), then there emerge two beams of light, at right angles to each other; each vibrates only in one plane. Interposition of a "Nicol prism" removes one of these, and so one beam of "plane polarised light" can be obtained. As long ago as 1815 the French physicist

Biot observed that many naturally occurring substances—such as sugars, terpenes and camphors—were capable of rotating the plane in which such polarised light is vibrating, when it was passed through their aqueous solutions.

A rotation is effected when such light is passed through a sheet of mica. In this case it is considered that the arrangement of the molecules in the solid mica gives a twist to the light rays. This explanation obviously cannot be applied to solutions, in which we



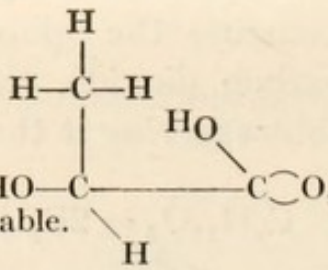


FIG. 3.—Model of lactic acid, HO—C———C⁀O, and its mirror image. They are not superimposable.

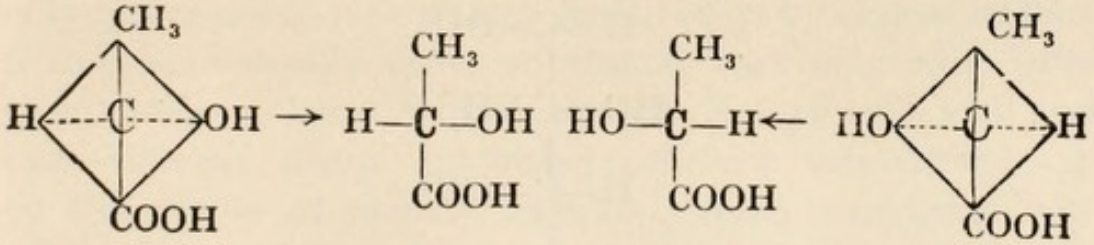
imagine the molecules as occupying every conceivable position and being in constant movement.

Pasteur, from a study of the differing crystal forms of the "right-handed" and "left-handed" varieties of tartaric acid, deduced the conclusion that the power of rotating the plane of polarisation of polarised light, the *optical activity*, possessed by certain molecules, must be attributed to a certain lack of symmetry, a certain degree of *asymmetry*, in the molecules that possess this power. In 1874 Le Bel and van't Hoff traced this asymmetry to one or more of the carbon atoms in such molecules. A carbon atom has four *valencies*, which we may imagine acting at the four points of a tetrahedron, and satisfied by linkage with four groups—with four radicals. If these four are all of different kinds, such

linkage can take place to give two different configurations in space, which are mirror images of each other, but which are not superimposable on one another. Such an arrangement is pictured in Fig. 3.

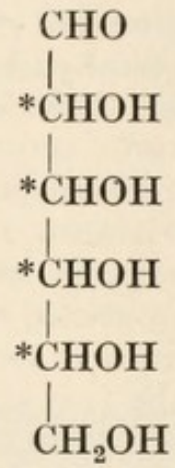
It is found that, provided some one of the four attached radicals is sufficiently massive, there is always the possibility of optical activity. If a compound is found without this property, but possessing asymmetric carbon atoms, then it is usually possible to break it up into equal mixtures of two active forms of the same compound, the activity of the two being opposite in sign, but equal in degree, so that the effect of every molecule of the one kind is exactly neutralised by the corresponding effect of a molecule of the other kind in the original compound, which is termed a *racemic* mixture.

Lactic acid is one of the simplest examples of an optically active substance. Since we cannot easily use solid figures to illustrate the asymmetry, we may imagine them projected on one plane, and thus get, for lactic acid,

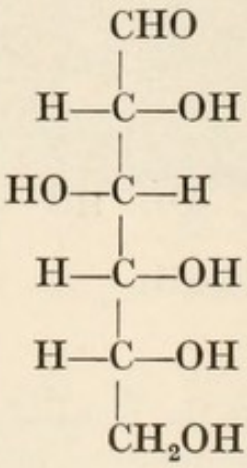


Substances which rotate the plane of polarisation clockwise, to the right, are called dextro-rotatory, and written, for example, as *d-lactic acid*. Those which rotate counter-clockwise, to the left, are termed lævo-rotatory, and the corresponding example is written *l-lactic acid*. The racemic or inactive mixture of lactic acid is written *i-lactic acid*. The so-called " sarco-lactic acid," which can be extracted from muscle, is *d*-lactic acid. When cane-sugar is fermented by certain bacteria *l*-lactic acid is formed. The ordinary lactic acid from sour milk is *i*-lactic acid, or *dl*-lactic acid.

All the sugars have asymmetric carbon atoms, and all are therefore potentially optically active. In the formula that has been written for glucose there are four asymmetric carbon atoms, indicated below by asterisks :



An immense amount of research work with glucose and similar sugars has shown that the (projected) formula of *d*-glucose, the active form that occurs naturally, is



It is on account of this dextro-rotation that glucose is frequently termed *dextrose*. The name is not fitting, however, since many other sugars possess dextro-rotation, while the corresponding lævo-glucose can be prepared, and its properties are exactly those of ordinary glucose, with the exception of the direction of rotation. Such a term as lævo-dextrose would obviously be a misnomer.

Each optically active substance produces a rotation effect the degree of which depends on the *specific* effect of the molecules of that substance, and the number of molecules of it that are acting. Consequently we are able to speak of the

specific rotatory power, $[\alpha]_D$, (D being the sodium line of the spectrum that is used for accurate measurements), which can be calculated from the formula

$$[\alpha]_D^{t^\circ} = \frac{\alpha_D^{t^\circ}}{p \,.\, l}$$

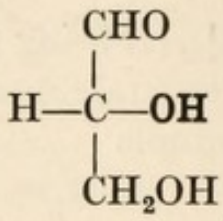
where α is the observed rotation at t° of p grams of substance, dissolved in 1 c.c. of liquid, and l is the length of tube, containing the solution, in decimetres. For the actual method of measurement practical textbooks must be consulted.

The specific rotation of glucose is $+ 52{\cdot}5^\circ$ at 20° C.

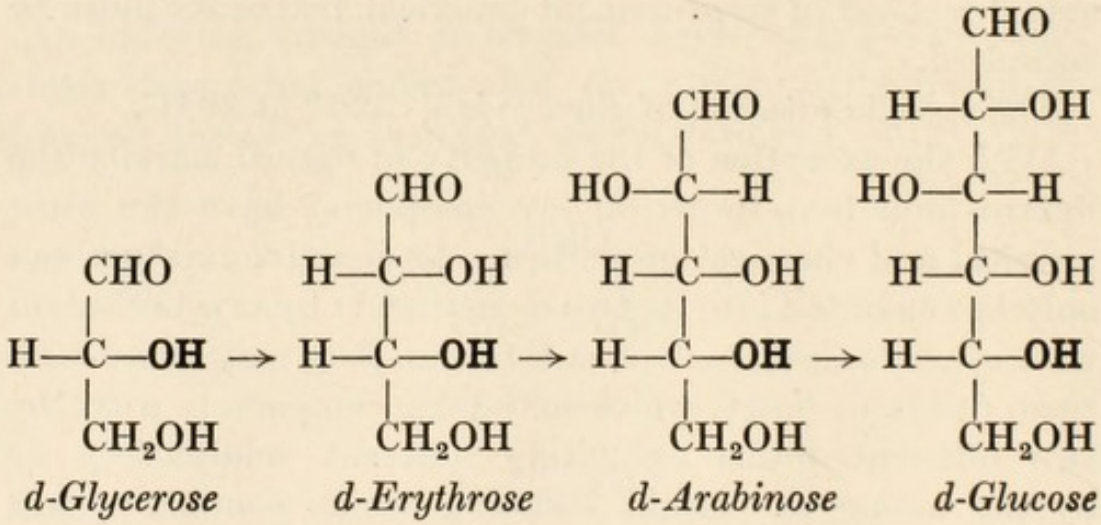
With the exception of the property of optical activity the dextro- and lævo-forms of any compound have the same physical and chemical properties. An inactive mixture can only be separated into its two constituents by crystallisation with other optically active substances that may unite with them (acids or bases, which may give compounds with the two different forms exhibiting different solubilities), or by the action of certain bacteria, which sometimes will only act on one form and leave the other, or by mechanical means, as by separating by hand the different crystalline forms.

Most of the chemical compounds that are associated with living processes are optically active, and the optical activity of a series is frequently of the same kind. Such activity is associated with the fact that the biochemical catalysers which react with these compounds, the enzymes, are themselves optically active, and can only react with one of two optically active isomers.

The one-plane configuration of the carbohydrates is now based on their relationship to *glycerose*, the simplest sugar to possess an asymmetric carbon atom. It possesses but one, and the hydroxyl group attached to it is *arbitrarily* written on the *right* for the *dextro*-compound.



All sugars derivable from *d*-glycerose are termed dextro-sugars, *whatever their rotation*, and all derived from *l*-glycerose are termed lævo-sugars. The relationship between *d*-glycerose and *d*-glucose, all the steps of which have been clearly traced and proved, is shown below :



The Oxide (*Lactone*) *Structure of Glucose.* We have not yet, however, arrived at the true configuration of most of the molecules of glucose in solution.

When crystalline glucose is dissolved in water it is found that the optical rotating power of the solution changes for many hours, sometimes increasing, but more usually decreasing, until finally that equilibrium is reached for which

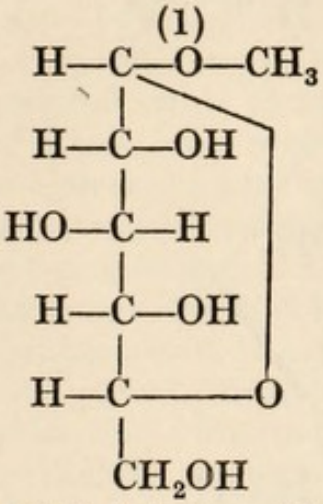
$$[\alpha]_D = +52{\cdot}5°.$$

This phenomenon of changing rotation has been called *mutarotation* (L. *mutare*, to change).

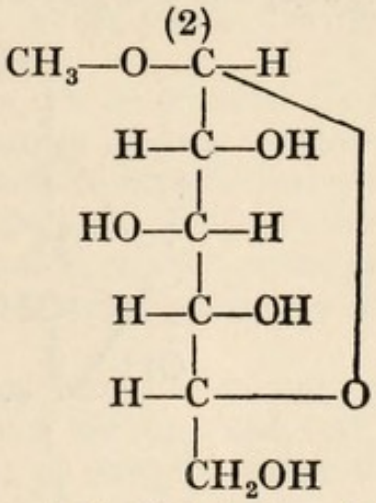
Such an effect at once suggests that there are present in the solution at least two different optically active substances, which are gradually changing, one into the other. And it

has been proved that two different forms of *d*-glucose do exist, *isomers*, but differing in rotatory power. One, *α-d-glucose*, crystallises at ordinary temperatures from 70 per cent. ethyl alcohol, and has a molecular rotation of $[\alpha]_D = + 110°$. The other, *β-d-glucose*, crystallises from aqueous solutions at temperatures above 98°, and for it $[\alpha]_D = + 19°$. If glucose contained only four asymmetric carbon atoms, then the existence of these two forms would be impossible, since with four asymmetric atoms there are only possible sixteen different arrangements, and the sugars corresponding to these are all known. Evidently, therefore, *d*-glucose in solution has in reality *five* asymmetric carbon atoms. This has been proved.

Two methyl-*d*-glucoses (simple *glucosides*) have been prepared, which are believed to be :

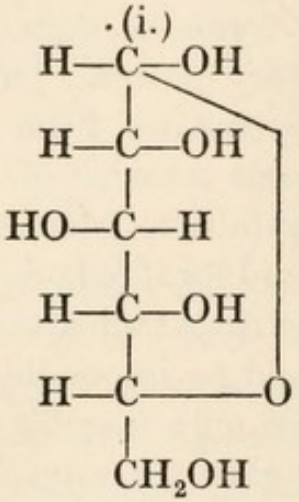


α-Methyl-d-glucoside

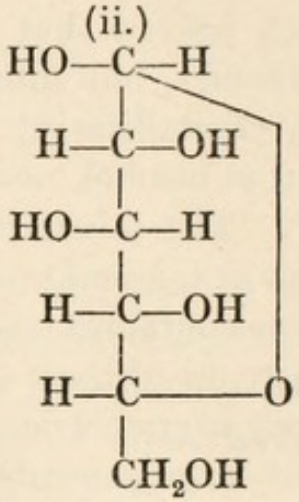


β-Methyl-d-glucoside

Each of these is hydrolysed by an appropriate enzyme. The first, the α-compound, yields a glucose of high rotatory power. On adding a drop of ammonia the rotation rapidly falls to the equilibrium value of ordinary glucose. When the second is hydrolysed, glucose of low rotatory power is produced, and when ammonia is added the rotation rapidly increases. Hence we may consider that the formulæ of these two *d*-glucoses are :

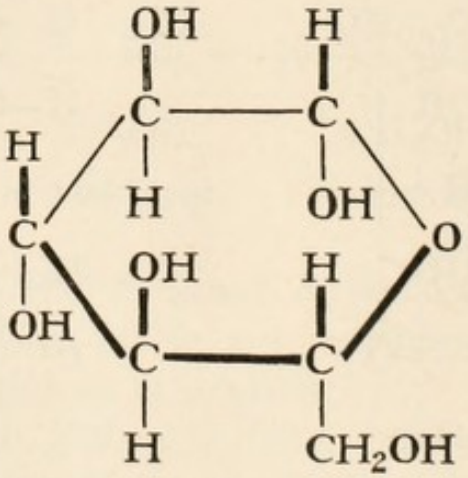


α-d-Glucose



β-d-Glucose

The position of the α- and β-OH groups attached to the first carbon atom is based on physico-chemical considerations, which cannot be dealt with here. The actual spacial arrangement is most aptly illustrated by such formulæ as the following for *β-d-glucose* (after Haworth),



From the equilibrium rotation value it can be calculated that an equilibrium solution contains 37 per cent. of the α-form of glucose, and 63 per cent. of the β-form.

Most Sugars Exhibit Mutarotation. Since, nevertheless, they behave as aldehydes (reducing alkaline solutions of metallic oxides, and yielding osazones) it seems evident that at least some of the molecules are in solution in the aldehyde form. In the case of glucose we may regard its equilibrium solution as a mixture of the α- and β-forms, *and* the alde-

hyde form, the latter being present in negligible amount. During reduction, or osazone formation, as fast as the aldehyde reacts the equilibrium is upset, and more of the α- and β-forms pass into the aldehyde form, so that ultimately most of the glucose is used up. This explains why such reactions require a definite time interval, and also why, in methods which utilise a reduction procedure for estimating glucose quantitatively, we cannot deduce the quantitative relationships from any equation, but, since the reaction is never complete, can only ascertain them empirically.

Armstrong considers, however, that it is quite possible that glucose can react in its ring form, without the intermediate production of an aldehyde compound; if this prove to be the case, then the aldehyde formula of glucose will merely be of historic interest.

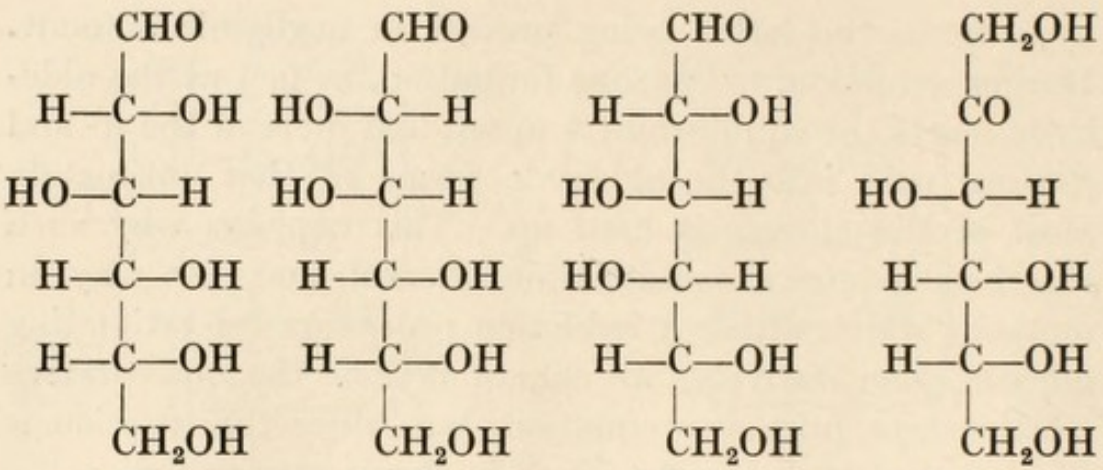
The α- and β-glucoses are said to exhibit an oxide structure. (They also resemble the cyclic esters termed "lactones," formed by elimination of water from two hydroxyl groups of such compounds as γ-hydroxybutyric acid, which yields the ring compound γ-butyrolactone.)

The ring formation in the sugars can be regarded as brought about by a molecular re-arrangement, a hydrogen atom shifting from the hydroxyl group of the fourth or fifth carbon atom, and becoming attached to the oxygen atom of the first carbon atom, a simple transposition in the condensed spacial arrangement, in which the atoms attached to the fifth carbon atom of a series are in close juxtaposition to the first.

The most recent research on sugar structure strongly supports the *amylene* ring formation, in which the first and fifth carbon atoms are linked through an atom of oxygen, rather than the *butylene* ring grouping (first and fourth carbon atoms).

The Hexoses

Although the sixteen possible *stereo-isomers* of glucose with four asymmetric carbon atoms all exist, all of which are alcohol-aldehydes (*aldoses*), and, in addition, there are a number of *ketoses* (alcohol-ketones) known, the only important ones from a biochemical standpoint are *d-glucose*, *d-mannose*, *d-galactose*, and *d-fructose* (which is lævo-rotatory). Their aldehyde formulæ are shown :



d-Glucose (aldose) | *d-Mannose* (aldose) | *d-Galactose* (aldose) | *d-Fructose* (ketose)

The corresponding lævo-compounds of these four have been prepared in the laboratory, but do not occur in nature, and are only of theoretical importance.

As is easily understood from their formulæ, *mannose, fructose and glucose give the same osazone*, glucosazone.

Mannose can be prepared from various plant seeds, which contain an anhydride condensation product, a *mannan ;* this, on hydrolysis, yields mannose. Mannose is a hard, colourless solid, deliquescent, easily soluble in water, slightly soluble in alcohol, slightly sweet.

Galactose does not usually occur free in nature, though it has been observed as a crystalline efflorescence on ivy-berries after the first sharp frost of the autumn. In animals the mammary glands construct it from blood glucose, thereafter building it up with more glucose to milk-sugar. During digestion lactose is broken down to glucose and galactose. Galactose forms a complex anhydride, *galactan*, in some sea-weeds, and its radical is present in certain glucosides (such as plant saponins, and the cerebrosides in brain tissue).

Fructose (also termed fruit sugar, and lævulose, the latter term being, like dextrose, unsuitable) occurs in certain fruits, especially tomatoes and mangoes, and, mixed with glucose, in honey.

After an unusually long heat, followed by a sudden frost, there has been observed on half-ripe tomatoes an excrescence

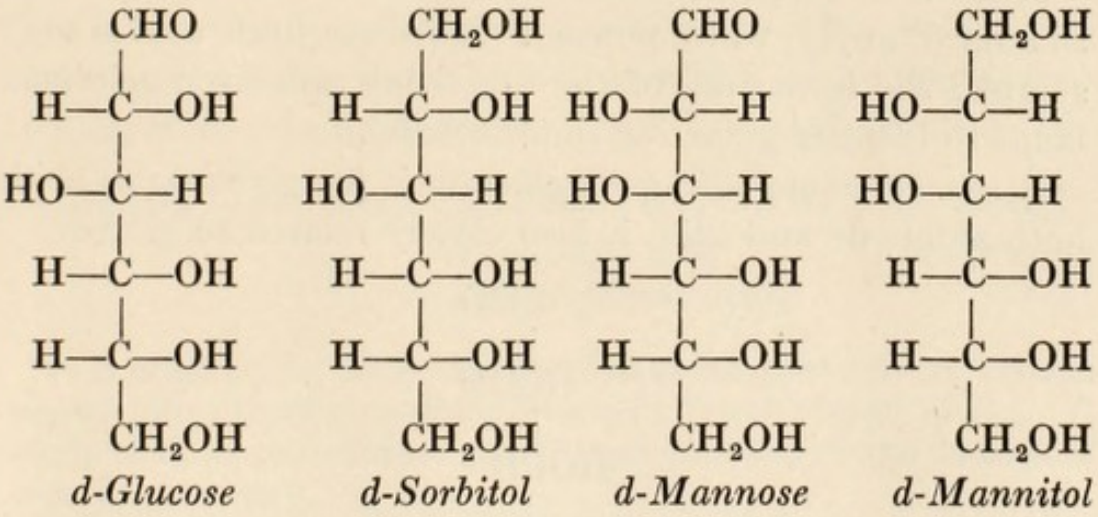
consisting of a mucilaginous nucleus permeated with a multitude of pointed needles. These crystals consisted of pure fructose.

In the sap of the young sugar-cane fructose, glucose and cane-sugar occur in about equal amount, but as the plants age the percentage of fructose decreases; the sap of the adult plant only contains traces of it. Honey contains equal amounts of glucose and fructose, along with a little cane-sugar and dextrin.

The naturally occurring fructose is strongly *lævo-rotatory*, in spite of which fact it is termed *dextro-fructose*, in order to emphasise the fact that it corresponds in configuration to dextro-glucose (and since it is a derivative of dextro-glycerose).

Derivatives of the Hexoses

Each sugar corresponds to an alcohol, from which it can be derived by oxidation, and to which it can be reduced by the action of sodium amalgam. The relationships between glucose and sorbitol, and mannose and mannitol, are shown by their formulæ:

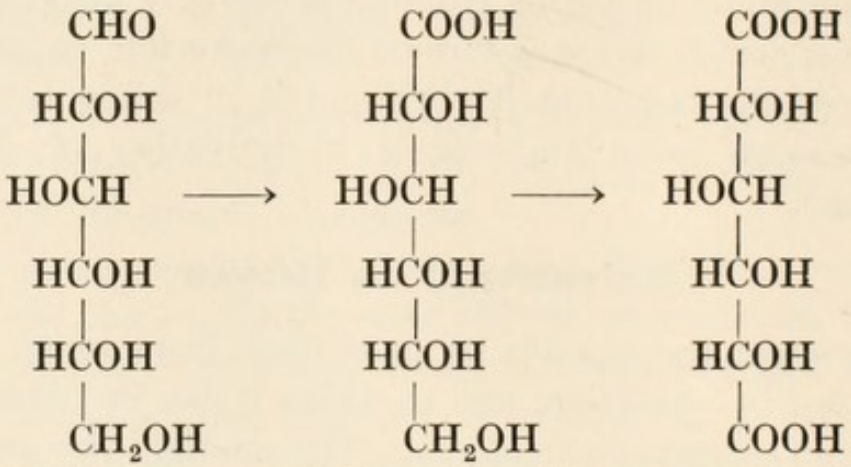


Similarly, galactose is related to dulcitol. Fructose, obviously, is related to sorbitol.

These alcohols occur in plants, and mannitol especially is widely distributed. They are sweet, white crystalline solids;

they are not fermented by yeast. In some fungi there is more mannitol than glucose.

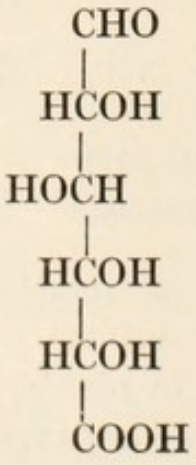
Oxidation of the aldoses gives rise to two series of acids, mono- and dibasic. Any of the ordinary reactions in which glucose plays the *rôle* of reducing agent are examples of methods for its conversion into gluconic acid. This conversion is brought about most simply by oxidising it with bromine water. Heating it with nitric acid converts it into the dibasic saccharic acid :



d-Glucose *d-Gluconic acid* *d-Saccharic acid*

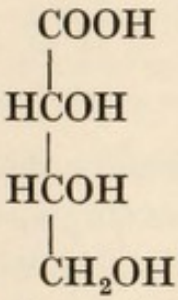
Nitric acid oxidises galactose to *mucic acid*, which separates as a hard, gritty, white powder. Since saccharic acid is very soluble, the formation of the much less soluble mucic acid helps to identify galactose (and lactose).

A second monobasic acid, *glucuronic* (or glycuronic) acid, both aldehyde and acid, is also closely related to glucose.

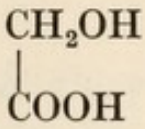


This compound is formed in the body to neutralise certain toxic compounds that are absorbed from the intestinal tract ; these are then excreted as *glucuronates* (*glycuronates*). Glucuronic acid can be prepared from glucose by the oxidising action of hydrogen peroxide.

It will have been observed that the aldoses, when oxidised, give rise to acids with the same number of carbon atoms. The ketoses, on the other hand, yield acids in which the number of carbon atoms has been decreased by one or more. Thus fructose yields trihydroxy-butyric acid and glycollic acid :



Trihydroxy-butyric acid



Glycollic acid

When the hexoses are heated for long periods with dilute hydrochloric acid (but not with nitric acid) they yield a mixture of lævulinic and formic acids, along with *humin substances* (which are dark brown, insoluble compounds, containing relatively more carbon than do carbohydrates).

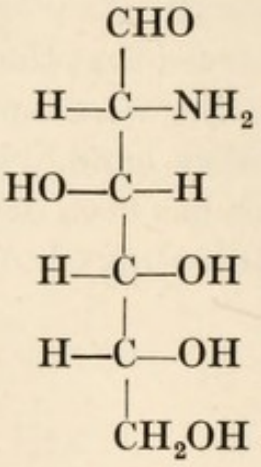
$$C_6H_{12}O_6 \longrightarrow \underset{\substack{\textit{Lævulinic acid}\\(\textit{Acetyl-propionic acid})}}{CH_3.CO.CH_2.CH_2.COOH} + \underset{\textit{Formic acid}}{H.COOH}$$

It is possible, by different chemical means, to convert various sugars into others containing more, or fewer carbon atoms. A study of these procedures belongs more properly to the domain of organic chemistry.

In presence of dilute alkali any one of glucose, mannose, or fructose, is converted into a mixture of all the three. But more concentrated alkali brings about decomposition with formation of *lactic acid* (we shall see later that the formation

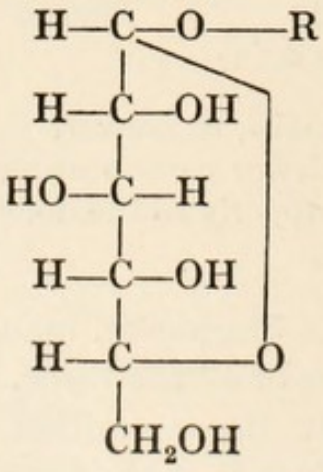
of lactic acid from glucose is biochemically of great importance).

Glucosamine (or *chitosamine*) is an important *amino*-derivative of glucose, with the formula :

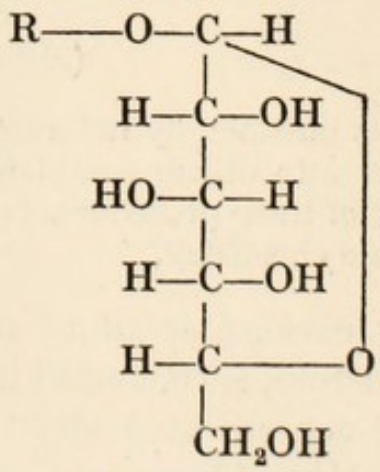


It can be readily prepared in considerable quantities from the exo-skeletons of crustacea, as, for example, the shells of lobsters. These shells consist largely of *chitin*, which on boiling with concentrated hydrochloric acid is broken down by hydrolysis, glucosamine being the chief product. Glucosamine is an important constituent (as a radical) of *mucins* and *mucoids* (proteins found in mucous secretions). *Chondrosamine*, built up into *chondroitin sulphuric acid* of cartilage, is the corresponding derivative of galactose.

Glucosides are glucose derivatives (or, more exactly, derivatives of hexoses and pentoses), whose typical formulæ are :



α-Glucoside



β-Glucoside

The linkage is a typical "ether linkage," easily hydrolysed, and in which the radical "R" may represent an alcohol, acid, aldehyde, or phenol group, etc., as the following illustrations show :—

Arbutin, $C_{12}H_{16}O_7$, hydrolyses to glucose and hydroquinone, dihydroxy-benzene, $C_6H_4(OH)_2$, an alcohol (or phenol).

Amygdalin, $C_{20}H_{27}O_{11}N$, hydrolyses to glucose, hydrocyanic acid, and benzaldehyde.

Digitalin, $C_{35}H_{56}O_{14}$, hydrolyses to glucose, digitalose, and digitaligenin.

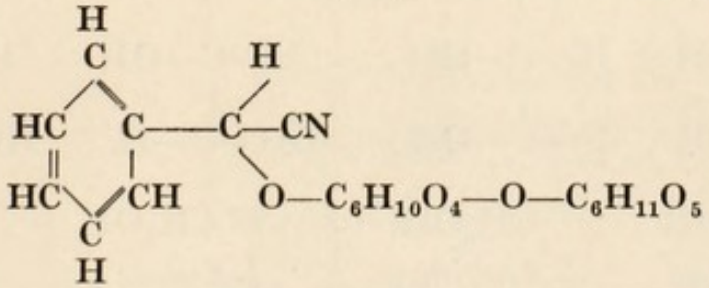
Saponins hydrolyse to glucose, galactose, and sapogenins.

Strophanthin hydrolyses to glucose, cymarose (a methyl hexose), and strophanthidin.

Vernin, $C_{10}H_{13}O_5N_5$, hydrolyses to *d-ribose* (a pentose), and guanine (a derivative of nucleic acid).

Several of the disaccharides possess the chemical nature of glucosides, and we can regard all glucose derivatives which possess the type formulæ given above as glucosides, from the two simple methyl glucosides to the complex *nucleotides* obtained from nucleic acid. The cerebrosides of brain tissue are glucosides in which the galactose radical is present, united with complex fatty compounds.

Amygdalin, which can be considered as typical of many naturally occurring glucosides, occurs in the kernels of cherries and almonds, and is hydrolysed by the enzyme *emulsin* to glucose, hydrocyanic acid, and benzaldehyde, its formula being :



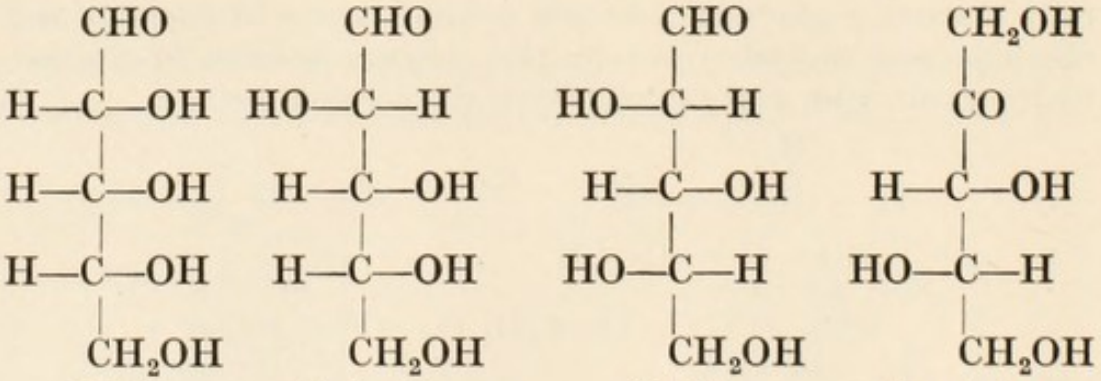
Many glucosides of great pharmacological importance are obtained from leaves and seeds of such plants as *Digitalis purpurea*, *Strophantus*, and *Scilla*. They are generally prepared by extraction with water or alcohol, and most of them are colourless, lævo-rotatory, crystalline, and bitter. They can usually be hydrolysed by enzymes present in the same tissue, but in adjacent

cells. Since the hydrolytic products usually include some toxic compound, *the purpose of the glucosides appears to be to exert a protective action against insects when the plant is bruised*, bringing the enzyme into contact with the glucoside.

Enzymes which hydrolyse glucosides are known as *glucosidases*. The best known is emulsin, which hydrolyses β-glucosides, derivatives of β-glucose, and is therefore an example of a β-glucosidase. Maltase is an α-glucosidase, and hydrolyses α-glucosides, of which maltose is an example.

Pentoses

Pentoses are not, for the most part, found as such in living tissues. In plants complex carbohydrates, *pentosans*, are widely distributed; these on hydrolysis yield pentoses. Plant nucleo-proteins and certain animal nucleotides yield the pentose *d-ribose* on hydrolysis with dilute mineral acids. Pentoses occur in small quantities in normal urine after ingestion of large amounts of certain fruits, and occasionally occur constantly in certain abnormal urines. The principal pentoses of biochemical importance are *d*-ribose, *d*- and *l*-arabinose, *l*-xylose, and *l*-xyloketose.

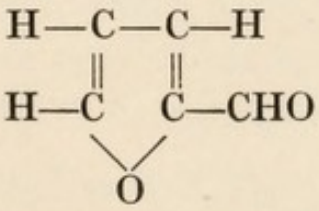


d-Ribose (lævo-rotatory) — *d-Arabinose* (lævo-rotatory) — *l-Xylose* (lævo-rotatory) — *l-Xyloketose* (dextro-rotatory)

The rotation-terminology used to describe them relates them to *d*-glycerose. It will be observed that their actual rotation is usually opposite.

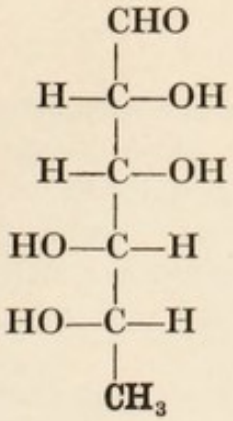
l-Arabinose is obtained from *arabans* by hydrolysis with dilute sulphuric acid. Such arabans are cherry gum, peach gum, and gum arabic. Arabinose crystallises in prisms and has a sweet taste. Wood gums, in the cell-walls of plants, are *xylans*, and yield xylose on hydrolysis. Prepared thus, the xylose is inactive. *d*-Arabinose and *d*-xyloketose have been identified in certain abnormal urines.

These pentoses all give crystalline osazones, those from ribose and arabinose being identical, and crystallising in long needles. The pentoses are not fermented by yeast, but are all "reducing sugars." They yield acids and alcohols on oxidation or reduction. On prolonged boiling with mineral acids they yield, not lævulinic acid, but furfural,



which can be distilled off, and colours aniline-acetate paper red, affording a delicate test for pentoses and pentosans.

Methyl-pentoses can be prepared by hydrolysis of many plant tissues. Typical of them is *l-rhamnose*.



whose radical occurs in many glucosides.

The cell-walls of marine algæ contain a polysaccharide *fucosan* which on hydrolysis yields the methyl-pentose *fucose*.

Sugars, with two, three, four and seven carbon atoms, dioses, trioses, tetroses and heptoses, are not of biochemical importance.

References

PRYDE. "Recent Advances in Biochemistry," 3rd. ed., Chap. VI. (London, Churchill, 1931.)

HAWORTH. "Constitution of Sugars." (London, Arnold, 1929.)

CHAPTER V

THE COMPLEX CARBOHYDRATES

The Disaccharides

THE following disaccharides are of biochemical importance: sucrose, maltose, lactose, cellose, trehalose, gentiobiose (isomaltose and isolactose). The first three are by far the most important.

Boiling any of these compounds with dilute mineral acids hydrolyses them to one or more of the hexoses glucose, fructose and galactose, according to the equation :

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose, saccharose, or cane-sugar, occurs in the sap and tissues of many plants, such as carrots, beets, sweet fruits as the banana, strawberry, and pineapple, the sap of the sugar maple, and of the sugar-cane. The last named contains about 20 per cent. of sucrose. It is both a valuable food and a condiment.

It is prepared by treating the sap or expressed juice with milk of lime to neutralise free organic acids, then boiling to remove proteins, removing excess of calcium by carbon dioxide, decolorising the solution with animal charcoal or sulphur dioxide, boiling, filtering, and evaporating under greatly reduced pressure. The sugar crystallises out, leaving an impure sugar solution (molasses).

It is usually prepared commercially from sugar-cane sap or from the sugar-beet. Manufacture from the cane, grown in the tropics, is economically preferable. Cultivation of beet in temperate climates lessens production of grains ; such grains cannot be cultivated successfully in the tropics.

Sucrose is a typically sweet substance, crystallising well, readily soluble in water, but less so in alcohol. It hydrolyses to a mixture of glucose and fructose in equal quantities. Heated to 160° it yields a glass-like substance—barley sugar—and at 200° the brown mixture we call *caramel.*

Sucrose does not reduce alkaline copper solutions, nor does it form an osazone, so that it is neither actually nor potentially an aldehyde nor a ketone.

Sucrose is dextro-rotatory, $[\alpha]_D^{20^\circ} = +66{\cdot}5^\circ$. When its solution is hydrolysed the rotation of the mixed hexoses is lævo-rotatory, so that an *inversion* of the rotation has taken place, and we speak of the process as *the inversion of cane-sugar.* The explanation lies in the fact that the specific rotation of fructose is much more strongly to the left ($[\alpha]_D = -92{\cdot}0^\circ$) than that of glucose is to the right ($[\alpha]_D = +52{\cdot}5^\circ$), so that, as an equal mixture of the two is produced, the net result as observed is a rotation to the left. A specific enzyme *sucrase*, found in yeasts, moulds, and some of the higher plants, and in the intestinal juice of mammals, is sometimes named, on this account, *invertase.*

Sucrose, treated with acetyl chloride, yields an octo-acetate, indicating that the sugar has eight free hydroxyl groups. Its constitutional formula is given on p. 68. It was recently synthetised by Pictet and Vogel (1928) and this constitutional formula confirmed.

Lactose, or milk-sugar, occurs in the milk of all mammals, and is the only sugar and the only carbohydrate in the diet of sucklings.

Cow's milk contains 4 per cent. of it, human milk 5 to 7 per cent. When the whey of milk is concentrated, lactose, which is not very soluble, crystallises out in hard and gritty crystals. It is, as we know from experience, not very sweet, and if milk contained an equal quantity of sucrose it would prove a nauseating food.

Lactose is not found in plants.

Lactose reduces alkaline copper solutions, and gives a

specific osazone (see Plate I.), suggesting that it contains a free, or, at any rate, a potential, aldehyde group. It is not fermented by pure yeast. It hydrolyses to a mixture of equal quantities of glucose and galactose. It is dextro-rotatory, its specific rotation being nearly the same as that of glucose.

Maltose, or malt-sugar, according to recent researches, is present in distinct amounts in the tubers of certain climbing plants. It is produced by the hydrolysis of starch or glycogen by the enzyme amylase. (If acid is used for the hydrolysis the maltose cannot be separated but is immediately hydrolysed further to glucose.) Maltose is hydrolysed by the specific enzyme *maltase* (present in intestinal juice) to two molecules of glucose.

It is not so sweet as sucrose, an idea of its sweetness being obtained by holding masticated bread in the mouth for two or three minutes until the salivary amylase has produced some amount of maltose.

It reduces alkaline copper solutions, gives a specific osazone, and ferments readily with yeast, the first two properties suggesting a potential aldehyde linkage.

Cellose, or cellobiose, is obtained by the hydrolysis of cellulose and of lichenin, complex polysaccharides. It hydrolyses to two molecules of glucose, and is a β-glucoside.

Gentiobiose is obtained from gentianose, a trisaccharide occurring in gentian roots, which on hydrolysis with invertase from snails yields gentiobiose and fructose. Gentiobiose also hydrolyses to two molecules of glucose.

Trehalose is found in fungi, yeast, and some marine algæ, in which it replaces sucrose. It hydrolyses to two molecules of glucose.

Isomaltose is believed to be identical with gentiobiose.

A mixture of *isolactose* and lactose is synthetised when *kefir grains* are allowed to act on a concentrated solution of equal parts of glucose and galactose. (These contain a specific "kefir lactase"; they are a symbiotic union of a yeast and bacteria and are used in the Caucasus to produce the drink *kefir* by fermenting milk.)

Melibiose is obtained by hydrolysing the trisaccharide raffinose with dilute acids. It is slowly hydrolysed by emulsin to glucose

and galactose, but is unacted on by maltase, invertase, and lactase.

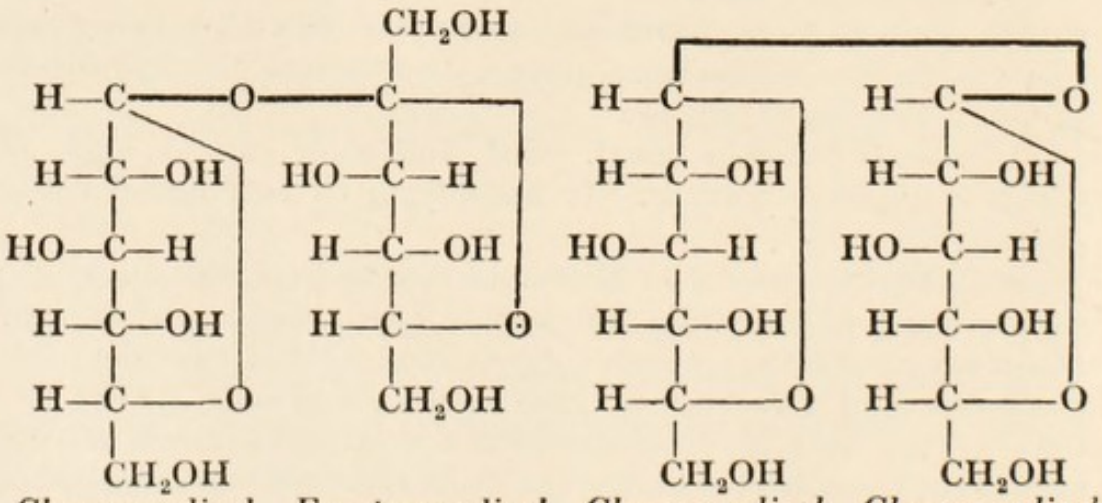
With the exception of sucrose and trehalose all these sugars exhibit muta-rotation. Studies of their constitutions show that they are built up variously from the α- and β-forms of the three hexoses, and that most of them can be regarded as α- or β-glucosides. The actions of enzymes on them depends on the type of glucosidic union, emulsin acting only on β-glucosides, maltase only on α-glucosides.

(It is not so certain that the synthetic activity of these enzymes is quite so specific. There is some evidence, for example, that when maltase is allowed to act on a concentrated solution of glucose, not only maltose but also some isomaltose—a β-glucoside —is formed, though this evidence may be based on work with impure enzyme preparations.)

The hydrolyses brought about by the α- and β-glucosidases emphasise the specificity of the *action on particular linkages*, rather than on particular compounds, and illustrate the delicacy of the action, which for any particular biochemical catalyst is not only confined to *one* optically active isomer of a compound, but further to only one of the α- and β-forms of this compound where both exist.

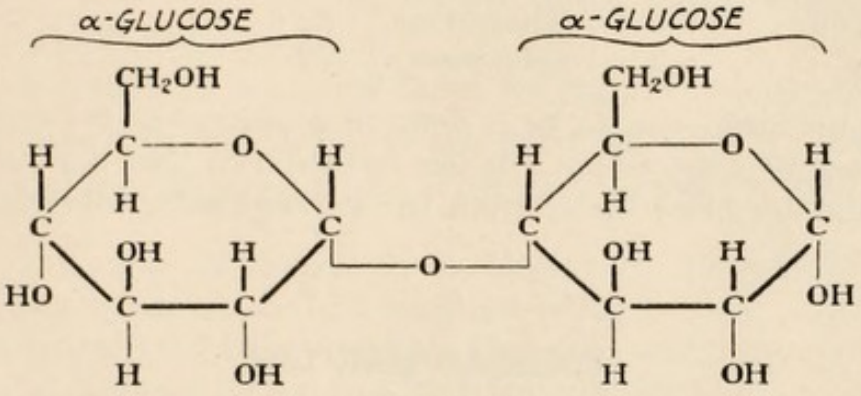
The comparison with a lock and key, frequently made to illustrate the nature of enzyme action, seems quite appropriate, provided we consider the most elaborate form of lock, which can only be turned by one correspondingly elaborate key.

The evidence at present available favours the following constitutional formulæ for the disaccharides :

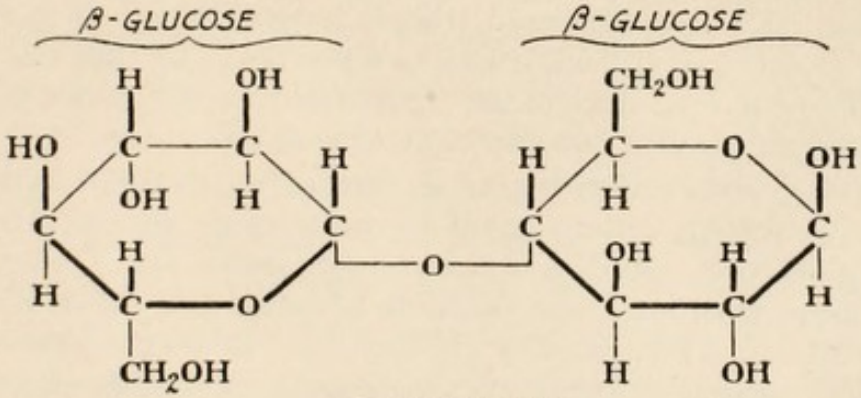


SUCROSE TREHALOSE

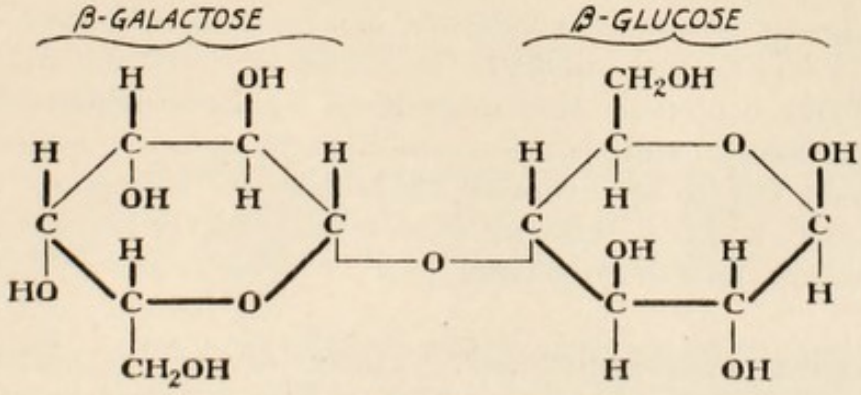
The following formulae for maltose, cellobiose and lactose (after Haworth) are suggestive of the actual molecular arrangement of these compounds in space (*cf.* p. 54).



α-MALTOSE



β-CELLOBIOSE



β-LACTOSE

The Relative Sweetness of Sugars. An attempt has been made recently to contrast the relative sweetness of different sugars. Taking sucrose as standard, and assigning it the value 100, the following figures were obtained :—

Fructose	173·3	Xylose	40·0	Raffinose	22·6
Invert sugar	127·4	Maltose	32·5	Lactose	16·0
SUCROSE	100·0	Rhamnose	32·5		
Glucose	74·3	Galactose	32·1		

There would seem to be a definite relationship between taste and spacial configuration. It has been shown that derivatives of β-glucose are more bitter than the corresponding derivatives of α-glucose.

POLYSACCHARIDES

Simpler Polysaccharides. *Raffinose*, a trisaccharide, is found abundantly in many plant tissues and products, especially molasses, eucalyptus manna, wheat, barley, fungi, bacteria, and yeasts. Cotton seed meal contains 8 per cent., so that the annual yield of cotton seed cake in the United States of America has been estimated to contain 100,000 tons of raffinose.

Raffinose unites with two molecules of water when hydrolysed by strong acids, yielding equal amounts of *d*-glucose, *d*-fructose, and *d*-galactose. Hydrolysed by weak acids, and by invertase, it combines with only one molecule of water and yields melibiose and fructose. Hydrolysed by emulsin it yields sucrose and galactose, so that the three hexose radicals are evidently linked :

galactose-glucose-fructose.

Gentianose, also a trisaccharide, can be extracted from gentian roots by 95 per cent. alcohol. It is faintly sweet, colourless, and crystallises in plates. It is hydrolysed by weak acids to fructose and gentiobiose, and therefore contains two glucose radicals.

Haricot beans and similar seeds contain *stachyose*, a tetrasaccharide, which is built up from a molecule of fructose, one of glucose, and two of galactose.

Complex Polysaccharides. These occur in plants and animals as reserve supplies of food material, that is, as stored potential energy. In plants and in some of the lower animals certain polysaccharides are important constituents of the

supporting framework or protective covering. The gums and mucilages of plants serve, at least in part, to close up wounds and protect them during the healing process.

These polysaccharides consist of large molecules of an unknown degree of complexity. They include the *starches*, *glycogen*, *dextrins*, *inulin*, *pectins*, *humins*, *celluloses*, *gums*, and *vegetable mucilages*.

With regard to many of these we cannot yet say with certainty whether the terms in use refer to a single compound, or to a group of compounds with very similar properties.

A terminology is frequently employed for the polysaccharides based on the nature of their products of hydrolysis. Thus starch, which hydrolyses to glucose, is termed a *glucosan*, and inulin, which hydrolyses to fructose, is termed a *fructosan*.

Most, if not all, of the complex polysaccharides do not reduce metallic oxides in alkaline solution, and none give osazones, so that they do not possess free or potential aldehyde groups. They are optically active, usually dextrorotatory, white, and amorphous, and they do not possess a sweet taste, though those of them which are hydrolysed by amylase, when kept long enough in the mouth, develop a sweet taste through their conversion to maltose.

Starch occurs in plants in the leaves, seeds, fruits, and tubers. Fifty to 70 per cent. of the dry weight of grains may consist of starch. It constitutes between 15 and 30 per cent. of the wet weight of potatoes. In the cells it forms characteristic granules.

It can be prepared from potatoes or grain by grinding up the material, filtering it through sieves, suspending the filtrate in water, and allowing the suspended starch granules to settle.

Starch is an amorphous white powder, insoluble in cold water, and giving with warm water an opalescent solution. It gives a blue colour with iodine solutions, which disappears on heating, and, if the heating is not continued too long,

reappears on cooling. When it is boiled with dilute acids it is broken down by stages to glucose, the known stages being erythrodextrin, achroodextrin, maltose, glucose.

Action of very dilute mineral acid in the cold slowly produces " soluble starch," which appears to differ from the ordinary form only by possessing the property of solubility in water. If this action is continued for several weeks, or, with stronger acid (4 per cent. sulphuric acid) for an hour at 80°, then *amylodextrin* (L. *amylum*, starch ; *dexter*, right hand), or erythrodextrin (Gk. *erythros*, red), which gives a port-wine colour with iodine, is first formed, and on further hydrolysis gives place to achroodextrins (Gk. *achroos*, colourless), which give no colour with iodine.

It seems not improbable that at each stage beyond that of soluble starch a molecule of maltose is set free. The evidence concerning the exact series of decompositions of starch is conflicting, and indeed the homogeneity of starch preparations is by no means certain. Starch granules contain a proportion of " amylopectin," and this contains phosphate radicals.

Starch is a very important food compound, and is also used in the arts for stiffening. The application of heat during this process produces a certain proportion of dextrins, whence the stiffness that results.

The properties of the *dextrins* have been indicated. They are soluble in water. Hydrolysis with mineral acids converts them into maltose and, finally, into glucose.

Glycogen, or *animal starch*, is present in relatively large amounts in the liver and muscle tissues of animals, and in certain plant cells, such as the yeasts. It closely resembles the erythrodextrins in its chemical and physical properties, is a white amorphous powder, soluble in water, and gives a port-wine colour with iodine.

It can be distinguished from dextrin by adding to their solutions a few drops of 0·5 per cent. orseillin BB in 90 per cent. alcohol ; glycogen gives a red colour, erythrodextrin none.

Celluloses, with other compounds, constitute the walls of plant cells. Like the starches, they hydrolyse to glucose, but they must be regarded as more complex in structure

than starch, are much less soluble, and more resistant to chemical agents. They bear the same relation to cellobiose that starch does to maltose. Ordinary white blotting-paper or filter paper is almost pure cellulose.

Inulin occurs in the sap of various plants, and constitutes from 10 to 12 per cent. of dahlia tubers. It is soluble in hot water. It gives no colour reaction with iodine. Hydrolysis converts it to fructose. It appears to contain between 20 and 30 fructose units in its complex molecule, its molecular weight being of the order of 4,000.

Various other plant products, such as *amylin*, *lavosin*, *cerosin*, and *secalin*, resemble starch or inulin more or less closely, yielding on hydrolysis either glucose or fructose. From *Lupinus luteus* is obtained *galactin*, which on hydrolysis yields only galactose. *Lichenin*, from lichens, yields glucose.

Pectins are found in apples, pears, beets, carrots, flax, etc. On mild hydrolysis they are converted into *pectic acids*, the calcium salts of which cause fruit juices to jell. If these acids are hydrolysed with mineral acids they yield *d*-galactose and *l*-arabinose. The latter is not improbably formed from galacturonic acid during hydrolysis, and recent work suggests that di-galacturonic acid, formed from two molecules of galacturonic acid by loss of water, is the simple unit-nucleus of the pectin molecule.

The *mucilages* obtained from algæ, lichens, and mosses are *galactans*.

Gums are usually *pentosans*.

It is to be noted that phosphoric acid is usually associated with these complex carbohydrates, and cannot be separated from them ; this suggests that hexose-phosphate radicals may be present in the polysaccharide molecule. We shall see later that hexose-phosphates play an important *rôle* in metabolism.

Both starch and cellulose are believed to be built up from somewhat simple units. Over 80 per cent. of starch can be converted into maltose, and at least 50 per cent. of cellulose can be converted into cellobiose. These facts suggest structural formulæ in which the greater part of the starch molecule is made up of conjugated maltose units, and at least half the cellulose molecule is made up similarly of conjugated cellobiose units. Various other theories of the unit structure of these complex polysaccharides have been put forward.

The hexagonal formula for the hexose unit suggests that in the various complexes formed from such units the hexagons are pieced together to give a structure resembling the cross-section of a honeycomb.

The specific rotations of the various sugars, and the melting points of their osazones, are shown in Table IV.

TABLE IV.

Carbohydrate.	Specific Rotation.	M.P. of Osazone.
Pentoses—		
d-arabinose . . .	− 104·5°	160° C.
d-ribose	—	160° C.
l-xylose	− 19·0°	160° C.
Hexoses—		
d-fructose . . .	− 92·0°	208° C.
d-glucose	+ 52·2°	208° C.
d-galactose . . .	+ 80·5°	193° C.
d-mannose . . .	+ 14·6°	208° C.
Disaccharides—		
lactose	+ 55·3°	200° C.
maltose	+ 136·0°	206° C.
sucrose	+ 66·5°	—
Trisaccharides—		
gentianose . . .	+ 31·2—33·4°	—
raffinose	+ 104·0°	—
Complex Polysaccharides—		
dextrin	+ 195·0°	—
glycogen	+ 197·0°	—
soluble starch . . .	+ 196·0°	—

References

For further details of the carbohydrates, the student should consult Armstrong, "The Simple Carbohydrates and the Glucosides," 2nd ed. (London, Longman's, 1924); or, Pryde, "Recent Advances in Biochemistry," 3rd ed., Chap. VI. (London, Churchill, 1931.) Also Haworth, "Constitution of Sugars" (London, Edward Arnold & Co., 1929).

CHAPTER VI

COLLOID SUBSTANCES AND THEIR PROPERTIES

THOMAS GRAHAM, Professor of Chemistry in University College, London, about sixty years ago carried out a series of experiments on the diffusion of substances in solution through animal membranes such as parchment paper. He found that certain compounds which all crystallise well, such as sodium chloride, cane-sugar and urea, would, when dissolved in water, pass freely through such membranes, whilst others, such as gelatin, albumin, gum or starch, would only pass through very slowly, if at all. He therefore differentiated between these two classes, calling the first *crystalloids*, on account of the ease with which they could be obtained in crystal form, and the second *colloids*, from their apparent similarity to glue (Gk. *kolla*, glue; *eidos*, form). *All the sugars are crystalloids; most of the polysaccharides are colloids.*

A sharp differentiation into the two classes cannot now be approved, since different solutions are known which exhibit every gradation between complete and rapid diffusibility and total non-diffusibility. Further, *the essential properties of colloidal substances are referable not to their chemical composition, but to their physical state.* Solutions of the same compound prepared by one method may exhibit crystalloidal properties (in Graham's sense), and by another method colloidal properties. Again, certain true colloids, such as a few proteins, can be obtained in crystal form.

The essence of the possession of colloidal properties depends on the size of particles in solution, or, more exactly, on the relative sizes of the molecules of the solvent, and the

molecules (or molecular aggregates or particles) of the substance dissolved (or suspended) in the solvent. When these are not greatly different the solution behaves as a *true solution*. When the molecule (or molecular aggregate) of the dissolved (or suspended) substance is very much larger than those of the solvent the solution exhibits definite colloidal properties. All sizes of molecules are known from that of hydrogen, with a molecular weight of 2, to those of starch and the proteins, with molecular weights of over 10,000. It is not, therefore, surprising that all grades of solutions exist. Bearing in mind the importance of the size of the molecule (or " particle," representing some aggregation of molecules), the following properties exhibited by colloidal solutions are easily understood :—

1. Colloidal solutions do not readily pass through parchment membranes. The simplest explanation of this, though perhaps not the most correct, is a mechanical one—the inability of the large molecules or particles to pass through the smaller interstices in the membrane.

2. Colloidal particles do not readily diffuse—for similar causes. (A molecule of albumin, a protein, diffuses less than one-tenth as rapidly as one of glucose.)

This behaviour is easily illustrated by experiment. Half-fill three test-tubes with 1 per cent. agar solution, and allow it to set to a jelly. Fill the tubes respectively with coloured solutions of copper sulphate and potassium permanganate (crystalloidal compounds) and congo red (a colloid). At the end of twenty-four hours the congo red will scarcely have penetrated the jelly, while the tubes containing the crystalloids will show marked penetration.

3. Colloidal solutions are generally slightly turbid. This can often be seen by holding them against a dark background, when the turbidity is apparent. Such turbidity is due to the large particles of the solute. These solutions nearly always exhibit the " Tyndall phenomenon " ; if a beam of light is passed through them in a dark room it becomes visible through being reflected in all directions by the large particles.

What, actually, are the sizes of these *large* molecules and molecular aggregates, large by comparison with those of liquid water (which are chiefly H_6O_3 and H_4O_2)? Our ordinary linear measurements are too unhandy for such sizes; new ones have been introduced. The thousandth part of a milli-

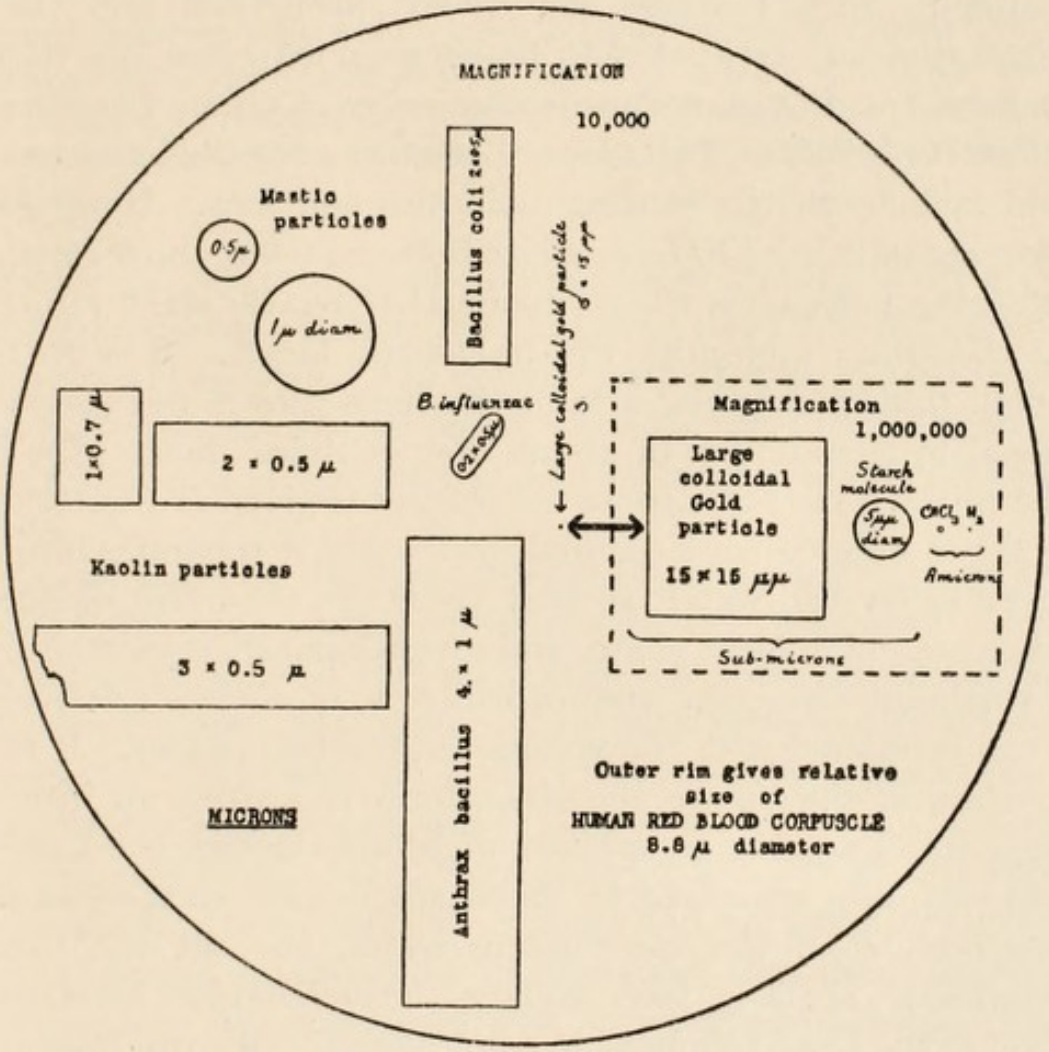


FIG. 4.—Scheme illustrating various sizes of particles and molecules. (Modified from W. Ostwald.)

metre is termed one μ (equal to 0·001 mm. $= 1 \times 10^{-3}$ mm.), and the millionth part is termed one $\mu\mu$ (equal to 0·000001 mm. $= 1 \times 10^{-6}$ mm.). Particles which can be seen under the microscope, but not by the naked eye, are termed *microns*, and have a diameter of at least 0·1μ. These form suspensions in water and other solvents (provided, when immersed in

these, they do not break up into smaller particles). Many particles which are invisible microscopically can still be detected in the ultramicroscope (in which a bright beam of light causes diffraction haloes round such particles; when they are viewed through a microscope placed at right angles to the beam of light the haloes, larger than the particles, are visible). Such particles are called *submicrons*, and their dimensions are estimated to be between $0{\cdot}1\mu$ and $1\mu\mu$ (that is, from 1 to $100\mu\mu$, a considerable range in size). *These constitute the colloids.* Particles still smaller are called *amicrons*, and include all the smaller molecules and ions. Hydrogen ions are estimated to have a size between 0·067 and $0{\cdot}159\mu\mu$, while the molecule of water vapour (H_2O) has the size $0{\cdot}113\mu\mu$. Liquid water molecules will be a little larger. It is to be noted that the spacial size of the molecules is not proportional to the number of atoms they contain. Some idea of the relative sizes of various substances is given in Fig. 4.

When chloroform is poured into water it separates into a layer below the water, so that there are then two distinct *phases*, a chloroform phase, and a water phase, separated by a single interface, the area of which is, of course, relatively small compared with the volumes of the two liquids. If the mixture is shaken up the chloroform is broken up into a large number of small spheres, *dispersed* through the water, and each now separated by its outer spherical surface, as an interface, from the surrounding water, so that the total interfacial surface has become tremendously increased though the total volumes have not altered. We can imagine the process continuing until the chloroform has broken up to molecular proportions; all the time the interface area is increasing.

Some idea of the rapid increase in the area of the interface is given by the following calculation. If a glass cylinder of 100 sq. cm. cross-section contained 100 c.c. of chloroform, and 1,000 c.c. of water at equilibrium, then the area of the interface would be 100 sq. cm. If the mixture were then so shaken that the chloro-

form was broken up into 100 globules, each of 1 c.c. volume, then the surface area of each of these spheres would be a little more than 4·83 sq. cm., whence that of them all would be just over 483 sq. cm. The area of the interface would therefore have increased nearly five times. If the division were still greater, into 10,000 spheres each of 0·01 c.c. volume, then the total area of the interface would be about 2,245 sq. cm., and the area of the interface would have increased over twenty-two times. Since these minute spheres still would have a diameter over 25,000 times greater than the largest colloid particle, when subdivision had taken place to an extent comparable with that of colloid particles, the relative increase of interface would be in the neighbourhood of 1,000.

Such an idea of gradual *dispersion* of one substance through another explains certain terms constantly employed in colloid chemistry. The colloid particles are referred to as the *dispersoid* or *internal phase*, while the fluid in which they are suspended is termed the *dispersion medium* or *external phase*.

Considering the *interface* further, it is obvious that interfaces between two phases in which the discrete particles are of the same order of size, as in true solutions, are not likely to be of more importance than interfaces between two molecules of the solvent itself. But when the particles of the dispersoid are much larger than those of the solvent, as in true colloid solutions, then there exists a vast interface between two very different phases, and all sorts of phenomena such as are exhibited in *adsorption* may occur (in fact there exists, sub-microscopically, a system comparable with, say, a gas in contact with animal charcoal).

Colloids are usually divided into *suspensoids* and *emulsoids*. The former are usually considered to be suspensions of solid particles in a solvent, the latter suspensions of liquid particles. The distinction is obviously crude and inexact. The relative properties of the two depend on the relative size of the suspended particles, of the dispersoid.

Emulsoids behave more like true solutions. The solution volume is less than the combined volumes of solute and solvent.

The surface tension is less than that of the solvent, and the viscosity greater. The solution exhibits a distinct osmotic pressure.

Suspensoids, as their name suggests, are more closely related to true suspensions, in which the discrete particles (solid or liquid) are visible microscopically. The volume of their " solutions " is the sum of those of the suspensoid and solvent, so that no contraction takes place on adding them together. The surface tension and viscosity are those of the solvent, uninfluenced by the presence of the suspensoid, and a negligible osmotic pressure, or none at all, is exhibited.

We have thus an artificial grading, true solutions, emulsoids, suspensoids, and suspensions, with every intermediate stage existing.

Brownian Movement. An interesting property of colloidal solutions and one that is easily explained when we remember the relatively large sizes of the particles concerned, is that of Brownian movement. In 1827, R. Brown, a botanist, observed that when pollen grains are suspended in water and examined under the microscope they appear to be in constant motion. This movement is shown by all suspended particles if they are sufficiently small. The maximum size seems to be about one-hundredth of a millimetre diameter, such particles just showing movement. The movement is oscillatory, and the extent and rapidity of it depend on the size of the suspended particle and the viscosity of the medium. All colloidal solutions exhibit it, though with small particles the simple oscillations are replaced by zigzag movements. The paths taken can be considered as a summation of the molecular motion due to the intrinsic energy of the molecule (existing in gases and solutions, and even extending to the larger particles) and those movements resulting from continued impacts of the smaller molecules of the solvent in their continued molecular motions, on these larger particles.

The viscosity of colloids is, as has been mentioned, chiefly exhibited by the emulsoids. The viscosity of a solution may be considered as due to the force required to cause its particles to flow past each other. We measure it by timing the passage of a definite amount of the solution through a capillary tube, and the layer of solution next the glass can be regarded as stationary, so that the time of flow of particles past similar particles is actually being measured. With emulsoids the larger particles exhibit greater friction, which requires greater force to overcome it, and the viscosity is therefore so much the greater. With suspensoids

the still larger particles are no longer subject to such molecular forces, or else occupy so little space relative to that of the solvent that their own effect is negligible.

(With suspensions in which the number of large particles is relatively very great, however, the viscosity of the system is markedly affected. Thus the viscosity of whole blood, containing five million red cells per cubic millimetre, is about four times that of pure water, while that of blood serum, freed from corpuscles, is less than twice that of pure water.)

Velocity of Sedimentation. In a solution containing only crystalloidal particles, amicrons, their diffusions due to their own intrinsic energies counteract any external force such as gravity, and bring about or maintain complete intermixture. Particles of colloidal size are not so completely independent of external forces. The gradual fall of microscopic particles suspended in a solution can be seen and the rate of fall measured. The sizes and densities of the particles are controlling factors.

From Stokes' law, defining the frictional force opposed by a liquid medium to a slowly moving sphere, it has been calculated that particles of a gold emulsion (to take one example) of diameter 0·2 mm. fall, under the action of gravity, 1 cm. in 2·5 seconds, of 2 μ diameter, the same distance in 4·2 minutes, of 200 $\mu\mu$ diameter, in seven hours, and of 20 $\mu\mu$ diameter (that of a typical colloidal particle) in 29 days.

Centrifuges can be employed to produce sedimentation at a much faster rate. Svedberg and his associates within recent years have developed an ultracentrifuge capable of 44,000 revolutions per minute, at which speed a centrifugal force up to 110,000 times that of gravity is developed. In these machines cells are placed containing the material that is being studied ; these are viewed visually or photographically through slots ; a photographic method involving refractive indices has been introduced for quantitative measurements of the rate of fall.

By measuring the sedimentation velocity in such instruments Svedberg has been enabled to ascertain the molecular weight and particle size of a number of compounds of colloidal size with an accuracy of which the experimental error is estimated at about 3 to 5 per cent. He has studied proteins especially, and has shown that their molecular weights are, in most cases, either 34,500 or that figure multiplied by some simple number. He is also able to derive from his results some information concerning the shape of such molecules. He concludes that proteins whose molecular weights are 34,500, or 34,500 $\times$ 6, are spherical, while

those with weights 34,500 × 2 or × 3, are non-spherical. Some of his results for particular proteins are quoted in Chapter VIII.

The Electrical Properties of Colloid Solutions. In aqueous solution electrolytes dissociate, and an equal number of ions carrying positive and negative charges result. Non-electrolytes, such as cane-sugar, do not dissociate and carry no electric charge. Colloids, on the other hand, usually carry an electric charge, and they can be divided into two groups according to the sign of that charge. To ascertain which group they belong to an electric current can be passed through solutions containing them. Those carrying positive charges will migrate to the cathode, those carrying negative charges to the anode, just as do electrolytes. The process is known as *electrophoresis* (Gk. *phoretos*, borne, carried). The origin of the electric charge on the colloid particle is not yet fully accounted for, but it seems probable that it is due to ordinary ionic dissociation of either one large colloidal molecule (as in proteins) or of one molecule of a colloidal aggregate (as in colloidal solutions of ferric hydroxide).

Neutralisation of the electric charge causes coagulation (and precipitation) of suspensoids, but only to a lesser extent of emulsoids. Such neutralisation can be brought about by addition of electrolytes, the ions carrying the opposite electric charge producing the effect (and being precipitated with the colloid). This effect is governed in degree by the valency of the active ion, bivalent ions being more active than monovalent, and trivalent ions still more active. The effect can also be produced by the addition to one suspensoid solution of another carrying the opposite electric charge. The effect is not solely one of reaction between equal quantities of two electric charges. Thus when a quantity of electrolyte which, when added all at once, is capable of producing the complete precipitation of a suspensoid, is added little by little, it is ineffective. The precipitation seems to depend on the production of temporary inequality and irregular distribution of the electric charges.

The Donnan Equilibrium. Donnan has established a law which has a considerable degree of application to biochemical phenomena. Whenever two solutions containing electrolytes are separated by a membrane through which one of the ions cannot pass, then, when an equilibrium has been set up on the two sides of the membrane, there exists an inequality in the distribution of the diffusible electrolytes. This is governed by the relationship that *the product of the concentrations of any pair of diffusible cations and anions on one side of the membrane is equal to the product of the concentrations of the same pair on the other side.*

Large-sized molecules, with the physical properties of colloids, cannot (easily) pass through animal membranes. If they are capable of any degree of ionic dissociation, then they will give rise to a diffusible and to a non-diffusible ion. The latter will hold on the same side of the membrane an ion of equal, but opposite, electrical charge. If such a non-diffusible ion be represented by R^+, then a simple Donnan equilibrium may be represented by the scheme—

Membrane	
$R^+ \quad Cl^-$	
$x\ Na^+\ x\ Cl^-$	$y\ Na^+\ y\ Cl^-$
(I)	(II)

From the governing relationship (proved from thermodynamical considerations) there results the equation—

$$(Na^+)_I \times (Cl^-)_I = (Na^+)_{II} \times (Cl^-)_{II}.$$

Obviously the concentrations of sodium and of chloride ions in (II) will be equal, but in (I) that of chloride will exceed that of sodium, so that

$$(Cl^-)_I > (Cl^-)_{II}$$

and

$$(Na^+)_I < (Na^+)_{II}.$$

Donnan has shown that, as a result of such different concentrations, a potential difference must exist between the

two sides of the membrane. Throughout the organism such membranes exist, with inequality of non-diffusible ions between the two sides, and resulting inequality in the distribution of electrolytes.

Surface Tension. Whereas molecules within a liquid are subjected to equal forces of attraction in every direction, at the surface these forces act on one side of the molecule only, and so tend to pull such molecules inwards into the liquid. This results in a strain on the surface, pulling it together to occupy the least possible area, and this is the force we call surface tension. The surface behaves as if stretched.

This force can be illustrated by dipping a circle of wire in a soap solution, and then floating a fine loop of silk in the soap film. If the film within the loop is removed by touching it with filter paper, the loop immediately becomes stretched to a circle.

With solutions of crystalloids surface tension effects are exerted at the surface in contact with air, at the surface between solution and container, and at the interface between solution and any solid placed in it. In colloidal solutions the surface of a colloidal particle may itself be so large as to permit surface tension effects between the particle and the solvent.

Most salts and strong alkalies increase the surface tension of a solution ; ammonia and strong acids decrease it. Most organic compounds dissolved in water lower the surface tension a little, but certain of them, such as the bile salts, produce a very marked effect. This is of great significance in connection with the intestinal digestion of fats.

Adsorption. By adsorption is meant literally a drinking up (L. *ad sorbere*, to drink up) ; the substance that is drunk up adheres to the surface, and does not penetrate the adsorbent. In a system consisting of two or more phases, and being therefore heterogeneous, it is often found that the concentration of a particular compound is either greater or less at the surface of contact than it is in either phase. This difference of concentration at the phase boundary is what we call adsorption. If the concentration of the compound is greater at the interfacial surface than it is in either phase positive adsorption has occurred, and if it is less, negative adsorption. Whenever we put on clothes we adsorb the clothing (Mathews). The term implies nothing as to the cause or causes which produce the phenomenon. These may be chemical, cohesional, electrical, magnetic, gravitational, or other forces.

A long known and outstanding example of adsorption is the power possessed by charcoal, with its relatively huge expanse of surface, to soak up carbon dioxide and other heavier gases from air, and to remove coloured compounds from solution. The property is essentially associated with large surface, such as is possessed by any solid when in a state of fine powder.

Adsorption in the case of colloidal solutions may be defined as the local concentration or condensation at the interface between two phases. From what has been stated as to the relatively very great interfacial area in such solutions, it is obvious that adsorption can play a great *rôle*, and induce many effects. It is considered that adsorption plays a large part in the actions of enzymes, the combinations of toxins with antitoxins, the sensitising of leucocytes by opsonins, and the subsequent ingestion of bacteria by the sensitised leucocytes, and the formation of adsorption compounds as between inorganic salts and proteins, and complex phosphatides. It is possible that the distribution of a substance in protoplasm is largely governed by its concentrations at different interfacial boundaries.

Sols and Gels. A *sol* is a colloid solution, actually liquid, such as a solution of gelatin. A *gel* is a colloid solution that has solidified, taking on a jelly-like form, such as an actual jelly. Sometimes simple processes such as warming will convert a gel into a sol. This process is called *solation*, and the gel is then spoken of as a *reversible gel*. But the treatment in the case of other gels merely produces precipitation. Some sols (as gums) never set in a jelly, nor coagulate, but always form more or less viscous solutions. Gelatin and agar are examples of reversible gels.

In a sol we can regard the dispersoid as actually being dispersed in the solvent, but during gelation the positions become reversed, and in the solid gel the solvent must be considered as dispersed through the solid dispersoid. In agreement with this it is found that after a gel has *set* very great pressure is required to squeeze water from it, indicating that the water no longer forms a continuous phase, but is enclosed in vesicles formed of more solid material. The dispersoid has taken up a condition

resembling the solid network of a sponge, holding the solvent within it.

Imbibition is the power possessed by a *gel* of taking up relatively large quantities of water without actually forming a liquid solution. Gelatin exhibits this phenomenon, as do also many dried tissues of plants and animals. The mass of material increases considerably, though not quite to the extent of the water taken up. The process may involve considerable transfer of energy, and can proceed in spite of great force exerted against it. Seeds in swelling can lift heavy weights, while rocks can be shattered by pouring water upon wooden wedges driven into them. Imbibition has undoubtedly to be taken into consideration in dealing with the mode of action in many living processes, such as those of growth, the passage of water into cells, etc.

Free and Bound Water. In the presence of colloidal substances water exists in two forms, in part as free and mobile molecules of H_4O_2 or some other multiple of H_2O, and in part " bound " by the colloids, and to that extent immobilised. The proportion of " bound " water differs in different tissues and under different conditions. In various plant tissues it varies from a trace to over 30 per cent. In mammalian muscle it is said to amount to 20 or 30 per cent. of the total water content. Such " bound " water appears to play an important *rôle* in imbibition, and also plays some part in various pathological conditions such as oedema.

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CHAPTER VII

THE LIPIDES, OR LIPOIDS

THE second great class of food compounds, the *fats*, forms part of a larger group that has usually been referred to as the *lipoids* (Gk. *lipos*, fat ; *eidos*, resemblance), has sometimes been termed *lipins*, and, more recently, *lipides*. The lipides include a number of different classes of chemical compounds, which are much more closely related by physical than by chemical properties. They are all insoluble in water, and can all be extracted from plant and animal tissues by the so-called *fat solvents*, such as alcohol, ether, and chloroform. They have been classified in various ways. A simple classification is :—

1. *Simple lipides*, which are all esters of the fatty acids with various alcohols.

A. Fats (oils), all *glycerides*.

B. Waxes, esters with higher alcohols.

C. Cholesterol esters.

2. *Complex* or *compound lipides*, which are compounds of fatty acids with alcohols, but contain, in addition, other radicals.

D. Phospholipides, or phosphatides, including the lecithins, kephalins and sphingomyelins.

E. Glucolipides, which contain carbohydrate radicals, and nitrogen, but no phosphorus. The cerebrosides are examples.

F. Aminolipides, sulpholipides, etc.

3. *Derived lipides*, which are compounds derived from the above groups by hydrolysis, and which still possess the general physical properties of lipides.

G. Saturated and unsaturated higher fatty acids.

H. The higher monatomic alcohols, including the sterols.

The Simple Lipides

The Fats are widely distributed in plants and animals, their content varying in different cells from less than 0·1 to over 90 per cent. They are the most compact form of stored energy, and, at the same time, since they conduct heat very poorly, they act as insulators to an organism, preventing undue loss of heat through conduction at its surface.

When hydrolysed the fats break down to the trihydric alcohol *glycerol*, $CH_2OH.CHOH.CH_2OH$, and mixtures of the two completely saturated acids, *stearic acid*, $CH_3.(CH_2)_{16}.COOH$ (or $C_{17}H_{35}COOH$, or $C_{18}H_{36}O_2$), and *palmitic acid*, $CH_3.(CH_2)_{14}.COOH$ (or $C_{15}H_{31}.COOH$ or $C_{16}H_{32}O_2$), and the unsaturated acid *oleic acid*, $CH_3.(CH_2)_7.CH:CH.(CH_2)_7.COOH$ (or $C_{17}H_{33}COOH$, or $C_{18}H_{34}O_2$), and other closely related acids.

Obviously a mixture of the three different fats, *tristearin*, *tripalmitin*, and *triolein* is possible.

$CH_2.O.CO.C_{17}H_{35}$	$CH_2.O.CO.C_{15}H_{31}$	$CH_2.O.CO.C_{17}H_{33}$
$CH.O.CO.C_{17}H_{35}$	$CH.O.CO.C_{15}H_{31}$	$CH.O.CO.C_{17}H_{33}$
$CH_2.O.CO.C_{17}H_{35}$	$CH_2.O.CO.C_{15}H_{31}$	$CH_2.O.CO.C_{17}H_{33}$
Tristearin	*Tripalmitin*	*Triolein*

There are possible also internally mixed fats of the type

$$\begin{array}{l} CH_2.O.R' \\ | \\ CH.O.R'' \\ | \\ CH_2.O.R''' \end{array}$$

where R′, R″, and R‴ represent the three different acid radicals. A number of such internally mixed fats have been found in animal fats.

Of the pure fats tristearin and tripalmitin are white, waxy solids at ordinary temperatures, and triolein is an oil. The corresponding fatty acids are respectively very similar in

physical properties. Generally speaking, the physical properties of a natural fat depend on the relative proportions of tristearin, tripalmitin, and triolein that it contains. The more oleic acid that can be derived from it the more liquid will it be. Thus beef fat contains more olein than mutton fat and has a lower melting point. Olive oil is almost pure olein (89 to 98 per cent.).

The specific gravity of these fats is less than that of water (upon which they therefore will float, so that a fat person requires relatively less energy to keep afloat). They are all insoluble in water, but soluble in the characteristic fat solvents. They crystallise from alcohol-ether mixtures in somewhat characteristic forms, such as fine, curved needles. Each pure fat has a specific melting point, which helps to identify it.

Tripalmitin melts at 65·5° C., tristearin at 71·6°. Triolein solidifies at — 6° C.

When heated with alkali or acid, or even treated with superheated steam, fats are hydrolysed. This particular hydrolysis is usually referred to as *saponification*, because when alkalies are used *soaps* are formed (L. *sapo*, *-onis*, a word of Celtic origin, is stated by Pliny to be a soap used by the Gauls as a pomade for the hair). With sodium hydroxide the process can be represented :

$$\begin{array}{l} CH_2.O.CO.C_{17}H_{35} \\ | \\ CH.O.CO.C_{17}H_{35} \\ | \\ CH_2.O.CO.C_{17}H_{35} \end{array} + 3\,NaOH = \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + 3\,NaO.CO.C_{17}H_3$$

Tristearin *Glycerol* *Sodium stearate (a soap)*

Actually, soaps are made by this process, and are *salted out* from the mixed products formed by heating fats and alkali by addition of sodium chloride ; the soaps being less soluble in the saturated brine form a scum, and can be skimmed off.

Although the fats are so insoluble in water, yet in the

presence of emulsifying agents, which reduce surface tension, such as soaps, bile salts, saponins, etc., they form stable emulsions, a fact of considerable importance for their proper digestion.

Certain other fats occur in nature. Thus butter contains 80 per cent. of fats, of which 7 per cent. are glycerides of the lower fatty acids—

Myristic acid,	$CH_3 . (CH_2)_{12} . COOH$	$C_{14}H_{28}O_2$
Lauric acid,	$CH_3 . (CH_2)_{10} . COOH$	$C_{12}H_{24}O_2$
Capric acid,	$CH_3 . (CH_2)_8 . COOH$	$C_{10}H_{20}O_2$
Caprylic acid,	$CH_3 . (CH_2)_6 . COOH$	$C_8H_{16}O_2$
Caproic acid,	$CH_3 . (CH_2)_4 . COOH$	$C_6H_{12}O_2$
Butyric acid,	$CH_3 . (CH_2)_2 . COOH$	$C_4H_8O_2$

It will be observed that all these acids contain an even number of carbon atoms. Fats from acids containing an odd number of carbon atoms are not known in nature. An interesting fat of this type is sold commercially under the name " intarvin," and is a derivative of margaric acid, $C_{16}H_{33}.COOH$. It has been used in the treatment of diabetes mellitus, with but doubtful success.

The lower fats are somewhat more soluble in water. Their corresponding acids have more marked properties, including an extremely penetrating and unpleasant odour, typified by the smell of rancid butter : butter that has become partially decomposed with the liberation of a small proportion of these acids.

Castor oil consists chiefly of the glyceride of ricinoleic acid, $C_{17}H_{32}(OH).COOH$, a hydroxy derivative of oleic acid. Cod-liver oil consists chiefly of a mixture of the glycerides of a great variety of saturated and unsaturated acids.

Glycerol is not a lipide, but a note on it may be conveniently introduced here. It is a trihydroxy alcohol, liquid at ordinary temperatures, and miscible in all proportions with water. It has a sweet taste.

When glycerol is heated with potassium hydrogen sulphate, two molecules of water are eliminated leaving the aldehyde acrolein, with an evil-smelling, penetrating odour. This test

serves to characterise glycerol, and also the fats, since they all contain glycerol radicals.

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} - 2H_2O = \begin{array}{l} CHO \\ | \\ CH \\ \| \\ CH_2 \end{array}$$

The *Waxes* are fatty acid esters of *monatomic* alcohols. They are found in various tissues and tissue products. They are characterised by their melting points, which are higher than those of the fats, and the fact that they are hydrolysed by alkalies with much greater difficulty than are the fats. The fat-splitting enzymes, the lipases, have no action on them. They are insoluble in water, have a characteristic "greasy" appearance, and are used as polishing agents and for water-proofing. Examples of the waxes are *spermaceti* and *beeswax*.

The skull of the white whale or cachelot, *Physeter macrocephalus*, contains a large cavity filled in life with an oily fluid. At death, with consequent cooling, this partially solidifies to a crystalline mass, spermaceti, which can be pressed free from the residual oil, spermacetic oil, and can be purified by recrystallisation. The spermaceti thus obtained is a mixture of waxes, consisting chiefly of *cetyl palmitate*, the palmitic acid ester of cetyl alcohol, $C_{16}H_{33}OH$, but containing also slight amounts of the esters of lauric, myristic, and stearic acids with the monatomic alcohols, lethal, $C_{12}H_{25}OH$, methal, $C_{14}H_{29}OH$, and stethal, $C_{18}H_{37}OH$. This mixture melts between 30° and 50°, is insoluble in water, but easily soluble in fat solvents.

Beeswax is a digestion product of the honey-bee, elaborated by special glands, the production of honey and wax by these animals being in inverse proportion, so that production of 1 gram of wax diminishes the yield of honey by from 10 to 14 grams. The chief constituent of beeswax is *myricyl palmitate*, the palmitic acid ester of myricyl alcohol, $C_{30}H_{61}OH$. Chinese wax consists chiefly of ceryl cerotate, the ester of ceryl alcohol, $C_{26}H_{53}OH$, and cerotic acid, $C_{25}H_{51}COOH$.

Obviously the waxes are not compounds utilisable in a diet.

The *cholesterol esters* of fatty acids are widely distributed in the animal kingdom, being found in blood, lymph, the medullated sheaths of nerves, the adrenal cortex, the gall-

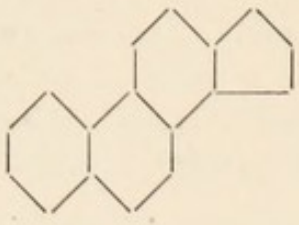
bladder (especially in certain pathological conditions), and in the secretion of the sebaceous glands.

Lanoline, the fat or grease of sheep's wool, consists almost entirely of a mixture of cholesterol stearate, palmitate, and oleate, with water. (Such cholesterol esters exhibit the phenomenon of *imbibition*.)

Cholesterol esters are not easily saponified, and, hence, do not become rancid, resembling in this particular waxes, and differing from fats. They hydrolyse to cholesterol $C_{27}H_{45}OH$, an important sterol, and fatty acids.

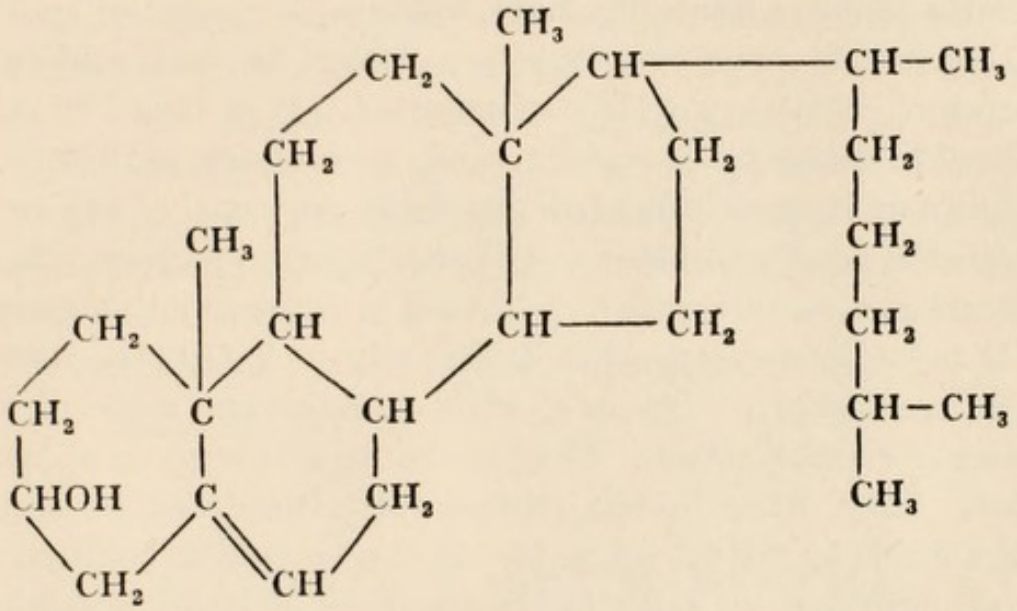
The *sterols* and the *bile acids* together make up an important group of naturally occurring compounds, the *cholane series* (Gk. *chole*, bile).

The gradual elucidation of the nature of these compounds has been brought about by a vast amount of work carried out during the past twelve or fifteen years by Windaus and Wieland and their respective collaborators. Within the past year or two ideas of the underlying skeletal structure have required revision following Roentgen-ray examinations of crystals of these structures by Bernal, and the applications of his ideas by Rosenheim and King. It seems now to be finally agreed by Windaus and by Wieland that the skeleton of (i.) cholesterol and the sterols, (ii.) various cholic acids, and (iii.) the endocrine compounds secreted by the gonads and a number of compounds closely related to them, has the four-ring structure :

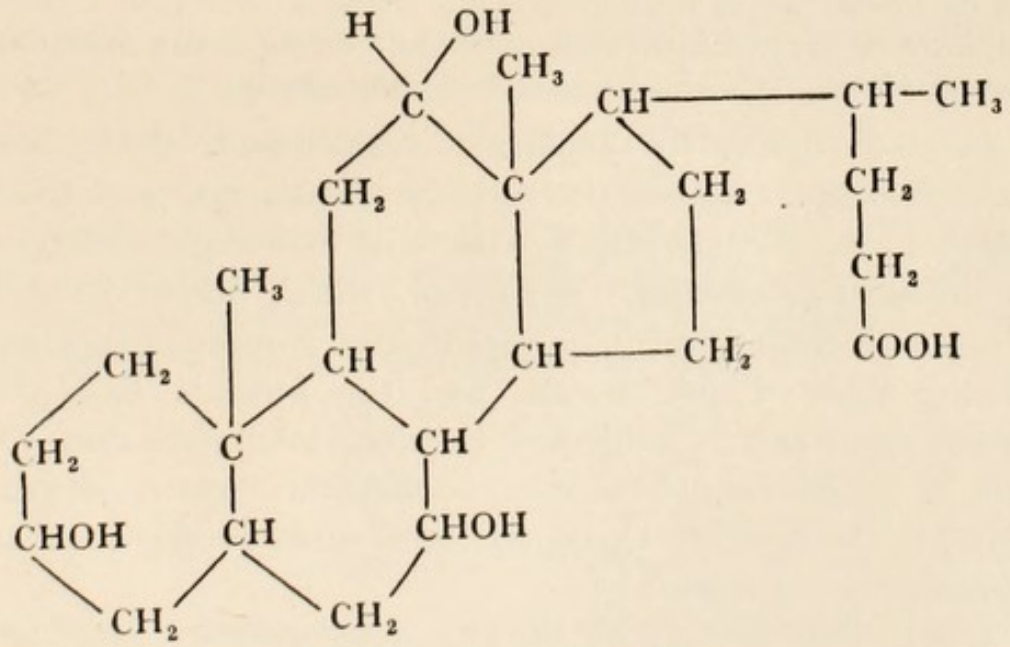


The exact constitution of the individual compounds has not yet been finally settled. The formulae which are given here are at any rate close approximations and are probably accurate.

Cholesterol is an unsaturated monohydric alcohol, with the probable constitution :



The *bile acids* are compounds of certain " cholic acids " with taurine and glycine, and their salts play an important *rôle* in digestion (*cf.* pp. 156, 158). The formula of *cholic acid*, $C_{24}H_{40}O_5$, is probably :



The closely related endocrine compounds secreted by the ovaries and testes are dealt with in Chapter XIX.

Cholesterol is found mixed with animal fats, and in small quantities in bile, blood, milk, yolk of egg, the medullated sheaths of nerve fibres, the liver, kidneys, and adrenal bodies, and, generally, wherever its esters occur. In small amounts, therefore, it is very widely distributed. It is found in considerable quantity in cod-liver oil, and under pathological conditions it constitutes from 64 to 98 per cent. of the commonest type of gallstones ; it is also found in ætheromata of the arteries, in tubercular cysts, and in carcinomatous tissue.

It crystallises very characteristically in flat plates with a re-entrant angle. These crystals contain one molecule of water of crystallisation. They are white and waxy in appearance. Like other lipoids cholesterol is insoluble in water, but soluble in the fat solvents. It can be held in solution or as an emulsion in water in the presence of soaps, saponins, bile-salts, or lecithin.

Cholesterol gives some very striking colour reactions which serve to identify it easily. Thus if a few drops of acetic anhydride and then of concentrated sulphuric acid are added to its solution in chloroform a red colour develops, which changes to blue, and finally bluish green, while if to a few crystals are added a drop of sulphuric acid, and then a drop of very dilute iodine solution, a play of violet, blue-green and red colours results.

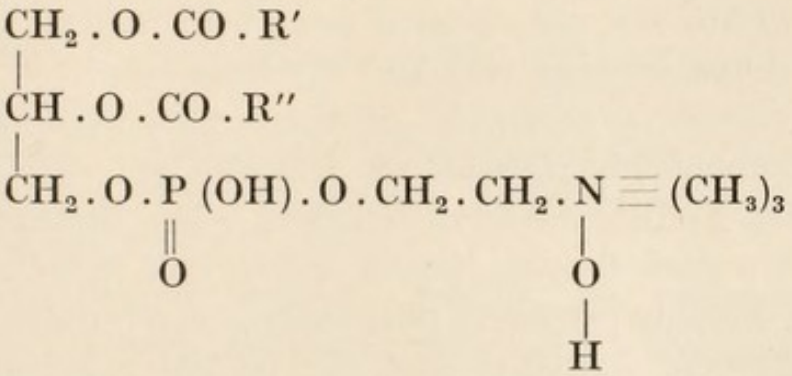
In plant tissues the *phyto-sterols* are found, closely allied to cholesterol. *Sitosterol*, found in wheat, rye, and linseed oil, is an isomer, also $C_{27}H_{45}OH$, with but slight differences in physical properties. *Ergosterol*, which occurs in traces along with cholesterol in animal tissues, is more unsaturated, having three double bonds, and the formula $C_{27}H_{41}OH$. When ergosterol is subjected to ultraviolet radiation *Vitamin D* is formed. This compound, now termed *calciferol*, also has the formula $C_{27}H_{41}OH$, and must be regarded as a stereoisomer of ergosterol.

The Complex Lipides

The *phosphatides* or *phospholipides* are those esters of phosphoric acid which resemble fats in their physical properties.

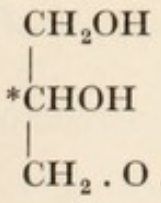
All the three groups, the lecithins, kephalins, and sphingomyelins, are soluble in hot alcohol. Acetone, in presence of fat, extracts principally but incompletely unchanged kephalin and lecithin, while ether extracts both of these and also products of their partial hydrolysis. Sphingomyelin is almost insoluble in ether. The groups can therefore be partially fractionated by such solvents, and, thereafter, tediously purified through formation of specific compounds with such salts as cadmium chloride.

The *lecithins* all have the general formula :



The radicals of the lecithins are all built up through ester linkages, which give their molecules a certain lability. On hydrolysis the lecithins yield glycerol, phosphoric acid, *choline*, and fatty acids. Most lecithin molecules contain a saturated and an unsaturated fatty acid radical.

The above formula is considered correct, and not the alternative formula in which the phosphate-choline radical is attached to the middle carbon of the glycerol radical, since not only are all lecithins dextro-rotatory, but the derived *glycerophosphoric* acid is also active, and must therefore have the formula :



All lecithin preparations contain several different lecithins. Such mixtures yield on hydrolysis both stearic and palmitic acids, and two or more unsaturated acids according to the source of the preparation. That from egg-yolk yields oleic

acid, $C_{18}H_{34}O_2$, linolic acid, still more unsaturated, $C_{18}H_{32}O_2$, and arachidonic acid, $C_{20}H_{32}O_2$. The proportions of the last two present are small. Individual samples of egg-lecithin show definite differences in the content of these two acids.

Lecithins from brain yield oleic and arachidonic acid, but linolic acid has not yet been isolated from them. Lecithins from liver yield oleic and arachidonic acids, the former predominating. Soy-bean lecithin yields oleic, linoleic, and linolenic acid, $C_{18}H_{30}O_2$.

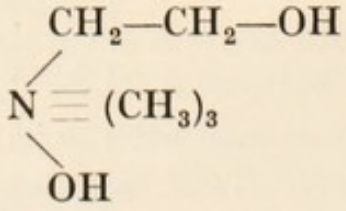
Thus liver contains at least four lecithins, each with one saturated and one unsaturated fatty acid radical, brain four and, perhaps, six, egg yolk and soy-bean six.

On account, presumably, of the unsaturated fatty acid radicals invariably present, the lecithins are very unstable, and easily hydrogenated or oxidised. The lecithin of commerce is a dark brown, impure mixture of these and other lipoids. Freshly prepared, pure lecithin is a brownish, greasy solid. Levene considers that the typical physical characteristics, the brownish appearance and very soft consistency, are due to the presence of the highly unsaturated fatty acid radicals.

The lecithins are hydrolysed by lipases (or, probably more correctly, esterases, usually found along with lipases) to fatty acids, glycerophosphoric acid, and choline.

Cobra venom contains an enzyme which has the very specific property of hydrolysing lecithins (and kephalins) in such a way that only the unsaturated fatty acid group is split off. The resulting *lysolecithins* (and *lysokephalins*) very powerfully hæmolyse (break up) red blood cells, and also combine in equimolecular proportions with cholesterol.

Choline, or trimethylhydroxyethylammonium hydroxide, *is not a lipide.*



It is a syrupy, strongly alkaline liquid, miscible easily with water or alcohol. It is precipitated from such solution by a solution of iodine in potassium iodide, while boiling its aqueous solution decomposes it into trimethylamine, ethylene oxide, and ethylene glycol. It yields crystallisable salts with hydrochloric acid and with platinum chloride.

The *kephalins* all have the general formula :

$$\begin{array}{l} CH_2—O—CO—R' \\ | \\ CH—O—CO—R'' \\ | \\ CH_2—O—PO(OH)—O—CH_2—CH_2—NH_2 \end{array}$$

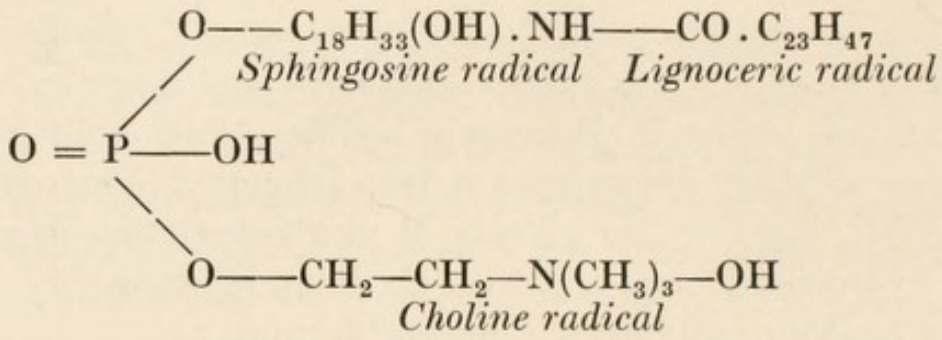
Thus they are lecithins in which the choline radical has been replaced by that of amino-ethanol, or amino-ethyl alcohol, or *cholamine*, $HO.CH_2.CH_2.NH_2$.

Most molecules of kephalin contain the radical of a saturated and of an unsaturated fatty acid. The saturated radical is usually that of stearic acid, though other acids possibly occur.

In brain-kephalins at least three unsaturated acids have been obtained, oleic, linoleic and arachidonic. So that evidently a number of different kephalins exist. It is not known whether the saturated or unsaturated radical is in juxtaposition to the phosphate radical (and this is also true for the lecithins).

Aminoethanol, cholamine, is, like choline, not a lipoid. It can be obtained by hydrolysis of kephalins. It is a colourless, viscous oil, miscible with water and with alcohol in all proportions. It is strongly alkaline, and gives various crystalline double salts.

The *sphingomyelins* are not glycerides. They yield two molecules of base and only one of fatty acid on hydrolysis, and may be described as diamino-monophosphatides. The "sphingomyelin fraction" from brain contains three of these compounds of which one is :



In the other two the corresponding fatty acid radicals are those of stearic acid, and the unsaturated nervonic acid, $C_{24}H_{46}O_2$.

Sphingomyelin is a common constituent of the cellular material of the animal kingdom—for example, brain, kidney, liver and egg-yolk—but has not yet been found in the plant kingdom. It crystallises in very thin plates, which often congregate in rosette forms. When dried it gives a white powder of waxy character. Its principal impurities are galactosides. Pure sphingomyelin gives a negative test for galactose.

Sphingosine (not a lipoid) is an unsaturated amino-alcohol. It has not yet been obtained from plant material. Its constitution is probably

$$CH_3 . (CH_2)_{12} . CH : CH . CH(NH_2) . CHOH . CH_2 . OH.$$

All the phosphatides possess amphoteric properties, which is to say that they are capable of acting as both base (with acids) and acid (with bases). In the free state they are only moderately soluble in water. They are not diffusible through such membranes as are impermeable to proteins. Like proteins they probably have isoelectric points (for the explanation of which see the chapter dealing with Proteins).

In 1913–14 Mayer and Schaeffer published work leading to the conclusion that the phosphatide content is a constant for any type of living cell, and apparently independent of physiological factors such as growth and state of nutrition (except to a slight extent in the liver).

It has been claimed by various writers that tissues contain other similar phosphatides. Such claims have been disproved. Thus *cuorin*, a preparation from heart tissue, has been shown to be a mixture of kephalin and its decomposition products. *Heparphosphatide*, from liver, is a similar mixture.

Kephalin is an important factor in the clotting of blood, as Howell has proved. Delezenne and Fourneau have demonstrated that a cleavage product of lecithin is an active agent in hæmolysis.

The *Glucolipides* all contain a carbohydrate radical. The *cerebrosides* can be considered as typical of this class of compound; they are all galactose derivatives, *Galactolipides*. These are important constituents of the white matter of nervous tissue (whence their name), and have also been found in the spleen, pus, and egg-yolk. They are much less soluble than other lipoids, being extracted from tissues that contain them by boiling alcohol, but being insoluble in cold alcohol, cold and hot ether, and water. Four are known, phrenosin, kerasin, nervone, and hydroxynervone. Treatment of either with baryta splits off a fatty acid (specific for each), leaving *psychosine*, a crystalline compound of galactose and sphingosine radicals joined through an ether linkage which leaves unaffected the amylene oxide structure of the sugar radical.

Phrenosin, or *cerebron*, has the formula $C_{48}H_{93}NO_9$. It hydrolyses to galactose, sphingosine, and cerebronic acid (α-hydroxylignoceric acid).

Kerasin, $C_{48}H_{93}NO_8$, hydrolyses to galactose, lignoceric acid, and sphingosine.

We may imagine the molecule of kerasin as built up in some such fashion as

$C_6H_{11}O_5$——O——	$C_{18}H_{33}(OH).NH$——	$CO.C_{23}H_{47}$
Galactose radical	*Sphingosine radical*	*Lignoceric acid radical*

In brain phrenosin is present to a much greater extent than kerasin.

In a case of splenomegaly (Gaucher type), Lieb has recently isolated kerasin to the extent of 10 per cent. of the dry substance of the spleen. In a second case 8 per cent. was found, but no trace of phrenosin.

Nervone yields nervonic acid; hydroxynervone the corresponding α-hydroxy acid (see below).

The *amino-lipides* and *sulpho-lipides* still require detailed examination and classification.

The Derived Lipides

The *saturated acids* have been dealt with. It will have been observed that we are chiefly concerned with stearic and palmitic acids, whose radicals occur in neutral fats, in waxes, and in phosphatides.

The brain fatty acids are mainly either C_{18} or C_{24} acids, multiples of C_6, and it has been suggested that they are derived from hexoses. Of the fatty acids derived from the ether-insoluble fraction of brain over 90 per cent. are C_{24} acids, chiefly in cerebroside combination. Lignoceric acid (*a*) and α-hydroxylignoceric acid (*b*) are saturated, nervonic acid (*c*) and α-hydroxynervonic acid (*d*) are unsaturated :

(*a*) $CH_3 . (CH_2)_7 . CH_2 . CH_2 . (CH_2)_{12} . CH_2 . COOH.$

(*b*) $CH_3 . (CH_2)_7 . CH_2 . CH_2 . (CH_2)_{12} . CHOH . COOH.$

(*c*) $CH_3 . (CH_2)_7 . CH : CH . (CH_2)_{12} . CH_2 . COOH.$

(*d*) $CH_3 . (CH_2)_7 . CH : CH . (CH_2)_{12} . CHOH . COOH.$

The lower fatty acids are found as glycerides in small amounts in butter (and therefore are present in milk). It has been pointed out that all the naturally occurring fatty acids, saturated and unsaturated (with the exception of the derived fatty acid, cerebronic acid), possess an even number of carbon atoms. This has an important bearing on their synthesis and degradation in the organism.

Of the *unsaturated fatty acids* oleic acid is present in by far the greatest amount. Linoleic acid, $C_{18}H_{32}O_2$ (which, like oleic acid, hydrogenates to stearic acid, and which has two unsaturated linkages), and arachidonic acid are found linked in the phosphatides. Arachidonic acid, $C_{20}H_{32}O_2$, has four

unsaturated linkages, and hydrogenates to arachidic acid, $C_{20}H_{40}O_2$, which occurs in combination as a fat in earth-nut oil, and probably in cacao butter.

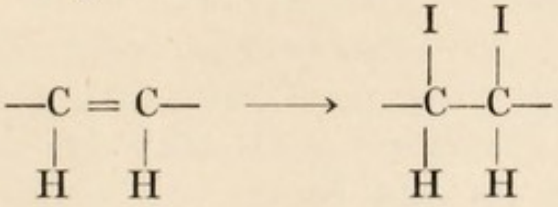
Fats built up of such unsaturated acids are widely distributed in the organism. Thus in the dog the arachidonic acid content of the liver is 0·18 per cent., of the pancreas 0·10, of the kidney 0·11, of the lung 0·05, of the spleen 0·06, of muscle 0·01–0·02.

A still more unsaturated acid, $C_{22}H_{36}O_2$, has been obtained from a Japanese alga, *Sargassum sajamianum*. When spread on the skin this develops the characteristic fish odour.

A trace of an elaidic acid, " vaccenic acid," isomeric with oleic acid but with the double bond in an unsymmetrical position— $CH_3.(CH_2)_9.CH : CH.(CH_2)_5\ COOH$—has very recently been isolated from cow-fat, lard and tallow.

The number of unsaturated acids detected in the animal organism is increasing.

The determination of the *iodine number* is a useful method to ascertain the degree of unsaturation of such acids, and similar compounds with unsaturated linkages, since these unite with iodine, decolorising it, and the amount of iodine taken up from solution by a known quantity of such a compound establishes the number of double bonds, as is indicated by the change—



Of the *higher alcohols* cholesterol and the related sterols have already been discussed, and their lipide properties mentioned.

Cetyl alcohol (ethal), $C_{16}H_{33}OH$, *ceryl alcohol* (cerotin), $C_{26}H_{53}OH$, and melissyl or *myricyl alcohol*, $C_{30}H_{61}OH$, are all white crystalline compounds, insoluble in water, but easily soluble in alcohol and ether, so that they may also be considered as lipoids.

Glycerophosphoric acid is a strong, dibasic acid. Its presence in animal tissues and fluids in traces results probably only through decomposition of lecithins or kephalins. It is a syrupy liquid strongly resembling glycerol in physical properties, and not a lipoid.

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CHAPTER VIII

THE PROTEINS AND AMINO-ACIDS

THE term *protein** is derived from the Greek *protos*, first, and proteins may be regarded as the most important of the three classes of foodstuffs, proteins, fats, and carbohydrates, since carnivorous animals, at any rate, can subsist on a diet of protein without fat and carbohydrate, and can form from protein the fat and carbohydrate they require.

Proteins all contain carbon, hydrogen, nitrogen and oxygen; most of them also contain sulphur. Some contain phosphorus in addition. Analysis shows that the extremes in which these elements are present are:

C	50·6—54·5	per cent.	
H	6·5— 7·3	,,	
N	15·0—17·6	,,	
O	21·5—23·5	,,	
S	0·3— 2·2	,,	(when present).
P	0·4— 0·9	,,	(when present).

The German name for protein is *egg-white substance* (Eiweiss-stoff), so that evidently the proteins present in egg-white may be considered as typical of the class. They may be prepared by the following processes:

The whites of absolutely fresh hen's eggs are carefully separated from the yolks, then beaten up, separated from the foam produced, and mixed with an equal volume of saturated ammonium sulphate. The precipitate which is formed—*egg-globulin* (L. *globulus*, globule)—is filtered off. The filtrate is allowed to concentrate slowly at room temperature, and after a while solid separates. This is dissolved in water, and ammonium sulphate solution is added until a slight cloudiness appears. On standing, *crystals* of *egg-*

* The International Committee on Biochemical Nomenclature has suggested that the term "protein" be replaced by "*protide*." The suggestion has been but little adopted.

albumin (L. *album*, white) separate. These can be purified further by dissolving them in water, and dialysing the solution in collodion bags against distilled water until it is free from inorganic salts. On concentrating the dialysate under very low pressure the egg-albumin separates as an *amorphous white powder*.

Egg-globulin can be most easily prepared by diluting egg-white with several volumes of water. The globulin is held in solution in the egg-white by the concentration of mineral ions present (chiefly sodium and chloride ions). On dilution the concentration of salt becomes too small to retain the globulin in solution, and most of it is precipitated. It can be purified by filtering it off, re-dissolving it in 1 per cent. sodium chloride solution, and dialysing away the salt, when the globulin gradually separates, also as a white amorphous powder.

There is some evidence that egg-albumin is not a homogeneous single compound, but is a mixture of two or more albumins. However, for the purpose of considering the properties of albumins, we may regard it as typical.

There is stronger evidence that egg-globulin is not homogeneous, and may indeed be a mixture of globulins and proteins that are not globulins at all. As a typical globulin, therefore, we may consider *edestin*, easily obtained from the seed of the hemp plant.

Crushed hemp-seed is extracted with a 5 per cent. solution of sodium chloride at 60° C., and the hot solution is filtered through a paper moistened with the same salt solution. On cooling the solution very slowly the globulin separates in small octahedral crystals. If its solution in dilute sodium chloride is dialysed the edestin separates as a white amorphous powder.

A third protein, *casein* or *caseinogen** (L. *caseus*, cheese),

* *Caseinogen*, in the British nomenclature, is termed *casein* in the American literature ; the latter name is given, in the British system, to the initial product of digestion of " caseinogen " (that which gives rise to casein). The compound actually secreted and present in milk should be given the key-name, and the American terminology seems therefore more appropriate, and will be used in this text.

can be prepared very easily from cow's milk in the following fashion: The milk is diluted with four times its volume of water, and acetic acid is added to make a 0·1 per cent. solution. Impure casein is precipitated. It is purified by redissolving it in water containing a trace of alkali, filtering from undissolved impurities, again precipitating by adding a trace of acetic acid, and finally washing the precipitate with water. So obtained, it is also a white amorphous powder.

One other protein may be selected for comparison. If we cut up white fibrous connective tissue, such as the *tendo Achillis* of the ox, into small pieces, wash them for a long time with running water, and then shake them up with excess of half-saturated lime water for twenty-four hours, a complex protein (*tendomucoid*) passes into solution, leaving a residue of which the most important constituent is *collagen*. If this residue is boiled with excess of water for several hours the collagen is changed into the protein *gelatin* (L. *gelare*, to congeal). On concentrating and cooling a typical gelatin jelly will be formed, and this can be dried in air. If then powdered it also is obtained in the form of a white amorphous powder.

Thus, when pure, these four proteins are all white amorphous powders, tasteless, though with characteristic faint odours, which are probably due to slight traces of impurities. Although they are scarcely distinguishable in appearance, they possess very different physical and chemical properties. Thus, to deal first with their solubilities, egg-albumin and gelatin are soluble in water, the latter on the application of heat (*pure* gelatin is, however, only slightly soluble). If their solutions are boiled, that containing the albumin will *coagulate;* the albumin will be precipitated. But gelatin will not be precipitated by this treatment; on concentrating and cooling it forms a *gel*, being the only one of the four to do so. Edestin is not soluble in water, but is soluble in dilute solutions of sodium chloride. Its solution is coagulated on warming. Casein is insoluble in water and dilute neutral salt

solutions, but is soluble in dilute alkali; on boiling its alkaline solution it is not precipitated.

If we add to each of their solutions an equal volume of saturated ammonium sulphate the globulin, gelatin, and casein are precipitated, but the albumin remains in solution. Complete saturation with ammonium sulphate precipitates albumin.

All four compounds behave in solution as colloids; gelatin is somewhat more diffusible than the others. All four are optically active, but to different degrees.

Element analysis shows that all four contain carbon, hydrogen, nitrogen, oxygen and sulphur. Casein contains, in addition, phosphorus. The percentage figures are approximately:

	C	H	N	S	O	P
Egg-albumin .	52·8	7·1	15·5	1·6	23·0	0·0
Edestin . .	51·4	7·0	18·6	0·9	22·1	0·0
Cow-casein . .	53·0	7·0	15·7	0·8	22·8	0·7
Gelatin . .	50·5	6·7	17·9	0·6	24·3	0·0

Early attempts to ascertain the molecular weights and probable empirical formulæ of such proteins, based on chemical methods, such as the content of some particular element or radical, led to weights which were undoubtedly far too low, *e.g.*, for egg albumin, 5,739, and for casein, 8,800. The empirical formulæ corresponding to these numbers are respectively, $C_{250}H_{409}N_{67}O_{81}S_3$ and $C_{394}H_{618}N_{100}S_2P_2O_{126}$. Obviously such formulæ give little information of value beyond suggesting the very large size of the protein molecule. More recently, physical-chemical methods have led to results of greater accuracy; amongst the best of such methods are Sørensen's determinations of the osmotic pressures of carefully purified proteins, and Svedberg's measurements of molecular sedimentation produced in centrifuges of very high velocity (see Chapter VI.). Svedberg and Nichols have recently shown that purified egg albumin has a molecular weight of

34,500 ± 1,000, confirming Sørensen's earlier figure of 34,000. Some of Svedberg's results are shown in Table V.

TABLE V. MOLECULAR WEIGHTS AND PARTICLE SIZES OF PROTEINS IN SOLUTION

Protein.	Class.	Source.	Molecular Weight.	Spherical Radius.
				μμ
Egg albumin .	Albumin .	Egg-white	34,500	2·18
Hæmoglobin .	Chromoprotein.	Blood .	66,800	2·44
Serum albumin	Albumin .	Blood .	68,000	2·39
Serum globulin	Globulin. .	Blood .	103,000	2·75
Legumin .	Globulin. .	Vetch .	208,000	3·96
Edestin. .	Globulin .	Hemp-seed	208,000	4·16
Amandin .	Globulin. .	Almond .	208,000	3·94
Excelsin .	Globulin .	Brazil-nut	212,000	3·96
Casein .	Phosphoprotein	Milk .	375,000	Non-spherical
Hæmocyanin .	Chromoprotein	Snail .	5,000,000	12·00

The proteins give a series of marked colour reactions. The most important of these will be dealt with in turn.

The Biuret Reaction. When the compound *biuret* (which is one of the products formed when urea is sublimed) is dissolved in water, and the solution is mixed with an equal volume of concentrated sodium hydroxide, and a trace of dilute copper sulphate solution is then added, a red-purple colour results. Solutions of egg-albumin, edestin and casein tested in this way give a purple colour, gelatin a blue-pink. The purple colour is given by most soluble proteins. A suspension of insoluble protein is coloured purple at its surface.

Biuret has the formula $NH_2.CO.NH.CO.NH_2$, and on account of the similarity of the colour reactions it is concluded that proteins contain a similar series of linkages.

Millon's Reaction. Millon's reagent is prepared by dissolving mercury in strong nitric acid. When a few crystals of the amino-acid *tyrosine* are suspended in water, and a few drops of the reagent added, and heat applied, the liquid

gradually becomes dark red in colour. Solutions of egg-albumin, edestin and casein give this reaction very definitely, forming at first a white precipitate, which turns red on application of heat. Gelatin only gives the reaction feebly.

It may be concluded that the tyrosine radical is present in all four proteins, though only to a small extent in gelatin.

The Xanthoproteic Reaction (Gk. *xanthos*, yellow). Addition of concentrated nitric acid to a protein solution or suspension results in a white precipitate, which on warming turns yellow and dissolves to a yellow solution. Addition of alkali deepens the yellow colour to orange. All the four proteins under discussion give this reaction ; it is also given by the amino-acids tyrosine, phenylalanine and tryptophane, and is characteristic of the phenyl radical C_6H_5 present in these acids. Evidently such groups are present in the proteins.

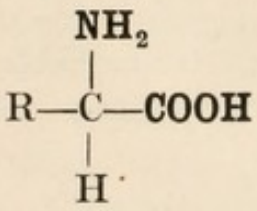
The Glyoxylic Acid Reaction (or Hopkins-Cole reaction) is carried out by mixing with the protein solution in a test-tube an equal volume of solution of glyoxylic acid, $CH(OH)_2.COOH$, and adding carefully, so that mixture is avoided, a little concentrated sulphuric acid. At the junction between the two phases a purple ring slowly forms. This is characteristic of the amino-acid tryptophane, and signifies the presence of its radical in the protein molecule. Gelatin does not give this test. Its molecule contains no tryptophane radicals.

Such tests evidently indicate distinct differences in the four proteins under discussion.

When the proteins are heated in a reflux condenser with dilute mineral acid a series of hydrolyses takes place, resulting in the gradual breaking down of the large protein molecule into smaller ones. At first *proteoses* are formed. These still give a purple biuret reaction though the purple colour is less marked. They are all soluble in water, but are precipitated by saturation with ammonium sulphate. As hydrolysis proceeds further *peptones* are formed. These are still more

soluble in water, give a *pink* biuret test, and are not precipitated by ammonium sulphate, though they are precipitated by phosphotungstic acid and by tannic acid. Finally a mixture of amino-acids is produced and the biuret reaction is no longer given.

The amino-acids can be separated by various complex procedures, and so far, from different proteins and tissues, somewhat more than twenty have been obtained and definitely identified. They are essentially both substituted ammonias, RNH_2 (in one or two instances RNHR′), and carboxyl acids, and most of them have the type-formula :



so that, since the amino-group is attached to the carbon atom adjacent to the carboxyl group, the α-carbon atom, they are α-amino-acids.

In virtue of this double property of being at the same time base and acid the amino-acids are all *amphoteric* (Gk. *amphoteros*, on both sides), reacting as bases with acids, and as acids with bases. They are " amphoteric electrolytes " or " ampholytes " and ionise very slightly in solution in two ways. Glycine, $NH_2 . CH_2 . COOH$, the simplest, typifies the behaviour of all—

i. $NH_2 . CH_2 . COOH \rightarrow NH_2 . CH_2 . COO^- + H^+$.

ii. $COOH . CH_2 . NH_2 + HOH \rightarrow COOH . CH_2 . NH_3OH \rightarrow COOH . CH_2 . NH_3^+ + OH^-$.

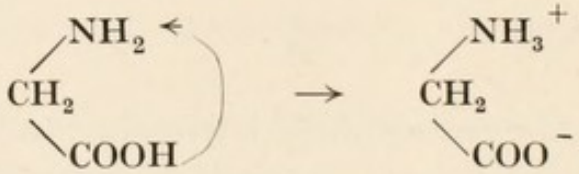
The second series of changes parallels the formation of ammonium ions—

$$NH_3 + HOH \rightarrow NH_4OH \rightarrow NH_4^+ + OH^-.$$

The extent to which these two different types of ionisation

take place respectively depends upon the *p*H of the solution. If the reaction be alkaline, then the first is the main change, and from such solutions alkaline salts of the amino-acids will crystallise. If the reactions be acid, then the second type of ionisation predominates and from such solutions acid salts will crystallise, such as glycine hydrochloride. At some intermediate point ionisation is reduced to a minimum. This is known as the *isoelectric point* and has a different and definite value for each of the amino-acids. It is of great importance for the similarly amphoteric proteins.

It has been suggested that in solutions of amino-acids the hydrogen of the carboxyl group migrates to the amino group, so that, for example, the glycine molecule becomes an ion carrying both positive and negative charges.



The resulting "ionised internal salt" has been termed a *zwitterion* (Germ. *Zwitter*, hybrid, hermaphrodite). Harris has recently put forward evidence supporting this theory.

A list of these acids, with some of their most important physical properties, is given in Table VI. Micro-photographs of certain of them are shown in Plate II.

The optical activity of certain of the amino-acids varies considerably with the nature of the solvent, and the concentration of the amino-acid. Except where stated, the figures in Table VI. refer to solutions in water.

Additional Notes on the Amino-acids

Glycine (or *Glycocoll*) is obtained in large quantities from such proteins as gelatin, silk-fibroin, elastin and spongin. In alkaline solution glycine dissolves freshly precipitated cupric hydroxide to a blue solution, which is not reduced on boiling. On concentrating and cooling blue needles of copper-glycine

separate. (Similar reactions are given by a number of the amino-acids.) Glycine gives a well-crystallised hydrochloride,

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

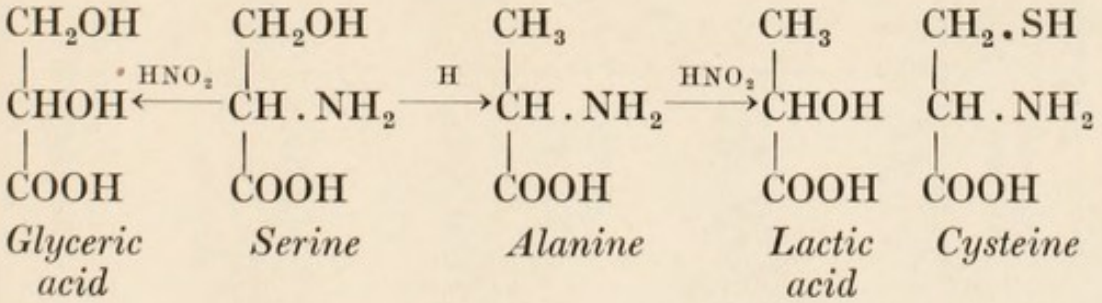
which is readily soluble in water. When glycine is shaken with benzoyl chloride in an alkaline medium *hippuric acid* results. This reaction can be used for the identification and separation of glycine :

$$C_6H_5 . COCl + HNH . CH_2 . COOH = C_6H_5 . CO . NH . CH_2 . COOH + HCl.$$

A very similar reaction takes place in the body during the detoxication of benzoic acid. Glycine has no asymmetric carbon atom, and consequently cannot be optically active.

Alanine is found in large amount in the hydrolysed products from certain sclero-proteins (albuminoids), such as fibroin from silk, and keratin from horn.

Serine is present in small amounts in the hydrolysed products from many proteins. Its relationship to a number of other important compounds is readily seen from the formulæ :



Glyceric acid — *Serine* — *Alanine* — *Lactic acid* — *Cysteine*

This scheme illustrates an important reaction which is given by all α-amino-acids. Addition of nitrous acid results in the liberation of nitrogen as gas, and the substitution of the amino- by a hydroxy- group.

Cysteine is not a primary hydrolytic product of proteins,

TABLE VI. THE AMINO-ACIDS AND THEIR PHYSICAL PROPERTIES

Amino-acid.	Empirical Formula.	Constitutional Formula.	Crystal Form.	Solubility		Taste of Aqueous Solution.	Specific Rotation.
				In Water.	In Alcohol.		
Glycine . . *Amino-acetic acid.*	$C_2H_5NO_2$	$NH_2 \cdot CH_2 \cdot COOH$	Rhombs and 4-sided prisms.	Marked	Insol.	Sweet	Nil.
d-Alanine . . *α-amino-propionic acid.*	$C_3H_7NO_2$	$CH_3 \cdot CH(NH_2) \cdot COOH$	Needles.	Marked	Insol.	Sweet	+ 2·7°
l-Serine . . *β-hydroxy-α-amino-propionic acid.*	$C_3H_7NO_3$	$OH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	Thin leaves.	Fair	Insol.	Sweet then flat.	−6·8°
l-Cysteine . . *β-thio-α-amino-propionic acid.*	$C_3H_7NSO_2$	$SH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	Cryst.	Sol.	—	—	−1·0°
l-Cystine . . *Di-cysteine.*	$C_6H_{12}N_2S_2O_4$	$S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ $S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	6-sided tables.	Insol.	Insol.	—	−242·6° [1% sol. n 0·1 *N* HCl]
l-Methionine . *γ-methylthiol-α-amino-butyric acid.*	$C_5H_{11}SNO_2$	$S \cdot CH_3 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	White 6-sided plates.	Sol.	Sol.	—	−7·2°

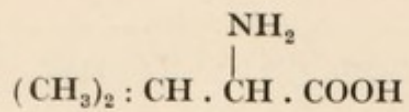
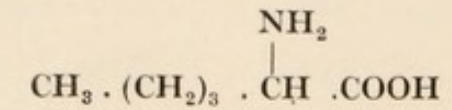
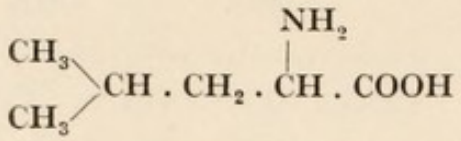
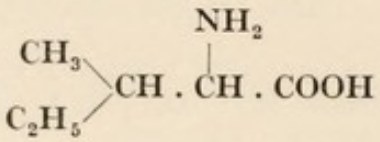
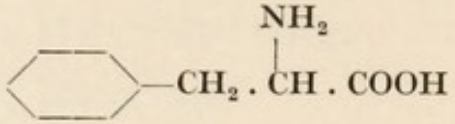
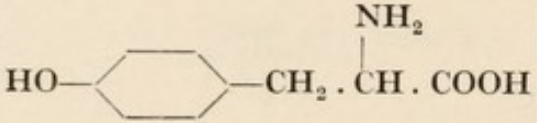
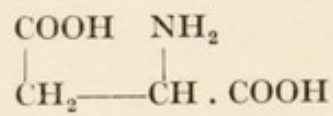
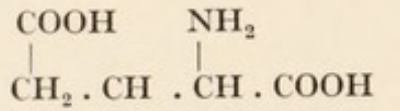
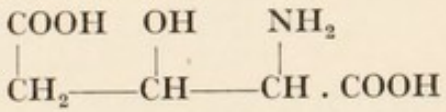
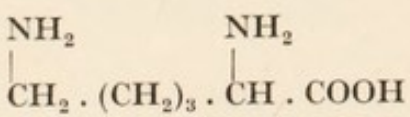
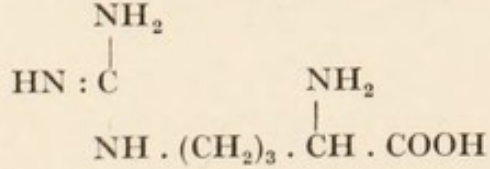
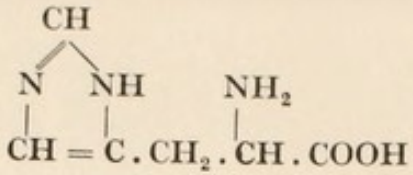
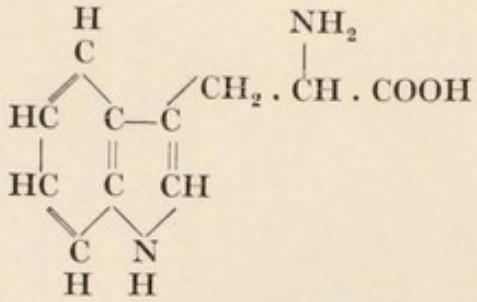
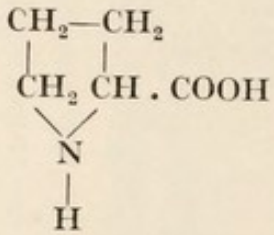
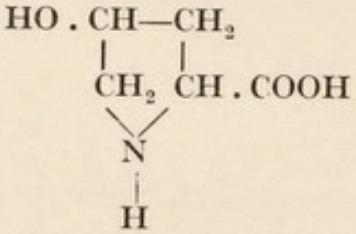
d-Valine . . *α-amino-isovalerianic acid.*	$C_5H_{11}NO_2$		Microscopic leaves.	Fair	—	Bitter sweet.	+6·4°
d-Caprine or Norleucine. *α-amino-normal-caproic acid.*	$C_6H_{13}NO_2$		6-sided leaves.	Very slight.	Insol.	—	+4·5°
l-Leucine . . *α-amino-iso-caproic acid.*	$C_6H_{13}NO_2$		Shining white thin leaves.	Slight	Slight in cold, marked in hot.	—	−10·4°
d-Isoleucine . *α-amino-β-methyl-β-ethyl-propionic acid.*	$C_6H_{13}NO_2$		Leaves and tables.	Slight	Insol.	Bitter, astringent.	+ 9·6°
l-Phenylalanine . *β-phenyl-α-amino-propionic acid.*	$C_9H_{11}NO_2$		Small bright leaves or needles.	Slight in cold, marked in hot.	—	Slightly bitter	− 35·1°
l-Tyrosine. . *β-parahydroxy-phenyl-α-amino-propionic acid.*	$C_9H_{11}NO_3$		Needles.	Very slight.	Insol.	—	− 13° [In dil. HCl]

TABLE VI.—*continued*

Amino-acid.	Empirical Formula.	Constitutional Formula.	Crystal Form	Solubility		Taste of Aqueous Solution.	Specific Rotation.
				In Water.	In Alcohol.		
l-Aspartic acid . *Amino-succinic acid.*	$C_4H_7NO_4$		Rhombic prisms.	Slight	—	—	[*l* in alk., *d* in acid sol.]
d-Glutamic acid . *α-amino-glutaric acid.*	$C_5H_9NO_4$		Rhombic tetra-hedra and octa-hedra.	Slight	Insol.	Acid with peculiar after-taste.	+ 12·0°
d-β-Hydroxy-glutamic acid.	$C_5H_9NO_5$		prisms.	Marked	Insol.	—	+ 0·8°
.....	.....	.....	.....	.....	.....	.....	.....
d-Lysine . . *α-ω-diamino-caproic acid.*	$C_6H_{14}N_2O_2$		Needles.	Marked	Insol.	—	+ 14·6°
d-Arginine . *α-amino-δ-guanidino-valerianic acid.*	$C_6H_{14}N_4O_2$		Tabular rosettes or thin prisms.	Marked	Almost insol.	—	+ 21·3°
.....	.....	.....	.....	.....	.....	.....	.....

l-Histidine . *β-iminazolyl-α-amino-propionic acid.*	$C_6H_9N_3O_2$		Colourless needles and tables.	Marked	Slight	—	− 39·7°
l-Tryptophane . *β-indole-α-amino-propionic acid.*	$C_{11}H_{12}N_2O_2$		Rhombic or 6-sided leaves.	Marked in hot water.	Very slight.	Slightly bitter.	− 33°
l-Proline . . *Pyrrolidine carboxylic acid.*	$C_5H_9NO_2$		Flat needles.	Marked	Marked	Sweet	− 77·4°
l-Hydroxy-proline. *γ-Hydroxy-pyrrolidine carboxylic acid.*	$C_5H_9NO_3$		Colourless tables.	Marked	Slight	Sweet	− 81°

though on account of the ease with which it is converted to *cystine* it is by no means certain that the cysteine radical may not occur as such in the protein molecule and be converted to cystine during hydrolysis. It can be prepared from cystine by the action of zinc dust and acid (nascent hydrogen). It is certainly present free in many tissue cells, in which it can be detected by its reaction with sodium nitroprusside and alkali, the production of a marked purple-red colour. It oxidises to cysteic acid,

$$\begin{array}{l} CH_2 . SO_3H \\ | \\ CH . NH_2 \\ | \\ COOH \end{array}$$

from which, by loss of carbon dioxide, taurine

$$\begin{array}{l} CH_2 . SO_3H \\ | \\ CH . NH_2 \\ | \\ H \end{array}$$

is derived.

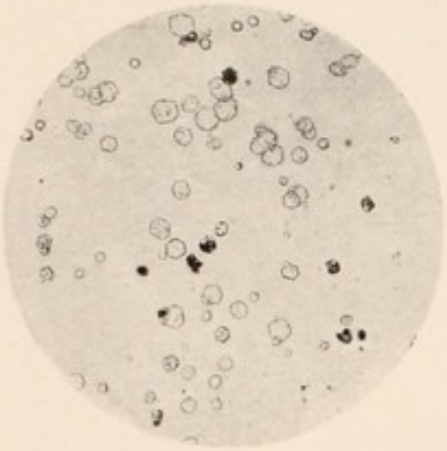
Cystine is an important constituent radical of many proteins, and may be considered as responsible for most of the sulphur present in such compounds, though a second amino-acid containing sulphur has recently been isolated (see below). Phosphotungstic acid precipitates cystine quantitatively from sulphuric acid solution.

Methionine is the most recently discovered amino-acid definitely shown to be present in the protein molecule. Its radical exists in casein (3·5 per cent.), egg-albumin, and edestin, and it has been found in the products of hydrolysis and a number of other proteins.

Valine has been obtained to the extent of over 7 per cent. from caseinogen.

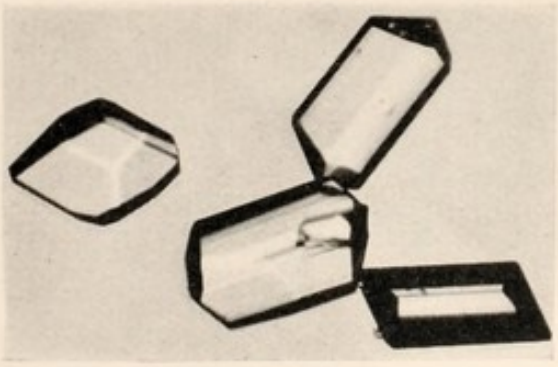
Leucine is one of the amino-acids most easily liberated from the protein molecule, and, on account of its slight solu-

PLATE II



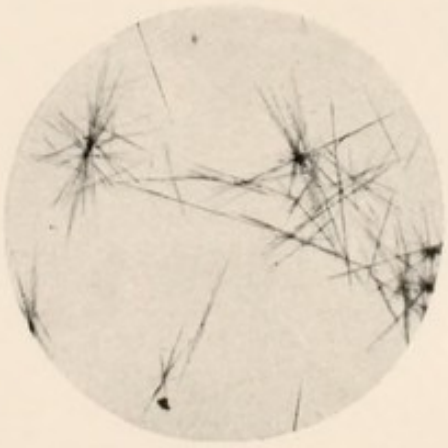
(*a*) Cystine.

(*b*) Leucine.



(*c*) Glycine.

(*d*) Aspartic acid.



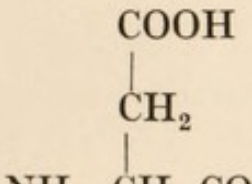
(*e*) Tyrosine.

Micro-photographs of recrystallised amino-acids, to illustrate their differing crystal habits. *Magnification* × 45.

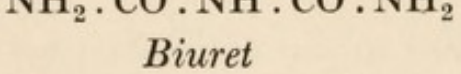
[*To face p.* 116.

bility, it was one of the first to be separated and studied. It is difficult to purify, and its insolubility increases with its purity. It is easily soluble in acids and alkalies.

Aspartic acid has an amide derivative, *asparagine*, a compound widely distributed in plants. Asparagine gives a blue-violet biuret reaction, and its formula presents some points of resemblance with that of biuret itself :



Asparagine



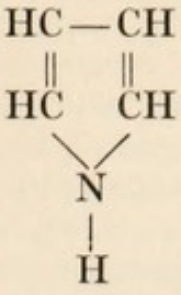
Biuret

Glutamic acid is one of the chief products of hydrolysed proteins ; hydrolysed casein yields 21 per cent., and certain plant proteins yield over 40 per cent.

Hydroxyglutamic acid, although one of the most recently discovered of these acids, is evidently present in radical form in large amounts, since Dakin, its discoverer, obtained 10·5 per cent. from casein.

Tyrosine is yielded by hydrolysis of most animal proteins in amounts between 10 and 13 per cent. of the total hydrolysate. On account of its slight solubility, it, along with leucine, was one of the first amino-acids to be discovered and studied.

Proline and *hydroxy-proline* are *imino-acids* closely related to *pyrrole*,



and they differ from the amino-acids by their marked solubility in alcohol.

Histidine (and *tyrosine*) give Pauli's *diazo-reaction*, the development of a red colour with diazo-benzene sulphonic acid in presence of mild alkali. On acidification with hydrochloric acid, reduction with zinc dust, and, finally, addition of strong ammonia, a characteristic golden colour is produced with histidine, a bright rose red with tyrosine.

Arginine, when hydrolysed with baryta water, breaks down to *ornithine* and urea—

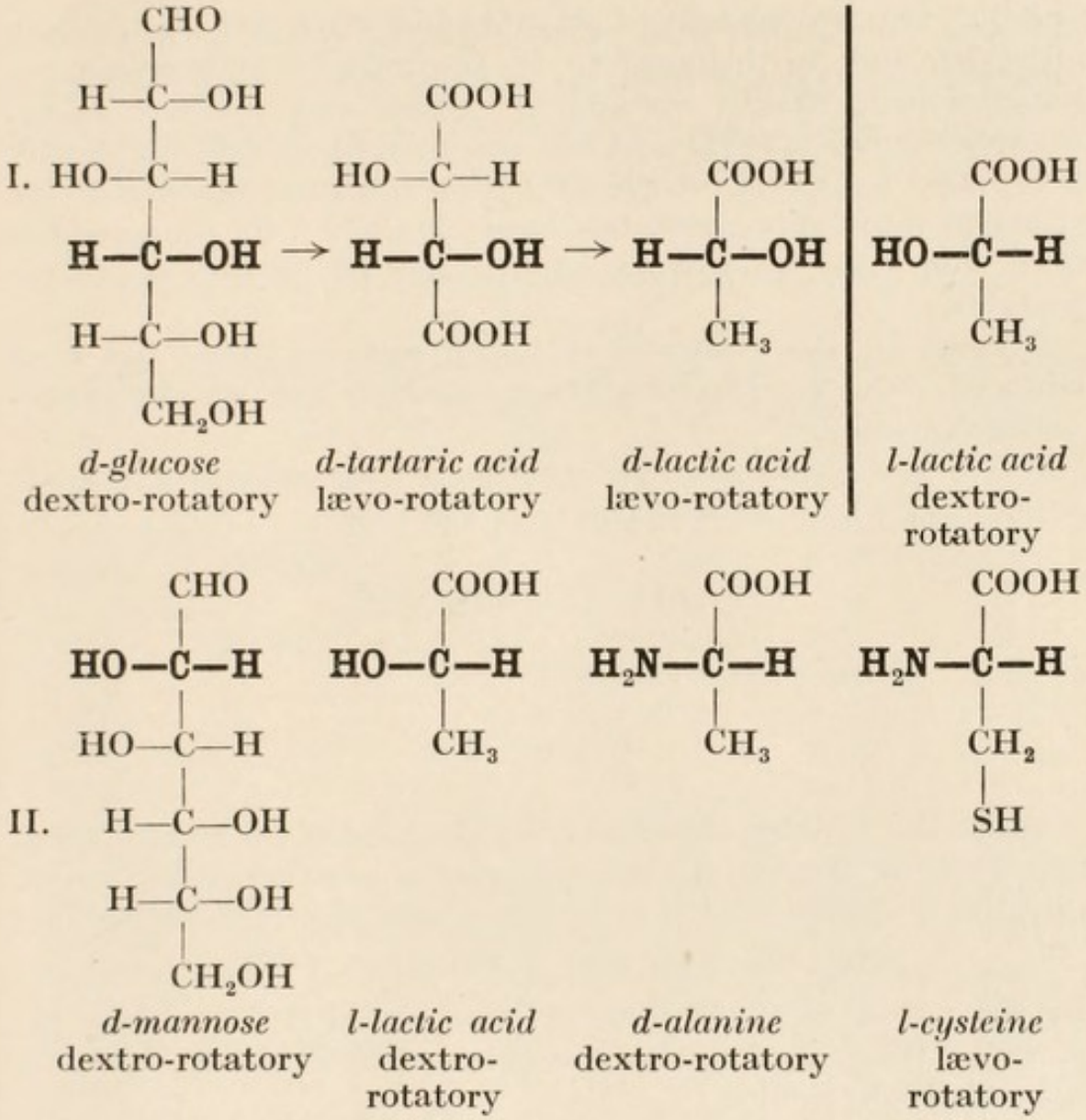
$$NH_2 . (CH_2)_3 . CH(NH_2) . COOH$$

Ornithine

Just as certain colour reactions typify histidine, tyrosine, and tryptophane, so Sakaguchi has recently found one typical of arginine. When to a solution of arginine is added a trace of alcoholic solution of α-naphthol, and some drops of sodium hypochlorite solution, in presence of alkali a red colour develops. This is also a delicate test for the presence of arginine radicals in proteins.

Many of these amino-acids exhibit different optical properties in acid and in alkaline solutions, and their activities sometimes vary according to the degree of acidity or of alkalinity. In some cases, consequently, only an approximate specific rotation can be stated. The majority of these compounds are lævo-rotatory.

Levene and others have worked out a scheme of derivation from tartaric acid similar to that relating the sugars to glycerose. Dextro-tartaric acid (actually lævo-rotatory) can be derived from *d*-glucose, and this fixes its configuration. All hydroxy-acids that can be derived from *d*-tartaric acid are termed *d-acids*, and those derived from *l*-tartaric acid *l-acids*, whatever their actual rotation. All naturally occurring amino-acids are configurationally related to the corresponding *l*-hydroxy-acids, and the amino- or hydroxy- groups in these acids have actually the same spacial position as the hydroxyl group on the second carbon atom in mannose. These relationships are illustrated in the following schemes, in which the essential groups are shown in heavy type :—



Karrer has recently suggested that Greek prefixes might be employed to indicate the configurational relationships of such compounds, Roman characters indicating their actual rotation. According to his suggestion dextro-rotatory lactic acid should be written λ-*d*-lactic acid, and naturally occurring alanine λ-*d*-alanine. The corresponding lævo-rotatory lactic acid would be written δ-*l*-lactic acid.

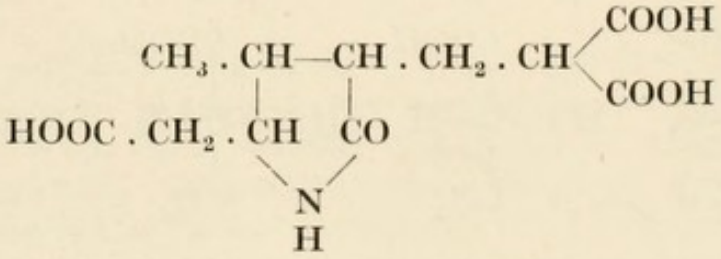
Other amino-acids have been obtained from the hydrolysis of highly specialised tissues. Examples are thyroxine (from the thyroid), diiodotyrosine (from thyroid tissue, sponges, and corals), and dibromotyrosine (from corals).

Probably numerous other amino-acids are built up into natural proteins, but the existence of such acids cannot yet be regarded as finally proved. From the sclera of the whale, after hydrolysis, an amino-butyric acid has been isolated. By hydrolysis of an oat

glutelin, two amino-acids, $C_4H_9NO_3$ and $C_5H_{11}NO_3$, have been obtained, and are believed to be respectively hydroxy-amino-butyric acid, closely resembling serine, and hydroxy-valine. Hydroxy-amino-butyric acid has also been obtained from casein. From acid hydrolysis of isinglass from the swim-bladder of the sturgeon β-hydroxy-lysine has been obtained ; its presence has been shown in the hydrolysed products of edestin and other proteins.

Others, for the existence of which some evidence has been adduced, are 3-4-dihydroxyphenyl-alanine and *d*-α-amino-nor-valerianic acid.

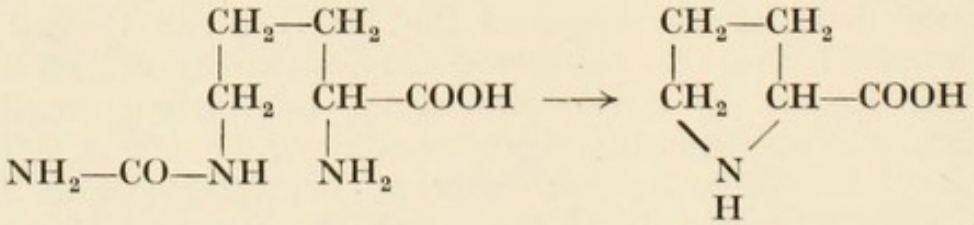
Dakin and West have isolated from liver extract a tribasic amino-acid whose constitution is of the type :



Wada has isolated *citrulline* from the juice of the water-melon, and also, by tryptic digestion in *neutral* solution, from casein. Citrullin is closely related to arginine, and has the constitution :

$$NH_2 . CO . NH . (CH_2)_3 . CH(NH_2) . COOH.$$

By the action of dilute alkali it is converted into ornithine (*cf.* p. 338). When heated with concentrated hydrochloric acid it is converted into proline :



Citrullin *Proline*

Wada suggests that when proteins are hydrolysed by acid, part of the proline in the hydrolysate may arise from citrullin.

Distribution of the Amino-acids in Proteins

Careful analysis shows that the amounts of the different amino-acids which can be obtained from proteins are different with every protein examined. Considering the four

proteins that have already been dealt with, our present knowledge shows that 100 gm. of these proteins contain the weights in grams of the respective amino-acids shown in Table VII. :—

TABLE VII. DISTRIBUTION OF AMINO-ACIDS IN PROTEINS

	Egg-albumin.	Edestin.	Cow Casein.	Gelatin.
(Ammonia) . .	1·34	—	1·61	0·4
Glycine . . .	0·0	3·8	0·45	25·5
Alanine . . .	2·2	3·6	1·85	8·7
Valine . . .	2·5	Present	7·93	0·0
Serine . . .	—	0·3	0·5	0·4
Cystine . . .	0·9	1·0	0.3	0·17
Methionine . . .	Present	2·1	3·5	—
Caprine . . .	—	—	—	?
Leucine . . .	10·7	20·9	9·35	9·2
Isoleucine . . .	—	—	1·43	0·0
Phenylalanine . .	5·17	3·1	3·88	1·4
Tyrosine . . .	4·2	4·5	6·5	0·01
Aspartic acid . .	6·2	10·2	4·1	3·4
Glutamic acid . .	13·3	19·2	21·77	5·8
Hydroxy-glutamic acid	—	—	10·5	0·0
Lysine . . .	5·0	2·2	7·7	5·9
Arginine . . .	5·6	15·8	5·2	9·1
Histidine . . .	1·4	2·1	2·6	0·9
Tryptophane . .	1·3	1·5	2·2	0·0
Proline . . .	3·56	4·1	7·63	9·5
Hydroxy-proline . .	—	2·0	0·23	14·1
Total . .	63·4	96·4	99·2	94·5

Few of the amino-acid totals from protein hydrolysates add up to 100 per cent. of the weight of protein hydrolysed. Actually, since water is taken up and chemically combined during the hydrolysis, a figure higher than 100 should be obtained. The difficulties inherent in the separation may account for part of the discrepancy. And colorimetric methods of estimation, which do not necessitate isolation

of the amino-acids, give much higher figures for lysine, arginine, and histidine. Some part of the discrepancy is also probably to be explained by the exclusion of still undiscovered amino-acids, and of radicals of other compounds that are not amino-acids. According to Pryde, zein, from maize, can at present be regarded as the most completely analysed protein. The amino-acids obtained from it represent 94·4 per cent. of its actual weight. Vickery and Shore consider that the most accurate analyses of the basic amino-acids of egg albumin indicate that it contains 3 histidyl, 11 arginyl, and 12 lysyl radicals.

It is next necessary to consider in what way the amino-acids are built up into the protein molecule, and, further, what other units may take part in the formation of such a complex structure.

The Constitution of the Protein Molecule

Our knowledge of the constitution of the protein molecule is largely due to the work of Emil Fischer and his pupil Abderhalden, and to that of Albrecht Kossel.

Since the amino-acids are at the same time acids and derived ammonias, two molecules of the same or of different acids should be able to unite, giving a *di-peptide*, as, for example :

$$\underset{\textit{Glycine}}{NH_2.CH_2.COOH} + \underset{\textit{Glycine}}{HNH.CH_2.COOH} = \underset{\textit{Glycyl-glycine}}{NH_2.CH_2.CO.NH.CH_2.COOH} + HOH$$

$$\underset{\textit{Alanine}}{CH_3.CHNH_2.COOH} + \underset{\textit{Glycine}}{HNH.CH_2.COOH} = \underset{\textit{Alanyl-glycine}}{CH_3.CHNH_2.CO.NH.CH_2.COOH} + HOH$$

and generally :

$$\underset{\textit{Any amino-acid}}{R.CHNH_2.COOH} + \underset{\textit{Any amino-acid}}{HNH.CHR'.COOH} = \underset{\textit{Any di-peptide}}{R.CHNH_2.CO.NH.CHR'.COOH} + HOH$$

Such dipeptides will be also amphoteric; they also contain amino- and carboxyl-groups, and so should also

unite with amino-acids to form still more complex compounds, *tri-peptides.*

$$\underset{\textit{Alanyl-glycine}}{CH_3 . CHNH_2 . CO . NH . CH_2 . COOH} + \underset{\textit{Alanine}}{HNH . CH(CH_3) . COOH} =$$

$$CH_3 . CHNH_2 . CO . NH . CH_2 . CO . NH . CH(CH_3) . COOH + HOH$$

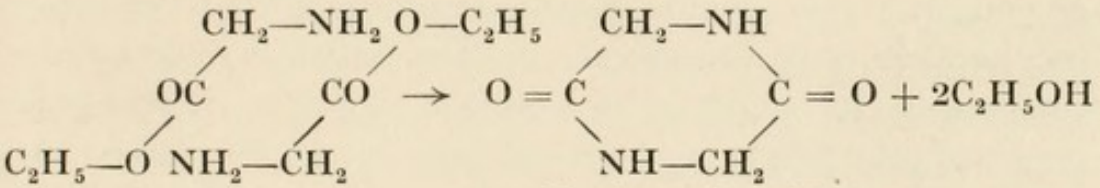
Alanyl-glycylalanine

The actual methods by which the dipeptides and tripeptides are built up from the amino-acids in the laboratory are, of course, not quite so simple as the above equations might suggest. Several methods have been devised, of which the following are examples :

The ethyl ester of glycine in aqueous solution forms, to a considerable extent, the anhydride of glycine.

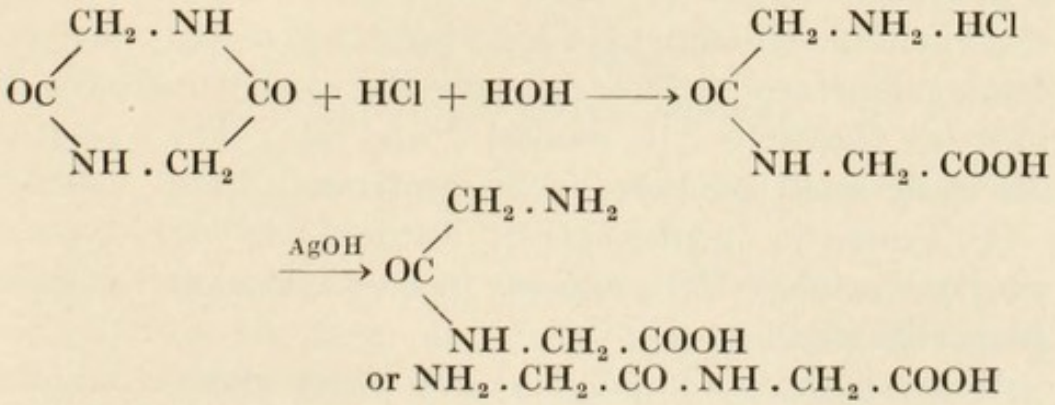
$$NH_2 . CH_2 . COOH + C_2H_5OH = NH_2 . CH_2 . COOC_2H_5 + HOH$$

Glycine ethyl ester



Glycine anhydride

If this anhydride be boiled for a short time with concentrated hydrochloric acid, it changes to glycyl-glycine-hydrochloride, which, with silver oxide, gives (precipitated) silver chloride and free glycyl-glycine :



If, in the original treatment with hydrochloric acid, an alcohol medium is employed, the ethyl ester of glycyl-glycine is obtained. If this is heated with chlor-acetyl chloride, $Cl.CH_2.COCl$, chlor-

acetyl-glycyl-glycine ester is obtained, and the chloracetyl derivative can be set free from this by saponification :

$$Cl . CH_2 . COCl + HNH . CH_2 . CO . NH . CH_2 . COOC_2H_5$$
$$\rightarrow Cl . CH_2 . CO . NH . CH_2 . CO . NH . CH_2 . COOC_2H_5 + HCl$$
$$\rightarrow Cl . CH_2 . CO . NH . CH_2 . CO . NH . CH_2 . COOH.$$

If this compound be treated with concentrated aqueous ammonia, the chlorine is replaced by an amino-radical, and diglycyl-glycine results :

$$NH_2 . CH_2 . CO—NH . CH_2 . CO—NH . CH_2 . COOH$$

In such ways a large number of di-, tri- and higher polypeptides have been built up.

The importance of these synthetic polypeptides lies in their similarity to the proteins, and their partially broken down products, the proteoses and peptones.

Most of the tripeptides which contain other radicals than glycine, triglycyl-glycine (a tetrapeptide), and all the higher polypeptides give the biuret reaction, which is more intense the greater the length of the polypeptide chain. These polypeptides give the typical protein colour reactions, of course, only when they contain the amino-acid radicals to which these are respectively due, *e.g.*, tyrosine, phenylalanine, and tryptophane. The most complex that have been so far prepared, *l*-leucyl-triglycyl-*l*-leucyl-triglycyl-*l*-leucyl-octaglycyl-glycine, containing eighteen amino-acid radicals, and having a molecular weight of 1,213, and *l*-leucyl-triglycyl-*l*-leucyl-triglycyl-*l*-leucyl-triglycyl-*l*-leucyl-penta-glycyl-glycine, containing nineteen amino-acid radicals, closely resemble the proteins themselves in general properties. Their solutions are opalescent, like those of the proteins.

A synthetic pentapeptide, *l*-leucyl-triglycyl-*l*-tyrosine, closely resembles the proteoses in its physical and chemical properties.

When hydrolysed, polypeptides break down to amino-acids, and many of them are broken down in this way by the various *proteases*, the enzymes which normally act on proteins.

Further, various di-, tri-, and tetrapeptides have been isolated from the mixture obtained by the incomplete hydrolysis of proteins.

All these facts strongly suggest that the ordinary protein molecule consists of a long chain of amino-acid radicals joined through a long series of *peptide linkages.*

$$—NH\,.\,CO—.$$

This peptide linkage is, of course, an amide linkage in which the hydroxyl group of the acid radical is replaced by a substituted amino-group. Is it the sole method of combination ?

It must be remembered that many of the amino-acids contain more than one amino-group, while several contain two carboxyl-groups. Kossel and his co-workers have shown, however, that the terminal amino-group in lysine and arginine does not give rise to a peptide linkage, but is still free in the protein molecule (to contribute to its amphoteric properties). No branching side-chains can therefore occur through such radicals. There is ground for belief that the protein molecule may branch wherever radicals of the dicarboxylic acids occur. Thus a tripeptide has been prepared from asparagine having the constitution :

$$NH_2\,.\,CH_2\,.\,CO——NH\,.\,CH\,.\,CO——NH\,.\,CH\,.\,(C_4H_9)\,.\,COOH$$
$$\Big|$$
$$CH_2\,.\,CO\,.\,NH_2$$

glycyl-l-asparaginyl-l-leucine

Similar compounds have been prepared from glutamic acid.

Such compounds as the asparagine-tripeptide yield on hydrolysis free ammonia, and since many proteins also yield a certain amount of free ammonia when hydrolysed, this would seem to suggest a certain number of acid-amide linkages in the protein molecule.

There is strong evidence that, while the chief linkage in

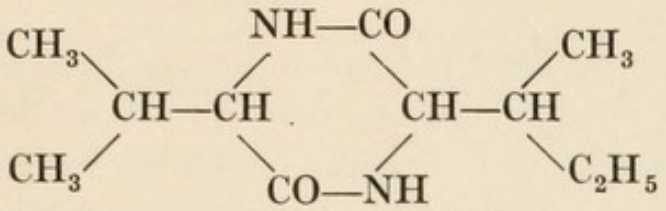
protein molecules is the peptide linkage —CO.NH—, yet, actually, this behaves as if it were changed to its *enol* form :

Changes of this kind are known to occur readily in many simpler compounds, and such a change explains the possession by these proteins of a considerably greater power to neutralise acids than would be supplied by a few free amino-groups (since the hydroxyl group can react with acids).

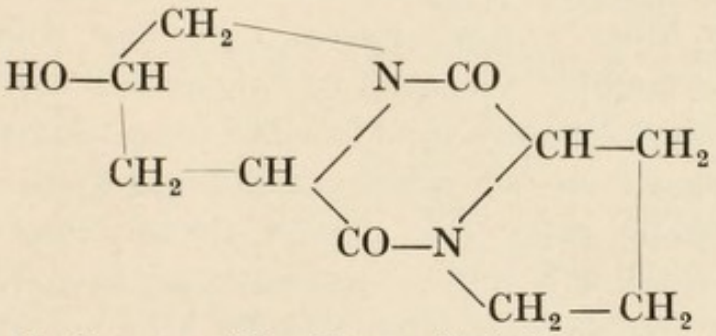
But while this peptide linkage undoubtedly is the commonest form of amino-acid junction in the proteins—Hunter, in a recent critical examination of this problem, estimates that it accounts for 70 per cent. of the combinations—yet other types of linkage undoubtedly occur. Pepsin of the gastric juice hydrolyses many proteins to proteoses and peptones, setting free from 20 to 25 per cent. of the amino-groups in the process. Equivalent amounts of $—NH_2$ and —COOH are produced, suggesting that the peptide linkage is split. Yet pepsin will not decompose synthetic polypeptides built up solely by peptide linkages, from which one would infer the precisely opposite conclusion. It seems possible that pepsin can only act on very long polypeptide chains. Such an explanation would harmonise these apparently conflicting facts.

Abderhalden emphasises the probable formation of anhydrides formed from closely adjacent amino-acid radicals. Several of these have actually been isolated from protein hydrolysates. Dakin has obtained isoleucylvaline anhydride from the acid hydrolysis of casein in amounts greater than 1 per cent. This compound separates from the crude butyl alcohol extract of the hydrolysate in woolly masses of fine needles, often more than 1 cm. in length. He has also obtained from the acid hydrolysis of gelatin as much as 2·75

per cent. of the compound hydroxyprolyl-proline anhydride, readily soluble in water and alcohol, sparingly soluble in pure ether, and reacting faintly acid to litmus.

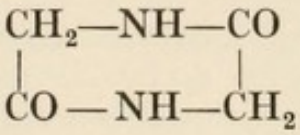


Isoleucylvaline anhydride



Hydroxyprolylproline anhydride

The inherent anhydride ring in such compounds is known as the *α-diketopiperazine ring*, the simplest *diketopiperazine* being



Such compounds can easily be prepared by warming together the corresponding amino-acids, so that evidently in the ordinary course of hydrolysis they may well be formed. But Abderhalden has obtained still more complex anhydrides, such as one built up from one molecule of tyrosine, one of alanine, and two of glycine, from hydrolysis of silk, and such compounds as these are not formed directly from the corresponding polypeptides by heating. He has also found specific colour tests for such complexes, and finds that their parent proteins also give these colour tests, indicating that the actual complexes are present *as such* in the protein molecules. Pre-formed anhydride linkages undoubtedly do occur in the protein molecule of a particular type—the class

of scleroproteins which are especially resistant to the action of digestive enzymes, and of which silk fibroin is a good example. Some other types of linkage have also been suggested.

It has already been pointed out that the total weight of amino-acids obtainable from most proteins adds up to less than 100 per cent. of the weight of protein hydrolysed. This undoubtedly is largely due to the complex methods inherent in their separation, but in many cases evidence of the presence of other radicals exists, especially those containing sulphur. Compounds, such as thiolactic acid, $CH_3.CH(SH).COOH$, have actually been isolated from the hydrolysates.

Occurrence of such acids, containing sulphur and containing no amino-group, may explain the apparent fact that the protein sulphur is present in two types of combination, one of which reacts immediately with lead acetate, indicating loosely combined sulphur, and the other only being detectable after fusion and oxidation of the protein molecule (when sulphate can be precipitated by addition of barium salts).

There is evidence that in the protein molecule certain nuclei exist from which radiate long side chains. The side chains are rapidly split off and broken up by enzyme hydrolysis ; the nuclei are much more resistant to enzyme action.

We possess, therefore, a considerable volume of evidence as to the main types of linkage in the protein molecule, but require much more data before the whole story can be written accurately.

Classification of Proteins

Two classifications are in use, fairly similar, one employed by British, the other by American biochemists. If they be written side by side, their points of resemblance are at once seen (see p. 129).

Both the British and American classifications are imperfect, since they are based too largely on differences of solu-

British.	American.	Properties.
Simple proteins.	*Simple proteins.*	
Albumins.	Albumins.	Soluble in pure water; coagulated by heat.
Globulins.	Globulins.	Insoluble in pure water; soluble in salts of strong bases with strong acids.
Glutelins.	Glutelins.	Insoluble in all neutral solvents; readily soluble in dilute acids and alkalies.
Alcohol-soluble proteins.	Prolamins.	Insoluble in water, absolute alcohol, and other neutral solvents, but soluble in 70–80 per cent. alcohol.
Scleroproteins.	Albuminoids.	Very insoluble in all neutral solvents.
Histones.	Histones.	Soluble in water; insoluble in dilute ammonia. Basic, and yield excess of di-amino acids on hydrolysis.
Protamines.	Protamines.	Soluble in water, uncoagulated by heat, and strongly basic in reaction, yielding chiefly di-amino acids on hydrolysis.
	Conjugated proteins.	
Phosphoproteins.	Phosphoproteins.	Compounds of the protein molecule with some phosphate compound other than nucleic acid or a phosphatide.
Conjugated proteins.		
Nucleoproteins.	Nucleoproteins.	Compounds of protein molecules and nucleic acid.
Glucoproteins.	Glucoproteins.	Compounds of proteins with substances containing a carbohydrate group.
Chromoproteins.	Hæmoglobins.	Coloured compounds of protein with hæmatin or some similar compound.
—	Lecithoproteins.	Compounds of proteins with phosphatides.
Products of Protein Hydrolysis.	*Derived Proteins* (1) *Primary Protein Derivatives.*	
—	Proteans.	Products resulting from the incipient action of water, very dilute acids, or enzymes.
—	Coagulated proteins.	Insoluble products resulting from the action of heat or alcohol on protein solutions.
Infraproteins.	Metaproteins.	Soluble products formed by the action of acids or alkalies: they are soluble in dilute acids and alkalies, insoluble in salt solutions.
	(2) *Secondary Protein Derivatives.*	
Proteoses.	Proteoses.	Soluble in water, non-coagulable by heat, precipitated by saturation with ammonium sulphate.
Peptones.	Peptones.	Soluble in water, non-coagulable by heat, and not precipitated by saturation with ammonium sulphate.
Polypeptides.	Peptides.	Definitely characterised combinations of two or more amino-acids.

bility and other physical considerations, and insufficiently on chemical properties and constitution. The different classes will be discussed very briefly.

Albumins are almost neutral in reaction. Typical are egg-albumin, serum-albumin (from blood), lactalbumin (from milk), and leucosin (from wheat, rye and barley seeds). The amounts of albumins in plants are relatively small. They are more readily precipitated by ammonium sulphate than are animal albumins, and it is doubtful if they can be properly differentiated from vegetable globulins. Animal albumins contain no glycine radicals. Plant albumins contain a small amount, of the same order as that in plant globulins.

Globulins are typified by serum-globulin, ovoglobulin (from egg-yolk), edestin (from hemp-seed), excelsin (from brazil nuts), amandin (from almond, peach, plum and apricot nuts), and legumin (from the pea, horse-bean, vetch and lentil). Many of the plant globulins are readily crystallisable. They are somewhat more soluble and less readily coagulable than are animal globulins.

Glutelins are obtained only from plants. Glutenin from wheat is the only well-characterised representative of this group that has so far been obtained.

Prolamins are so called on account of the relatively large amount of proline (10 to 14 per cent.) that can be derived from them. They are soluble in 70 per cent. alcohol, but not in absolute alcohol, whence the British name for them—alcohol-soluble proteins—is not a good one. They are solely of plant origin. Typical are zein (from maize), hordein (from rye), and gliadin (from wheat). Zein contains no tryptophane radicals.

Scleroproteins (Gk. *skleros*, hard), or albuminoids (not a satisfactory name), do not occur in plants. One of the chief differences between them and albumins and globulins is their pronounced insolubility. As has been mentioned, they especially seem to contain anhydride linkages within them.

A further chemical differentiation is the larger amounts of glycine (up to 37 per cent.), of alanine (up to 24 per cent.), and of tyrosine (up to 10 per cent.), obtainable from them than from most other proteins, though many of them contain but traces of one or other of these radicals. Typical are silk-fibroin, elastin (from tendinous material), spongin (from sponges), and keratin (from horn and hoof). Gelatin is usually included in this group for convenience, though it is extremely soluble, and is, perhaps, preferably to be regarded as a derived protein.

Histones are basic proteins, intermediate between albumins and protamines. They do not occur in plant tissues. As might be expected from their basicity, the di-amino acids predominate in their hydrolysed products. Typical of histones are the globin of hæmoglobin and thymus-histone.

Protamines are the simplest of all the proteins and contain no sulphur. They are derived from animal sperm. On hydrolysis they yield very few amino-acids, and the di-amino-acids predominate. Typical protamines, and those which have been most completely studied, are salmine (from Rhine

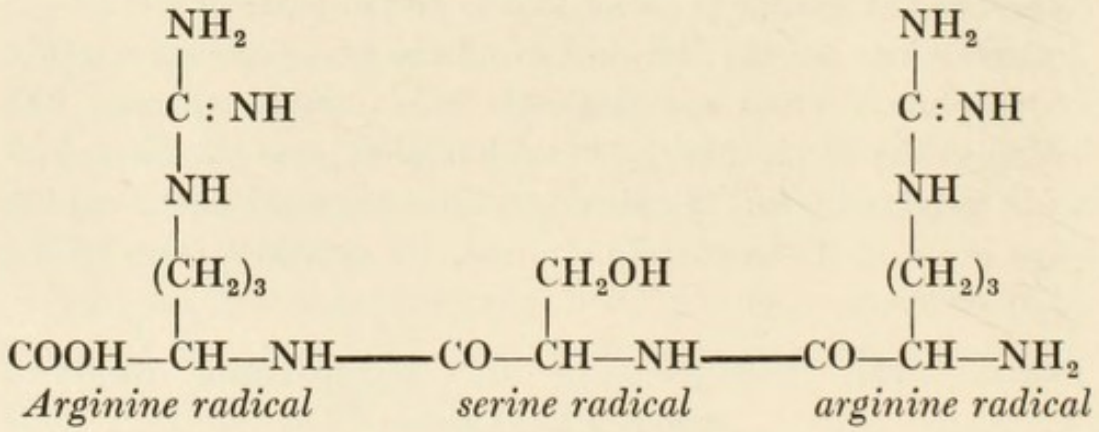
TABLE VIII. PERCENTAGES OF AMINO-ACIDS DERIVED FROM PROTAMINES

	Salmine.	Sturine.	Clupeine.	Scombrine.
Alanine . . .	trace	trace	trace	trace
Valine . . .	4·3	—	trace	—
Leucine . . .	trace	trace	—	—
Serine . . .	7·8	—	trace	—
Proline . . .	11·0	—	trace	trace
Arginine . . .	87·4	58·2	82·2	87
Lysine . . .	0·0	12·0	0·0	0
Histidine . .	0·0	12·0	0·0	0
Total	110·5	83·1	82·2	87

salmon sperm), sturine (from the sturgeon), clupeine (from herring), and scombrine (from the mackerel). For most of our knowledge concerning the protamines we are indebted to Kossel. The amino-acid contents of these four are shown in Table VIII.

With such combinations it can be calculated that 100 gm. of protein should yield on hydrolysis 115 to 120 gm. of amino-acids.

According to Kossel protamines are hydrolysed first into *protones*, which are compounds containing two radicals of arginine (or corresponding amounts partially replaced by lysine or histidine), united with one of alanine, or serine, or proline, or valine, so that a typical protone will have the constitution :



The alkalinity of the protamines can be fully accounted for by the free amino-groups.

Felix and Lang (1930) consider that clupeine is a mixture of four different protamines ; all four have two molecules of arginine to each molecule of mono-amino-acid. Two, with molecular weights of 817 and 873, are believed to have four molecules of arginine united respectively to two C_3 and two C_5 acids, while the third is a compound of the first two, less a molecule of water, and the fourth is believed to be derived similarly from two molecules of the third. To the hydrochloride of the third Felix and Lang ascribe the formula $C_{64}H_{135}N_{36}O_{17}Cl_9$; they state that it is built up from eight molecules of arginine and one each of alanine, serine, amino-valerianic-acid, and proline, there being only one free —COOH group. Although such statements obviously

require confirmation by other workers, they illustrate the steady advances that are being made by Kossel's pupils in ascertaining the constitution of the simplest type of protein molecule.

Conjugated Proteins. These are proteins containing a *prosthetic* group (Gk. *prosthetos*, added to), which is not amino-acid in character. We should probably include the phosphoproteins more correctly amongst the conjugated proteins.

Phosphoproteins are protein compounds containing some phosphorus (presumably phosphate) radical which is neither of phosphatide nor of nucleic acid nature. They are typified by the casein of milk, which contains from 0·7 to 1·0 per cent. of phosphorus (different caseins from milks of different animals). Hydrolysis sets free the phosphorus of casein as phosphoric acid.

The phosphorus of casein, and probably also of other phosphoproteins, is present as a phosphate ester combined through the hydroxy group of hydroxy-amino-acids, such as hydroxy-glutamic acid, serine, and hydroxy-aminobutyric acid. Most of these phosphate radicals in casein are localised in a relatively small part of the molecule.

Nucleoproteins are compounds of one or more molecules of protein with *nucleic acid*, and are found in all cell nuclei.

Lecithoproteins are certain *presumed compounds*, rather than mixtures, found in blood serum and in fish eggs, from which treatment with alcohol does not remove the lecithin as easily as it would with mixtures, so that it is believed that there is actually a chemical combination between the protein and phosphatide radicals. Theoretically, such combinations are possible between a substituted phosphoric acid (the lecithin) and a substituted ammonia (the protein).

Glucoproteins contain a prosthetic group which includes a carbohydrate radical. (A carbohydrate radical is also present in nucleic acid, but the nucleoproteins are excluded from this class.) Glucoproteins include the mucins (from various

secretions such as saliva) and the mucoids (from bone, tendon, etc.).

The actual prosthetic group in these " mucoproteins " is built up, according to Levene, in equimolecular proportions from sulphuric acid, acetic acid, glucuronic acid, and, in most mucins, glucosamine (chitosamine), which in the mucoids of tendon and cartilage is replaced by galactosamine (chondrosamine).

The work of Fränkel and of Levene indicates that the carbohydrate group in ovomucoid from egg-white is built up from four trisaccharide units, each of which contains one glucosamine and two *mannose* radicals.

Chromoproteins contain a coloured prosthetic group, and are themselves also coloured. The typical representative is hæmoglobin, built up from a histone, globin, united to *hæmatin* (or *hæm*), a compound containing several pyrrole rings, and an atom of iron (*cf.* Chapter XV.).

The Derived Proteins. These are properly divided into two groups, those in which the protein molecule is but slightly altered and those consisting of break-down products of the protein molecule.

(1) *Primary Derived Proteins.* When very dilute acids are allowed to act on certain proteins they become insoluble, forming *proteans.* Thus edestin gives rise to edestan. Further action of dilute acids or alkalies produces *infraproteins* or *metaproteins,* which are insoluble in water but soluble in dilute acids and alkalies. Hydrolysis of proteins by acids and alkalies probably normally proceeds through this stage. Coagulated proteins are formed by the action of heat or of alcohol on soluble proteins, as in the coagulation of egg-white on boiling. It is believed that the main change consists in the formation of internal rings, internal anhydrides, similar to the diketopiperazine rings already dealt with, and produced through a considerable degree of dehydration, since in the formation of each ring a separate molecule of water is eliminated.

Proteins changed by such processes are frequently termed *denatured.* It has been found that the peptic digestion of such

denatured proteins is unaltered, but that the tryptic digestion is accelerated, suggesting that part of the change is in the nature of a degradation.

(2) *Products of Protein Hydrolysis.* These are the proteoses, peptones and polypeptides. The proteoses and peptones have been subdivided into various groups, but little advantage is to be gained by such subdivisions, since they are not based on chemical differences. It is, further, by no means certain that proteoses are more complex than peptones; they may be merely built up from different amino-acids and show different properties in consequence.

Since many of the proteins, as casein, react acid, whilst others, as the histones and protamines, have an alkaline reaction, and since these will react together to give insoluble protein "salts," it seems very possible that similar compounds of protein with protein may exist in living tissues.

Colloidal Behaviour of Protein Solutions

It is obvious that proteins all contain large molecules, and it is not, therefore, surprising that their solutions show colloidal properties. Since it was found that these colloidal solutions were not precipitated by salts and did not react with acids and alkalies in the definite gravimetric proportions that are usually found with solutions of chemical compounds, it became customary to insist on the tendency of colloids to form aggregates of molecules, such aggregates being termed micellæ (L. *micella*, a small morsel, a crumb).

Zsigmondy, for example, has written, "The essential and characteristic constituents of colloidal solutions are very small ultramicroscopic particles, the dimensions of which lie between molecular and microscopic size. . . . These have the same significance for colloidal solutions as the isolated molecules have for crystalloidal solutions."

Such conceptions led to a quagmire in the study of protein solutions. Jacques Loeb has shown that the inherent error arose in neglecting to consider the hydrogen-ion concentra-

tion in such solutions. When this is taken into account it can be shown that protein solutions obey the ordinary laws of aqueous solutions in their behaviour with ions, and we can regard them at any rate as containing, as a rule, isolated protein molecules or ions, which react stoichiometrically with acids and alkalies, forming highly dissociable metal proteinates or protein-acid salts. There is for each protein a definite *p*H value at which the protein exists practically in a non-ionised condition, and forms metal proteinate and protein-acid salt to a minimal (and equal) extent. This is the *iso-electric point* of that protein. If to a solution of protein at its iso-electric point we add acid, so increasing the hydrogen-ion concentration, acid salts are formed, and ionise, and correspondingly, if the solutions are made alkaline, metal proteinates are formed and ionise. *At its iso-electric point a protein is least soluble*, and advantage is taken of this property in the preparation of pure proteins from solution. Crystallisable proteins crystallise most readily from solutions having a *p*H close to their iso-electric point.

The iso-electric point of a protein can be determined by electrophoresis. At this point, since equal ionisation takes place, an electric current produces no movement of protein ions.

Typical iso-electric points are, approximately :

	Hydrogen-ion concentration.	*p*H.
Glutenin	$3{\cdot}2 \times 10^{-5}$	4·5
Serum albumin	2×10^{-5}	4·7
Gelatin	2×10^{-5}	4·7
Casein	2×10^{-5}	4·7
Egg-albumin	$1{\cdot}6 \times 10^{-5}$	4·8
Serum globulin	4×10^{-6}	5·4
Oxyhæmoglobin	$1{\cdot}8 \times 10^{-7}$	6·7
Edestin	$1{\cdot}3 \times 10^{-7}$	6·9
Gliadin	1×10^{-9}	9·0

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CHAPTER IX

OTHER CONSTITUENTS OF FOODSTUFFS

Such other constituents are inorganic salts, organic salts and acids, vitamins and water.

Inorganic Salts. Of these, that present in largest amount is sodium chloride. Some idea of the ratio of sodium chloride to total inorganic salt is given by ash determinations, as shown in Table IX. (The ash, however, includes sulphur of organic origin, as sulphate.)

The other ions present to yield the total ash are, principally, potassium, magnesium, calcium, phosphate, carbonate and sulphate. These may be considered as combined, along with the sodium and chloride ions, in every way possible.

Foodstuffs also contain manganese, in what form of combination is not yet known, minute traces of fluorine, iodine (partly as iodide), bromine (as bromide), arsenic and silicon.

Organic Compounds. Foodstuffs contain iron, chiefly in organic combination. Meats contain it in hæmoglobin, their iron content being about 0·0033 per cent. The iron content of vegetables varies from 0·0003 to 0·0041 per cent. The approximate figures for those containing most are, for barley, 0·0041 ; spinach, 0·0036 ; eggs, 0·0030 ; and dates, 0·0030.

A number of organic salts and acids are ingested, especially with fruits. Of these the most important are citrates and malates ; tartrates, oxalates and benzoates are also so obtained.

Vitamins. These are organic compounds of, as yet, unknown composition. At least six exist. They are ingested

TABLE IX. ASH, SODIUM CHLORIDE, AND WATER CONTENTS OF DIFFERENT FOODS

Food material.	Ash.	NaCl.	Water.
	Per cent.	Per cent.	Per cent.
Fresh lean beef	0·8–1·1	0·1–0·3	67–70
Fresh lean mutton	1·0–1·1	0·1–0·2	66–71
Hen's eggs—			
White	0·6	0·3	87
Yolk	2·0	0·04	47
Cow's milk	0·75	0·07	87
Cheddar cheese	2·6	1·8	34
Bread—			
White	1·8–2·1	0·8–0·9	41–45
Brown	1·8–2·7	0·9–1·4	44–48
Fruits—			
Apples	0·3	0·03	85
Grapes	0·3	0·024	85
Oranges	0·5	0·06	87
Strawberries	0·5	0·1–0·2	90
Honey	0·3	—	18·3
Walnuts (fresh)	1·8	0·02	11·4
Cane-sugar	0·0	0·0	0·0

along with all foodstuffs that have not been specially "purified." The "purer" the foodstuff the less is its vitamin content. Since the methods used in studying vitamins require the study of abnormal and pathological organisms, and since these compounds do not appear to undergo change during digestion, they will be dealt with later (see Chapter XXVIII.).

Enzymes. Foodstuffs ingested raw will obviously introduce their special enzymes into the alimentary tract. Most are probably destroyed during digestion, and in any case (except those present in bacteria) it is doubtful if, under normal conditions, they bring about any but negligible changes in the animal economy. However, advantage is

sometimes taken of their presence in experimental work; dogs from whom the pancreas has been removed can have the digestive function of the pancreas maintained by the continued feeding of raw ox pancreas.

Water. Water constitutes about three-fourths of the material of foodstuffs, as Table IX. shows. In addition, a great deal is consumed in the various beverages.

CHAPTER X

THE EFFECT OF PRELIMINARY TREATMENT OF FOODSTUFFS ON THEIR COMPOSITION

ALL the processes of cooking must be considered as a part of digestion. Starch is partly changed into soluble starch and dextrins. Hard connective tissue is changed, at least in part, into collagen and gelatin. Some of the other proteins may be partly changed to proteoses, and the fats may be partly hydrolysed. Cooking in water leads to a marked loss of mineral constituents.

Previous auto-digestive changes (see Chapter XX.) may affect meat and eggs, generally producing some degree of hydrolysis. In the ripening of fruits a number of changes occur, with increase in sugar content and neutralisation of organic acids.

Cooking tends to increase the solubility of the foodstuffs, making the subsequent digestion in the alimentary tract easier, while the appearance, taste and odour of the foods are improved, leading to increased physiological reflex stimulation of the digestive juices. Cooking also kills bacteria and parasites. (On the other hand, a repetition of the process increases the difficulty of digestion of meat proteins.)

The process of cooking frequently involves marked changes in the composition of individual foods. Thus potatoes are frequently cooked with fat, and then such cooked potatoes contain marked amounts of fat, while raw potatoes contain none. Such changes are exemplified in Table X. (In the final column of this table are given the heat values for each of the foodstuffs considered; these will be interpreted in Chapter XXX.)

We are now ready to consider what happens to these various foodstuffs during digestion in the alimentary tract.

Table X. Percentage Composition of Typical Cooked and Uncooked Foods

Group.	Foodstuff.	Water.	Ash.	Protein.	Fat.	Carbo-hydrate.	Cals. per 100 gm.
	Foodstuffs of animal origin—						
1.	Raw beef, sirloin steak	61·9	1·0	18·9	18·5	0·0	249
	Broiled loin steak	54·8	1·2	23·5	20·4	0·0	287
2.	Raw hindleg of mutton	63·2	1·0	18·7	17·5	0·0	239
	Roast leg of mutton	50·9	1·2	25·0	22·6	0·0	313
3.	Raw hindleg of lamb	58·6	1·0	18·6	22·6	0·0	298
	Roast leg of lamb	67·1	0·8	19·7	12·7	0·0	198
4.	Fresh ham	50·1	0·9	15·7	33·4	0·0	375
	Fried smoked ham	36·6	5·8	22·2	33·2	0·0	400
5.	Fresh oysters	86·9	2·0	6·2	1·2	3·7	52
6.	Uncooked hen's eggs	73·7	1·0	13·4	10·5	0·0	159
	Boiled hen's eggs	73·2	0·8	13·2	12·0	0·0	169
7.	*Cow's milk	87·6	0·7	3·3	3·6	4·8	67
	Butter	11·0	3·0	1·0	85·0	0·0	795
	Cheddar cheese	27·4	4·0	27·7	36·8	4·1	473
8.	Gelatin, as purchased	6·4	2·1	91·4	0·1	0·0	376
	Calf's foot jelly	77·6	0·7	4·3	0·0	17·4	89
9.	Honey	18·2	0·2	0·4	0·0	81·2	335
	Foodstuffs of plant origin—						
10.	Raw cabbage	91·5	1·0	1·6	0·3	5·6	·32
	Cooked cabbage	97·4	1·5	0·6	0·1	0·4	5
11.	Raw onions	87·6	0·6	1·6	0·3	9·9	49
	Cooked onions	91·2	0·9	1·2	1·8	4·9	42
12.	Raw green peas	74·6	1·0	7·0	0·5	16·9	102
	Cooked green peas	73·8	1·5	6·7	3·4	14·6	119
13.	*Celery, edible part	93·7	1·0	0·5	1·0	3·8	19
14.	*Mushrooms	90·7	1·1	4·7	0·2	3·3	31
15.	Raw potatoes	78·3	1·0	2·2	0·1	18·4	85
	Boiled potatoes	75·5	1·0	2·5	0·1	20·9	97
	Chip potatoes	2·2	4·5	6·8	39·8	46·7	589
	Mashed and creamed potatoes	75·1	1·5	2·6	3·0	17·8	111
	*Potato flour	12·9	0·2	0·3	0·0	86·6	356
16.	Entire wheat flour	11·4	1·0	13·8	1·9	71·9	369
	*Ordinary wheat flour	11·3	0·8	10·1	1·6	76·2	366
	*Bread, white	42·3	1·8	7·2	0·2	48·5	229
	*Bread, brown	43·2	2·3	7·0	0·4	47·1	223
	Bread rolls	29·2	1·1	8·9	4·1	56·7	308
	Whole wheat bread	38·4	1·3	9·7	0·9	49·7	251
	Toasted wheat bread	24·0	1·7	11·5	1·6	61·2	313
	Soda crackers	5·9	2·1	9·8	9·1	73·1	424
17.	*Coarse oatmeal	7·0	1·8	12·3	8·2	70·7	413
	*Rolled oats	8·5	1·8	13·1	6·5	70·1	399
18.	Tomatoes	94·3	0·5	0·9	0·4	3·9	23
19.	Apple, edible portion	84·6	0·3	0·4	0·5	14·2	64

TABLE X.—*continued.*

Group.	Foodstuff.	Water.	Ash.	Protein.	Fat.	Carbo-hydrate.	Cals. per 100 gm.
	Foodstuffs of plant origin—contd.						
20.	Banana, edible portion	75·3	0·8	1·3	0·6	22·0	101
21.	*Grapes (average)	84·7	0·5	0·6	0·1	14·1	60
22.	*Grape fruit	91·9	0·3	0·6	0·1	7·1	27
23.	Orange, edible portion	86·9	0·5	0·8	0·2	11·6	53
	*Marmalade	27·9	0·2	0·2	0·0	71·7	282
24.	Peaches, edible portion	89·4	0·4	0·7	0·1	9·4	42
	*Peach jam	26·6	0·2	0·2	0·0	73·0	298
	Canned peaches	88·1	0·3	0·7	0·1	10·8	49
25.	*Strawberries	90·1	0·5	0·7	0·1	8·6	38
	*Strawberry Jam	29·3	0·3	0·3	0·0	70·1	273
26.	(Chicken soup)	84·3	2·0	10·5	0·8	2·4	61
	(Meat stew soup)	84·5	1·1	4·6	4·3	5·5	81
	Cream of corn soup	86·8	1·0	2·5	1·9	7·8	59
	Consommé soup	96·0	1·1	2·5	0·0	0·4	12
27.	Cane-sugar	0·0	0·0	0·0	0·0	100·0	410
28.	*Chocolate	1·0	1·4	4·8	31·1	61·7	554
29.	*Brazil nuts	2·9	3·3	13·2	70·4	10·2	742
30.	*Chestnuts	44·3	0·9	3·0	1·9	49·9	228
31.	*Coconut flesh	37·3	0·8	4·2	48·5	9·2	500
	* ,, milk	92·6	0·6	0·2	0·0	5·2	22
32.	*Peanuts	4·1	2·5	20·1	47·6	25·7	616
33.	Walnuts (dried)	2·5	1·7	18·4	64·4	13·0	728

References

The figures in the table marked by asterisks are taken from R. H. A. Plimmer's " Analysis and Energy Value of Foods " (London, H.M. Stationery Office, 1921). The remainder are from Atwater and Bryant's Bulletin, No. 28, 1906, U.S. Department of Agriculture, as abstracted by E. A. Locke (" Food Values," New York and London, D. Appleton & Co., 1911).

SECTION III

THE CHEMISTRY OF DIGESTION, THE CIRCULATION, AND THE EXCRETA

CHAPTER XI

DIGESTION IN MAMMALS (OTHER THAN RUMINANTS)

Digestion consists essentially of a series of processes by which the insoluble compounds of a diet are converted into soluble compounds whose molecular size is relatively small enough to permit their passage through an animal membrane. A series of "colloidal" compounds is thereby converted into "crystalloidal" compounds.

These changes are brought about by the actions of five dilute solutions manufactured in the body by specific glands and poured out of the body into the alimentary tract. These solutions are the saliva, the gastric, pancreatic, and intestinal juices, and the bile.

The Digestive Enzymes and their Actions

Saliva, the mixed secretion of the parotid, submaxillary and sublingual glands, is essentially a very dilute solution of an *amylase*, *i.e.*, a starch-splitting enzyme, which contains also the necessary co-enzymes of this enzyme, *phosphate* and *chloride* ions, and a gluco-protein, a *mucin*. The purpose of the mucin is simply to aid, by virtue of its viscous nature, in the mastication and balling together of the solid food, prior to swallowing. Traces of a number of other compounds are present in saliva. These must be considered as having diffused through the salivary glands from the blood circulating

in these glands, and their presence is incidental and not functional. Amongst these is a trace of maltase, which, perhaps, produces a very slight action on some of the maltose set free through the action of the amylase. There is also present a slight trace of thiocyanate (of the order 0·05—0·1 mg. per 100 c.c.). Diffusions of this type account for the presence of traces of many compounds in the different secretions of the body, and it is desirable to emphasise the *accidental* nature of their presence. The ease with which inorganic compounds especially can diffuse into these fluids is shown by the simple experiment of swallowing a capsule containing potassium iodide. If the capsule is made of solid gelatin, this will be unacted on in the mouth, but will dissolve in the stomach within a few minutes and liberate the iodide. Almost immediately iodide can be detected in the saliva, indicating a very rapid passage from the stomach *viâ* the intestines and circulation.

Salivary amylase, formerly called ptyalin (Gk. *ptyalon*, saliva), produces on starch an action very similar to that produced in its hydrolysis by acids. Erythrodextrin, achroodextrin, and maltose are successively produced, but the amylase does not carry the action beyond the stage of maltose. Some maltose is possibly liberated at each successive stage of the digestion, as in acid hydrolysis (*cf.* p. 72).

Certain writers believe in a separate *dextrinase*, responsible for the hydrolysis of the dextrins, but since all enzyme action is essentially on a type of linkage rather than on a type of compound, there is no necessity to pre-suppose such a complexity of action until much further evidence is adduced by these writers. We must suppose that the hydrolysable linkages in the dextrins are of the same type as those in the similar but slightly more complex starches.

The precise nature of the changes from starch to maltose is not yet known.

The amylase is without action in the absence of salt ions. Those activate it in the order (*a*) Cl^-, Br^- ; (*b*) I^-, NO_3^- ;

(*c*) SO_4^{--}, $CH_3.COO^-$, PO_4^{---}. Obviously, for salivary amylase, the chloride ion must be regarded as the essential co-enzyme. (Phosphate radicals are apparently always present in the starch molecule that is being acted on.) The action of the amylase is largely controlled by the hydrogen-ion concentration of the medium. It is active in slightly alkaline solution, more active in neutral solution, and still more active in very slightly acid solution. In the presence of chloride or bromide ions the activity is greatest in solution of *p*H 6·7, and the range of activity extends from *p*H 4 to *p*H 9. The enzyme is destroyed when these limits are much exceeded. Largely through the *buffer action* (see Chapter XVI.) of the carbon dioxide of the expired air the *p*H value of the saliva is kept very close to that required for its optimal action.

The optimum temperature for the amylase activity lies between 40° and 50° C., and the enzyme will not withstand a continuous temperature of 70°. With amylase, as with most enzymes the presence of the product (in this case maltose) slows the action. Some of the maltose probably remains in chemical combination with the enzyme, which is in that way removed from action.

Amylase is absent from the saliva of the true carnivora. Dog's saliva is stated to possess definite but very weak amylolytic activity, and if this be true it is very probable that the amylase comes from the pancreas through the circulation, and is not secreted by the dog's salivary glands. Horse's saliva is said to contain the zymogen of amylase, which is changed to the active enzyme during mastication.

The *gastric secretion* can be regarded as essentially a 0·4 per cent. solution of hydrochloric acid, containing the enzymes *pepsin* and *rennin*, and traces of the enzyme *gastric lipase*. Mixed with food, much of the hydrochloric acid unites with the amphoteric protein, so that during digestion the hydrogen-ion concentration is equivalent to 0·2 per cent. of this acid. This change may be accelerated by reflux passage of alkaline fluid from the duodenum, and such

passage may also bring into the stomach traces of intestinal erepsin, bile, etc.

The first direct observations on the secretion of gastric juice in the human stomach were made by an American army surgeon, Dr. Beaumont, on a Canadian coureur du bois, Alexis St. Martin, in whom the surgeon established a gastric fistula, following perforation of the stomach wall by accidental discharge of a musket. These observations, though made in 1822, were wonderfully accurate, and have been many times confirmed. Beaumont established the acidity of the juice, and its digestive action on meat, and, further, that the mucous membrane of the stomach during rest normally reacted neutrally or slightly alkaline. Previous to Beaumont's observations doubt existed as to whether the gastric secretion was or was not acid ; investigators who detected acidity attributed it to various organic acids, or to phosphoric acid. Silliman, of Yale University, detected hydrochloric acid in samples of gastric juice obtained by Beaumont from St. Martin.

The secretion of gastric juice, like that of the saliva, is under nervous control, excitement to secretion being induced through nervous channels by various causes, such as actual presence of food in the stomach, presence of food in the mouth (reflex stimulation), and even sight or smell of food (psychic stimulation). The secretion can be obtained for examination from experimental animals by means of the stomach tube, or by gastric fistulæ (artificial openings on to the abdominal wall), or by fistulæ opening into a Pawlow pouch, which consists of a part of the stomach surgically separated from the rest, which is sewed up and allowed to carry on its normal function, this separated pouch being sewed to the abdominal wall and so allowed to act as its own fistula.

Hydrochloric acid is believed to be secreted by the oxyntic (Gk. *oxyein*, to make acid) or parietal (L. *paries*, wall) cells in the stomach mucosa. It seems very unlikely that any cell has the power of setting free within itself such a strong acid, and the theory has been put forward that actually

ammonium chloride (or some organic derivative) is secreted from the cell, and in leaving the cell reacts with some organic substance which retains ammonia, leaving the free acid :

$$NH_4Cl - NH_3 = HCl$$

The evidence regarding the existence of free acid within the cell is based on staining reactions. While most of the experimental work suggests that there is no such free acid, very recent work by Dawson and Ivy led them to the conclusion that acidity can occur within the parietal cells to the extent of *p*H 3.

The source of the chlorine of the acid is readily ascertainable. During the secretion of gastric juice the chloride concentration of the blood may be lowered to the extent of 7 per cent.

Hollander (1929) claims to have obtained evidence that when the skin of a frog that has been recently killed is arranged to separate two portions of the same solution of sodium chloride, there is a shift across this membrane, so that acidity develops on the one side, and alkalinity on the other, in accordance with the equation—

$$NaCl + HOH = HCl + NaOH.$$

He presents the hypothesis that hydrochloric acid is secreted into the vacuole of the parietal cell by a similar process, and is then concentrated by reabsorption of water from the vacuole, until it is isotomic with blood.

Pepsin is formed in the " chief " cells of the stomach mucosa, or, more accurately, the precursor, *pepsinogen*, is there formed. The existence of this precursor can easily be demonstrated. An aqueous extract of the stomach mucosa shows none of the activity of pepsin. If it be made acid with hydrochloric acid peptic activity is at once present. This test might merely demonstrate the fact that before the activity can occur a definite acidity is necessary. But pepsin is quickly decomposed in an alkaline medium. Pepsinogen is more resistant. It is possible to add alkali to an aqueous extract of stomach mucosa, allow it to remain such a length of time that any pepsin would certainly be destroyed, and then acidify, when the activity of pepsin can at once be demonstrated.

Pepsin (Gk. *pepsis*, digestion) is a powerful protease, and digests proteins in an *acid* medium. The essential factor for its action is a favourable hydrogen-ion concentration. Thus, in presence of an amount of hydrobromic acid corresponding to that of hydrochloric acid in the juice, its action is just as strong. The action of pepsin on proteins is very similar to that of hot dilute acid, a series of hydrolyses resulting. The action is much more limited, however, seeming not to pass the peptone stage. As has been pointed out (Chapter VIII., p. 126), pepsin appears to act on a very specific linkage, which is not the simple peptide-linkage. As a result of this it has no action on the protamines salmine and clupeine. It has been suggested that an ether, an ester, or an anhydride linkage is attacked by pepsin, but the problem has still to be solved. Its action on most insoluble proteins is rapid; these pass rapidly into solution as proteoses and peptones. As the action proceeds a certain amount of the enzyme appears to unite with the hydrolysed products.

Very powerful pepsin preparations have been obtained by Fenger, Marston, Forbes, and Northrop. Northrop believes his crystalline preparation is pure pepsin. An account of his work has been given in Chapter II.

Rennin, rennet, or chymosin (Gk. *chymos*, juice), possesses the property of causing milk to clot. The actual clotting is a double process. Rennin converts *casein* into *paracasein* (or, in the British terminology, caseinogen into casein; see footnote on p. 104). This process probably consists in the breaking up of the large casein molecule into two smaller paracasein molecules by hydrolysis. Paracasein then unites with the calcium ions present in milk to form insoluble calcium paracaseinate. This insoluble compound separates as a bulky precipitate, holding the whey of the milk in solid suspension, so that a clot is formed. Calcium caseinate is obviously soluble or else a clot would form in fresh milk.

The double nature of the process of clotting can be easily demonstrated. If the calcium ions of milk are removed by the

addition of potassium oxalate solution, so that insoluble calcium oxalate is precipitated, then addition of rennin causes no clot. Hence calcium is necessary for the clot-formation. If this solution is then boiled (so that the enzyme rennin is destroyed) and then a calcium solution is added, a clot forms. Hence the rennin can produce its action in absence of calcium, yielding soluble paracasein; calcium is required to clot this paracasein.

The clotting of milk can be brought about by pancreatic juice, intestinal juice, secretions of many bacteria, pineapple juice, and other plant juices. Wherever proteases exist in nature, their solutions appear to be able to coagulate milk. From the widespread distribution of such active juices, and from the fact that plants do not normally digest milk, it would seem justifiable to conclude that the clotting of milk can be brought about by the proteases themselves, through their formation of paracasein by cleavage (with the subsequent reaction between paracasein and calcium ions) rather than that wherever a protease exists there must a rennin exist also.

It is, consequently, a matter for discussion as to whether the normal action of the gastric secretion in clotting milk is not due to pepsin. Are not pepsin and rennin one and the same compound? The question is difficult to resolve finally; the experimental evidence is conflicting. Clotting can take place in practically neutral solutions, while the proteolytic activity of pepsin requires a markedly acid medium. It does not follow that pepsin cannot clot milk in neutral solution, especially since it has been shown that the rate of clotting is accelerated by addition of acid. However, apparently the two enzymes have been separated by Hammarsten, who has shown that pepsin is completely precipitated by lead acetate or magnesium carbonate, but that the solution, though its proteolytic activity has vanished, will still produce clotting (still contains rennin). He showed also that heating to 40–45° C. in 0·3 per cent. hydrochloric acid almost completely destroys the rennin action, though the solution still has proteolytic properties. Further, there seems to be a difference in the action of gastric juice of the young and adult animal. That from the calf's stomach has a powerful clotting action (and is the source of commercial rennet), but has only a weak proteolytic action on egg-white. That from the adult pig's stomach has only weak clotting action, but powerful proteolytic action. An extract from a dog's stomach contains probably no rennin, the clotting it produces being entirely due to pepsin.

Fenger has added considerably to the solution of this problem.

He states that while the suckling calf stomach contains both enzymes, the stomach of the adult hog contains pepsin only. He finds that pepsin is coagulated by heat and is colloidal in nature, while rennin is a decomposition product of protein of the acid-albumin type, is not coagulated by boiling, and is not proteolytic in action. Finally, Tauber and Kleiner have shown that rennin is completely digested by pepsin and trypsin (though not by erepsin), so that it follows that rennin is a protein quite distinct from the protein pepsin.

Fenger's results illustrate the enormous catalytic activity of these enzymes. He has found that pepsin will digest at least 25,000 times its own weight of freshly coagulated and disintegrated egg-albumin in 2·5 hours at 52° C. Rennin will coagulate more than two million times its weight of fresh milk at 40° C. in ten minutes.

The cumulative evidence in favour of the separate existence of the two enzymes pepsin and rennin can now be considered convincing. It is important to remember that *the clotting of milk by rennin can take place in a neutral medium.* This property is essential in the digestion of milk by sucklings.

Gastric lipase is unable to produce much effect on non-emulsified fats, but the slight action produced by it in the stomach results in the stomach contents containing a slight amount of free fatty acid by the time they pass on into the intestine.

That gastric lipase exists as such, and is not merely a trace of pancreatic lipase that has passed upwards into the stomach, is shown by the fact that it can be extracted from the stomach mucosa, and, further, that its optimum activity takes place at a different (more acid) hydrogen-ion concentration.

The gastric juice, in virtue of its acidity, has a slight degree of hydrolysing action on the complex carbohydrates, and especially on that one which is most easily hydrolysed, sucrose. In virtue of its marked acidity it also has an antiseptic action, killing all forms of life except spores, *providing the period of action be long enough.* But it must be remembered that fluid passing rapidly through the stomach, in

absence of solid food, will travel on into the intestine before sufficient acid has been secreted to sterilise it.

The *pancreatic juice* contains two important enzymes, *pancreatic amylase* (formerly called *amylopsin*, from L. *amylum*, starch, and Gk. *pepsis*, digestion), and *pancreatic lipase* (formerly called *steapsin*, from Gk. *stear*, tallow, and *pepsis*), and also *inactive trypsin* or *trypsinogen* (Gk. *tryein*, to rub down, and *pepsis*), an incompletely activated enzyme. In addition, a small amount of chloride and much smaller amounts of phosphate and sulphate are present. The reaction of the juice is distinctly alkaline, the *p*H value approximating to 8.

The *intestinal juice* contains an important zymo-excitor, *enterokinase*, which reacts with trypsinogen, converting it into active trypsin, and a number of enzymes, *erepsin*, *sucrase maltase*, *lactase*, and others concerned with the digestion of nucleic acid, etc. The juice contains much protein, and also appears to act in part as an excretory channel. It also is distinctly alkaline in reaction, with an average *p*H value of 7·7.

The *bile*, a secretion of the liver, is a mixture of secretory and excretory products. Its contents may be divided into three groups : (*a*) *the bile salts*, (*b*) *cholesterol*, *phosphatides*, *mucin* and *soaps*, and (*c*) *the bile pigments*. The members of the first group have a specific function in aiding digestion, the second are largely, and the third wholly excretory. The colour is green or golden (on account of the pigments present), and the taste bitter (from the bile salts). Bile is usually neutral or faintly alkaline in reaction, but may be slightly acid.

Bile stimulates intestinal peristalsis, and thus accelerates the passage of the intestinal contents. In this way it diminishes bacterial action indirectly, although bile itself is a good medium for bacterial growth.

We are only concerned in this chapter with those constituents of the bile that aid digestion.

The secretion of the pancreas is of two distinct kinds, an internal secretion which is poured directly into the blood circulating through that organ and an external secretion poured out through two ducts into the duodenum. *The external secretion is in great part controlled by a purely chemical mechanism.* The mucosa of the duodenum, and to a lesser extent of the jejunum, contain a substance called *secretin.* According to Bayliss and Starling, who discovered its existence, as soon as acid enters the duodenum secretin is formed from a precurser, *prosecretin,* and is passed into the capillaries of the mucosa and so reaches the circulation. Mellanby's recent work suggests that, as soon as bile reaches the duodenum, bile salts are in part absorbed through its mucosa, and carry preformed secretin along with them into the portal circulation. Whichever is the mechanism, it is certain that the action is entirely independent of nervous control, and takes place when the nerves to the duodenum (and jejunum) have been severed. Within a minute or two thereafter, that is, practically as soon as any of the secretin can have reached the pancreas, this organ commences to secrete its juice. Bile secretion is also stimulated, though to a smaller extent. Secretin is thus an example of the " internal " secretion of a specific chemical compound. It will be discussed more fully in Chapter XIX.

It has been shown that by stimulating certain nerves to the pancreas a flow of juice can be evoked, indicating that to some extent nervous reflexes may play a part in the production of the secretion. It has been further suggested that the actual formation of the constituents of the juice is under nerve control and that the action of secretin is merely to release the secretion.

Samples of pancreatic juice can be obtained by fistulæ from the pancreatic duct.

The intestinal juice is stated to be mainly secreted by Lieberkühn's follicles. Its secretion commences as soon as the acid contents of the stomach enter the duodenum.

Fistulæ from the duodenum permit collection of the juice for study of its contents and properties.

The secretion of bile by the liver is continuous, though normally, in the intervals of digestion, it is stored in the gall-bladder. As has just been stated, secretin accentuates the flow of bile.

Properties of the Specific Compounds Present in the Pancreatic and Intestinal Juices and the Bile

Pancreatic amylase has a powerful action on starch, very similar to that of salivary amylase ; dextrins and, finally, maltose (and some isomaltose) are produced. Under its action a small amount of glucose is set free, though this is probably due to the presence of a trace of pancreatic maltase. Pancreatic amylase also requires the presence of chloride and phosphate as co-enzymes, the dialysed juice having no amylolytic action. Pancreatic amylase acts best in solutions having a *p*H value of 7, while salivary amylase requires for optimum action the slightly different value of 6·7. The optimum temperature for its activity is also slightly different, 35° C.

Pancreatic lipase has a powerful action on neutral fats, hydrolysing them to fatty acids (or soaps, in a slightly alkaline medium) and glycerol. Since the lipase can only attack the fat globule at its surface, the action is vastly accelerated by substances which will lower surface tension, and so promote emulsification. The traces of soap from gastric lipase action, and from the bile, and to a much greater extent the bile salts, promote emulsification through their effect on surface tension, and, in the absence of bile in the intestine through pathological or experimental causes, fats are but very slightly acted on.

"*Inactive Trypsin*" or *trypsinogen*, as secreted by the pancreas, has been shown by Waldschmidt-Leitz to be, not an inactive precursor of the enzyme, as was customarily

considered, but an enzyme showing specific but incomplete activity. It will hydrolyse peptones and protamines such as clupeine, but will not act upon gelatin or casein, until it has been changed to " active " trypsin by the enterokinase (Gk. *enteron*, gut ; *kinein*, to move) of the intestinal juice.

Enterokinase can be extracted from the mucous membrane of the duodenum by dilute solutions of sodium bicarbonate. Since it is destroyed by boiling, and since a small amount of it will activate a relatively large quantity of trypsinogen, it may perhaps be itself an enzyme. Its enzymic nature is not yet definitely established.

Casimir Funk has shown that trypsin can be greatly concentrated by precipitation with picric acid and removal of the latter by nitron. Repetition of this process gives material fifty times more active, weight for weight, than the original extract, but the treatment at the same time seems to effect a partial separation into trypsinogen and the " kinase." Such a result suggests an actual combination of the two compounds rather than an enzyme action.

Trypsin is a more powerful enzyme than pepsin, rapidly hydrolysing proteins or the proteoses or peptones that have been formed from them by pepsin action, and carrying the hydrolyses to the polypeptide and amino-acid stages. It seems to act specifically on the peptide linkages (although some recent evidence suggests that very pure trypsin will not attack simple peptides). The optimum hydrogen-ion concentration for its activity depends on the nature of the substrate. Thus, for gelatin, the optimum *p*H value is 9·7, for caseinogen and peptones 8. It is interesting to note that, if it be correct that trypsin acts best in such slightly alkaline media, the normal conditions in the intestine, which approximate to neutrality, are not optimal for its action.

The action of the intestinal enzyme *erepsin* is definitely established as the splitting of the peptide linkage. It is usually stated to act powerfully on proteoses and peptones, but Waldschmidt-Leitz finds that pure erepsin preparations

will only attack relatively simple polypeptides, setting free their constituent amino-acids. Erepsin is destroyed at a temperature of 59° C. Its optimum activity is at a *p*H of 8, though it is able to act in a very slightly acid medium.

Pancreatic juice has been stated also to contain an erepsin. The term " erepsin " or *ereptase* is applied generally to enzymes that have the same type of action as intestinal erepsin. Different erepsins vary somewhat in their actions.

Sucrase has a specific action on the α-fructoside linkage, and so hydrolyses sucrose.

Sucrase from the human intestine seems to be limited in its action to sucrose. That from lower forms, such as the enzyme present in the intestinal tract of the snail, can also act on raffinose and stachyose.

Maltase has a specific action on α-glucosides, and therefore hydrolyses maltose to glucose.

Lactase has a specific action on β-galactosides, and, in consequence, hydrolyses lactose to glucose and galactose.

The *bile salts* are the neutralised salts (whether by sodium, potassium, or other ions is immaterial) of the bile acids, and possess the property of considerably lowering the surface tension of any aqueous solutions to which they are added. In addition, solutions containing them are able to dissolve relatively large quantities of cholesterol and its esters.

The bile acids are two in number, *glycocholic acid* and *taurocholic acid.* They are white crystalline compounds. The first is sparingly soluble in water, and has a bitter, with a sweet after taste. Taurocholic acid is much more soluble, and has a very bitter taste. These acids are easily hydrolysed by warming with mineral acids, yielding *cholic acid* and, respectively, *glycine* and *taurine.* Glycine is one of the amino-acids formed during hydrolysis of proteins, and taurine is a sulpho-acid which can be readily formed by oxidation from cysteine (see p. 116).

Table XI. Composition of the Digestive Secretions

Mixed Human Saliva, Specific Gravity, 1·002 to 1·008.

Average composition—

Water	99·41 per cent.	
Solutes, etc.	0·59 „	
Mucin and epithelium		0·213 per cent.
Soluble organic compounds		0·152 „
Urea	0·022 per cent.	
Uric acid	0·001 „	
Thiocyanate	Trace.	
Inorganic compounds		0·219 „
Na	0·021 per cent.	
K	0·100 „	
Cl	0·040 „	
PO_4	0·056 „	
Ca	Trace.	

Human Gastric Juice, Specific Gravity, 1·002 to 1·006.

Normal limits—

Water	99·20–98·94 per cent.	
Solutes	0·80– 1·06 „	
Organic compounds		0·34–0·47 per cent.
Inorganic compounds		0·46–0·59 „
HCl	0·35–0·45 per cent.	
Total Cl	0·49–0·56 „	
NH_3	0·05–0·07 „	
K, Ca, Mg.	Traces.	

Dog's Intestinal Juice, Specific Gravity, 1·01 to 1·011.

Normal limits—

Water	98·78–97·59 per cent.	
Solutes, etc.	1·22– 2·41 „	
Organic compounds		0·8 per cent. or more.
NaCl		0·5 „

Human Pancreatic Juice, Average Specific Gravity, 1·007.

Average composition—

Water	98·7 per cent.	
Solutes	1·3 „	
Organic compounds		0·6–0·7 per cent.

Inorganic ions include Na, Cl, and much smaller amounts of K, SO_4, and PO_4.

Human Bile, Specific Gravity, fistula bile, about 1·012; gall-bladder bile, 1·027.

	Fistula bile.	*Bladder bile.*
Water	96·47–97·48	85·9–86·0
Solutes, etc.	3·53– 2·52	14·1–14·0
Bile salts	0·90– 1·82	7·2– 9·1
Mucin and pigments	0·10– 0·12	2·66–2·98
Cholesterol	0·06– 0·16	0·16–0·26
Lecithin, fats, soaps	0·14– 0·24	0·32–0·92
Inorganic salts	0·73– 0·83	0·65–0·77

Cholic acid (or cholalic acid), $C_{24}H_{40}O_5$, is a white, crystalline, very bitter compound. Its probable constitution has been given on p. 94. Perhaps one of the functions of the liver is to convert a certain proportion of cholesterol into cholic acid, and then to conjugate the acid with glycine and taurine to form the bile acids (*cf.* p. 393).

Human bile contains conjugated bile salts not alone derived from cholic acid, but also from the related compounds deoxycholic acid, $C_{24}H_{40}O_4$, chenodeoxycholic acid, $C_{24}H_{40}O_4$, and lithocholic acid, $C_{24}H_{40}O_3$.

It is difficult to estimate the average amounts of the various secretions produced per twenty-four hours by normal adult man. The amount of saliva will vary widely with the time of chewing, the character of the food, etc. It may total 1,500 c.c. Probably about the same volume of gastric juice is secreted for *each* meal. A somewhat lesser amount of pancreatic juice is probably secreted, and still smaller amounts of bile and intestinal juice. The average composition of these juices is shown in Table XI. It will be noted that there is a marked difference between fistula bile and bladder bile. The former is more dilute than freshly secreted bile, since its constituents are entirely of fresh formation; no bile salts have been conserved to the organism by absorption from the intestine, the normal procedure. The latter is more concentrated than freshly secreted bile, since during storage in the gall-bladder much water is absorbed, and some part, perhaps, of the cholesterol.

The Digestion of Food in the Alimentary Canal

Food is taken into the mouth, moistened with saliva, masticated in varying degree according to its consistency and the habit of the individual, and swallowed. It usually remains in the mouth a few seconds only. Thus in the course of a meal there accumulates rapidly at the cardiac end of the stomach the usual mixture of the average repast, solids

reduced to a moderate state of subdivision, impregnated with saliva, and mixed with the water (or diluted water), which should be ingested *along with* the solid constituents *during* the meal, if the dietary be correct for a normal individual.

The stomach, which when at rest is a mere tube, is gradually distended from the cardiac end downwards by the food entering it. This distension starts peristaltic waves, which commence towards the cardiac end and pass downwards to the pylorus, not very rapidly—at the rate of one or two a minute. They gradually carry the front wave of food towards the pylorus. Unless it be very liquid in average consistency the food is not greatly intermixed, and the bulk of the meal is only slowly swept forward. Thus if we commence a meal with an *hors d'œuvre*, this travels as an advance guard through the stomach, and does not mix with the ice cream acting as rearguard.

There has been, for all practical purposes, no time in the mouth for the action of salivary amylase. The amount of starch hydrolysis occurring there has been negligible. But the amylase can continue its action in the food mass impregnated with saliva, lodged within the stomach entrance, and does so for from twenty to thirty minutes. *Salivary digestion occurs within the stomach.* The first part of digestion *within the stomach* results in formation of some dextrin and some maltose, from some of the starch (and dextrin) of the food.

In this way, within fifteen or twenty minutes of ingestion, about 75 per cent. of the starch of mashed potatoes and 60 per cent. of that of bread are converted to maltose, and an additional percentage to dextrins.

Sight and smell of the food, and its presence within the mouth, stimulate reflexly through nerve channels the secretion of gastric juice. Actual presence of food in the stomach accelerates the production of more juice. Acid of the juice converts pepsinogen into active pepsin. The acid fluid slowly permeates the food-mass. Production of protein-hydro-

chloride delays the acidification of the mixture. Any regurgitation from the duodenum accentuates this delay. As the hydrogen-ion concentration of the mixture increases the activity of the amylase gradually ceases. At *p*H 4 it ceases entirely, and with still greater acidity the amylase is decomposed. But for some length of time there co-exist gastric digestion in the forward mass of food, and salivary digestion in the remaining portion.

Through the action of pepsin, in presence of acid, insoluble protein changes to soluble proteose and some proteose changes to peptone. The forward wave becomes fluid, with few solid particles of appreciable size remaining. As soon as the continuous peristaltic contractions force forward to the pylorus—normally closed—succeeding waves of sufficient fluidity (and acidity) the pylorus opens, and liquid gushes into the duodenum. But as soon as this acid liquid comes in contact with the duodenal mucosa nervous reflexes are set up, resulting in the reclosing of the pyloric orifice. The remaining stomach contents continue to become more fluid and more acid.

The entrance of acid fluid into the duodenum not only is the cause of a reflex closing of the pylorus, but stimulates immediate secretion of intestinal juice from the glands lining the duodenal mucosa, the outpouring of bile, and, through the intermediation of *secretin*, the outpouring of pancreatic juice and more bile. Two of these juices, the intestinal and pancreatic juices, are invariably alkaline ; the bile is usually alkaline. The acid fluid from the stomach is quickly neutralised, and the guardian action of a high hydrogen-ion concentration on the pyloric gate is withdrawn. The fluid in the stomach again produces its effect, and another gush of fluid through the reopened pylorus follows. The alternating series of fluid stimulations on one side of the pylorus, and acid stimulations on the other, continues until most of the stomach contents have been discharged into the duodenum. Finally, undigested solid particles can also pass.

The time required for stomach digestion is very variable, the determining factor being the nature of the food mixture. Thus it has been shown that a slice of bread will completely pass through the stomach of a dog in one hour, but if the bread had been buttered four hours are required. Evidently fat delays the passage. In man the average meal takes from two to five hours for complete passage.

Fluid beverages (including alcoholic beverages) taken on an empty stomach pass through it to the duodenum rapidly, indicating that the essential factor in opening the pylorus is fluidity rather than acidity. Milk taken on an empty stomach is clotted. Rennin can produce its effect in an almost neutral medium; the milk is clotted before the amount of juice secreted by the stomach is great enough to produce a marked acidity. The importance of this is essentially for the young animal, the suckling, living solely on a milk diet; the milk so clotted can then have its casein and other proteins properly digested by pepsin. Otherwise, in the rapidity of the passage of this fluid through the intestine, though some clotting might occur (through the action of trypsin and erepsin), digestion would be incomplete and diarrhœa might occur. It is probably no exaggeration to say that on the power of rennin to clot milk in an almost neutral medium depends the life of the suckling.

What passes into the intestine? An acid fluid, containing proteoses, peptones (in smaller amount), fats, liquid at body temperature, and a trace of fatty acid (from the action of gastric lipase on any emulsified fat), undigested starch, and partially digested starch products (dextrins, maltose), sucrose and traces of glucose and fructose (from acid hydrolysis of sucrose), vitamins and various salts. In addition, the fluid carries with it undigested cellulose and other undigestible polysaccharides, traces of pentoses, and some still reactive pepsin. Some part of this pepsin, adsorbed or chemically combined with protein, may withstand the slight alkalinity of the intestinal contents, and may even carry on further

action in the ileum, wherever the medium becomes distinctly acid.

The mixture is practically sterile. In connection with the sterilising action of the acid it may be noted that people who have a copious and active acid gastric secretion are stated to be less liable to infection by typhoid and cholera than those with a less active juice, while any reduction of acidity in the stomach is apt to be followed by a bacterial or yeast fermentation which may produce irritating organic acid and gas.

Practically nothing is absorbed within the stomach except a trace of alcohol. (Certain alkaloids may be absorbed through the stomach wall, but these are not normal constituents of a diet.)

The mixture entering the intestine is now subjected to the combined action of trypsin, erepsin, pancreatic amylase and lipase, sucrase, maltase, lactase, and other enzymes, in the presence of bile salts.

Trypsin and erepsin continue the proteolytic hydrolyses begun by pepsin and carry them on more rapidly and further. Amino-acids are set free in increasing quantity. Amylase soon breaks down the starch and dextrin mixture to maltose ; maltase changes the product to glucose. The bile salts lower the surface tension of the fluid, and intestinal peristalsis emulsifies the fat droplets suspended in it. The minute fat particles are attacked by the lipase, and soaps and glycerol result. Sucrase converts sucrose into glucose and fructose ; lactose is split by lactase into glucose and galactose. The nucleo-proteins, partially split by pepsin, are broken down to protein and nucleic acid, and special enzymes hydrolyse the latter further. Other conjugated proteins are similarly broken down.

This continuous series of hydrolyses by these different fermenting agencies produces finally amino-acids, glucose (and smaller amounts of fructose and galactose), soaps and glycerol, with fragments of other complexes. Along with these are the unaltered salts and vitamins of the original

ingested food. The mixture of partially digested compounds and products gradually travels down the intestine, forced onwards by the peristaltic waves in that closed passage, and so continuously brought into contact with fresh mucosa, an animal membrane which will permit passage of almost all the smaller soluble molecules of the mixture. The chemical constituents of the digest may themselves facilitate the peristalsis. The amino-acids and sugars pass through to the capillaries of the membrane and so to the portal circulation; the soaps, in solution with the bile salts and glycerol, are absorbed, retransformed into fats, and pass through the lacteals of the villi to the lymphatics. Undigested and undigestible remnants are left behind, mixed with certain compounds excreted through the bile, or through the intestine itself. Salts and vitamins are apparently absorbed without difficulty.

Of course, water is also continuously absorbed, but watery secretions are continuously being added, and so the fluid consistency of the intestinal contents persists throughout the length of the small intestine.

In the large intestine the main change that occurs is absorption of water, the contents gradually assuming the consistency of fæces. The value of rectal feeding is obviously bound up with the degree of possible absorption in that part of the gut. Only salts, such as sodium chloride and, to a smaller extent, glucose, will pass in solution through the wall of the large intestine, so that feeding per rectum is practically only slow starvation; it can have but a very limited temporary value.

There is one important type of chemical action continuously proceeding in the intestine that has not yet been mentioned. Under what we at present call normal conditions bacteria abound in the intestinal canal, and these bacteria attack sugars and amino-acids, producing a great number of diverse compounds. These will be discussed in the next chapter.

Food residues commence to pass through the ileo-cæcal valve four or five hours after the commencement of a meal ; the passage continues for two or three hours. The concentration of fæces in the large intestine obviously will occupy a very variable time. Perhaps from twelve to twenty-four hours may be considered the average period. The movement in this part of the gut is forward *and* backward ; peristalsis and anti-peristalsis occur.

Variations of Hydrogen-ion Concentration in the Medium during Passage through the Alimentary Tract

According to Starr (1922) 86 per cent. of 228 normal human subjects gave salivas whose reaction varied between *p*H 6·35 and 6·8, and averaged 6·6. He emphasises the effect of the carbon dioxide in the expired air in controlling this value. Bunzell found that the average *p*H value is 6·4, that of children being slightly less acid, 6·6, and that of aged persons slightly more acid, 6·1. A few per cent. of the children, and a still smaller per cent. of adults, showed an alkaline reaction. Henderson and Millet (1927) found slightly higher values ranging from 6·2 to 7·6 (electrometric measurements). (There is no relation between the reaction of the saliva and the condition of the teeth.) The act of chewing—even paraffin—is said to decrease the acidity. Henderson and Millet demonstrated that saliva measured just after a meal always shows a distinctly increased *p*H value above that of a sample taken just before the meal, while subsequently the *p*H decreases to a value still more acid than the original one.

Neutral food, moistened with saliva, will obviously give a mixture very closely approximating to neutrality in reaction. If acids, such as vinegar, are ingested, the mixture will react more acid, and the acidity may be great enough to inhibit the salivary digestion of starch ; potato salads probably come within this category.

In the stomach the food gradually becomes more acid, up to a concentration usually corresponding to 0·2 per cent.

hydrochloric acid, *i.e.*, *p*H 1·3, through admixture with gastric juice, containing about 0·4 per cent. acid.

In infants, on artificial milk diets, much lower degrees of acidity exist, the reaction of the stomach contents approximating to *p*H 5. The function of hydrochloric acid secretion is incompletely developed during the first six months of infancy. With breast-fed children after seven months there is a fair degree of peptic digestion.

Stomach contents passing to the duodenum are rapidly neutralised by the three alkaline juices with which they are almost immediately mixed. (The duodenal contents during fasting vary in *p*H from 6·6 to 8·0.) Food obtained by duodenal tube from the duodenum in human subjects shows a *p*H varying between 4·9 and 8, and such variations may well occur, according to the stage of neutralisation when the actual sample is taken. No evidence of the presence of free hydrochloric acid is ever found. Duodenal contents from dogs, obtained by fistula, show similar variations. Fistula samples obtained from the lower part of the ileum in dogs on a normal mixed diet show *p*H values between 5·7 and 6·6. Evidently the mixture regains a certain degree of acidity (perhaps through bacterial action) before it leaves the small intestine.

The reaction of normal fæces seems to depend to some extent on the consistency. Hard fæces are alkaline, fluid fæces vary from slightly alkaline to slightly acid. The fæces of infants usually show an acid reaction.

CHAPTER XII

CHEMICAL ACTIONS PRODUCED IN THE INTESTINE BY BACTERIA

UNDER the present conditions of human existence the intestine contains many millions of bacteria which bring about a vast number of chemical reactions. These reactions are of service to the bacteria, but are seldom of service to their host.

It cannot be positively stated that our organism is designed to carry on its existence without this bacterial decomposition. It has been reported that the fæces of Arctic animals are sterile —contain no bacteria ; it is to be presumed that they do not in consequence live an abnormal existence. Experiments have been carried out in which young chicks have been hatched and reared under sterile conditions, and guinea-pigs removed from the mother by Cæsarean operation have been found able to grow and utilise sterile food. Banana-flies have been reared under sterile conditions and lived normally.

Such experiments seem to indicate that the bacterial actions of the intestine are not necessary for the good of the host. Yet it seems undoubted that ruminants derive a considerable amount of energy-yielding food-products from bacterial action on cellulose which otherwise would pass through their intestines unaltered, and it has been suggested that the intestines of such animals have actually become adapted by elongation to favour such bacterial action.

Man probably derives a small amount of energy-producing material through the action of his bacteria, but it seems almost certain that they are liable to do him more harm than good.

The number of bacteria in the human intestine is too vast to be imagined. Living and dead bacteria account for between 2·5 and 13·5 per cent. of the dried weight of fæces. One mg. of fæces has been found to contain four thousand million bacteria. They are present in the gut from close below the pylorus up to the rectum in constantly increasing numbers, so that they can produce their action throughout the intestine. According to Alvarez normal adults excrete daily 33 million million bacteria, and they account for 46 per cent. of the total fæcal nitrogen. Osborne and Mendel report that 70 per cent. of the nitrogen of the rat's fæces is present in bacteria.

As the fæces harden in the large intestine a large proportion of the bacteria die.

Many kinds of bacteria are present, and they vary very greatly under different conditions. Those of the *B. coli communis* type are commonest; this includes many toxic strains, while to the same group belong the pathogenic paratyphoid and similar strains. The chemical actions produced depend to a considerable extent on the strains of bacteria that are responsible for these actions, and perhaps to an even greater extent on the composition of the material on which the bacteria act. Many types of bacteria are present which can act both in presence and in absence of oxygen.

Although the fluid leaving the stomach is usually sterile, it is easy to understand how the intestine is continually being infected with fresh bacteria, since the mouth always contains large numbers, even with the best system of mouth hygiene, and fluids taken on an empty stomach will wash these through the stomach to a most favourable breeding-ground.

Bacteria attack carbohydrates and protein decomposition products; intestinal bacteria appear to have no action, or negligibly small action, on fats. *The substances produced from carbohydrates are non-toxic.* They include lower fatty acids of the type of butyric acid, lactic acid, alcohol, and gases such as carbon dioxide, hydrogen and methane. Over-production of such gases may, of course, lead to painful distension of the bowel.

Many of the same bacteria that produce these changes will, in the absence of sufficient carbohydrate, act on amino-acids, producing compounds with varying degrees of toxicity. They split off hydrogen sulphide. They form mercaptan (ethyl hydrogen sulphide) from cysteine, probably through thioethylamine:

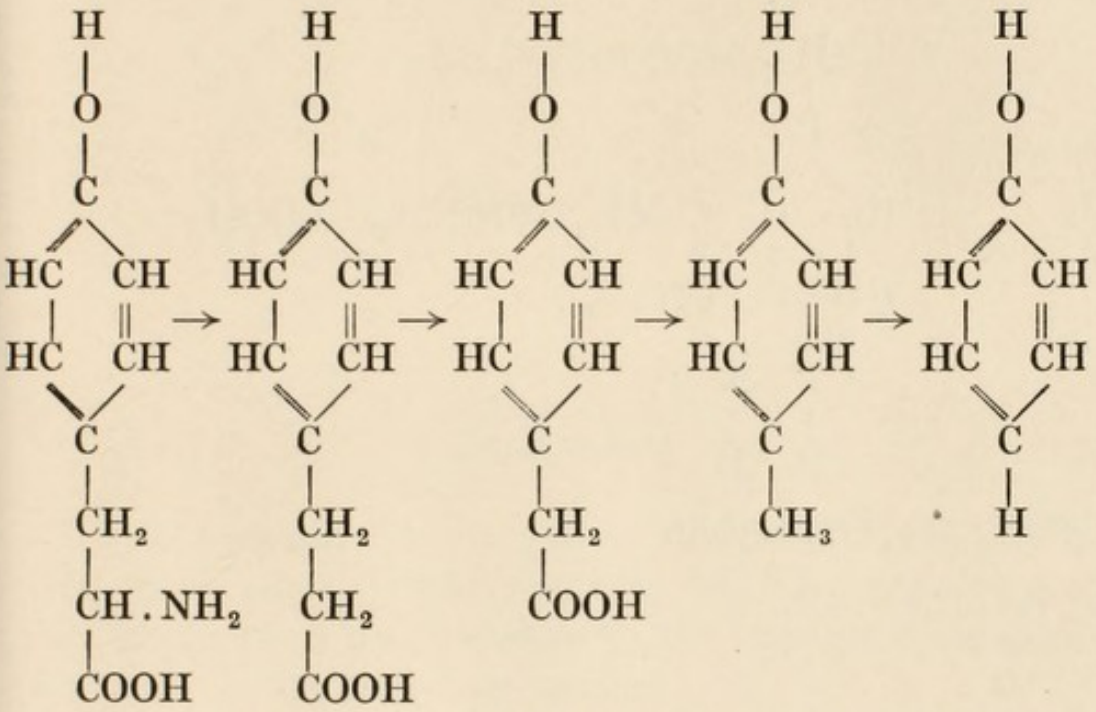
$$\underset{\textit{Cysteine}}{SH.CH_2.CH(NH_2).COOH} \rightarrow CO_2 + \underset{\textit{Thioethylamine}}{SH.CH_2.CH_2.NH_2}$$

$$\rightarrow \underset{\textit{Ethyl mercaptan}}{SH.CH_2.CH_3} + NH_3$$

Their actions on amino-acids are of two distinct types. They either at first deaminise the acid, producing ammonia and a derived fatty acid, and then subsequently split off carbon dioxide from this, leaving derived phenols, or else they split off carbon dioxide at once, producing a more toxic amine.

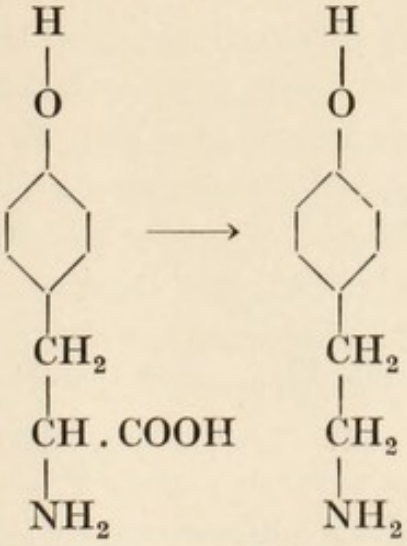
These actions can be illustrated with tyrosine. By the first method parahydroxy-phenyl-propionic acid, parahydroxy-phenyl-acetic acid, para-cresol, and phenol are successively produced, and by the second method tyramine (parahydroxy-phenyl-ethylamine) is formed.

(1)



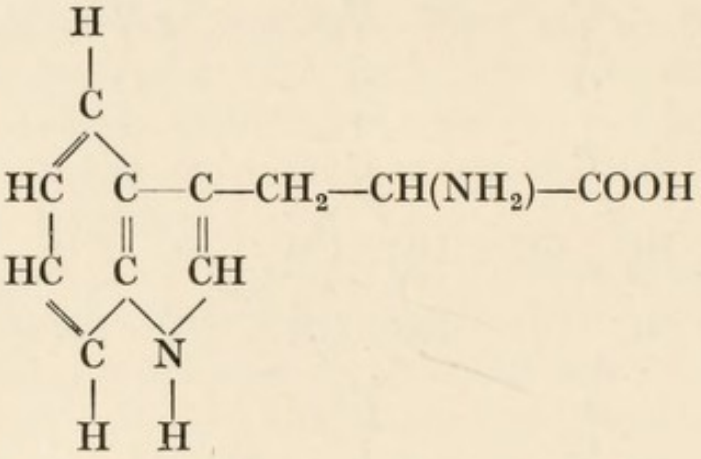
Tyrosine — *p-Hydroxy-phenyl-propionic acid* — *p-Hydroxy-phenyl-acetic acid* — *p-Cresol* — *Phenol*

(2)

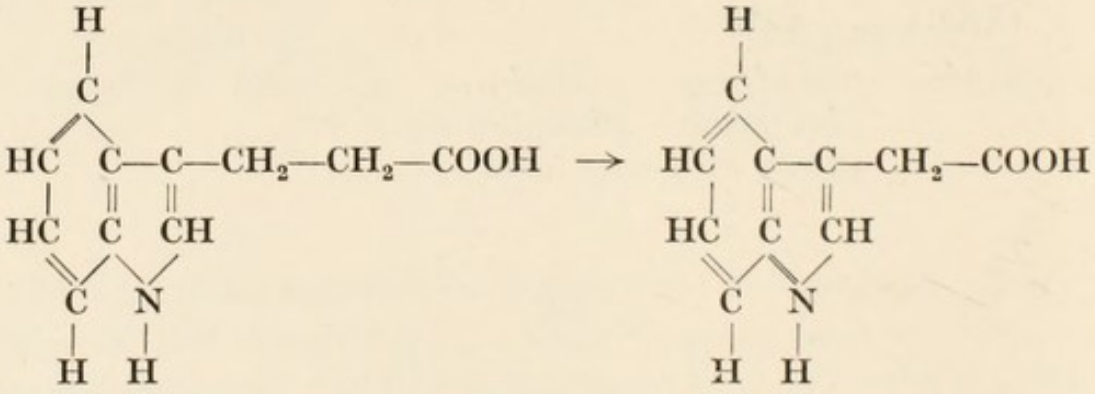


Tyrosine — *Tyramine*

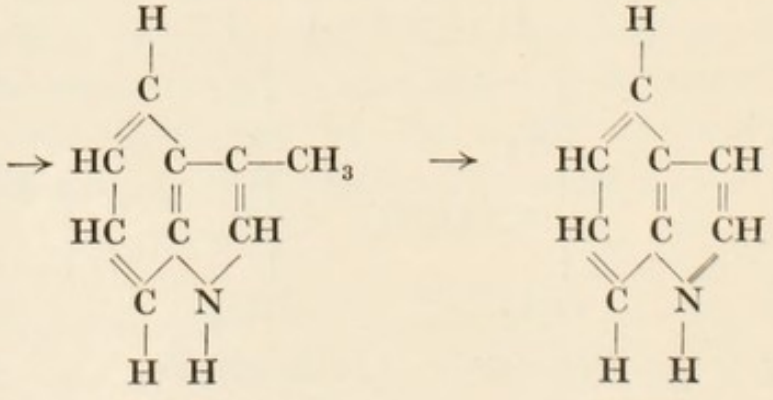
By the first method tryptophane gives rise to a very interesting series of compounds—



Tryptophane

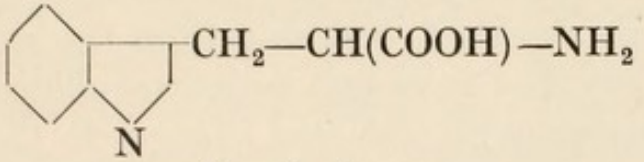


Indole-propionic acid *Indole-acetic acid*

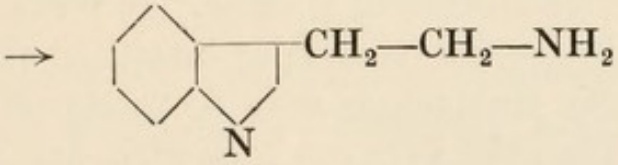


Skatole *Indole*

By the second method tryptophane gives rise to tryptamine (indole-ethylamine)—

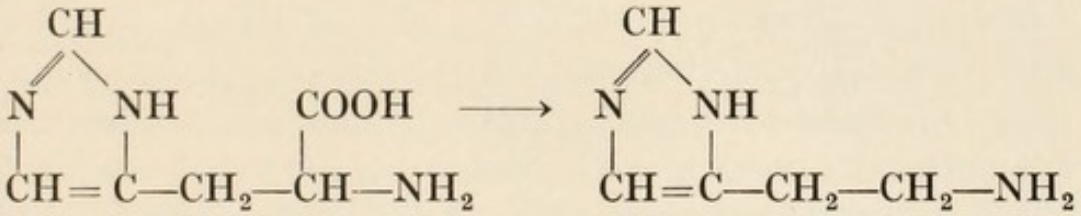


Tryptophane



Tryptamine

By this second type of action alanine, $CH_3.CH(COOH).NH_2$, gives rise to *ethylamine*, $CH_3.CH_2.NH_2$, arginine gives rise to *agmatine*, and histidine to the important compound *histamine* (β-iminazolylethylamine).



Histidine *Histamine*

Bacterial actions that can occur in the intestine are not limited to the final hydrolysed products of proteins. It has been shown that *B. coli* can decompose caseinogen, and various streptococci can act on proteoses and peptones.

The amines, acids and phenols formed by bacterial action are all absorbable through the intestinal wall, and some of them, or their derivatives, are usually present in urine, and, indeed, give a clue by their amount to the extent of bacterial action that is actually proceeding.

The ease with which these products can be absorbed suggests that they can be responsible for certain symptoms of toxicity in the organism. Thus it is known that inhalation of hydrogen sulphide and mercaptans in small traces leads,

if it be continuous, to headache and nausea. Cannot the continuous absorption of small amounts of these compounds through the intestinal wall produce similar effects ? The compounds so absorbed, resulting from the usual bacterial decompositions that have just been exemplified, are largely rendered innocuous by chemical changes to them produced in the liver, before they can reach the general circulation, *provided their concentration is small.* It seems possible that, at any rate in conditions such as intestinal stasis (constipation), the toxic compounds may be produced in concentration greater than the liver can cope with, and may then produce their pharmacological effects. What are these effects ?

Many of the amines, from ethylamine (producing very slight effect) up to tyramine, when injected into the blood stream produce a marked rise of blood pressure, acting as constrictors of the smooth muscle of the arterioles. This effect is produced to a still greater extent by the compound *adrenine*, a normal product of the adrenal glands, an imine, and a derivative of tyramine. All these compounds produce series of actions simulating those which result when the sympathetic nerves are stimulated (whence their action is called *sympathomimetic*). Since the most powerful of them is a normal product of the organism, it can scarcely be considered probable that the others, less active, will produce such actions in greater than physiological degree.

Tryptamine also produces effects of this type.

On the other hand, when histamine is injected into a vein the blood pressure falls, while there is an accompanying rise of body temperature and bronchial spasm. It stimulates smooth muscle, a dilution of one part in one million causing contraction of the uterus of the guinea-pig.

Para-cresol and phenol are not very toxic, and the liver has no difficulty in excreting them as conjugated sulphates (they form, in this way, part of the *ethereal sulphates* of the urine).

When indole is injected in large amounts into rabbits

death results. In man large amounts taken by mouth cause headache and restlessness. A small amount is continually being produced in the human intestine, and part of this is continually being absorbed. The odour of healthy fæces is due in large part to indole and skatole. The liver oxidises indole, and the product, indican, is excreted as a combined sulphate through the kidney. Skatole is less toxic than indole.

Evidently there are considerable possibilities of toxic actions through absorption of unusually large amounts of these compounds, and consequently, since such large amounts may well be formed during any marked degree of intestinal stasis, it is not uncommon to attribute the various symptoms accompanying constipation to the effects of these toxic products of bacterial action.

Nevertheless Alvarez claims that such symptoms can be traced, at any rate in large part, to pressure in the large intestine, and he states that the classical symptoms of the so-called " intestinal auto-intoxication " can all be induced by packing the rectum with absorbent cotton, such packings, and the pressure they cause, setting up numerous reflexes, including reversed peristalsis, and that these are responsible for the symptoms experienced in the condition.

We do not yet know the whole story. It seems very probable that bacteria acting on polypeptides of varying complexity can produce compounds of even greater toxicity than those described above. In fact we have many classical examples in the different toxins produced by bacteria of the type of *B. diphtheriæ*.

One of the most important recent discoveries in bacterial chemistry is that of A. I. Kendall, who has shown that the chemical action of any bacterium depends in great measure on the medium in which it exists, and that, provided this medium contains a sufficient proportion of carbohydrate, the products of the action are non-toxic. Thus *B. diphtheriæ*, in absence of carbohydrate, and having therefore to

act on protein derivatives to derive material for its energy requirements, produces as a by-product the dread diphtheria toxin. If, however, the medium contains much lactose, the same bacterium merely produces lactic acid, the harmless acid of sour milk.

Such an observation has an important practical application. Feeding of lactose in large amount, since it is not too rapidly broken down in the intestine (by lactase), and cannot be absorbed through the intestinal wall unaltered, produces a medium towards the lower end of the gut essentially carbohydrate in character, so that, as a result, non-toxic bacterial products predominate.

Since some strains of bacteria tend especially to react with carbohydrates rather than with protein products, a second type of treatment to lessen production of toxic compounds in the intestine is the feeding of these (*e.g.*, *B. acidophilus*) along with lactose.

The sole products of bacterial action that are of value to the organism are the fatty acids formed from carbohydrates, such as cellulose, which otherwise could not be utilised, and would pass through the intestine unaltered. In man 40 per cent. of the cellulose of young celery may be utilised through such action, since after absorption of the resultant fatty acids they can be oxidised, and so produce energy. This is but a slight advantage to offset the great possibilities of toxic action, and even with this the lower (volatile) fatty acids may act as intestinal irritants, and, in children, may cause diarrhœa.

Bacterial action in the intestine is, as far as man is concerned, an almost unmixed curse.

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CHAPTER XIII

COMPARATIVE DIGESTION

Digestion in Plants

THE great majority of green plants, growing normally, absorb their nutriment as gaseous carbon dioxide, and solutions of inorganic salts. The subsequent processes by which they produce from these simple compounds sugars and amino-acids, and then their starch, cellulose, and specific proteins, are synthetic processes of assimilation.

In considering the power of plants to *digest* nutrient material we are limited to a discussion of the processes of the germinating seed, and of bacteria, moulds, and the higher fungi, which lack the complete synthetic powers of green plants.

Seeds contain reserves of protein, and in inverse proportions of fat and carbohydrate. The germinating embryo utilises all these and develops the necessary protease, amylase, and lipase to reduce them to assimilable forms. These initial processes may therefore be regarded as a digestion.

The changes brought about by yeasts and moulds on their nutrient media must be considered as largely of the nature of intermediate metabolism, since in most of them there is not an initial decomposition designed to convert insoluble to soluble and absorbable compounds. The simple sugars afford them ideal sources of carbon, and the amino-acids are especially easily utilisable as sources of nitrogen. Most yeasts require their nitrogen in organic form or as ammonium salts; the moulds can utilise nitrates as well.

Bacteria may be divided into three classes, one of which, like the green plants, can obtain requisite nutriment from carbon dioxide and inorganic salts, the second requires organic carbon, but can utilise inorganic nitrogen, while the third, including the pathogenic bacteria, requires organic material for both carbon and nitrogen.

Many of these lower plant forms can manufacture special enzymes, such as sucrase, maltase, trehalase, raffinase, lactase, enabling them to utilise such compounds as sucrose, maltose, trehalose, raffinose, etc.

The bacteria especially are capable of decomposing the most resistent organic material, including cellulose itself. The specific enzymes they secrete, capable of attacking cellulose, are termed cytases, or cellulases. There are two different types of cytase action, presumably produced by different cytases, which are, perhaps, formed by different bacteria. In the one formation of hydrogen is the dominant feature, in the other methane ; the latter is the faster action. Some idea of the rapidity and extent of these actions, and of the nature of the products, is given by two experiments of Omeliansky.

In the first he showed that in thirteen months 3·47 grams of cellulose were almost completely decomposed, there remaining only 3·7 per cent. of unaltered material, while 64·5 per cent. of fatty acids, 28·6 per cent. of carbon dioxide, and 0·4 per cent. of hydrogen by weight were produced.

In the second, in 4·5 months, of 2·08 grams of cellulose only 3·6 per cent. remained unaltered, and 49·1 per cent. of fatty acids, 41·7 per cent. of carbon dioxide, and 6·6 per cent. of methane by weight were produced.

The fatty acids so produced included acetic, butyric and valerianic, and traces of formic acid. In the methane type of action acetic acid predominates.

These cytases are also present in higher forms. Certain wood-boring fungi secrete them, while the embryos of certain starch-rich seeds contain an enzyme which can act on the cellulose of the cell-walls of the seeds.

Bacterium chitinvorus, a marine organism, secretes enzymes which can decompose chitin and keratin, and so render them utilisable for food, but this bacterium has no power to digest starch or cellulose.

Digestive lipases are not widely distributed. They are found in the developing embryos of fatty seeds, in certain soil and pathogenic bacteria, and in some moulds ; the latter have been shown capable of being grown on butter, utilising the glycerol and part of the fatty acids of the hydrolysed fat.

Bacteria can extrude proteases which are capable of acting on coagulated egg-white and similar proteins. They belong to the trypsin type of enzyme, since amino-acids are produced by their action. Yeasts and the higher fungi also secrete such proteases, whilst certain moulds, bacteria, and some of the higher plants, have been shown to contain nucleases capable of attacking nucleo-proteins.

The most interesting, if unusual, type of protease digestion of

plants is exhibited by the so-called *insectivorous plants*. These are green plants, capable of assimilating carbon dioxide, but are furnished with specific mechanisms for catching and digesting insects. Typical of them are the sundew, *Drosera rotundiflora*, and the pitcher plant, *Nepenthes*, both found growing in bogs. The leaves of *Drosera* are covered with pin-shaped tentacles of a glandular nature, which secrete a sticky fluid. When an insect alights upon a leaf the tentacles fold over it and secrete a copious flow of an acid liquid containing a protease of presumably pepsin-like nature which digests the insect. The nature of the acid has not been ascertained, though it is an organic acid, and not improbably formic acid. The secretion can also digest fibrin and egg-white fairly rapidly.

In *Nepenthes* a part of the petiole is modified into a pitcher-shaped structure, to which the leaf-blade acts as a cover. The inside of this cup has a definite secretion zone, and in absence of external stimuli the cup is partly filled with a liquid of neutral reaction. Cells at the entrance to the cup secrete a sticky, sweet fluid, with a slight aroma that attracts insects. These, caught by this secretion, finally fall into the liquid below, and this stimulus, or the stimulus of some soluble extractive from the insects, leads to further secretion of acid and a protease which digests them.

The fluid secretions of both these plants have a definite antiseptic action.

Digestion in Single-celled Animals, the Protozoa

The great majority of protozoa—amœbæ, foraminifera, helizoa, and most radiolarians—require their diet to be in the solid form of specific organisms,—diatoms, protococci, algæ, or other protozoa. The chemotaxis, which attracts certain of them to their food, is outside the scope of this chapter. Whether they engulf their food by flowing round it, as does the amœba, or whether the engulfing process is aided by means of cilia and a rudimentary mouth, the method of digestion is essentially the same. The engulfed material is surrounded by fluid in a temporary *vacuole*, a temporary stomach created by the protozoan for digestive purposes, and into this are secreted protease, and, when necessary, amylase and lipase. Digestion ended, and the products absorbed, the amœba flows away from the residue, while more highly developed species eject it. The liquid in the vacuole reacts at first acid, and subsequently slightly alkaline, and it is still a matter of dispute whether the protease is of pepsin or of trypsin nature, or whether, as seems

theoretically possible, enzymes of both types are consecutively produced. When fat and carbohydrate are fed, the acid reaction predominates.

Certain insectivorous plants, as has just been mentioned, assume animal methods in digesting animal food. On the other hand, a very few protozoa, the green Vorticellæ, according to Engelmann, possess diffused through their protoplasm a green-coloured material with chlorophyll functions, since in the presence of light it can liberate oxygen, and may, therefore, be assumed to utilise carbon dioxide in the construction of carbohydrate.

Digestion in Many-celled Organisms—the Metazoa

A. Digestion in the Invertebrates

With the gradual morphological development of an alimentary canal there is a gradual change from intracellular to extracellular digestion. In the lowly developed *sponges* and *cœlenterates*, while the food is enticed or hastened within a partially enclosed body cavity, in the one case impingement upon the interior surface of this cavity is at once followed by enclosure of the prey within the sponge cells, whilst in the other, although the cells of the entoderm (lining layer of the cavity) show an amœboid character in the *hydroid*, yet intracellular action is limited by the size of organism undergoing digestion. Nevertheless digestion of relatively large organisms by the hydroid is not truly extracellular, but rather contact-digestion, since it takes place where the surface of the food-organism is in contact with entodermal cells.

Concerning the nature of such types of digestion we have few actual facts to guide us. We must assume that, as usual, proteases, amylases, and other enzymes are present. Bodansky and Rose have ascertained that *extracts* of medusæ contain numerous enzymes, though this in no way indicates that these are used in digestive, rather than in subsequent metabolic processes. These authors have also found that the normal food-proteins of medusæ (fish-proteins) are more easily hydrolysed by them than are strange food-proteins, such as egg-albumin, beef-fibrin, and caseinogen.

The *worms* and *echinoderms* exemplify the change from one type of digestion to the other. Worms, for the most part carnivorous, show a gradually developing alimentary tract, and a gradually developing system of secretion of digestive juices into this tract, although many species of worms undoubtedly carry on an intracellular digestion. Certain parasitic (intestinal) worms

exhibit a retrograde step, subsisting largely or entirely on predigested food, and thus presenting no digestive process.

In the *Nemerteans* the alimentary canal begins to show functional subdivisions, the pharyngeal enlargement or pouch being chiefly responsible for digestion, the intestinal enlargement for absorption.

Extracellular digestion certainly occurs in the *star-fishes* and *sea-urchins ;* its occurrence is not so certain in the *holothurians*. The digestion, wherever it occurs, is brought about chiefly by proteases and amylases. Protease actions in both worms and echinoderms usually take place in an alkaline medium, and appear to be of tryptic character, although the alimentary tract, or at any rate the upper part of it, is stated by various observers to yield an acid secretion in certain oligochæte worms.

Various species show specific developments. An example is seen in the *leeches*, whose mouth secretion not only contains a protease, but also an anti-coagulant, which permits the easier digestion of their food—blood—and apparently keeps it sterile for long periods.

An invertase is apparently first met with in the secretion of the " pyloric cæca " of the radial arms of the starfish.

The further structural development shown by crustaceans, arachnids, insects, and molluscs is not accompanied by any marked general forward step in the chemical processes, though for specific purposes there may be marked species and genus development.

The " liver pouch " gradually becomes a separate gland, taking on definite secretory functions, initially apparently more resembling those of the mammalian pancreas, and slowly losing absorptive functions, and still more slowly—if at all—gaining the excretory functions of the mammalian liver. Even in the starfishes, however, the " liver " property of forming and storing glycogen is present.

Many crustaceans have some development of the upper part of the alimentary canal furnished with chitinous apparatus to facilitate a mastication of their food.

Spiders exhibit a regression of digestive method, since the digestion, or at any rate the initial digestion, of their prey is brought about outside themselves by a digestive fluid they secrete on to their caught food-material. They await its action and suck in the fluid product.

Insects show great variation in their food requirements. Many are typical omnivora, others are food *specialists* of the most

limited kind. Some are pure vegetarians, others flesh-eaters. The specialists are found in both these groups. Examples of this specialisation are presented by the wood-boring beetle, which appears to have a specific development for digesting cellulose—though it is possible, if perhaps unlikely, that this action on cellulose may be accounted for by bacteria present in the alimentary canal of the beetle. Another example is the larva of the clothes-moth, which appears to have developed a special power to digest the very undigestible keratin of the fatted hair of wool or fur on which it feeds.

The marked dietary specialisation of such insect-larvæ is stressed by the fact that, when they are fed upon a strange diet, their development may be arrested and metamorphosis may not take place.

Ants possess the power of community feeding in virtue of an enlargement at the upper end of the canal, which acts as a food reservoir, normally shut off from the rest of the digestive tract, and from which food can be rapidly transferred from animal to animal ; by this means the slow acquiral of food by a few animals is succeeded by its rapid distribution to a number. The ant only truly feeds when it passes food from this reservoir downwards to its true digestive apparatus.

The bee illustrates the same habit of collection, storage and disgorgement, though during storage definite changes occur in the material stored, since the nectar of its diet contains sucrose, while the honey it disgorges contains none, so that a definite invertase action has proceeded. The bee is able to utilise sucrose, maltose, trehalose and melizitose, but cannot transform to its requirements mannose, galactose, lactose, raffinose, dextrin, starch and the pentoses.

The honey-bee further illustrates the power of a diet to modify the individual. Differentiation between the queen and the worker-bee is produced solely through the different diets of their larvæ ; the former contains more protein and fat. Thus—

	Queen-larvæ.	Worker-larvæ.
N-compounds .	45·1 per cent.	40·6 per cent.
Fat . . .	13·6 „	6·0 „
Carbohydrate .	20·4 „	31·5 „

Snails illustrate a definite specificity of enzyme-development to meet the needs of diet, since their invertase can digest not only sucrose, but also tri- and tetra-saccharide derivatives of fructose,

such as raffinose and stachyose, a power that has been lost in the vertebrates. The " hepato-pancreas " of most invertebrates contains a lichenase, which can hydrolyse lichenin to glucose, and it would seem almost certain, therefore, that this enzyme is present in the secreted juice of the gland.

The digestive enzymes generally present include protease, amylase, lipase, etc. The nature of the protease is no more definitely known than in still lower forms, but protein digestion to the amino-acid stage has been shown for divers species of the four families under discussion. The reaction of the medium in which this protease action takes place also requires much more accurate work, but it would certainly appear that a degree of acidity corresponding to that of free hydrochloric acid is never attained in these invertebrates during digestion, and that the type of protease they secrete must act in a neutral or very weakly acid medium.

Extracts of the digestive organs of many of these invertebrates —jelly-fish, sea-anemone, honey-bee, scorpion—possess a rennin-like action, the purpose of which is difficult to comprehend, though it must be remembered that such an action is possessed to some extent by proteases generally that can act in neutral or weakly acid solution.

Amongst the lamellibranchs (mussels) is found another type of wood-borer, *teredo*, in whose digestive fluid a cytase would appear to be definitely present. Extract of its hepato-pancreas contains this enzyme, so that, even if bacterial action may assist this animal, such assistance is not requisite for the destructive processes it so rapidly brings about, and from which it appears to derive at least a part of its nutriment. Similar facts have been established for the related *Bankia* bivalve. (With these may be contrasted still another method of cellulose digestion. Cleveland has shown that most species of termites live on wood through a symbiotic union with their intestinal protozoa, *Trichonympha* and *Pyrsonympha*. These, presumably through a cytase, can digest cellulose, and their hosts live on the products. When their parasites are removed by various treatments the hosts only live from ten to twenty days. They cannot utilise the food they ingest. When " re-infected " with the protozoa, they live indefinitely.)

Mussels generally contain a peculiar *crystalline style*, either in a blind, pouch-like extension of the stomach, the free-end extending into the stomach to a greater or lesser degree, or practically entirely within the stomachic enlargement itself. This consists

of about 67 per cent. water, 32 per cent. of organic material, and 1 per cent. of inorganic material. It contains marked quantities of amylase, and an invertase. Its function has not yet been finally determined, but recent experiments suggest that it may be in part connected with oxygen supply, rather than ordinary digestion, since in the absence of air the style rapidly disappears from the animal; it is rapidly regenerated when normal aerated conditions are re-established, and contains a powerful oxidase, which presumably assists in maintaining life during conditions of oxygen deficiency, conditions under which, as Collip has shown, such animals can carry on for some length of time an anaerobic existence.

B. Digestion in Vertebrates

Fishes. In fishes the mechanical accessories of digestion are improved. Teeth and jaws appear. The liver becomes comparable to that of mammals, but is relatively larger, and in the selachians has an enormous fat content. Most fishes possess a relatively large stomach—it is unusually large in the selachians. The pancreas shows its whole range of development, from glandular tissue scattered through the mass of the liver, with common ducts, to a separate concrete organ. The gall-bladder appears in the selachians and a true bile is secreted.

In a few species of fish the stomach is absent. For example, Babkin and Bowie have recently reported that the mudfish *Fundulus*, with a very short alimentary canal, and with the œsophagus and duodenum directly joined, carries out every phase of digestion in an alkaline medium, pepsin and hydrochloric acid being absent.

The diets of different species exemplify all possibilities. While most species are carnivorous, herbivorous and omnivorous species are common.

The gastric juice of selachians, and of the bony fishes, shows a markedly acid reaction, though, according to several observers, after long gastric digestion the reaction may become neutral or, possibly, even alkaline. It is usually stated, though not yet conclusively proved, that the acidity is due to free hydrochloric acid. Certain investigators consider that the acidity may be due to the presence of an organic acid. The gastric protease definitely breaks down proteins to peptones, and appears to be of true pepsin nature. According to Bodansky and Rose, the gastric protease of the dogfish *Squalus* has an optimum activity at *p*H 3.

The main difference between the gastric protease of fishes and mammalian pepsin seems to lie in the lesser resistance of the former to the action of heat, a temperature of 40° C. decomposing it fairly rapidly. It digests effectively a larger variety of proteins than do the digestive proteases of the invertebrates, but shows less power of digestion than mammalian pepsin.

The published results of investigations are by no means fully in accord, however, concerning many points of this gastric digestion. Thus Svolima (1919) has reported that the gastric acidity of the shark, determined on liquid withdrawn by a fistula, may, during prolonged digestion, reach after forty-eight hours a value corresponding to 1·6 per cent. of hydrochloric acid, which certainly indicates presence of much free mineral acid and a much more marked acidity than the results of Bodansky and Rose would infer for the related selachian. Svolima states that digestion is relatively slow, may last for five days, that the gastric secretion contains no lipase, and that no absorption takes place in the stomach of the shark. Other observers have considered that some absorption may occur, especially of fats.

There is some evidence that an amylase is secreted by the mucosa of the œsophagus of the fish, though if this be so the enzyme is certainly destroyed by the marked acidity of the stomach contents. In many fishes the gastric secretion exhibits rennin-like activity ; in others none is exhibited, and the distribution of this property is quite irregular.

Intestinal secretion, whether due solely to enzymes secreted by the pancreas, aided as usual by the bile, or in part to a (possible) intestinal secretion, would appear to be of the usual mammalian type. In certain species blind pouches—pyloric appendages—contain the enzymes usually present in the pancreatic secretion of the higher vertebrates, suggesting a localisation of intestinal digestion.

While the markedly forward step in digestion processes is obvious, yet very many important details still have to be ascertained.

Other Vertebrates. In general, the digestive processes of the higher vertebrates are markedly similar ; this is especially true for the different mammals, as has been assumed in the general comparisons in Chapter XI. Hence only the points of differentiation need stressing.

Salivary digestion is merely mechanical for the lower vertebrates, being essentially a moistening with a sticky secretion to aid the act of swallowing by forming a bolus. In some mammals

a chemical adjunct appears—the salivary amylase. However, the saliva of carnivorous animals, such as the dog and cat, contains no amylase, and is not a digestive secretion. And while Palmer (1917) states that the mixed saliva of the horse readily digests both raw and cooked starch, an inactive pro-enzyme being activated by a special enzyme from the mouth glands, Scheunert and Trautman (1921) deny that it is present in the parotid secretion of the horse and sheep, while Swarz (1924) states that no pro-enzyme is present in the saliva of the horse and steer. The latter author states that the amylase activity of the hog is only about one one-hundredth of that of man. The *p*H value of the saliva of the hog, dog, horse and cow, according to the same author, ranges, for average values, from 7·3 to 8·1, the saliva being invariably alkaline.

As a result, presumably, of the different materials and consistency of their diets, the vertebrates exhibit marked variations in the anatomical build of the *stomach*. In most birds a part of this organ, furnished with extremely strong musculature (the crop), is separated off for the particular purpose of macerating their food. The stomach of herbivorous animals is usually relatively larger than that of carnivorous. In the ruminants it is divided into several portions, the fore-part, unfurnished with glands, being a collecting reservoir, receiving the slightly chewed and moistened food, which remains in a neutral or alkaline medium, and whose cellulose is bacterially decomposed. Regurgitation permits more complete mastication and moistening with saliva before the food, again swallowed, is passed on to the true digestive portion of the stomach.

Gastric digestion in these higher vertebrates is invariably carried out by a pepsin in presence of free hydrochloric acid.

Certain predatory birds, such as the goshawk, can digest the keratin of the beaks, claws, and feathers of their prey.

Amphibia, especially frogs, are furnished with glands discharging a pepsin-containing juice into the œsophagus ; presumably this supplements the normal gastric secretion. Comparative studies of the peptic digestion of cold and of warm-blooded animals show that the differences are essentially due to temperature and not to any variation in the nature of the gastric juice. Thus Flaum (quoted by Biedermann) introduced bits of protein into the stomachs of frogs, and kept some of the animals at room temperature, others on ice. Next day the stomachs of those kept at room temperature showed no trace of undigested protein, while the ice-frogs after fourteen days showed no trace of action on the protein. Such cooled frogs, if then brought back to

room temperature, exhibited very rapid gastric digestion of the protein.

Some remarks on different rennin-actions in different species were made in Chapter XI.

The variations in the structure of the *intestines* appear to be chiefly governed by the exigencies of different diets, and consist chiefly of changes in relative length, especially of the large intestine, and in the size of the cæcum (which is large and double in most birds). The large cæcum seems especially designed to facilitate bacterial action on cellulose ; this is well exemplified in the horse. In those species possessing an unusually long large intestine it is probable that (in addition to absorption) actual digestion occurs in this portion of the gut to a much greater extent than in man.

There appear to be no material points of difference between the secretions of the pancreas, liver and intestine in the various genera of animals under consideration. It is to be noted that many species of mammals—mouse, horse, etc.—do not possess a gall-bladder, though this merely affects the rate of discharge of bile during the initial stages of digestion.

It is to be emphasised that all these variations in the processes of digestion, of which the extremes are exemplified in the amœba and in man, are governed by the same underlying principle, the conversion of insoluble food material into a form in which it can be absorbed and utilised by the organism.

References

The material presented in this chapter has been taken from the weighty article by Biedermann in Winterstein's " Handbuch der vergleichenden Physiologie " (Band 2, erste Hälfte, pp. 1–1563), supplemented by recent papers in the literature.

CHAPTER XIV

CHEMICAL ACTIONS BROUGHT ABOUT BY THE LOWER FORMS OF PLANT LIFE

In Chapter XII. a brief account has been given of certain chemical processes which bacteria effect on amino-acids and sugars in the intestinal tract, in their efforts to obtain nutrient material and energy for their continued existence and development. Actions of this type are common to all the lower forms of plant life, which are saprophytic (nourished by dead organisms), or parasitic (nourished by living organisms), or either, according to their (varying) habitat. Such are the slime fungi (myxomycetes), the bacteria, and the higher fungi (including especially the moulds and yeasts). All of these, with the exception of a few bacteria, possess no chlorophyll, and must therefore obtain their carbon in organic form.

It is of interest to compare and contrast the actions of these lower forms of plant life.

Actions on Amino-acids

The same two types of action that have been discussed in Chapter XII. are exemplified. Either there is an initial decarboxylation, with formation of an amine, or an initial deaminisation, with formation of ammonia, and a fatty or aromatic acid, which may or may not be further changed.

In Table XII. compounds that theoretically may be, but are not yet, definitely shown to be formed are given in parentheses, those products definitely formed in experiments with individual amino-acids are given in italics, and those inferred to be formed from certain amino-acids, since they occur amongst the products of protein decomposition by such agents, are given in ordinary type.

The agents that have been used in bringing about such changes are indicated by the following letters: "B." stands for bacteria, "M." for moulds, "Y." for yeasts, and "F." for fungi other than moulds and yeasts; "Mi." indicates unspecified micro-organisms.

TABLE XII. DECOMPOSITION OF AMINO-ACIDS BY SAPROPHYTIC AND PARASITIC PLANTS

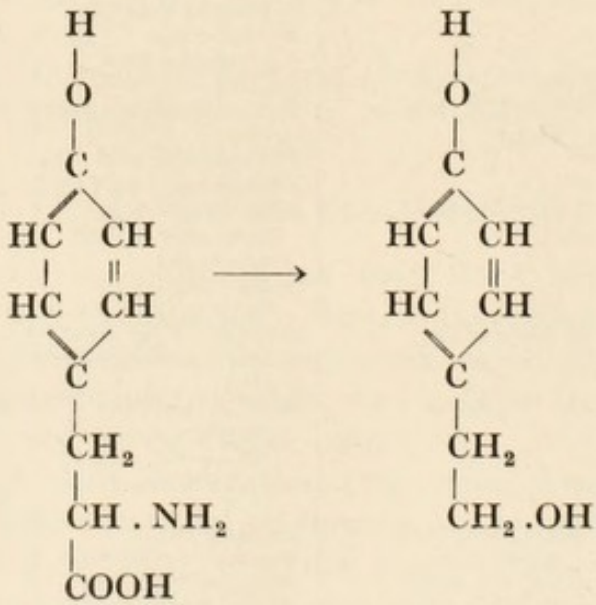
Amino-acid.	Decarboxylation Product.	Deaminisation Products.	
		Acids.	Other than acids.
Glycine . .	(Methylamine).	*Acetic acid* (B.Y.)	*Ammonia* (B.M.Y.)
Alanine . .	(Ethylamine)	*Propionic acid* (B.) *Acetic acid* (B.)	*Acetaldehyde* (Y.)
Serine . .	(Cholamine)	*Propionic acid* (B.) *Formic acid* (B.)	*Ethylene-glycol* (Y.)
Cysteine .	(Thioethylamine)	—	*Methyl-Mercaptan* (B.) *Ethyl sulphide* (B.) *Hydrogen sulphide* (B.) *Sulphate* (B.)
(α-Amino-butyric acid)	—	*Butyric acid* (B.)	—
Valine . .	*Isobutylamine* (Mi.)	*Isovalerianic acid* (Mi.) *Isobutyric acid* (Mi.) *Acetic acid* (Mi.) *Formic acid* (Mi.)	
Leucine . .	Isoamylamine (? Y.B.)	*Isovalerianic acid* (B.) *Butyric acid* (B.) *Isobutyric acid* (B.)	*Isoamylalcohol* (M.F.Y.)
Isoleucine . .	—	*Methyl-ethyl-propionic acid* (Mi.)	*Active Amylalcohol* (Y.)
Phenylalanine .	Phenylethylamine (Mi.)	Phenylpropionic acid (B.) *Phenylacetic acid* (B.) *Phenyl-lactic acid* (Y.)	*Phenylethylalcohol* (Y.)
Tyrosine .	Tyramine (Mi.)	*p-Hydroxyphenyl-propionic, Acetic acids* (B.) *p-Hydroxy-phenyl-lactic acid* (Y.)	*Tyrosol* (Y.) p-Cresol and Phenol (Mi.)
Aspartic acid .	*β-Alanine* (B.)	*Succinic, Propionic,* and *Formic acids* (B.)	—
Asparagine .	—	*Malic, Succinic, Butyric, Fumaric, Propionic, Acetic,* and *Formic acids* (B.)	*Ammonia* (B.)
Glutamic acid .	γ-Aminobutyric acid (Mi.) γ-Butyro-betaine (? Mi.)	(Glutaric acid) *Succinic acid* (Mi. Y.) *Butyric, Propionic, Acetic,* and *Formic acids* (Mi.)	
Lysine . .	*Cadaverine* (B.)	*ε-Aminocaproic acid* (Mi.)	
Arginine . .	Agmatine (B.) *Putrescine* (B.)	*δ-Aminovalerianic acid* (Mi.)	*Urea* (B.)
Histidine .	*Histamine* (B.)	*β-Iminazolylpropionic acid* (B.)	*β-Iminazolylethyl alcohol* (Y.)
Tryptophane .	*Tryptamine* (B.)	*Indolepropionic* and *Indole-acetic acids* (B.) *Indole-lactic acid* (Y.)	*Tryptophol* (Y.) *Skatole* and *Indole* (B.)
Proline . .	(Pyrrolidene)	(δ-Aminovalerianic acid) *n-Valerianic acid* (B.)	—

Whilst many of these agents, especially amongst the bacteria, may bring about the same reaction, a certain degree of specificity of action is by no means absent.

Such a particular type of action specific to one species is well exemplified by the production of derivatives of lactic acid by the fungus *Oidium lactis*, but, so far as has been ascertained, by no other species. It converts tyrosine into hydroxy-phenyl-lactic acid, phenylalanine into phenyl-lactic acid, and tryptophane into indole-lactic acid, the change in all of these being simply the replacement of an amino- by a hydroxy-group.

Again, while many bacteria can attack tyrosine, certain of them (*B. bifermentans*, *B. histolyticus*, *B. centrosporogenes*, *B. tyrosinogenes*) do not possess that power, since when they are allowed to act on protein containing tyrosine radicals tyrosine accumulates in the medium, indicating incapacity to decompose it.

The yeasts attack the amino-acids in a way characteristically different to that of bacteria, producing alcohols—the amyl alcohols, tyrosol, and tryptosol—by decarboxylation and replacement of the amino-group by a hydroxyl-group, as, for example :



Tyrosine *Tyrosol*

This type of action is discussed more fully in Chapter XXI. That decarboxylation is the initial step may be inferred from the fact that yeasts and moulds can transform isoamylamine into isoamyl alcohol, and tyramine into tyrosol. Phenyl-ethyl alcohol, produced in such a way from phenylalanine during alcoholic fermentation by yeast, is stated to be the essential constituent of the perfume of the rose.

Whilst almost all strains of bacteria can convert tryptophane into indole-acetic acid, the majority must rest their attack at this stage. Only a small proportion can further break down the molecule to skatole and indole

The moulds (*Aspergillus*, *Penicillium*) can deal more drastically with phenylalanine than can the bacteria, rupturing the benzene ring and producing simple products.

The decomposition of arginine frequently takes place through an initial cleavage into ornithine and urea, brought about by a specific enzyme arginase (see Chapter XXIII.). This is present in several bacteria, and in a number of fungi, which also contain the specific enzyme urease, capable of decomposing urea to ammonia, and so furnishing the plant with nitrogen in a generally utilisable form (see Chapter XVII.). According to Ivanov (1927) fungi can store urea even to the extent of 13 per cent. of their dry weight. Presence of carbohydrate in their medium materially decreases this amount. *A. niger*, grown on peptone medium, excretes urea as a waste product and manufactures no urease. When carbohydrate is added to the medium the mould produces urease and utilises the ammonia produced by the enzyme.

The two diamines, putrescine, or tetramethylene diamine, $NH_2.(CH_2)_4.NH_2$, derivable from arginine through ornithine, and cadaverine, or pentamethylenediamine, $NH_2.(CH_2)_5.NH_2$, derived from lysine, have been long known to occur in putrescent meat, fish, human corpses, and have also been isolated from cheeses.

It is to be observed that the simpler amino-acids, glycine and alanine, seem the most resistant to action of micro-organisms.

It has been pointed out in the preceding chapter that many bacteria have the power of digesting proteins themselves. Their actions on the less complex polypeptides do not appear to have been extensively studied.

While many putrefying bacteria can attack histidine, but few of these can decompose its derivative carnosine (see Chapter XVIII.). *B. pyocyaneus* attacks both readily, forming from carnosine ammonia, acetic acid, propionic acid, and other non-toxic compounds. It may be inferred that carnosine, invariably ingested as part of meat, is not decomposed to histidine, and cannot give rise to histamine, being either completely broken down or unaffected.

Actions on Carbohydrates

Just as it will be shown later for mammals that proteins, through their amino-acids, provide the nitrogen requirements of

the body, while excess supplies of amino-acids can be used for energy production, and, on the other hand, carbohydrates essentially provide energy through their oxidation, so, too, in the lower forms of plant life, the same truth holds.

We may imagine that the plant utilises directly, as far as it requires it, any amino-acid supplied to it. It is obvious that by deaminisation ammonia is set free, which can subsequently be transformed into any required nitrogen-containing compound. In addition, various acids are produced which through oxidation, or processes corresponding to oxidation, can provide energy.

The processes by which carbohydrates are decomposed must be regarded as mainly designed to provide energy and simple carbon compounds for the plant's own metabolism. As a provision of energy such a procedure is uneconomical, though frequently essential in absence of any, or of sufficient, enzyme-power capable of utilising gaseous oxygen.

The uneconomical nature of the procedure is exemplified by a comparison of the amounts of heat developed by direct oxidation of glucose, and its fermentation to alcohol and carbon dioxide, shown respectively in the two equations—

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \quad (1)$$
$$C_6H_{12}O_6 = 6CO_2 + 2C_2H_5OH \quad (2)$$

Since 1 gm. of glucose will furnish, when completely oxidised, 3·74 calories, while 1 gm. of ethyl alcohol will furnish 7·10 calories (see Chapter XXX., Table XXII.), it can be calculated from these two equations that 1 gram-molecule of glucose, completely oxidised, will furnish 673 calories of heat, while the amount of heat potentially producible from the alcohol it yields is 652 calories, so that the corresponding heat actually made available from 1 gram-molecule of glucose by the reaction shown in the second equation is only 21 calories. Since, in any actual fermentation of glucose, small amounts of other combustible products are formed, the disproportion is even greater.

The actions of the saprophytic and parasitic plants can largely be considered as a series of " fermentations " needed by them to provide energy, the energy being made available through interlocking these chemical changes with others essential to the plant metabolism.

One of the simplest anaerobic " fermentations " is carried out by *B. formicicum*, which converts calcium formate into carbon dioxide and hydrogen—

$$Ca(COOH)_2 + HOH = CaCO_3 + CO_2 + 2H_2$$

Acting on sugars, such as arabinose, glucose and lactose, or corresponding alcohols, the same agent produces carbon dioxide and hydrogen, along with varying amounts of lactic, acetic and formic acids, and ethyl alcohol.

Ethyl alcohol fermentation is produced characteristically by the yeasts, and also by various bacteria and moulds. Glycerol is a bye-product. Various sugars can be so transformed, especially glucose, fructose, maltose and sucrose (an invertase being available). Special yeasts (*e.g.*, Kefir yeast) can attack lactose. Such a fermentation actually involves a highly complex series of reactions, and yeast fermentation is discussed at some length in Chapter XXI.

"*Butyric acid fermentation*" is especially exemplified by the action of *B. amylobacter* on glucose, in complete absence of oxygen—

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + CH_3 . CH_2 . CH_2 . COOH$$

Lactic acid can also be transformed—

$$2CH_3 . CHOH . COOH = 2H_2 + 2CO_2 + CH_3 . CH_2 . CH_2 . COOH.$$

"*Lactic acid fermentation*" can be caused by various bacteria, and especially *B. lactici acidi* acting on lactose—

$$C_{12}H_{22}O_{11} + HOH = 4CH_3 . CHOH . COOH.$$

Acetic acid and other volatile acids usually are also produced, depending on the kind of bacteria acting, and the composition of their nutrient media. Certain other bacteria acting on sucrose, fructose and maltose, form lactic acid as the chief product. Usually inactive lactic acid is produced, but *Micrococcus acidi paralacti* produces *d*-lactic acid, presumably metabolising the lævo-form more easily, while *B. aceti lævolactici* produces *l*-lactic acid.

"*Acetic acid fermentation*" is more accurately a true oxidation taking place in presence of oxygen. Long used commercially in the production of vinegar, in the French process it is brought about by the combined actions of the three bacteria, *B. aceti*, *B. pasteurianum*, and *B. kuetzingranum*, and in the English process by *B. xylinum*. Alcohol is oxidised to acetic acid—

$$CH_3 . CH_2OH + O_2 = CH_3 . COOH + H_2O.$$

Another oxidation of interest is the quantitative conversion of glycerol into dihydroxyacetone by a bacterium of the *B. xylinum* type :

$$CH_2OH . CHOH . CH_2OH + O = CH_2OH . CO . CH_2OH + H_2O$$

While certain moulds can bring about formation of ethyl alcohol, the majority, such as the *Aspergillus* and *Penicillium* moulds, seem especially capable of producing from sugars citric and oxalic acids, the former being intermediate in the production of the latter. Certain strains of *A. niger* can decompose citric acid without producing oxalic acid (or else they transform the latter to simpler products as fast as it is formed). Other breakdown products occur, apparently unconnected with this type of transformation ; such include gluconic acid (from sucrose) and acetaldehyde (from glucose). (According to Hermann, *B. gluconicum*, which accompanies such moulds, converts glucose quantitatively into gluconic acid.)

These agents do not find appreciable amounts of the sugars to act upon in their natural habitats, and evidently can form them from the more complex carbohydrates. Moulds decompose cellulose energetically. *Cladosporium* has been shown to transform 25 per cent. of cellulose, in presence of air and mineral acids, to glucose in 256 days. The pentosans of corn silage and of rye straw are similarly rapidly transformed, and inulin is hydrolysed easily to fructose, which can then be utilised.

Bacteria are similarly capable of transforming cellulose to simple products, especially the simpler fatty acids, and (in some cases) lactic acid. Certain of them, *e.g.*, *B. mesentericus*, transform inulin to fructose, which is then decomposed as usual.

A species of *Penicillium* has been shown to transform glucose or sucrose or xylose with equal ease into a mixture of fats and sterols, the fatty acids produced including palmitic, stearic, oleic, and linolic.

Certain bacterial organisms produce in solutions of cane-sugar slimes or gums of a polysaccharide nature. *B. spongiosus* growing on an agar broth containing 20 per cent. cane-sugar produces an araban ; others have been shown to produce a galactan or a lævulan, while a gum identical with gum acacia has been produced by an organism isolated from the gum acacia tree when grown on potato juice containing some cane-sugar and tannic acid.

Actions on Lipides

It was stated in Chapter XII. that intestinal bacteria have no action, or a negligibly small action, on fats. It has been found that *B. typhi*, *B. coli*, *B. dysenteriæ* Shiga, *B. proteus*, and *Spirillium choleræ* attack neither fat nor lecithin. *B. prodigiosus* and *B. fluorescens liquefactans* split both. *Staphylococcus pyogenes*

attacks fats but not lecithins, and *B. piscium pyogenes*, *Spirillium dunbar*, and *S. El Tor* can attack lecithins but not fat.

Of the moulds *Aspergillus niger* definitely possesses lipase activity. It can utilise carbon of fats for its own needs when no other source of carbon is present. A fatty medium appears to favour the formation of lipase by this mould. The substances causing rancidity in cacao and palm oils are essentially methyl ketones (such as methyl-amyl ketone, methyl-heptyl ketone, and methyl-nonyl ketone) produced by the oxidative decomposition of fats by moulds. *Penicillium glaucum* and *Aspergillus* can produce such ketones from fats in presence of albumin, glycine, or gelatine, and from the ammonium salts of caprylic, caproic, butyric, capric, and lauric acids. Such methyl ketones are present in Roquefort cheese.

Special Types of Action

Certain micro-organisms, presumed to be bacteria, produce a turbidity in petroleum apparently due to the formation of asphalt. *Aspergillus*, grown on solid paraffin, utilises 75 per cent. of it without formation of fatty acids. It attacks waxes in a similar way.

B. pyocyaneus forms hydrocyanic acid in presence of oxygen, the optimum *p*H values being from 5·4 to 8·8. Apparently there is a possibility of an appreciable amount of this acid being formed by this agent in the animal organism.

Certain micro-organisms can attack such a resistant substance as pyrites, forming soluble sulphate.

Diatoms are believed to be capable of decomposing clay to obtain silica. Certain diatom cultures sown on colloidal clay in a nutritive medium devoid of silica develop rapidly, and aluminium hydroxide is liberated.

Special bacteria, acting in symbiosis with leguminous plants, can " fix " atmospheric nitrogen, while others, occurring in soils, transform ammonia into nitrites, and still others complete the transformation to nitrates.

The combined action of micro-organisms in attacking living and restoring dead organisms to the flux of nature is exemplified by the analysis of Schellenberg, who has shown that the biological decomposition of wood is dependent on the same three factors which condition the life of the plant generally, air, moisture and temperature. The filamentous fungi show three stages of attack

on wood, rust and rot fungi decomposing only sugar, starch and dextrin, the moulds attacking these, and also the pentosans, inulin, galactans, etc., and the Polyporeæ, Agaricaceæ and Ascomycetes destroying the membrane lining of the wood cell and attacking the true cellulose. Thus fungi first assimilate the sugars, then the dextrins and gums, and, finally, the celluloses. The organic substance of wood is first decomposed by fungi, a mixed flora of bacteria and fungi form the second line of attack, and, finally, a rich fauna of the lower animals complete it. Humin results.

The involved nature of many of the reactions is exemplified by the action of butyric acid bacteria of the type of *B. amylobacter*, which hydrolyses the proteins of " corn-starch " at the same time that it ferments the starch, in ninety-six hours transforming 50 to 60 per cent. of these proteins into peptides and amino-acids.

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CHAPTER XV

THE CHEMISTRY OF THE BLOOD

The Composition of Normal Blood

Blood, the chief circulating fluid of the body, amounts to about 8·8 per cent. of the body-weight. It is, at the same time, a carrier of nourishment to the tissue cells, and the sewer through which their refuse is carried to the excretory organs. We may expect, therefore, to find in it three groups of chemical compounds :—

(i.) Those compounds inherently connected with the functions of the blood, necessary, for example, in maintaining its requisite osmotic pressure, or required for carriage of oxygen and carbon dioxide.

(ii.) Nutrient material *en route* to the tissue cells from the alimentary canal, oxygen from the lungs, and the internal secretions, in carriage from the tissues that form them to those that use them.

(iii.) Oxidised waste products, on their way to the excretory organs from the tissue cells.

In the first group will also be included those substances necessary to clot blood ; the clotting properties inherent in blood are obviously necessary to prevent this fluid draining away from the body whenever the closed system containing it is opened by injury. Otherwise the merest scratch of the finger would result in death from hæmorrhage.

The blood contains formed elements, the *red* and *white corpuscles*, and the *platelets*, suspended in *plasma*. The white corpuscles are relatively few in number ; they contain

glycogen and specific enzymes, and have specific functions that can best be dealt with later.

Mammalian blood contains about 55 to 60 per cent. plasma and 40 to 45 per cent. corpuscles by volume. The cell volume tends to be higher in males than in females.

TABLE XIII. COMPOSITION OF NORMAL HUMAN BLOOD

	100 grams of red blood corpuscles contain	100 grams of plasma contain	100 grams of whole blood contain
Water	57–64 gm.	91–92 gm.	77–81 gm.
Solids	43–36	9–8	23–19
Functional constituents (including some nutrient material):			
Hæmoglobin	39–32 gm.	0 gm.	16–13
Plasma protein	0	6·7–8·2	—
"Serum albumin"	0	4·6–6·7	—
"Serum globulin"	0	1·2–2·3	—
Fibrinogen	0	0·3–0·6	—
Phosphatides	0·35–0·48 gm.	0·17–0·26 gm.	0·28–0·32 gm.
Cholesterol	0·13–0·17	0·15–0·18	0·14–0·17
Fats (as acids)	0·27–0·45	0·30–0·47	0·29–0·42
Inorganic constituents (ionised or in combination):			
Sodium	0	150–250 mg.	90–150 mg.
Potassium	410–440 mg.	16–22	160–200
Calcium	0 (?)	10–11	6–6·5
Magnesium	2–7	6–6	4·6
Chloride ion	130–165	350–380	270–300
Phosphorus as inorganic phosphate	3–5	1·5–4·5	—
Phosphorus as lipoid	40–75	5–12	—
Sulphate	—	—	2–4
Nutrient material:			
Amino-acid nitrogen	9·5 mg.	5·5 mg.	5–8 mg.
Glucose	100	103	70–120
Fats (see above)			
Salts (see above)			
Oxygen	—	—	13–23 vols. per cent.
Waste material:			
(Carbon dioxide)	—	—	45–65 vols. per cent.
Urea	28 mg.	28 mg.	25–32 mg.
Uric acid	1·9	3·9	1–3
Creatinine	2·5	1·2	0·5–2
Creatine	6	0·3	3–7
Ammonia	—	—	0·1
Acetone	—	—	2
Acetoacetic acid	—	—	1
β-hydroxybutyric acid	—	—	1
Undetermined nitrogen	19	2	—

It is not improbable that in severe winter climates there is a distinct diminution in the plasma volume, with a relative increase in that of the corpuscles. (There appears to be a definite increase in the number of red cells.)

The two systems, plasma and corpuscles, are in constant contact with each other, so that we may well contrast their composition as closely as possible. In Table XIII. this is done and for convenience the average figures for whole blood are included, though these obviously depend on the ratio of cells to plasma in the blood analysed. The data refer to venous blood.

The chloride content of blood is usually expressed in terms of sodium chloride, the average content of the cells being (so expressed) 0·3 per cent., of the plasma, 0·6 per cent., and of whole blood 0·48 per cent. The carbon dioxide extractable from blood is largely present in the blood as bicarbonate, the corresponding cations maintaining neutrality being sodium, potassium, and calcium. *Much of this bicarbonate is to be regarded as functioning and not merely as excretory material.*

Enzymes Present in Blood. Blood plasma contains an amylase which can act on glycogen, an invertase, proteases, ereptases, nucleases, lipase, cholesterolase, oxidase, and catalase. It is probable that these for the most part have diffused into the circulation from the glands that manufacture them. The white corpuscles have their own specific enzymes.

Other Constituents of Blood. The blood also contains anti-enzymes, or compounds which can inhibit enzyme action, and various complex substances, such as "antibodies" and "complement," of the chemical nature of which little is at present known (*cf.* Chapter XXXIII.).

The properties of certain of the blood constituents will now be dealt with in some detail.

Hæmoglobin

Hæmoglobin is the compound to which the blood owes its colour; it is present entirely in the corpuscles. There is some evidence that within the corpuscles it is united in still more complex union; and that " laking " the corpuscles sets it free. No recent work has been carried out to attempt to settle this point. The amount of hæmoglobin present in human blood is about 14 per cent., equivalent to 12·3 gm. per kilo. body-weight. The average man of 70 kilos. body-weight contains therefore about 860 gm. of hæmoglobin. It hydrolyses to *globin*, a histone and *hæm*, a compound containing iron.

Hæmoglobins from different mammalian species show a different elementary composition (though this may be in part due to difficulties of purification and therefore varying contamination with other compounds), and there is to be noted especially a different ratio between the iron and sulphur content. According to recent work of Vater and of Timár, in the cat this atomic ratio is 1 : 5, while in cows, horses, pigs, and dogs of pure race it is 1 : 3. These hæmoglobins also show definite differences in their crystalline structure, greater than could possibly arise from differences in composition of the media from which they crystallise, and the crystals from different species contain different amounts of water of crystallisation, so that there is fairly good evidence for believing that there are actually a large number of different compounds.

Assuming that each molecule of hæmoglobin contains one atom of iron, the molecular weight of the compound is of the order 16,000. An empirical formula $C_{759}H_{1208}N_{210}S_2FeO_{204}$ has been suggested; this corresponds to a weight of 16,669. But in all probability a molecule of hæmoglobin contains four atoms of iron, and the molecular weight is four times greater. This view is supported both by Anson and Mirsky's work (see below) and by the experimental ultracentrifugalisation determination of Svedberg and Fähraeus, which has led them to consider that the molecule of hæmo-

globin in aqueous solution contains four groups of weight 16,700, its total weight being therefore 66,800.

The amount of iron present in different hæmoglobins is very constant, about 0·33 per cent. One gm. of hæmoglobin will unite with 1·34 c.c. of oxygen (giving oxy-hæmoglobin), and this is in the exact ratio of one atom of iron to one molecule of oxygen, so that an equation may be written

$$HbFe + O_2 = HbFeO_2,$$

or, perhaps more accurately—

$$HbFe_4 + 4O_2 = Hb(FeO_2)_4.$$

Solutions of hæmoglobin are purple-red in colour, corresponding to venous blood ; those of oxy-hæmoglobin are scarlet-red, corresponding to arterial blood. Oxy-hæmoglobin, while readily soluble in water, is insoluble in alcohol, ether and other fat solvents. Hæmoglobin is still more soluble in water, and cannot therefore be as readily obtained in crystalline form. The crystals, when obtained, are, as a rule, isomorphous with those of the corresponding oxy-hæmoglobin.

When potassium ferricyanide is added to a concentrated solution of oxy-hæmoglobin, or to blood that has been shaken up with air, the colour changes to brown, through the formation of *methæmoglobin*. This compound is also formed when blood clots are allowed to remain for some time in contact with air. It crystallises in brown-red needles, prisms, or six-sided tables. According to Nicloux, if oxy-hæmoglobin is written $Hb(FeO_2)_4$ then methæmoglobin should be written $Hb(FeOH)_4$; Conant and Scott consider that the oxygen content is still less. The oxygen is removed with greater difficulty than that from hæmoglobin.

When oxy-hæmoglobin solutions are exposed to a partial vacuum oxygen is readily liberated. Methæmoglobin is not affected by this treatment. Both are readily converted into (reduced) hæmoglobin by treatment with a mild reducing agent such as ammonium sulphide.

Solutions of these compounds are characterised by yielding definite absorption spectra. Thus the spectrum of light passed through a dilute solution of oxy-hæmoglobin shows two definite dark bands in the yellow-green region, and through methæmoglobin less marked bands in the yellow green and a definite band in the red part of the spectrum. Reduced hæmoglobin produces a more diffuse single band in the yellow-green. Oxy-hæmoglobins from different species of animals show slight changes in the mid-positions of the two absorption bands.

When carbon monoxide (or coal gas, which contains it) is passed through a solution of hæmoglobin or of oxy-hæmoglobin the colour of the solution changes to cherry-red, due to the formation of carboxy-hæmoglobin. This gives a spectrum very similar to that of oxy-hæmoglobin, though each band has been shifted slightly towards the green region. Carboxy-hæmoglobin is much more stable, and does not easily give up the carbon monoxide, and so, when carbon monoxide or coal gas is breathed, even in dilute concentration, death follows from asphyxiation, through the gradual change of the bulk of the hæmoglobin and oxy-hæmoglobin into the carboxy-compound, and the consequent inability of the tissues to obtain their necessary supply of oxygen. (Treatment, accordingly, includes the administration of pure oxygen.) One gm. of carboxy-hæmoglobin contains united within it 1·34 c.c. of carbon monoxide, so that its formation can be represented by the equation—

$$Hb(FeO_2)_4 + 4CO = Hb(FeCO)_4 + 4O_2,$$

or

$$HbFe_4 + 4CO = Hb(FeCO)_4.$$

Crystals of carboxy-hæmoglobin are isomorphous (belong exactly to the same crystal system) with those of the corresponding oxy-hæmoglobin, but are less soluble and somewhat more blue-red in colour.

When any of these compounds are treated with nitric oxide,

the still more stable compound nitroxy-hæmoglobin, $Hb(FeNO)_4$, is formed.

The blood of all vertebrates contains hæmoglobin. It is present in the blood of many invertebrates, but its distribution is irregular. For example, it is present in only one species of starfish, in the larvæ of only two or three insects, in but one snail, *Planorbis*, and in some worms, but not in others. Other invertebrates contain some oxygen-carrying iron-protein of identical function. Certain crustaceans and molluscs, such as, for example, the king-crab, contain *hæmocyanin*, a copper- instead of an iron-protein. This, like hæmoglobin, contains a high percentage of histidine radicals. Oxy-hæmocyanin is blue; animals which contain it are therefore blue-blooded. (Reduced) hæmocyanin is colourless. This compound is not so efficient an oxygen-carrier as hæmoglobin. Different species contain different hæmocyanins. These have undoubtedly very complex molecules, and such different molecular weights as 73,400 (hæmocyanin from *Limulus*) and 5,000,000 (from *Helix*) have been suggested. The copper content of the compound from *Limulus* is 0·173 per cent. It seems well established that oxygen is taken up in the ratio of one atom for each atom of copper. There is some evidence that the prosthetic group of hæmocyanin is a copper porphyrin.

Certain polychæte worms contain in their plasma a solution of *chlorocruorin*, still another oxygen-carrier which is red in concentrated, green in dilute solution. The absorption bands of the oxidised and reduced forms resemble those of hæmoglobin, though nearer the red end of the spectrum. Different species of worms contain different chlorocruorins, which show different capacities for union with oxygen. These compounds also react with carbon monoxide. The prosthetic group contains iron, and strongly resembles hæm in general behaviour.

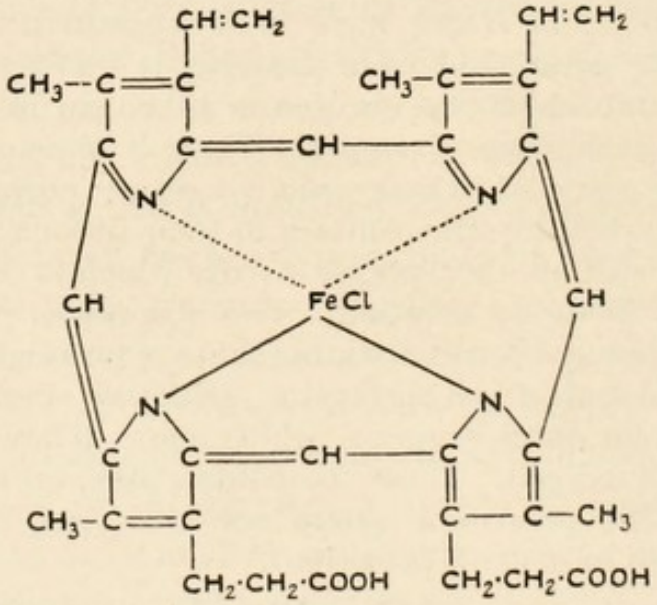
On hydrolysis hæmoglobin yields 94 per cent. of *globin*. Globin contains 55 per cent. of carbon, and 16·9 per cent. of nitrogen. It is very easily soluble in acids and alkalies, and behaves essentially like a histone. On hydrolysis it yields unusually large amounts of leucine (29 per cent.) and histidine (11 per cent.). Globin is colourless.

Addition of dilute alkali to hæmoglobin leads to the production of *hæmochromogen*; addition of acid to oxy-hæmoglobin results in the formation of *hæmatin*. Hæmochromogen also has a characteristic absorption spectrum. According to

the recent work of Anson and Mirsky it consists of a molecule of *hæm* united to one molecule of " denatured " globin. On adjustment to neutrality of the solution containing it hæmochromogen polymerises back to hæmoglobin.

When hæmoglobin is heated with sodium chloride and glacial acetic acid characteristic minute dark brown rhomboid crystals of hæmin, $C_{34}H_{32}N_4O_4FeCl$, are produced. The relationship between hæm and hæmin is expressed by the formulæ X . FeOH and X . FeCl. The corresponding bromine and iodine hæmins have been prepared.

Hæmin is built up from four pyrrole nuclei. It has been synthetised by H. Fischer and K. Zeile (1929), who give it the formula :



Hæmin

(It is still uncertain to which pair of the central nitrogen atoms is attached the iron atom.)

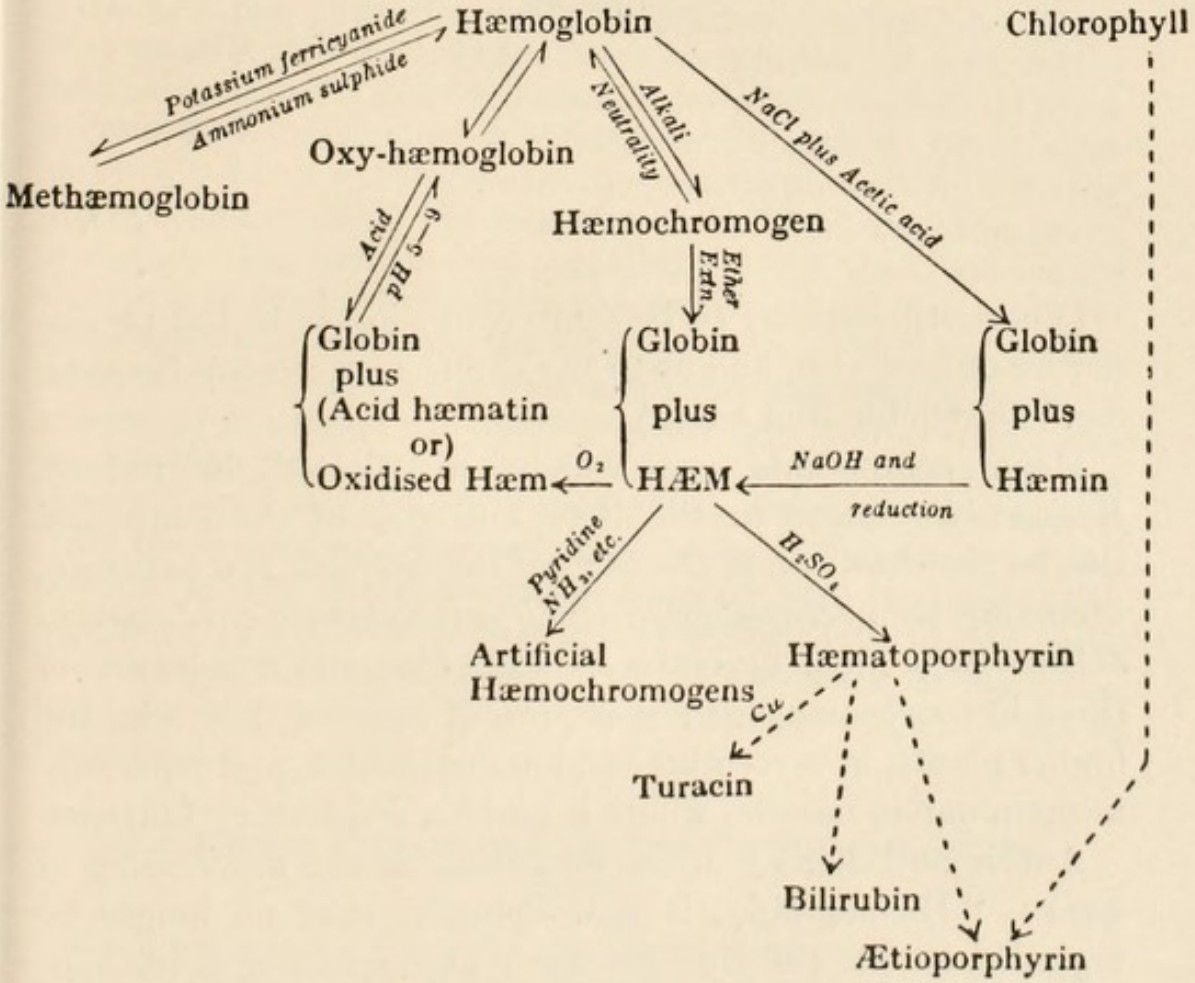
Hæm can combine with many nitrogen compounds to form " hæmochromogens " ; these are named from the particular nitrogen compound concerned. Such are cyanide, ammonia, amine, protein, pyridine, and nicotine hæmochromogens. Hæm itself is an oxidative catalyst, but such

unions render its catalytic action far more effective. The hæmochromogens have all very similar absorption spectra, with two sharply defined bands.

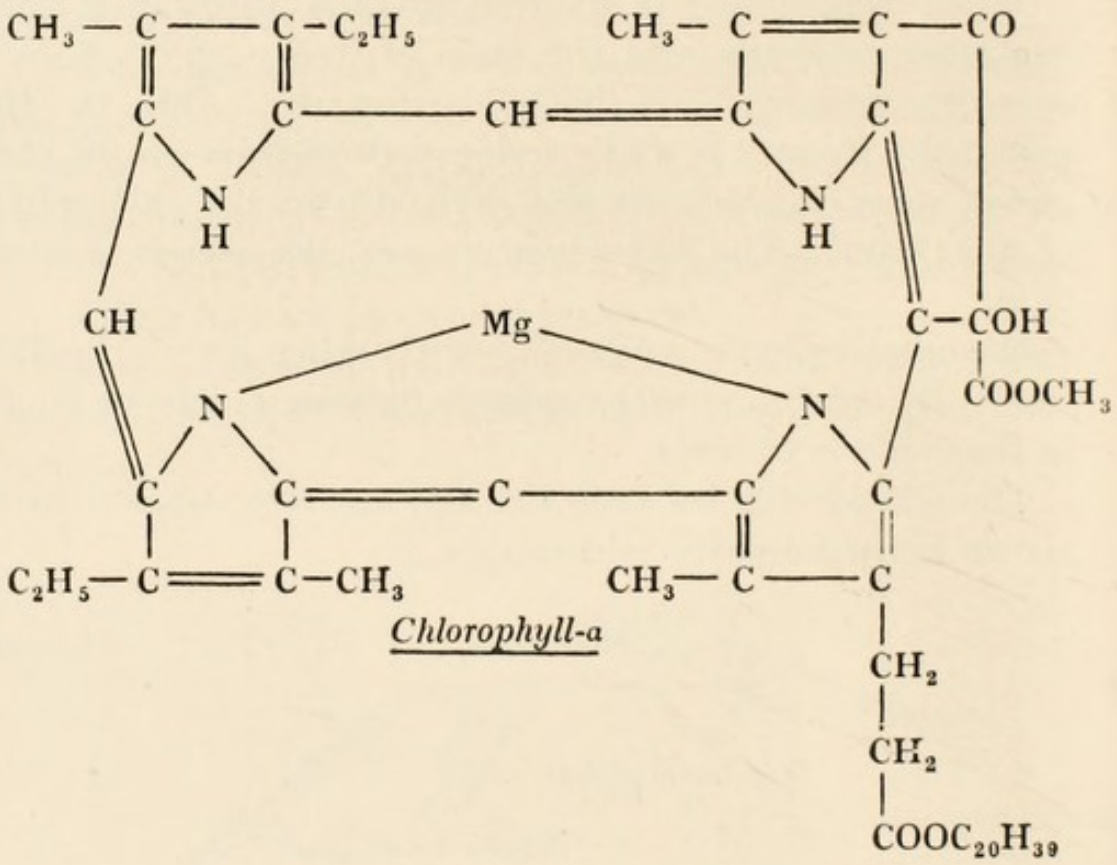
When hæm or any of its derivatives is dissolved in concentrated sulphuric acid the atom of iron is split off, and *hæmatoporphyrin*, $C_{34}H_{38}N_4O_6$, is formed. This is still coloured. From it a whole series of *porphyrins* can be prepared, some of which are also derived from the chlorophyll of the plant. The bile pigments are also closely related to it.

Hæmatoporphyrin will unite with other metals besides iron. Its compound with copper is *turacin*, a pigment found in the feathers of birds.

The relationship between the various hæm compounds is shown in the following scheme :



The close relationship between haem and plant chlorophyll is shown by comparison of the formula for haemin with that of chlorophyll-α :



Chlorophyll-a

(There still appears to be some confusion as to the precise nature of hæmatin, and as to the direct relationship between methæmoglobin and hæm.)

Hæm compounds are widely distributed in nature. *Helicorubin*, found in the liver and gut of the snail and similar molluscs and in the liver of the crayfish, is a naturally occurring hæmochromogen. The *actiniohæmatin* of certain actiniæ is a hæm derivative. *Cytochrome* is a mixture of three hæmochromogens ; it is present in yeast, bacteria, the higher plants, in vertebrates and invertebrates, and especially in mammalian muscle, where it plays a respiratory function.

Anson and Mirsky write as follows of the *universality of hæm :* " Hæmoglobin, it now appears, may no longer be regarded as the pre-eminent hæm pigment, nor is its hap-

hazard distribution now as mysterious. Hæmoglobin is merely an occasional specialised derivative of an iron substance hæm, which is much more widely distributed."

Hæmoglobin is probably formed by the tissues which manufacture the red blood cells that contain it. It is probably chiefly destroyed by the liver, though other tissues undoubtedly possess this power (see Chapter XVII., p. 235). Its function is to convey oxygen to the tissues in a form from which it can be easily set free, and to help to convey carbon dioxide from the tissues. The two processes are interrelated, since oxy-hæmoglobin is more acid—will combine with more of the potassium of the red corpuscle—than reduced hæmoglobin, so that coincident with the liberation of oxygen to the tissues base is set free to hold bicarbonate ions.

Barcroft has pointed out that there are marked advantages from the fact that hæmoglobin is enclosed in the red cells, rather than being simply a dissolved constituent of the plasma. If blood contained its hæmoglobin in solution in the plasma, the amount normally present would increase the osmotic pressure by 100 mm. pressure of mercury. The viscosity of the blood would be much increased. The hæmoglobin, propelled through the blood vessels according to the laws of stream-like motion, would to a considerable degree stagnate along the capillary walls, so that less oxygen could be carried from lungs to tissues in a given time by the same amount of hæmoglobin. Unless the capillary wall were less permeable than it usually is in mammals, and in consequence less efficient, some hæmoglobin would diffuse into the tissue spaces. "All these disadvantages are obviated by the enclosure of the pigment in cells commensurate in diameter with the bore of the capillary. In addition much is gained on the chemical side, since the intracorpuscular atmosphere may be adjusted to place the hæmoglobin in its most efficient environment which is not that of the plasma."

The Plasma Proteins and the Osmotic Pressure of the Blood

Since we usually designate the liquid portion of the blood as *plasma*, and the liquid exudate from clotted blood as *serum*, serum

containing practically the same solutes as plasma, with the exception of fibrinogen and prothrombin (see below), the proteins present in the plasma should more correctly be designated *plasma albumins* and *plasma globulins*, rather than, as is customary, *serum albumins and globulins.*

Both " serum albumin " and " serum globulin " are usually considered to be mixtures of at least two compounds, so that, *e.g.*, it has been customary to speak of serum globulin, euglobulin, and pseudoglobulin. Svedberg and Sjogren in 1928, by means of their high-speed centrifugal sedimentation method, found that both the albumin and globulin of plasma are homogeneous as regards their molecular weights and are probably single chemical individuals, their respective weights being approximately 68,000 and 103,000 (see Table V., p. 107) They are rather unstable, and are easily decomposed during purification.

It is probable that fractional precipitation methods used in attempting separation have suggested more than one albumin and globulin through formation of decomposition products during the fractionation process. Rimington's work indicates that both proteins contain a carbohydrate radical built up from glucosamine and mannose.

The place of formation of the two proteins is unknown. Their nature would appear to be specific ; they differ from the other albumins and globulins present in the body. Their function, or one of their functions, is to maintain a constant osmotic pressure. If the plasma did not contain some substances such as these which could not diffuse to any appreciable extent through the normal capillary wall, then the compounds with smaller molecules could gradually diffuse into the tissue spaces and blood could become a highly concentrated, almost stagnant, suspension of cells.

In making transfusion of normal saline solutions in conditions such as shock the sodium chloride rapidly passes from the blood by diffusion, so that the effect of the transfusion is very transient, unless, as Bayliss showed, a non-diffusible

substance such as gum arabic is added to the solution of sodium chloride.

The osmotic pressure of a molecule (or ion) is the pressure it exerts in the solution in which it is dissolved. It may be regarded as a driving force which causes the molecules of a dissolved substance to distribute themselves uniformly through the medium in which they are dissolved. Two solutions in contact through an animal membrane distribute the solvent between them, this distribution being governed by the respective osmotic pressures of the total solutes. If through diffusion the blood becomes concentrated, then the concentrated proteins will exert a greater osmotic pressure than before, and will, in consequence, attract water from outside the vascular system, so bringing about dilution of the blood and tending to restore its original concentration.

The presence of these plasma proteins also helps to give the blood a definite viscosity (although that viscosity is still more due to the presence of the red cells (*cf.* p. 81)). Further, they may play some part in keeping the hydrogen-ion concentration of the blood constant.

As Table XIII. shows, serum globulin is usually present in much smaller amount than is serum albumin. In certain infections or toxæmias these ratios may be definitely altered. In streptococcus and staphylococcus infections, and in nephrosis, the globulin may amount to as much as 80 or 90 per cent. of the total serum protein. Muscular activity increases the serum protein, chiefly the albumin portion.

Other Blood Constituents

The nutrient material contained in the blood can best be dealt with in treating of intermediate metabolism (Chapters XXII.—XXVII.), the excreta, in dealing with the excreta (Chapter XVII.), and the internal secretions in dealing with the composition of the tissues that secrete them (Chapter XIX.).

Fibrinogen and the Clotting of Blood

When blood is freshly drawn from an artery or vein it *clots* —forms a jelly-like mass—within three or four minutes. On standing the mass contracts, and colourless or faintly yellow *serum* exudes. The contracted clot consists of a mesh of *fibrin* holding within it the red blood cells. If freshly drawn blood is immediately " whipped " with a bundle of twigs it does not clot but a white protein gradually separates in strings on the twigs. This is fibrin. The unclotted blood, centrifuged, separates into serum and corpuscles.

Clotting can be prevented in various ways. Immediate addition of oxalate, citrate or fluoride prevents this change. Oxalate and fluoride precipitate inorganic calcium ; citrate forms a very slightly ionised calcium salt. *Clotting can* therefore *be prevented by any reagent which will reduce the calcium ions in the blood to a negligible quantity.*

Clotting can also be prevented by injecting into the circulation of the living animal solutions of peptones or proteoses, or extract of leech heads (hirudin), or certain snake venoms. Blood drawn subsequently from the animal does not clot. The mechanism of this inhibition is entirely different and will not be discussed further here.

If blood is drawn into a small amount of oxalate, and then centrifuged, and to the *plasma* is added an equal amount of saturated solution of sodium chloride, a precipitate of the protein *fibrinogen* separates. If this is washed with half-saturated sodium chloride, redissolved in dilute sodium chloride, re-precipitated by half-saturation with sodium chloride, and again dissolved in the dilute reagent, the pure solution of fibrinogen that is so obtained does not clot on standing.

If freshly formed strings of fibrin, obtained by whipping blood, are washed in cold water with constant kneading to remove any red blood corpuscles and other extraneous matter, and are then squeezed dry and cut up into small

pieces with scissors, and this finely divided material covered with 8 per cent. sodium chloride solution and allowed to stand in a refrigerator for forty-eight hours, the fibrin does not go into solution, but another compound does. This is termed *thrombin*. If the mixture is squeezed through a cheese cloth a viscous liquid is obtained. A few drops of this and a drop or two of calcium chloride added to the solution of fibrinogen prepared in the manner already described, cause immediate formation of a colourless clot. The solid so produced is fibrin. Addition of calcium chloride to thrombin produces no clot. Thrombin either contains no calcium or else such calcium is in organic combination and non-reactive.

Thus, *fibrin is produced by the action of thrombin and calcium ions on fibrinogen.*

If oxalated blood is centrifuged, and the plasma heated to 54° C., fibrinogen is coagulated (the ordinary coagulation of a protein by heat, not clotting). If the filtered plasma is treated with acetone a precipitate forms. This, collected on a filter and dried, and extracted with dilute sodium bicarbonate, yields a solution of *prothrombin*. Addition of this solution to pure fibrinogen solution, or to oxalated blood, does not produce clotting. But if to the prothrombin solution calcium chloride is added, and *then* the mixture is added to fibrinogen, a clot forms.

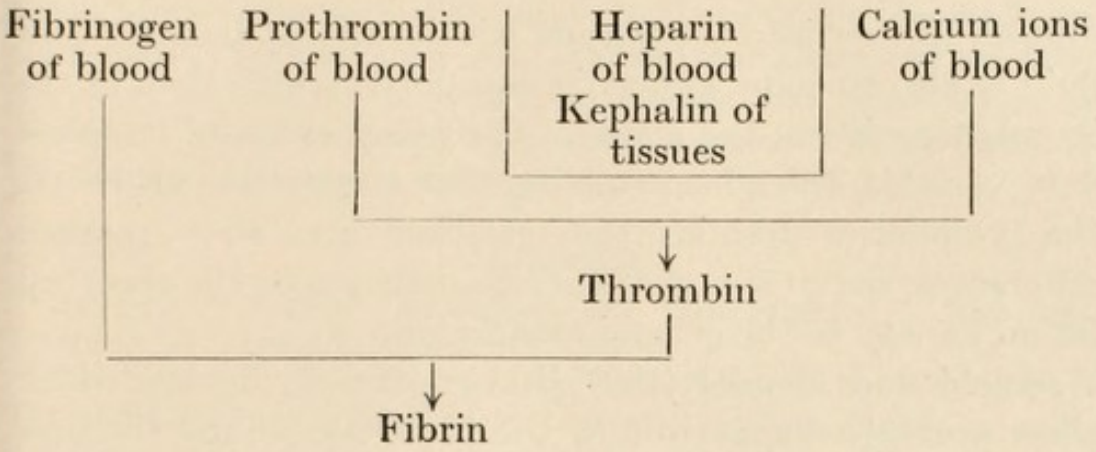
By the action of calcium ions on prothrombin thrombin is formed, and thrombin in presence of calcium ions converts fibrinogen into fibrin, and so produces a clot.

The circulating blood contains fibrinogen, prothrombin and calcium ions. Why, therefore, does it not clot? Evidently there is some additional factor necessary. Howell has produced strong evidence that this is the phosphatide *kephalin*, added prior to the clotting process to the freshly drawn blood either from the lacerated tissues over which the blood flows or from disintegration of the white cells or platelets of the shed blood. White corpuscles of birds and

amphibians do not disintegrate so readily after shedding as do those of mammals. If a paraffined cannula is introduced into an artery of a bird and the blood be collected in a paraffined centrifuge tube and immediately centrifuged, the plasma so obtained either does not clot at all or at most very slowly. Addition of many tissue extracts—extracts of leucocytes, of brain, of thymus, or of testis—to this plasma produces immediate clotting. The active substance of these tissue extracts is soluble in ether, not easily soluble in alcohol, and contains phosphorus and nitrogen. Kephalin possesses all these properties. Further, pure kephalin has the same power of producing clotting as have these tissue extracts, while the lecithins and sphingomyelins do not possess the property. Howell believes that in the presence of calcium ions kephalin permits the conversion of prothrombin into thrombin by removal of some inhibitory substance. He has obtained from the liver a preparation "heparin," which is very active in preventing clotting both *in vitro* and *in vivo*, and which, he states, is present in blood plasma. Heparin is probably the inhibitory substance which prevents the conversion of prothrombin into thrombin, so that it may be termed *antiprothrombin*.

It is still uncertain whether thrombin is, or is not, an enzyme. Heating it for a few minutes to 60° C. inactivates it, and a small amount of it will change a very large amount of fibrinogen. To this extent it resembles the enzymes. But the amount of fibrin produced depends on the amount of thrombin present; the interaction between thrombin and fibrinogen is quantitative. There is some evidence that thrombin contains a small amount of calcium (0·7 per cent.) in organic combination, and that in the reaction by which fibrin is produced this calcium passes into the serum, though still in organic combination.

The theory of Howell appears to account for nearly all the facts that we know regarding the normal clotting of blood. It can be written simply:



Fibrinogen is a globulin, precipitated by half-saturation with ammonium sulphate, but differing from the "serum globulins," because it is precipitated by half-saturation with sodium chloride. It seems to be produced in the liver.

Conditions associated with injury to, or insufficiency of, the liver, as phosphorus or chloroform poisoning, or hepatic cirrhosis, are accompanied by marked diminution of the fibrinogen content of the blood. In pneumonia and in septicæmia the blood fibrinogen may markedly increase.

Fibrin may be a compound of fibrinogen with thrombin, but it is probably not simply an additive compound.

Thrombin is possibly a proteose. It can be extracted from its aqueous solution by repeated treatment with chloroform. It gives the biuret and Millon's reactions, and all the tests for tryptophane, and is precipitated by half-saturation with ammonium sulphate.

Heparin (*antiprothrombin*) seems to be a relatively simple monobasic acid and a carbohydrate derivative. Study of its brucine salt has suggested the formula $C_{18}H_{32}O_{17},6H_2O$.

Chemical Composition of Lymph, Transudates and Exudates

Blood is not directly in contact with the tissue cells. These are bathed by a fluid in equilibrium with the blood plasma, or, more truly, which is constantly striving to attain to such an equilibrium, so that as the cells remove certain constituents from this fluid, these are replaced from the plasma, while material from the cells diffusing into this fluid then

diffuses further into the plasma within the capillaries. This fluid is also to some extent in equilibrium with that in the lymphatic system, the *lymph*. The composition of lymph is very variable, though resembling that of plasma. Of course the lymphatics draining the intestinal area show marked differences, since through them especially passes the absorbed fat on its way to the general circulation.

Arnold and Mendel (1927) have given us figures which allow accurate comparison of the blood-serum and thoracic duct lymph. These, expressed in terms of gm. per 100 c.c. fluid, are—

	Total solids	*Chloride-Chlorine*	*Ca*	*Inorg. P*	*Glucose*	*Non-protein N*	*Protein N*
Serum	8·3	0·39	0·0104	0·0043	0·123	0·027	0·9
Lymph	5·2	0·41	0·0092	0·0036	0·124	0·027	0·57

Their experiments showed also that interchanges take place between the blood and the lymph whenever fluctuations in the concentrations of the constituents occur, and that diffusible substances pass easily and rapidly at all times between the blood, the lymph and the tissues.

Cajori and Pemberton (1928) have shown that synovial fluid (the fluid within the synovial cavity between the opposing surfaces of joints) has a content of non-protein nitrogen, urea nitrogen, and amino-acid nitrogen almost identical with that of blood plasma, but a lower concentration of proteins.

Both transudes and exudates are fluids that have exuded from the blood vessels into the surrounding tissues, and a sharp differentiation between them probably does not exist. Roughly speaking, exudation is accompanied or preceded by inflammation, while transudation is not. Transudates certainly contain less protein than exudates, and may contain little or none. Their specific gravities are correspondingly lower, being usually below 1·015; those of exudates are usually above 1·018. The amount of protein differs in different exudates, varying both in different diseased conditions and in different parts of the body, and depending essentially on the particular pathological permeability of the vessels where the exudate is formed. Serum albumin, globulin and fibrinogen are all present. The crystalloidal contents are

similar in amount and composition to those of the blood plasma from which the fluid is derived. In old transudates and exudates proteoses and such amino-acids as leucine and tyrosine can be detected ; these are formed from the proteins of these fluids by the actions of autolytic enzymes (see Chapter XX.).

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CHAPTER XVI

THE CHEMISTRY OF RESPIRATION, PART I

The Chemical and Physical Mechanisms Associated with Gaseous Exchanges in the Organism

RESPIRATION (*L. respirare*, to breathe) is literally the act of breathing. By this process the tissue cells obtain oxygen and get rid of carbon dioxide. But the term is also used to describe the " combustion " within the tissues whereby by use of oxygen carbon dioxide (and water) are produced. This idea is old ; Lavoisier, victim of revolution, who not only laid accurate foundations for the study of quantitative chemistry, but also for that of quantitative biochemistry as well, wrote, late in the eighteenth century, " Respiration is only a slow combustion of carbon and hydrogen, which is similar in all respects to that which takes place in a lamp or lighted candle ; and from this point of view the animals which respire are truly combustible bodies which burn and consume themselves."

Any account of respiration, taking that term in the broader sense, therefore involves discussion of two very distinct series of phenomena, the chemical and physical mechanisms whereby the cells obtain oxygen from the air and contribute carbon dioxide (and water) to it, and the chemical changes involved in " tissue respiration," the oxidative changes within the tissue cells. For unicellular organisms the first series of changes can be dismissed with the explanatory term *diffusion*, but in multicellular organisms there have developed specially adapted and highly complex mechanisms. In this chapter some account will be given of these mechanisms

as exhibited by mammals and especially by man. In a later chapter tissue respiration and the biological oxidations which are involved in it will be considered in detail.

The gaseous exchanges between atmosphere and tissues involve three phases, first, the exchanges between the atmosphere and the blood, through the lungs and across the epithelial lining of their alveoli and the capillaries within the walls of these alveoli, second, carriage within the blood, and, third, exchanges between the blood and the tissue cells. Evidence will now be adduced to show that the first and third of these are brought about by diffusion, while the second involves chemical mechanisms.

Gaseous Exchanges Between the Atmosphere and the Blood. The lungs, through the movements of ribs and diaphragm, are continually receiving external air, and expelling part of their own contents into the atmosphere. Though this exchange takes place in a normal man about seventeen times per minute, it by no means brings the gas-content of the lungs into equality of composition with that of the atmosphere. This is largely because the volume of each normal inspiration is about 500 c.c., of which 150 c.c. is employed in filling the "dead space" of the trachea and bronchioles, while the lung space to which the other 350 c.c. is added already contains about 1,000 c.c. of "supplemental air" (air which can be expelled by prolonged forced expiration) and 1,000 to 1,500 c.c. of "residual air." Thus, with each inspiration, 350 c.c. of atmospheric air are mixed with 2,000 to 2,500 c.c. of *alveolar air*, and the following expiration only gets rid of 350 c.c. of this mixture. Since there is a continuous loss of oxygen from the alveolar gas to the blood, and gain of carbon dioxide from the blood, and since each exchange with the atmosphere introduces only one-sixth or one-seventh the lung volume of fresh air, and expels still less of the lung mixture that results, it is easy to see that there will be a distinct difference between the composition of the atmospheric air, the "expired air"

(which includes the 150 c.c. of atmospheric air from the dead space), and the true alveolar gas, of which a specimen can be obtained by collecting a sample *at the end of a forced expiration.* Expired air is also saturated with water vapour, while atmospheric air in temperate climates contains only traces of water vapour. In Table XIV., which shows the compositions of typical samples of these "airs," the water vapour content has been subtracted, and the figures refer to 100 volumes of dried gas :—

TABLE XIV. COMPARATIVE COMPOSITION OF THE RESPIRATORY GASES

	Inspired air.	Expired air.	Alveolar gas.
	Vols. per cent.	Vols. per cent.	Vols. per cent.
Oxygen . . .	20·95	16·02	14·59
Nitrogen . . .	79·02	79·60	79·70
Carbon dioxide . .	0·03	4·38	5·71

The total volume of expired air is less than the total volume of air inspired, which accounts for the difference in the figures for nitrogen. Oxygen is used up in the body in forming other oxidation products besides carbon dioxide, products such as water and urea. One volume of carbon dioxide corresponds to one volume of oxygen, and this oxidation does not lessen the total gas exchange. But the urea and water produced represent oxygen withdrawn from the inspired air without gaseous replacement.

Gaseous nitrogen is not affected in the body ; it does not take part in any chemical reaction within the body. The actual volume of nitrogen expired must be, therefore, equal to that volume inspired, and, hence, the difference in the percentage amounts can be used to calculate the amount of oxygen retained in the body and not accounted for as carbon dioxide (see p. 457).

The figures for alveolar gas represent the composition of the gas in the lung alveoli that is in close contact with the blood passing through the capillaries of the alveoli. During

this passage of blood through these capillaries a change from venous to arterial conditions is accomplished, the purplish-red venous blood becoming scarlet-red, and this is due to the conversion of most of the reduced hæmoglobin to oxy-hæmoglobin. In order that this change may take place by *diffusion*, and without any physiological secretion, the gas-tension (gas-pressure) of oxygen in the alveoli must be greater than that in the incoming venous blood, and at least as great as that in the arterial blood leaving the lungs, while the gas-tension of carbon dioxide in the alveoli must be less than that of the venous blood entering the lungs and not greater than that of the outgoing arterial blood.

To demonstrate this it is necessary to be able to measure the oxygen and carbon dioxide gas pressures in arterial and venous blood. How can we measure gas-pressure in a liquid ?

If gas is in contact with a solution of it in a liquid, when a condition of equilibrium is reached between the gas and the solution, as much gas will leave the liquid in a given time as passes into it in that time. If, then, the pressure of the gas is altered the equilibrium is upset. If the pressure is increased in the gas-phase more gas will pass into the solution ; if the pressure is lowered in the gas-phase gas will leave the solution. But an equilibrium will always be reached again, and will be reached the faster the greater the surface of contact between the gas and liquid phases. We can consider the pressure of the gas in the liquid therefore to be the same as the pressure of the gas in the gas-phase, once equilibrium is attained. Not only is this true for a single pure gas, but it is equally true for each constituent of a mixture of gases. Each such constituent will attain to its own equilibrium so that knowledge of the actual gas-pressure and the composition of the gas-phase will indicate the gas-pressures of the different gases in the liquid phase. This in no way determines the total volume of gas in the liquid phase, which depends on the solubilities of the different gases in the

liquid, and, further, on possible reactions between one or more of these gases and substances dissolved in the liquid.

Professor Auguste Krogh, of Copenhagen, has devised a *micro-œrotonometer* which is based on this principle. In this instrument he uses a minute bubble of air, about 2 mm. in diameter, and which, therefore, has a volume of about 0·004 c.c., a surface of about 0·125 sq. cm., and a ratio of surface to volume of about 30. Hence equalisation of tension between the bubble and surrounding liquid takes place very rapidly, even with only a small volume of liquid. Blood, venous or arterial, is caused to circulate rapidly round this bubble, whose volume is then measured by withdrawing it into a very fine, graduated, capillary tube. It is then subjected to treatment with dilute sodium hydroxide, and then with dilute alkaline pyrogallol solution, which dissolve respectively the carbon dioxide and oxygen, the changes in volume measured after each treatment showing the respective amounts of these gases present.

By the use of this device Krogh has shown that the tension of carbon dioxide in *arterial* blood is either identical with, or slightly greater than, that in the alveolar gas, while the oxygen tension of the blood is always lower than that of the alveolar oxygen by from 1 to 4 per cent. of an atmosphere.

It has also been calculated by less direct methods that the tension of oxygen in venous blood is only about 40 mm. mercury pressure, whilst that in the alveoli is over 100 mm., and that the corresponding figures for carbon dioxide are 46 and 40 mm. Hence there exists the necessary gradient of the gas-pressure in blood and alveolar gas to permit gas exchange by diffusion. Calculation has shown further that the observed differences of tension in the lungs, when allowed to act across a moist membrane such as the lung membrane is, will permit greater amounts of gas to diffuse in a given time than actually are known to diffuse in the lungs in such time. Hence *the laws of gaseous diffusion are entirely adequate to account for the gas exchanges in the lungs.*

Carriage of Oxygen and Carbon Dioxide in the Blood. It can easily be shown that the amounts of oxygen and carbon dioxide extractable from blood are much greater than would be accounted for by simple solution. If we expose a liquid to the zero pressure of a vacuum it will give up any gases it contains.

When arterial blood is so treated, from 100 volumes of blood are obtained about 18 volumes of oxygen, 50 of carbon dioxide, and 1 of nitrogen (at normal atmospheric pressure, *i.e.*, a pressure of 760 mm. of mercury, and at blood temperature, 37° C.). Venous blood under the same conditions yields about 13 volumes of oxygen, 57 of carbon dioxide, and 1 of nitrogen. Under the same conditions actual measurements show that pure water, shaken up with alveolar air of the composition given in Table XIV. (14·59 per cent. oxygen, 79·70 per cent. nitrogen, and 5·71 per cent. carbon dioxide) would dissolve (since each gas is dissolved in accordance with its own *partial pressure*) 0·35 volumes of oxygen, 3·14 of carbon dioxide, and about 1 of nitrogen. Addition to water of salts such as are present in blood *decreases* the amount of gas that can be held in solution. Evidently simple solution may account for the nitrogen present in blood, but can only account for a small fraction of the oxygen and carbon dioxide.

We know already that the red blood cells contain hæmoglobin which can unite with oxygen, and that while venous blood contains reduced hæmoglobin, arterial blood contains chiefly oxy-hæmoglobin. Here, then, is obviously the chemical mechanism for carriage of oxygen.

When solutions of hæmoglobin take up oxygen to form oxy-hæmoglobin it has been found that there is a peculiar dependence on the actual pressure of oxygen in contact with such solutions. If a series of solutions, each containing the same percentage of hæmoglobin, is brought into contact with atmospheres containing different pressures of oxygen, then, after equilibria have been attained, if the percentages of oxy-

hæmoglobin in the different solutions are plotted against the respective pressures of oxygen in contact with these solutions a curve such as that shown in Fig. 5 is obtained.

It might be expected that if the oxygen pressure were

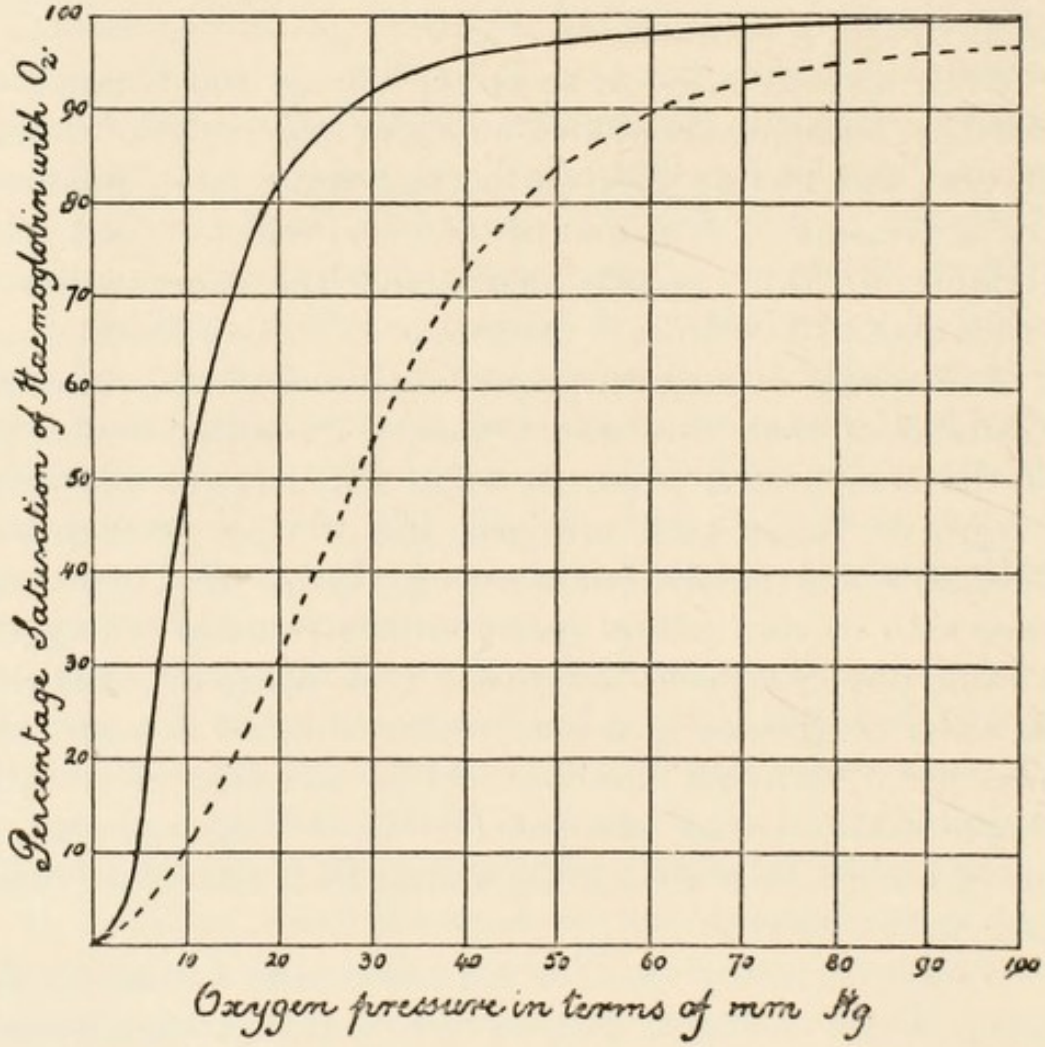

FIG. 5.—Dissociation curves of hæmoglobin. *Continuous line:* Hæmoglobin in contact with oxygen only. *Dotted line:* In presence of carbon dioxide at 40 mm. Hg pressure. (After Barcroft and Poulton, *J. Physiol.*, 1913, xlvi., Proc. v.; and Christiansen, Douglas, and Haldane, *ibid.*, 1914, xlviii., 262.)

doubled the percentage of oxy-hæmoglobin formed would also be doubled. This is evidently not the case. As the oxygen pressure is raised from 0 to 30 mm. mercury pressure most of the hæmoglobin changes to oxy-hæmoglobin, so that with a pressure of only 10 mm. mercury there is already 50

per cent. saturation. Increasing the pressure above 30 mm. mercury only very slowly increases the percentage of saturation. At 100 mm. pressure the saturation is over 99 per cent. If we start with a saturated solution of oxy-hæmoglobin in contact with oxygen at atmospheric pressure (760 mm. mercury), the same curve is obtained on gradually reducing the pressure of oxygen, scarcely any oxygen being liberated from the hæmoglobin until the oxygen pressure has been reduced to one-twenty-fifth of an atmosphere (30 mm. mercury).

The curve of saturation depends on certain factors. Increase of temperature depresses it ; the higher the temperature the less oxy-hæmoglobin is formed for the same oxygen pressure. Increase of the saline content of the solution also diminishes the percentage of oxy-hæmoglobin formed at the same pressure. The saturation curve is especially affected by the hydrogen-ion concentration of the solution. The tension of carbon dioxide will affect the hydrogen-ion concentration, since with greater carbon dioxide pressure more carbonic acid is formed, and, in consequence, more hydrogen ions. Increasing the pressure of carbon dioxide will therefore lower the oxygen saturation of hæmoglobin. This is shown in Fig. 5, and well illustrated by the following figures :

An oxygen pressure of 20 mm. mercury, and a carbon dioxide pressure of 5 mm. result in 67·5 per cent. of saturation.

An oxygen pressure of 20 mm. and a carbon dioxide pressure of 40 mm. result in 29·5 per cent. of saturation.

Actual experiment shows that venous blood will yield about thirteen volumes of oxygen per 100 volumes blood. The oxygen tension of this blood is about 70 mm. mercury, the carbon dioxide tension about 42 mm., and the hæmoglobin is between 72 and 80 per cent. saturated. Arterial blood yields about eighteen volumes of oxygen, its oxygen pressure is 91 mm., and its carbon dioxide pressure 40 mm.

The hæmoglobin is usually between 92 and 93 per cent. saturated.

The oxygen content of dried alveolar air may vary round 14 or 15 per cent., the carbon dioxide content averages about 5·5 per cent. The pressures corresponding to these figures (obtained by multiplying by 760/100) are respectively 106 and 114 mm., and 42 mm. Reference to Fig. 5 shows that an oxygen pressure of 106 mm. is quite sufficient even in the presence of carbon dioxide at 42 mm. pressure to transform venous to arterial blood whose hæmoglobin is over 90 per cent. saturated.

If blood is shaken up with air it will take up slightly more oxygen than is found in arterial blood, since the oxygen pressure of the atmosphere is greater than that of alveolar gas, while the carbon dioxide pressure is much less.

It has already been pointed out that the pressure differences of carbon dioxide in the blood and alveolar gas are sufficiently great to permit the passage of this gas from the blood to the alveoli. Venous blood loses during its passage through the lungs seven volumes of carbon dioxide. This is only 13 per cent. of the total carbon dioxide that it will yield on treatment with acid. It has been shown that this gas also cannot be held in the blood simply by solution. It is present chiefly as bicarbonate, and the following series of equations are believed to summarise the changes in blood in the lungs and in the tissues ; in the lungs the changes are from right to left, and in the tissues in the reverse direction :

Lung membrane and tissue cells—

$$CO_2 + HOH \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons H_2CO_3$$

Blood plasma—

$$H_2CO_3 + Na_2HPO_4 \rightleftharpoons NaH_2PO_4 + NaHCO_3$$

$$H_2CO_3 + \text{Na-Protein} \rightleftharpoons \text{H-Protein} + NaHCO_3.$$

Blood corpuscles—

$$H_2CO_3 + K_2HPO_4 \rightleftharpoons KH_2PO_4 + KHCO_3$$
$$2H_2CO_3 + 2KHbO_2 \rightleftharpoons 2KHCO_3 + 2Hb + HOH + 3O.$$

Gaseous Exchanges Between the Blood and Tissue Cells. Arterial blood enters the capillaries; venous blood leaves them. The lung exchanges are reversed, and, as in the lung exchanges, the processes across the extracellular fluid to and from the tissue cells are controlled and accounted for by diffusion. Oxygen and carbon dioxide in solution will diffuse in the direction of lower pressure. According to the most recent investigations the approximate average oxygen and carbon dioxide tensions in the different media under discussion are shown in Table XV.

TABLE XV. OXYGEN AND CARBON DIOXIDE TENSIONS BETWEEN ATMOSPHERE AND TISSUES

	Oxygen Tension.	Carbon Dioxide Tension.
	mm. Hg	*mm. Hg*
Atmosphere	159	Almost 0
Alveolar air	106	40
Arterial blood . . .	100	40
Venous blood . . .	50–40	46
Extracellular fluid . .	50 –20 or less	46–60 or more
Within the cell . . .	40–20 or less	

As the oxygen pressure in the blood diminishes through diffusion of oxygen from the blood oxy-hæmoglobin dissociates; carbon dioxide entering from the tissues facilitates the dissociation.

The series of changes in lungs, blood, and tissues is shown graphically in Fig. 6.

It can easily be demonstrated that oxidation takes place in the tissue cells and not in the blood. Ehrlich's experiment

illustrates the greed of the tissues for oxygen. A saturated solution of methylene blue (tetramethyl-aminophenthiazimium chloride) is injected into the circulation of a living animal. The animal is killed ten minutes later. On opening the body it is seen that the blood is coloured dark blue from the dye, but most of the organs show their natural colour. The exposure to the atmosphere is followed by rapid acquirement of the blue colour by all the organs. Hence in the ten minutes the tissues have reduced the methylene blue dye to its colourless leuko-base by removal of oxygen, and on exposure to the atmosphere, and therefore to excess of oxygen, the leuko-base recovers the lost oxygen and again becomes blue. The blood does not reduce the dye. Hence oxidation takes place in the tissues and not in the blood, and evidently the tissues contain practically no free oxygen.

A second method of demonstrating that the tissues are the seat of oxidation (and that the blood is not) is to wash out the blood of a frog with normal saline. The animal will then remain alive if kept in an atmosphere of pure oxygen. Its metabolism goes on as actively as before. It has no blood, so that evidently the metabolic processes requiring the utilisation of oxygen, and resulting in the production of carbon dioxide, must have their seat in the tissues.

"Tissue respiration" will be dealt with in Chapter XXXII.

The Hydrogen-ion Concentration of the Blood and the Buffering of Solutions

The hydrogen-ion concentration of the blood is extremely constant. That for venous blood averages a *p*H value of 7·34, and that for arterial blood is just measurably more acid with a *p*H value of 7·33.

While it is definitely established that arterial blood is very slightly more acid than venous blood, recent work by Earle and Cullen suggests that the average figures for each are a little higher than those just quoted. They find that the *p*H of venous blood varies in different normal individuals from 7·4 to 7·52. During the day the *p*H of any one individual increases very slightly (up to 0·07), with fluctuations during digestion and exercises.

The high degree of constancy of the *p*H of blood is governed by the blood-salts, especially the carbonates, and the blood

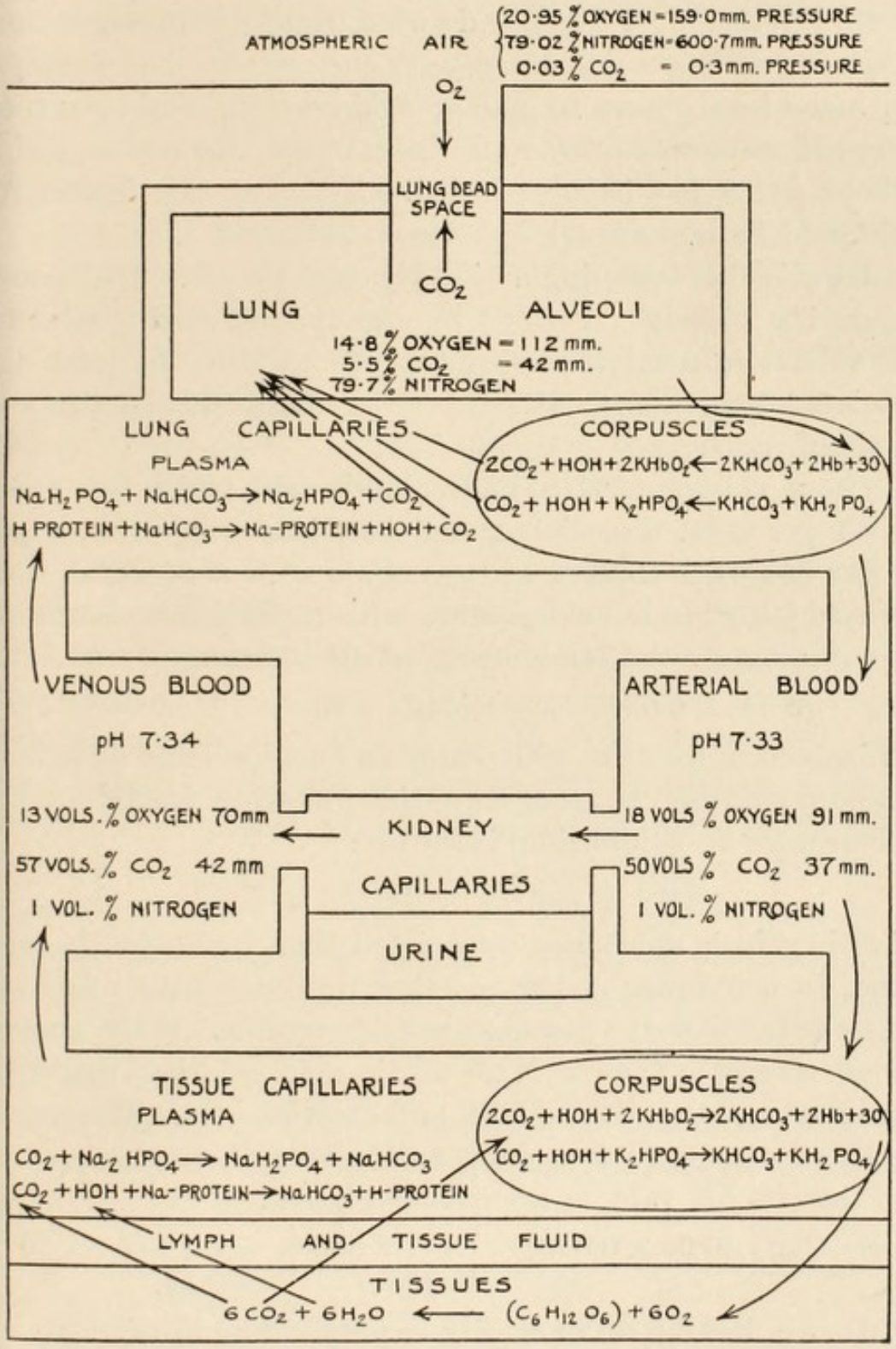

FIG. 6.—Graphic representation of respiratory exchanges in lungs and tissues. Gas in volumes per cent. Pressures in mm. Hg.

proteins, which act as a complex system of *buffers* (the term is self-explanatory), and owing to their buffering action, even in extreme acidosis, only on one occasion has a sample of blood been shown to give an acid reaction, and even then the *p*H value was 6·98, while in extreme alkalosis no value above *p*H 8 has been recorded. In health the figures for different individuals all lie between 7·3 and 7·5.

How is this buffering action brought about ? It depends upon the principle, verified by experiment, that a mixture of solutes in a solution always tends to form the least dissociated compounds. It can best be understood by study of a simple case.

If one volume of N/10 hydrochloric acid, which (see p. 34) is 91 per cent. dissociated, and has, in consequence, a *p*H value of 1·04, is diluted with an equal volume of water, then N/20 hydrochloric acid results, with a *p*H value, assuming approximately the same degree of dissociation, of 1·34.

$$\log\ 1/(0{\cdot}91 \times 0{\cdot}05) = \log 1/(4{\cdot}55 \times 10^{-2}) = 2 - 0{\cdot}66 = 1{\cdot}34.$$

If instead of dilution with water an equal volume of sodium acetate solution is used, then the following reaction takes place until an equilibrium is set up :

$$HCl + NaOAc = HOAc + NaCl.$$

Acetic acid is much less dissociated than hydrochloric acid, and, in consequence, the reaction proceeds until relatively little (dissociated) hydrochloric acid remains. If the change were complete then a N/20 acetic acid solution would be produced, along with sodium and chloride ions. This acetic acid solution only dissociates to the extent of 1·3 per cent., so that its *p*H value is approximately 3·2.

$$p\mathrm{H} = \log 1/(0{\cdot}05 \times 0{\cdot}013) = \log\ 1/0{\cdot}00065 = \log 1/(6{\cdot}5 \times 10^{-4})$$
$$= 4 - 0{\cdot}81 \qquad = 3{\cdot}19.$$

Hence, instead of an acidity corresponding to *p*H 1·34, we have one corresponding to about *p*H 3. Looking at the matter in another way, if to a volume of neutral water with a *p*H value of 7 we add an equal volume of N/10 hydrochloric

acid, the *p*H is changed to 1·34. If we first dissolve some sodium acetate in the water by the addition of the same volume of acid the *p*H is only changed to about 3. The sodium acetate has acted as a buffer.

The essence of the action of a buffer consists in damping down the effect of addition of an acid to a solution, by the production of an acid which ionises to a much smaller extent, or, conversely, of an alkali by formation of another alkali which ionises less.

In both blood and tissues the same three series of buffers, carbonates, phosphates and proteins are present, and the tissues also maintain a fairly constant, though not quite so constant, *p*H value. (Ocean waters also maintain a similar constant value through the agency of bicarbonates as buffers.)

A calculation of L. J. Henderson permits some idea of the tremendous damping effect of these buffers. If a kilogram of sodium carbonate is dissolved in 100 litres of water, and the solution allowed to remain in contact with an atmosphere containing 1 gm. of carbon dioxide per litre until equilibrium is attained (a considerable proportion of bicarbonate ions being produced), then the *p*H value of the solution is 7·244, that is, the solution is just alkaline. As long as contact with the atmosphere of dilute carbon dioxide is maintained, it requires 150 grams of pure hydrochloric acid to bring the solution to the acid side of neutrality, to a *p*H value of 6·967. The bicarbonate ion is obviously a very efficient buffer.

The bicarbonate solution can be represented as a mixture of $NaHCO_3$, H_2CO_3, and HOH; in this mixture the following equilibria are established:

$$NaHCO_3 \rightleftharpoons Na^+ + HCO_3^- \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$HCO_3^- + HOH \rightleftharpoons H_2CO_3 + OH^- \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$HOH \rightleftharpoons H^+ + OH^- \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$H_2CO_3 \rightleftharpoons HOH + CO_2 \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Carbonic acid ionises very little, so that equation (3) takes place to an almost negligible extent. Equation (2) determines that the solution shall have an alkaline reaction, the degree of alkalinity being determined by the extent of this reaction.

In such an equilibrium, whenever an ion or a molecule is added, it upsets the balance of all the reactions in which it takes a part, so that these reactions move in the opposite direction from that by which the ion or molecule is formed.

Thus if carbon dioxide is added to the solution, as, for example, from the tissues, it forms carbonic acid (5), which dissociates into H^+ and HCO_3^- ions (3). The H^+ ions will increase the formation of unionised water (4). The HCO_3^- ions from (2) and (3) react to increase the amount of unionised $NaHCO_3$ (1). The net result will be less ionised HOH and more unionised $NaHCO_3$, and the *p*H will remain practically unchanged.

Henriques and Ege have reported that in a series of cases the *p*H of the blood during normally regulated breathing averaged 7·31. Its value after the maximum period of holding the breath, when, therefore, carbon dioxide has been allowed to increase through non-ventilation of the lungs, fell only to 7·22. Its value after maximum forced breathing, that is, after maximum lung ventilation and removal of carbon dioxide, rose only to 7·52.

If the tissues furnish a stronger acid (such as lactic acid) to the blood, as they do, under many conditions, then, if we write such an acid *HX*, it will react in the following way :

$$HX + NaHCO_3 = NaX + H_2CO_3 (= NaX + HOH + CO_2),$$
$$\text{or} \qquad H^+ + X^- + HCO_3^- = X^- + H_2CO_3.$$

The net result will be a slight decrease in HCO_3^- ions, resulting in further ionisation of $NaHCO_3$ (1), and a greater CO_2 saturation, followed by increased loss of carbon dioxide through the lungs. The stronger acid will be eliminated as its neutral (sodium) salt, and the *p*H of the blood again will

be practically unaltered. We shall see later that this has an important bearing on acidosis.

Phosphates can produce similar effects through the following interrelated equilibria :

$$Na_2HPO_4 \rightleftharpoons Na^+ + NaHPO_4^- \rightleftharpoons Na + Na^+ + HPO_4^{--}$$
$$HPO_4^{--} + HOH \rightleftharpoons H_2PO_4^- + OH^-$$
$$NaH_2PO_4 \rightleftharpoons Na^+ + H_2PO_4^- \rightleftharpoons Na^+ + H^+ + HPO_4^{--}$$

Protein salts react similarly :

$$\text{Na-Protein} + HX \rightleftharpoons \text{H-Protein} + NaX.$$

Factors Affecting the Distribution of Water and Electrolytes between Cells and Plasma of Blood. The red corpuscles of the blood are normally impermeable to sodium and potassium and probably to all metallic ions ; the cause of this impermeability is unknown. On the other hand, water and anions, such as the chloride and bicarbonate ions, readily pass across the cell membrane in either direction. The gaseous exchanges which take place during the passage of blood through the capillaries of lungs and of other tissues are accompanied by such shifts of electrolytes and water. Within recent years L. J. Henderson has advanced explanations of these complex changes, and these have been amply confirmed by the experimental work of Van Slyke and his co-workers.

Since potassium and hæmoglobin cannot leave the red cell, while bicarbonate, chloride and hydrogen ions can diffuse in or out of it, the conditions for a Donnan equilibrium are present, and such equilibria assist in regulating the exchanges across each of these membranes. Further, hæmoglobin, in addition to all its other properties, is found to act as a polyvalent acid to such an extent that oxy-hæmoglobin can neutralise almost half the base present in the cell (which, in man, can be considered as almost entirely potassium). Reduced hæmoglobin is not quite so strongly acidic. The

rest of the base is neutralised almost entirely by chloride and bicarbonate.

The acidic property of hæmoglobin largely accounts for the fact that the concentration of chloride and bicarbonate in the cells is only about half that in the plasma (since the plasma proteins only combine with a small proportion of the plasma bases). The difference in acidity between oxy-hæmoglobin and reduced hæmoglobin explains the effect produced by oxygenation of blood (in the lungs) in facilitating release of carbon dioxide ; the effect is virtually that of adding more acid to the blood.

If the scheme for a Donnan equilibrium shown in Chapter VI. is applied to the conditions within and without the red cell, and if in the first place we consider only hydrogen, chloride and hæmoglobin, we can write :

	Membrane	
Cell		*Plasma*
Hb^-		
Cl^-		Cl^-
H^+		H^+

and from the theory of the equilibrium we have—

$$(H^+)_{cell} \times (Cl^-)_{cell} = (H^+)_{plasma} \times (Cl^-)_{plasma},$$

whence, since the concentration of plasma chloride is greater than that of cell chloride, it follows that the hydrogen-ion concentration is greater in the cells than in the plasma, in accordance with experimental fact.

The same reasoning can be extended to include bicarbonate and other diffusible ions, and it can be shown that the ratio between chloride and bicarbonate in cells and plasma in equilibrium with each other must be roughly the same.

Van Slyke has represented graphically many of the experimental facts ascertained by himself and his co-workers in illuminative diagrams, in which the details are conveyed by the heights of various columns. Fig. 7 is modified from one of his diagrams, and illustrates many of the main facts concerning the exchanges between cells and plasma.

Each vertical column is built up by summing together the milli-equivalents of the constituents in it present for 1 kg. of *water* (*not of solution*). Thus, column 1 indicates that in reduced blood there is associated with 1 litre of water in the cells 163 milli-equivalents of basic ions, which, expressed

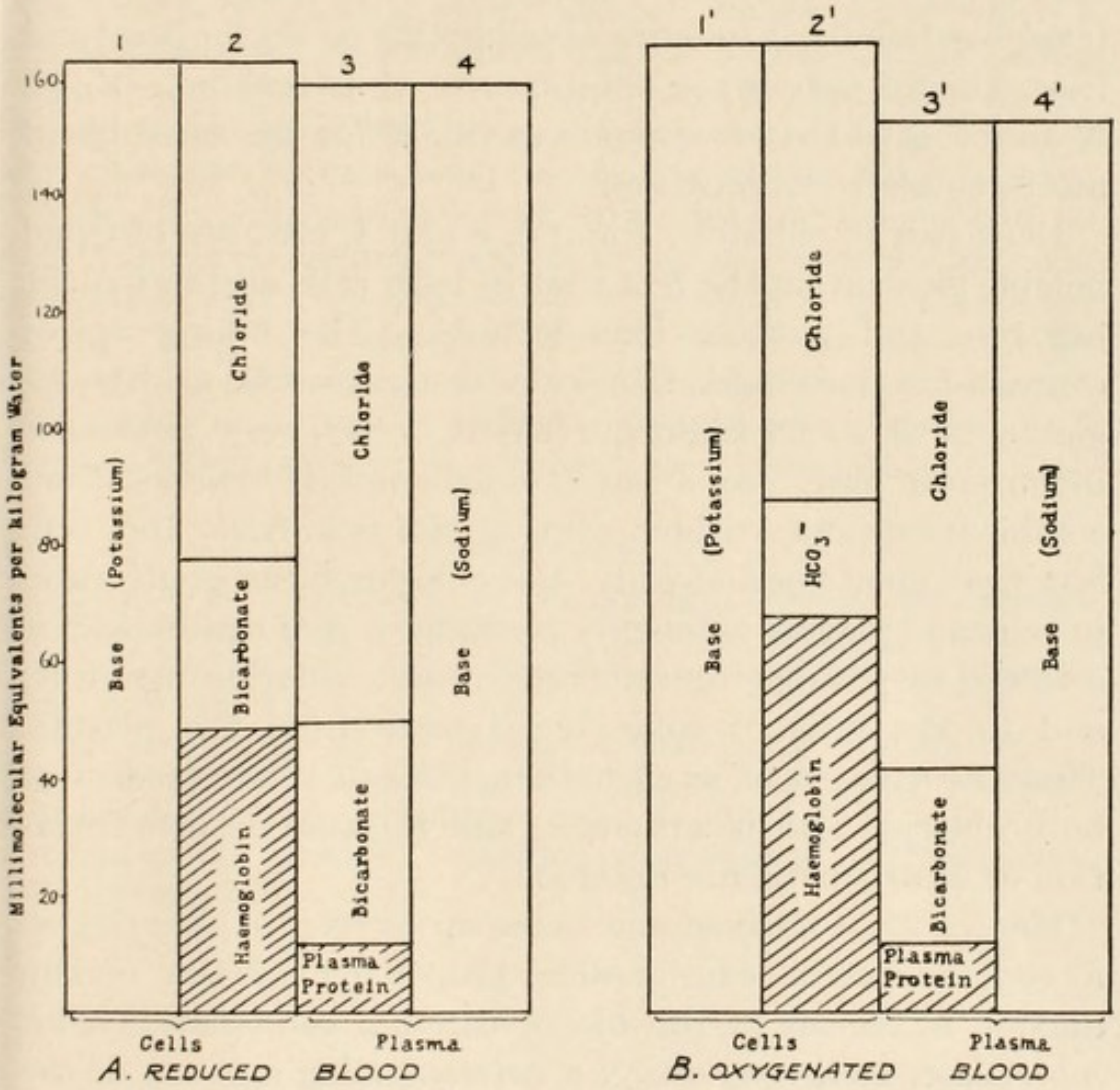

FIG. 7.—Distribution of base, bicarbonate and chloride in reduced and oxygenated blood. (After Van Slyke.)

as potassium, gives 0·163 × 39·1 gm. of potassium, *i.e.*, 6·4 gm. (Table XIII. shows that in the red cells somewhat more than 0·4 gm. of potassium is associated with about 60 gm. of water, a ratio which gives 6·6 gm. per litre of water.)

Column 2 shows that the potassium of the cell in reduced

blood is bound by 85 milli-equivalents of chloride ions, 29 of bicarbonate ions, and 49 of hæmoglobin. The hæmoglobin figures refer to the equivalent concentration of base found by actual experiment to be neutralised by the hæmoglobin.

Similarly, columns 3 and 4 give the corresponding concentrations of chloride, bicarbonate and plasma protein (expressed similarly in terms of combining power for base) and base (chiefly sodium) in the plasma, whilst columns 1′, 2′, 3′ and 4′ give the corresponding values for the same blood after complete oxygenation.

Each pair of columns, 1 and 2, 3 and 4, etc., are of equal height, illustrating the fact that in both cells and serum the negative and positive ions balance. The taking up of oxygen has increased the ionic concentration of haemoglobin in *B* to 68 milli-equivalents, which now neutralise much more base. As a result bicarbonate is loosened from combination and carbon dioxide diffuses from the cell. Since, as mentioned already, the chloride/bicarbonate ratio in cells and plasma is roughly constant, a new equilibrium is attained by passage from the cells of some chloride outwards, and to the cells of some bicarbonate from the plasma. Thus, as a result of oxygenation, the cell concentration of both chloride and bicarbonate, and the plasma concentration of bicarbonate, are lowered.

Hence, when hæmoglobin takes up oxygen, it sets free as a consequence a considerable proportion of the carbon dioxide in transit in the blood, through combination with more base, and, conversely, a corresponding amount of the carbon dioxide taken up by blood from the tissues is combined with alkali set free by loss of oxygen from haemoglobin.

The relative heights of the columns in *A* and *B* suggest that the concentration of ionic solute in the plasma of arterial blood is less, and that of the cells is greater, than the corresponding concentrations in venous blood. In other words, there is a shift of water from cells to plasma during oxygenation.

For further details of this aspect of the subject more specialised treatises must be consulted.

Acidosis and Alkalosis. The conditions of acidosis and alkalosis have been referred to (p. 226) and require further definition. Acidosis is a condition in which the body is less alkaline than it usually is under normal conditions; alkalosis is the opposite condition in which it is more alkaline. Conditions in which a transient acidosis exists include that of marked fatigue, when excessive amounts of lactic acid are formed in muscle and passed into the blood (*cf.* p. 321), there to be neutralised by blood bases and excreted (thus sometimes leading to depletion of blood base).

When the correct oxidation of fatty acids is interfered with, so that there is undue accumulation of β-hydroxybutyric acid and acetoacetic acid (and production of acetone) in the tissues (*cf.* p. 391), these acids also pass into the blood and are similarly neutralised and excreted; this condition is termed *ketosis*, and may or may not cause an acidosis. The presence of acetone and the neutralised acids in the urine is termed *ketonuria* (*cf.* p. 254).

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CHAPTER XVII

THE CHEMISTRY OF THE EXCRETA

Introductory Note. The blood receives from the intestines glucose, amino-acids, neutral fats, and certain other fairly simple chemical compounds, and carries these throughout the body. Along with them it carries to the various tissues a supply of oxygen obtained through the lungs. The tissues remove these constituents and replace them by excretory products. Logically, we should next consider the chemical changes by which the food material is transformed into these excretory products, but this is that portion of our subject about which at present we have least definite information. It will therefore repay us at this stage to study first these excretory products, and then, knowing the material furnished to the cells, and the material they ultimately produce, we shall be in a better position to consider how the one series of compounds is transformed into the other.

The blood carries these excretory products, carbon dioxide, urea, creatinine, etc., to special tissues from which they either diffuse or are secreted on to an outer surface of the body. There are five different channels of excretion, the *liver*, which excretes *bile* into the alimentary canal, the *intestinal mucosa*, the *lungs*, which excrete carbon dioxide and water, the *skin*, which excretes sweat, and the *kidneys*, which excrete a complex aqueous solution, the *urine*.

The Liver and Bile

Bile has already been dealt with (Chapter XI.) as a *secretion* containing bile salts and other compounds of service in

digestion. We have now to consider this liquid as an *excretion* containing the bile pigments and cholesterol.

The liver is a great warehouse of spare parts. Worn out red blood cells come to it from the spleen and are broken up. The hæmoglobin, freed from them, is split into its components, globin and hæm. Possibly some part of these is used in rebuilding fresh hæmoglobin after transference to the necessary site of that rebuilding. Certainly the iron contained is largely saved to the body for this operation. But some of the hæm, at any rate, is broken down to the bile pigments, and any cause—including such unusual ones as the inhalation of arseniuretted hydrogen, AsH_3—which leads to the freeing of hæmoglobin, leads to an increase of bile pigments from the liver. These bile pigments consist mainly of bilirubin and biliverdin.

Mann has recently measured the bilirubin content of arterial and venous blood in all the large organs and vascular areas, and finds the content of venous blood exceeds that of arterial blood most greatly in the circulation of the spleen and bone-marrow, whence he concluded that these two types of tissue are the important sites of bilirubin formation.

Bilirubin is a crystalline, golden-red compound, having the formula $C_{33}H_{36}N_4O_6$ or $C_{34}H_{38}N_4O_6$, and closely related to haem, $C_{34}H_{33}N_4O_5Fe$, and haematoporphyrin, the last compound having the same empirical formula as bilirubin itself. Bilirubin is easily oxidised to *biliverdin*, a green crystalline compound with the probable formula $C_{33}H_{36}N_4O_8$. Still further oxidation results in a series of coloured compounds, including *bilicyanin*, a blue pigment. The colour of faeces is only due to bilirubin and biliverdin when these rapidly pass through the intestine, as in diarrhoea. Usually bacterial action reduces them to *stercobilin*, a brown pigment, to which is due the colour of normal faeces.

When the skin is bruised the charming series of colour changes such as are well observed in a "black-eye," are due to the break-

ing down of hæmoglobin and the resulting conversion of hæmatin into compounds which are analogous to, or identical with, the bile pigments. But although many tissues seem capable of producing these compounds, whether the bilirubin and its derivatives are produced mainly in the liver, or elsewhere, they are in large part excreted through the liver by way of the bile. If the bile duct is obstructed experimentally by tying, the bile pigments leave the liver by way of the hepatic vein. In obstructive jaundice through the same cause the same result follows.

Liver extracts and skin extracts have been found to be equally potent in breaking down hæmoglobin, but a mixture of extracts of liver and spleen is still more potent.

The bile pigments are closely related to *urobilinogen*, a pigment present in urine, and undoubtedly derived from the small amounts of these compounds present in the circulation.

The other important excretory compound in the bile is *cholesterol*. Probably the gall-bladder exercises a regulatory action on this excretion. Undoubtedly during storage of bile in the gall-bladder it is greatly concentrated, and the solid content increases from about 3 per cent. up to a maximum of 20 per cent. (see Chapter XI.). It seems not improbable that some cholesterol is absorbed along with the water, so that there will thus be a certain degree of circulation of cholesterol. Bile is the essential channel of excretion of cholesterol on account of the presence in it of the bile salts (glycocholate and taurocholate), since their solutions (and bile can be regarded as a solution of these salts) are the only solutions in the body which can dissolve appreciable quantities of cholesterol. (If cholesterol is reabsorbed in the gall-bladder then probably some bile salts must also be reabsorbed with it.)

Cholesterol is found in the sterile fæces of the new-born and in fæces during starvation (when the bacterial content of the gut is greatly diminished), but normally in the intestine, through bacterial action, it is partly converted by reduction into *coprosterol*, a normal constituent of faeces.

Bile probably acts also as the excretory channel of certain toxic compounds and metallic poisons. The small proportion of bile salts which escapes reabsorption in the intestine is broken down, and the cholic acid reduced by bacterial action and excreted as *dyslysine*, $C_{24}H_{36}O_3$.

The Intestinal Mucus

During fasting the fæces contain a small but definite amount of fatty acids, which must, therefore, be regarded as a normal excretion, either directly through the intestinal mucus, or by way of the bile.

Salts of calcium, iron, and other metals, are excreted through the intestinal mucus. Excess of calcium is got rid of in this way to a greater extent than through the kidneys. It is excreted as phosphate or oxalate.

(Naturally the fæces will contain traces of protein and enzymes from the various digestive secretions poured into the intestine.)

N.B.—All the excreta from the bile and intestinal mucus, it must be remembered, are from *within* the body, and are to be carefully distinguished from food residues which have simply passed through the alimentary canal, and from products of bacterial action within the intestines and the living and dead bacteria of the fæces, which are not true excreta from the body itself.

The Lungs

The great part of the carbon dioxide formed in the body is excreted through the lungs. Since all the expired air is saturated with water vapour (though inspired air only contains traces of it) the lungs also form a channel of excretion of water which is by no means negligible. An adult person excretes per twenty-four hours through the lungs about 1·5 kilograms of carbon dioxide and 600 gm. of water.

The Skin, Sweat (and Sebum)

Sweat, the secretion of the sweat glands of the skin, is never quite free from epidermal cells and fat of the sebum. It is a liquid of specific gravity between 1·001 and 1·010, and is usually stated to be acid in reaction in man, though freshly secreted human sweat after pilocarpine injection and the sweat of domestic animals, such as the cat, goat and horse, is alkaline. It contains from 97 to 99·6 per cent. of water, between 0·3 and 1·4 per cent. of sodium chloride, and traces of urea, neutral fat, cholesterol, volatile fatty acids, etc. At high atmospheric temperatures the daily secretion of sweat is greatly increased, and with this increased secretion the amount of sodium chloride and urea excreted through the channel of the skin is also greatly increased. In certain pathological conditions, such as the anuria of cholera and uræmia, urea may be excreted in such amounts that crystals of it can be found on the skin surface. Glucose may be excreted through the skin in diabetes mellitus.

The main channels of water excretion from the body are the skin and kidneys, and the main effect of increase of external temperature on water excretion is diminution of urine volume and increase of sweat volume, the two channels acting reciprocally.

Various enzymes are present in the skin, a diastase, a lipase, a protease, and an ereptase having been identified.

Stale sweat is found to contain traces of formic, acetic, propionic, butyric, isovalerianic and caprylic acid. These are probably excreted in combination with glycerol, and if allowed to remain in contact with the skin are decomposed by an esterase (perhaps from bacteria), whence the odour of the great unwashed.

In amphibians, such as the frog, the skin is an important channel of respiration, and frogs can obtain at least 50 per cent. of their oxygen requirements and lose a corresponding amount of carbon dioxide through this channel. In mammals the oxygen

intake through the skin is very slight ; in man it is certainly less than 1 per cent. of that obtained through the lungs. At ordinary room temperature the amount of carbon dioxide excreted through human skin is negligibly small, but it shows a definite increase when the skin temperature rises above 33° C., at which point " visible sweating " commences. Between 29° and 33° the output is 0·35 gram, equivalent to 185 c.c. per hour, while at 38·5° it is 1·2 grams per hour.

Consequently there is a distinct increase with muscular exercise. The amount excreted per twenty-four hours is about 1·5 per cent. of that excreted through the lungs. A large part of this excretion must be regarded as taking place through the medium of a liquid saturated with the gas.

Sebum, the secretion of the sebaceous glands, is a secretion for skin protection, and consists in large part of cholesterol esters (*e.g.*, lanoline, from sheep's wool, is chiefly the mixed oleate, palmitate and stearate of cholesterol). Other compounds appear to be present in smaller amounts—esters of higher monatomic alcohols with higher fatty acids.

The Urine

The Secretion of Urine. The mechanism of kidney secretion probably belongs more to a course of Physiology than to one of Biochemistry. It may be mentioned very briefly that the prevailing theory is that the glomeruli of the kidney act as filters, which permit passage of all small molecules, and that the protein-free " plasma " so produced is then subjected to selective absorption while passing through the tubules, the greater part of the material still useful to the body being there reabsorbed. The mechanism of the selective absorption exercised by the tubular epithelium has not yet been explained.

The Reaction of Normal Urine. The reaction of a twenty-four hours' sample of urine is usually acid, though urine taken at varying times during the day may show a very varying degree of acidity, and is frequently slightly alkaline when

voided just after a meal—the so-called " alkaline tide." This change mirrors the necessary balance involved in the preservation of a constant *p*H in blood and tissues, along with the production of a markedly acid secretion, the gastric juice.

The acidity of the urine can be measured in two ways, " dynamically," so to speak, by finding out how much alkali is required to cause the solution to turn phenolphthalein red, *i.e.*, to bring the solution to a *p*H of 9, and " statically," by measuring the *p*H directly, by the colorimetric or electrometric methods. There is no direct relationship between the two results, since the urine contains varying amounts of phosphates, carbonates, etc., which all act as buffers, and which, present in different urines in different proportions, may require different amounts of alkali to produce the same change in *p*H value (from the actual value to that of the phenolphthalein reaction).

The normal variation of *p*H of urine is from 4·8 to 7·5, the average being about 6·0. In many pathological conditions the hydrogen-ion concentration is increased (the *p*H value is lowered). In the abnormal condition known as vegetarianism the average *p*H value is 6·6, *i.e.*, the reaction of the urine tends to approach that of herbivorous animals.

The Urine Specimen. In order to base any conclusions on the amounts of the various constituents present in a urine, they must be referred to a collection of at least twenty-four hours' duration, preserved by addition of a sufficiently powerful bactericidal agent such as toluene.

Inorganic Constituents of Urine. If urine were concentrated by boiling it down to small bulk, and then allowed to cool, sodium chloride would separate out as the chief inorganic constituent. We should be wrong, however, if we stated that sodium chloride is the chief inorganic constituent of the urine. Average normal urines contain about 1·5 per cent. of inorganic solids out of a total solid content of 4 or 5 per cent. At the actual dilution indicated by these figures most of the inorganic constituents are almost completely

ionised. It is therefore more correct to consider that these constituents are present chiefly as ions, and that there will be some slight amount of un-ionised molecules formed from every possible combination of these ions. The ions present are, in decreasing order of quantity, chloride, sodium, potassium, sulphate, phosphate, and smaller amounts of ammonium, calcium and magnesium, with traces of other elements.

We have seen that all these ions are present in blood, and, with the exception of ammonium, the amounts present show the same descending order.

It is interesting to remember that the element phosphorus was first discovered by the alchemist Brandt, of Hamburg, *in urine*, in 1669, while for a number of years this " phosphorus of urine " was prepared by a process elaborated by Robert Boyle, until Scheele discovered a method of preparing it from bone ash.

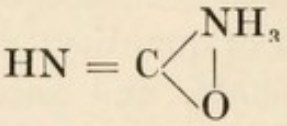
Organic Constituents of Urine. These are, in order of the amounts of them usually present, urea (2 to 2·5 per cent.), creatinine (0·1 per cent.), uric acid (0·05 per cent.), hippuric acid (0·05 per cent.), with much smaller quantities of thiocyanate, oxalate, indican, etc. In children, occasionally in women, and in many animals creatine is a normal constituent. The urine of most animals, other than man, contains allantoine, which largely replaces uric acid. Human urine contains but a trace of allantoine. In the urine of birds uric acid largely replaces urea.

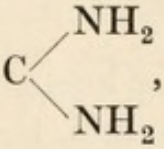
Many of these compounds contain nitrogen, and because of this the *total nitrogen* of the urine, and the *nitrogen partition* furnish important data in many metabolic studies and in many diseased conditions. The following figures may be taken as typical of a normal partition :

Urea-N, 84 per cent. ; ammonia-N, 3 ; creatinine-N, 7 ; uric acid-N, 4 : *rest*-N, 2 ; total, 100 per cent.

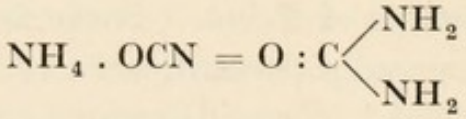
The most important constituents will now be dealt with.

Urea, CON_2H_4, is very soluble in water. It crystallises in long needles. According to E. A. Werner it has the constitution :

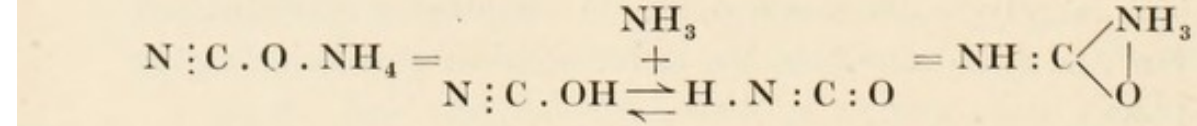


The old formula,  , suggests that it is the diamide derivative of carbonic acid, whence it was termed *carbamide*. This name, and this formula, must be discarded.

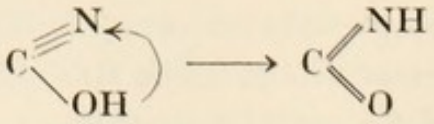
Wöhler originally synthetised urea from ammonium cyanate and represented the change as :



and suggested that this occurred by a simple molecular re-arrangement. Such a molecular re-arrangement would be by no means simple. Werner has brought forward strong experimental evidence that the actual change is :



which merely involves the slight molecular re-arrangement :

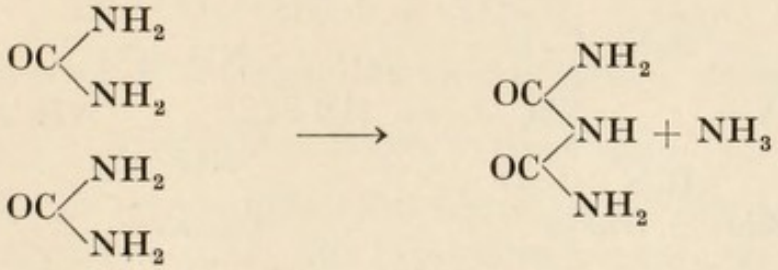


Enol-form of cyanic acid *Keto-form of cyanic acid*

Such a migration of hydrogen atoms, with formation of a keto- from an enol-form, or *vice versâ*, frequently occurs.

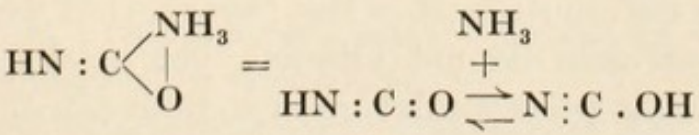
If Werner's theory is correct, the first change that occurs in the transformation of ammonium cyanate into urea is a dissociation into ammonia and cyanic acid. That such dissociation does occur is suggested by Liebig and Wöhler's own observation (which they omitted to interpret) that when ammonium cyanate was contained in a vessel *loosely covered with paper* within two days it was almost entirely converted into urea, *whilst ammonia was continuously given off* (so that dissociation must have occurred), but, on the other hand, if ammonium cyanate was left standing in a bell-jar over mercury and in an atmosphere of ammonia (which, through the law of mass action would tend to prevent such a dissociation), the cyanate remained unaltered after eight days. Further, in absence of water ammonium cyanate does not change to urea, and it is well recognised that water promotes, if it be not absolutely essential for, dissociation.

Formation of Biuret. The formation of biuret may be considered as additional evidence of the correctness of Werner's views. When urea is heated biuret is formed. According to the old structural formula of urea this change is easily accomplished :

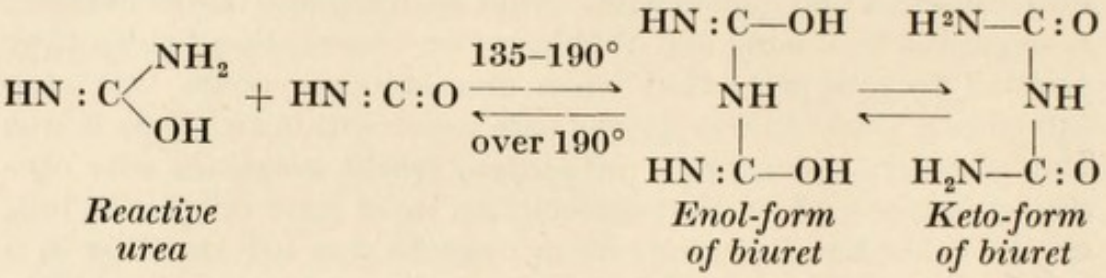


But biuret is only one of the products, though it is actually formed in relatively greatest amount provided the temperature does not exceed 190° C. In addition *cyanuric acid*, *cyanic acid*, and *ammelide* are produced ; their production cannot easily be explained by the old formula. Werner has shown that the enolic form of cyanic acid is stable only at low temperatures, while the keto-form is stable at high temperatures. His explanation for the series of changes is :

(i.) Application of heat breaks up the urea into ammonia and cyanic acid, and an equilibrium is established between the two forms of the latter :

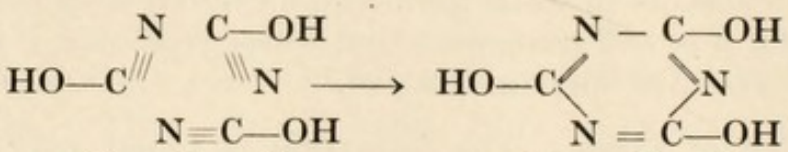


(ii.) Interaction of cyanic acid with unchanged urea gives biuret :



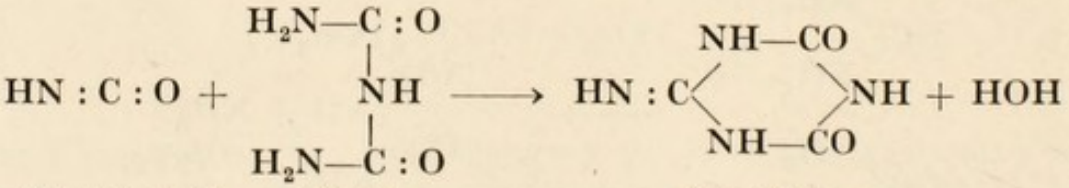
Reactive urea — *Enol-form of biuret* — *Keto-form of biuret*

(iii.) Some of the cyanic acid polymerises to cyanuric acid, which is more stable than biuret at high temperatures :



Cyanic acid (3 molecules) — *Cyanuric acid*

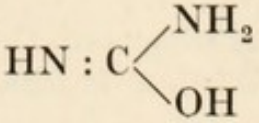
(iv.) Interaction of biuret and cyanic acid gives ammelide another fairly stable compound at higher temperature :



Cyanic acid — *Biuret* — *Ammelide*

When *biuret* is heated between 195° and 198° urea, cyanuric acid and ammelide are formed, and the above series of changes explains their formation.

Werner considers that in presence of strong mineral acid (that is, of a sufficiently high hydrogen-ion concentration) urea behaves according to the formula :



and points out, in support of this theory, that while urea and pure nitrous acid do not interact, yet in the presence of strong acid (as when a mixture of sodium nitrite and hydro-

chloric acid is used, as is usual, to produce the nitrous acid) there is a vigorous reaction. His " reactive " formula also explains why only one series of salts can be obtained from urea, as, for example, one urea nitrate,

$$HN : C\begin{cases} NH_2, HNO_3 \\ OH \end{cases}$$

and not two series, as the old formula would suggest is possible.

He has also shown that in the permanganate oxidation of amino-acids the formation of cyanic acid can easily occur through the intermediate stages of formamide, H . CO . NH_2, and oxamic acid.

Two important properties of cyanic acid must be stressed. At blood temperature it is, in the presence of water, quantitatively hydrolysed to urea :

$$2HNCO + HOH = CON_2H_4 + CO_2$$

In the presence of ammonia the yield of urea is doubled :

$$2HNCO + 2NH_3 = 2CON_2H_4.$$

Urea forms compounds with nitric and oxalic acids that are only slightly soluble, and crystallise in easily recognisable forms, and which therefore serve as an excellent test for urea. The formula for the nitrate has just been given. That for the oxalate is

$$\begin{array}{l} NH_2, HOOC \\ | \\ HN = C \\ | \\ OH \end{array} \qquad \begin{array}{l} COOH, H_2N \\ | \\ C = NH \\ | \\ HO \end{array}$$

Urea unites with two molecules of xanthydrol, $CHOH : (C_6H_4)_2 : O$, to form the extremely insoluble dixanthylurea. On this reaction is based an extremely delicate method for its estimation.

Urea reacts characteristically and almost quantitatively

with sodium hypobromite, a marked effervescence indicating the liberation of carbon dioxide and nitrogen :

$$CON_2H_4 + 3NaOBr \rightleftharpoons CO_2 + N_2 + 3NaBr + 2H_2O.$$

Decomposition of Urea by Urease. One other phase of the biochemistry of urea must be briefly discussed. When urine is left exposed to air without addition of an antiseptic it becomes strongly alkaline and strongly ammoniacal. The urea has changed to ammonia. It has been shown that this is due to a specific enzyme *urease*, which is not only present in the *Micrococcus ureæ*, the specific bacterium responsible for this fermentation of urine, but in many other micro-organisms, and in many plant tissues, such as those of the soy bean. Its presence has even been claimed in animal tissues, for Luck states that he has obtained very active preparations of the enzyme from the gastric mucosa of carnivores.

Werner considers that the action of urease is a specific example of the enzyme initiating a reaction. He explains the action as taking place through the intermediate stage of cyanic acid, and Fearon claims to have isolated the silver cyanate by addition of silver salts during the action. According to Werner urease adsorbs urea, and the latter then dissociates, the ammonia combining with the enzyme, and the cyanic acid reacting with water to give more ammonia. This theory is not generally regarded as proved. Yamasaki (1920) claimed that ammonium carbonate is formed, as the intermediate product, and his work has been recently confirmed by Sumner, Hand and Holloway (1931), who state definitely that cyanate is not formed.

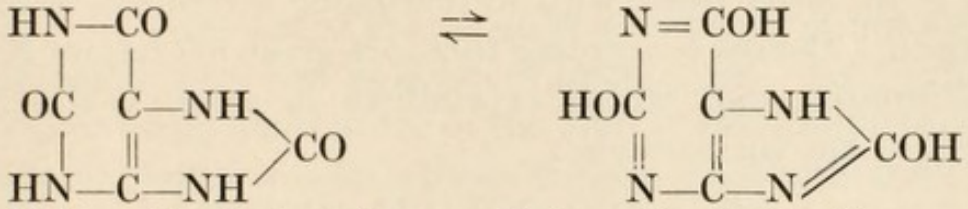
Mack and Villars have shown that urease acts on concentrated solutions of ammonium carbonate with formation of some urea. Nevertheless the process is almost quantitative in the direction of ammonia formation, and this quantitative reaction therefore is employed in the estimation of urea in urine and blood.

Urea is widely distributed in plants, many of which are capable of forming it. The same plants usually contain urease, and the function of the enzyme appears to be the reconversion of the useless urea (probably a by-product of some essential reaction) into ammonium compounds that can be utilised by the plant.

The wide distribution of urease permits the economic transference of nitrogen from animals to plants, completing the cycle of nitrogen transformations in living organisms.

Urease has been prepared in crystalline form by Sumner (1926), and is found to behave as a globulin. A microphotograph of urease crystals is shown in Fig. 1.

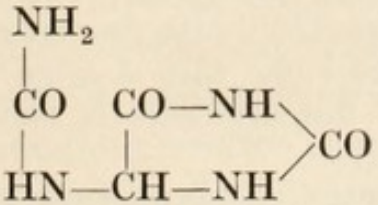
Uric acid, $C_5H_4N_4O_3$, is a purine derivative, which exists in solution in two forms, *lactam*, and *lactim*, in equilibria with each other :



Uric acid : lactam form *Uric acid : lactim form*

Uric acid is only soluble in water at blood temperature to the extent of one part in 15,000. It forms colourless crystals when deposited from such a solution, or by slightly acidifying an alkaline solution. When deposited from a distinctly acid urine it adsorbs uroerythrin (see p. 253) and so appears brownish-red. It gives two series of salts. The mono-urates are but slightly soluble, the dibasic salts more so ; one part of the dibasic sodium salt dissolves in 77 parts of water at 18° C.

In man uric acid is the final stage of oxidation of the purines liberated from digestion of nucleic acids ; most mammals can oxidise it still further to allantoine, $C_4H_6N_4O_3$:



Allantoine

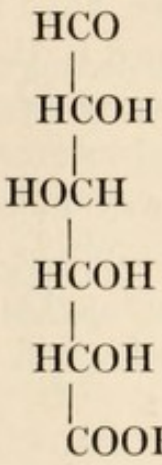
Allantoine is much more soluble than uric acid (to the extent of somewhat less than one part to 100 of water at 20° C.). The relations of uric acid and allantoine to other purine compounds will be dealt with in Chapter XXIV.

Creatinine, $HN=C\begin{cases}NH\text{———}\\ \\ N(CH_3)\text{—}CH_2\end{cases}\!\!\!

under different conditions, probably also brings about the synthesis.

When more is known of the comparative nitrogenous excretion in the different phyla of animals, it seems probable that considerable light may be thrown upon certain aspects of nitrogenous metabolism. In mammals the chief end-product is urea, in birds it is uric acid. Marine fishes excrete trimethylamine oxide, $(CH_3)_3N : O$, while the octopus excretes betaine $\overline{N(CH_3)_3.CH_2.COO}$

It has just been stated that a small percentage of ingested benzoic acid is excreted as a paired *glycuronate*. The formation of such compounds is one of the most important methods the body employs to render various toxic compounds harmless to itself. *Glycuronic acid*—



is a derivative of glucose (see p. 58), still retaining the aldehyde (or potential aldehyde) radical. The acid itself is dextro-rotatory, though most of its paired compounds are lævo-rotatory. In normal urine only traces (0·004 per cent.) of glycuronates are present. The output may be very greatly increased as a result of administration of antipyrine, camphor, morphine and similar compounds. In normal urine the chief representative is the derivative of phenol.

Most of the glycuronates reduce alkaline copper solutions, but they can be distinguished from glucose and similar sugars, since they are lævo-rotatory and are not fermented by yeast.

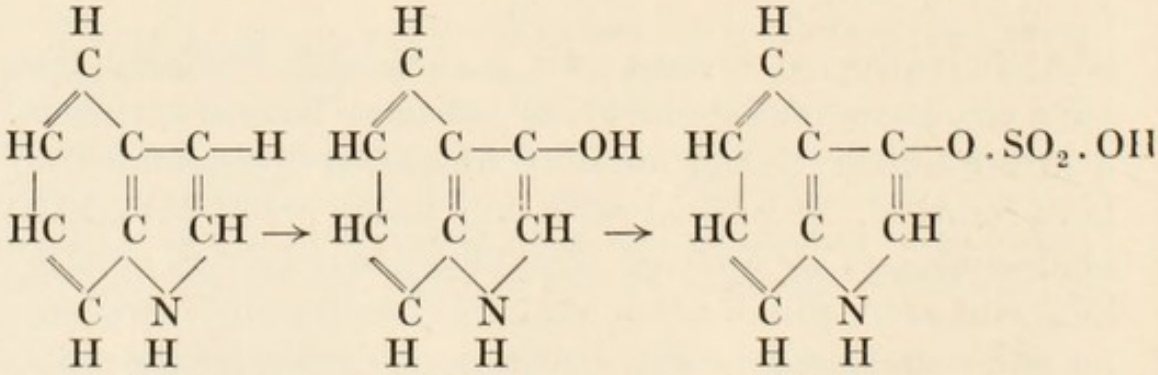
Ethereal Sulphates. If some barium chloride solution is added to urine, the inorganic sulphate present will be precipitated as barium sulphate. If this is filtered off, and the filtrate acidified with hydrochloric acid and warmed, after a minute or two more barium sulphate will be precipitated. This is due to the hydrolysis of the ethereal sulphates, and the change can be represented :

$$HO.SO_2.OR + HOH = H_2SO_4 + ROH$$

where *R* is an organic radical.

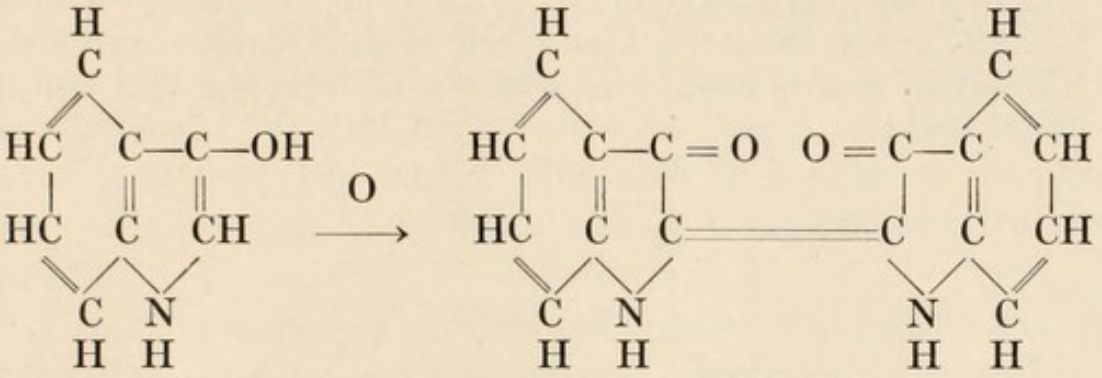
The total output of ethereal sulphates, calculated as SO_3, in normal urine does not exceed 0·25 gram per day.

The most important of these compounds present in urine are phenol- and *p*-cresol-sulphuric acid, and indoxyl- and skatoxyl-sulphuric acid. We have seen (p. 168) that the phenol and *p*-cresol are formed in the intestine by bacterial action on tyrosine. Of the amounts absorbed a considerable proportion is united to sulphuric acid in the liver and so detoxicated. In the same way indole and skatole, formed by bacterial action on tryptophane, are in part absorbed, and this part is oxidised to indoxyl and skatoxyl in the liver, conjugated with sulphuric acid, and excreted as the neutral salts of the acids so formed :



The amount of indican present in the urine gives some clue to the extent of bacterial action in the intestine ; there is a decided increase in conditions of intestinal stasis.

Indican can be easily tested for in urine by converting it into *indigo*. The urine is treated with concentrated hydrochloric acid, which splits off sulphuric acid, leaving indoxyl, and this, treated with an oxidising agent such as bleaching powder, is oxidised to *indigo blue*.



Indoxyl *Indigo-blue*

Urorosein. In various pathological conditions, such as pulmonary tuberculosis, typhoid and nephritis, and perhaps also to a very slight extent normally, urine contains a precursor of indole, *indole acetic acid* (see p. 170), also formed by bacterial action on tryptophane. This is the chromogen of urorosein, and is converted to this rose-red compound—whose constitution is not yet known—by the action of nitrous acid.

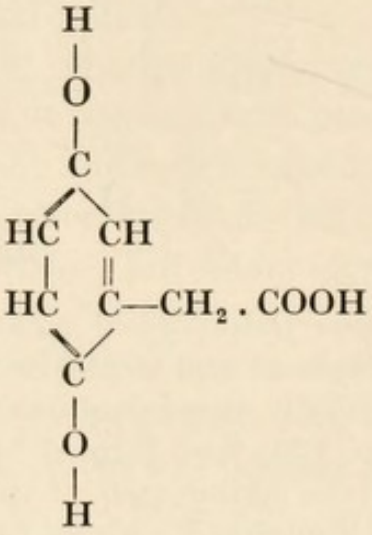
Neutral Sulphur Compounds. In addition to inorganic and ethereal sulphates, a few per cent. of the total sulphur excretion in urine is made up of the so-called " neutral sulphur compounds," the excreted sulphur from this source amounting to about 0·1 gram per day. These sulphur-containing compounds are cystine, oxy-proteic, and alloxy-proteic acid (intermediate oxidation products of proteins, which contain respectively 1 and 2 per cent. of sulphur), methyl mercaptan, $CH_3 . SH$. thiocyanates, taurine derivatives, etc.

Amino-acids are always present in small amount, from 0·4 to 1 gram per day.

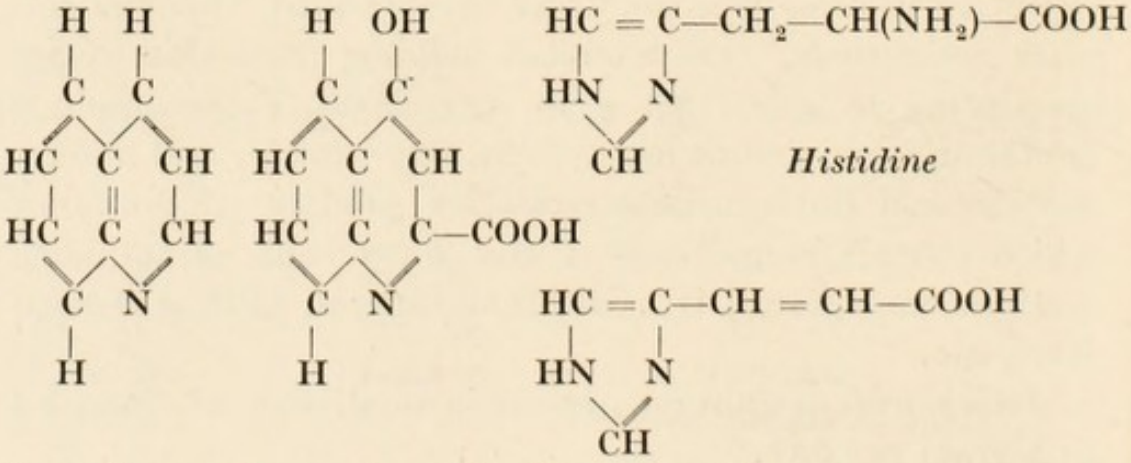
Hydroxy-acids and Related Compounds. Parahydroxyphenylacetic acid, $HO . C_6H_4 . CH_2 . COOH$, and parahydroxyphenyl, propionic acid, $HO . C_6H_4 . CH_2 . CH_2 . COOH$, are present in

urine in small amount, having been formed by bacterial decomposition of tyrosine in the intestine, and, to the extent to which they are absorbed, excreted through the kidneys.

The somewhat similar compound, *homogentisic acid*, is not a normal constituent of urine, but occurs in an abnormal (but not pathological) condition known as *alkaptonuria*, in urine, and is formed *in the body* from tyrosine. If to a urine containing this acid is added a little alkali the urine slowly turns greenish brown from the surface downwards. The acid can be regarded as a quinol acetic acid :



Two interesting acids, absent from human, but present in dog's urine, are kynurenic and urocanic acids. The latter is obviously derived from histidine, while the former is a derivative of quinoline :



Quinoline *Kynurenic acid* *Urocanic acid* *Histidine*

Raistrick has shown that urocanic acid is formed from histidine by bacteria of the coli-typhus type.

Oxalates and Citrates. In each twenty-four hours about 15 to 20 mg. of oxalic acid are excreted in the urine. Although calcium ions are present, and calcium oxalate is extremely insoluble, yet in the slightly acid phosphate medium of the urine calcium oxalate is not normally precipitated. The oxalate is in part derived from oxalate of the food, and is in part formed in the body (perhaps from other ingested organic acids, perhaps from carbohydrate catabolism). Normal urine contains traces of citric acid, averaging 0·07 per cent. This acid is present in minute amounts in most body fluids and is a normal constituent of milk.

Urine Pigments. The three important urine pigments are *urochrome*, *urobilin* and *uroerythrin*. Urochrome is present in greatest amount, and is chiefly responsible for the yellow colour of the urine. It is a derivative of urobilin, and can be derived from it by the evaporation of its solution in aqueous-ether, during which the transformation takes place. Urochrome may be identical with *lactochrome*, a yellow pigment present in milk whey.

In freshly voided urine occurs *urobilinogen*, a colourless precursor of urobilin, which is transformed to the latter apparently by the action of light. Urobilin is very similar to, if not identical with, hydro-bilirubin (stercobilin), a reduction product of bilirubin (see p. 235).

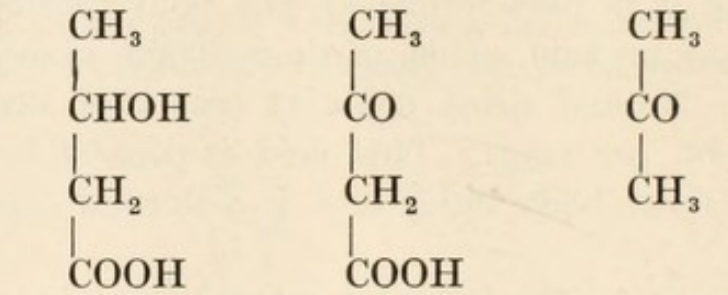
Uroerythrin is frequently present in small amount, and the red colour of urinary pigments is due to it. Little is known of its composition. It may be derived from melanins.

Pathological Constituents of Urine

Glucose is usually considered to be present in normal urine, but only in minute traces of the order 0·01 per cent., although such urine shows a reducing power equivalent to between 0·05 and 0·07 per cent. of glucose. The difference is due to traces of pentoses and other reducing substances. In

diabetes and certain other conditions glucose is excreted in large amount.

The "acetone bodies," *acetone*, *β-hydroxybutyric acid*, and *aceto-acetic acid*, are not detectable in normal urine, but are found present in many conditions in which an acidosis is present.



β-hydroxy-butyric acid — *Aceto-acetic acid* — *Acetone*

Lactic acid, $CH_3 . CHOH . COOH$, is excreted in various pathological conditions which involve diminished oxidation in the tissues, and also, normally, after prolonged fatiguing exercise, in which also an oxygen deficiency in the muscles can be assumed.

The *proteins* of the blood plasma are present in urine in such conditions of damaged kidney function as permit their passage through the kidney filter.

Reference.

WERNER, E. A., "The Chemistry of Urea." London, Longman's, 1923.

SECTION IV

INTERMEDIATE METABOLISM

CHAPTER XVIII

THE CHEMICAL COMPOSITION OF THE TISSUES

Some further assistance in a consideration of the intermediate metabolism of the body may be obtained from a knowledge of the most important compounds present in the different types of body-tissues.

As typical differing types will be considered (*a*) muscle tissue, (*b*) bone and tendon, (*c*) nerve, (*d*) glandular tissue, and (*e*) cutaneous tissue, etc. Since, further, our knowledge of the *internal secretions* is largely derived from study of the tissues that manufacture them, it will be convenient (in the succeeding chapter) to discuss the glands of internal secretion, and the secretions themselves.

The Constituents of Muscle

The compounds present in muscle tissue (from which all fat has been removed) may be conveniently divided into water, proteins, enzymes, and simple extractives.

The approximate composition is given in Table XVI.

Muscle Proteins. Muscle contains several proteins. About 70 to 75 per cent. of these appear to consist of *myosin*, a globulin, although only 25 to 35 per cent. can be extracted in that form. Some 25 to 30 per cent. of the proteins consists of a very soluble protein *myogen*.

Myosin forms the rod-like structural elements of the

TABLE XVI. COMPOSITION OF MUSCLE PER 100 GRAMS

	Mammalian Muscle.	Bird Muscle.	Frog Muscle.
	Gm.	Gm.	Gm.
Water	78·3–72·2	77·5–71·8	80·0
Solids	21·7–27·8	22·5–28·2	20·0
Inorganic compounds	1·0– 1·5	1·0– 1·9	1·0– 2·0
Organic compounds	20·7–26·3	21·5–26·3	18·0–19·0
Proteins	16·6–20·0	17·4–20·0	14·4–15·2
Creatine	0·3– 0·5	0·3– 0·5	0·2– 0·7
Creatinine	0·01		
Carnosine	0·2– 0·3		
Carnitine	0·02–0·03		
Purines	0·07–0·17	0·07– 0·13	0·05–0·09
Inosinic acid	0·01	0·01– 0·03	
Inositol	0·003		
Glutathione	0·06		
Glycogen	0·1– 3·7		

anisotropic discs of muscle. It can be extracted from ground mammalian muscle by cold dilute alkaline salt solution of *p*H 7·85, and can be purified by repeated precipitation and re-solution. Its solutions set into gels ; shaking transforms these to sols. On standing, gels again form.

Myosin has, between the *p*H limits of 6·4 and 7·3 (physiological conditions during muscular activity) a large capacity for binding acid or base, and is present in muscle in sufficient amount to neutralise half the lactic acid formed during fatigue.

Myogen is located in the sarcoplasm of muscle.

Muscle also contains proteins insoluble in water and neutral salts ; these include *muscle-stroma* (one or more proteins), and traces of nucleoprotein (from the cell-nuclei).

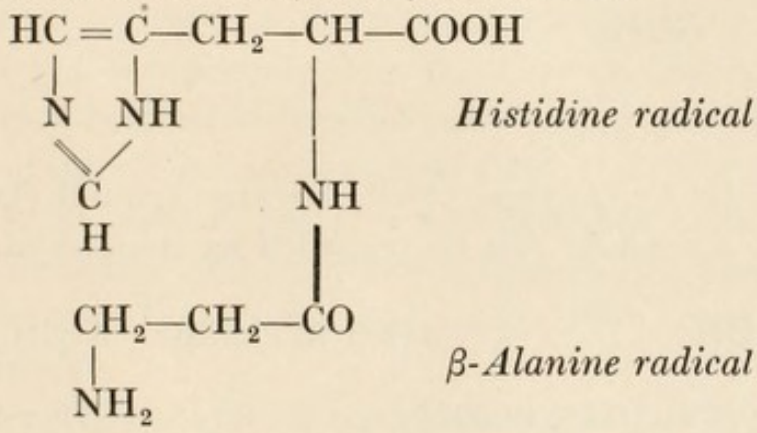
Muscle Enzymes. These include a catalase, a special diastase, a protease, a lipase, a xanthine oxidase, etc.

Simple Extractives from Muscle. A. *Nitrogenous Compounds.* The most important in point of the amount present

are, in order, creatine, carnosine, the purine bases, and carnitine. Traces of creatinine, urea, adenine nucleotide pyrophosphate (or adenine pyronucleotide), inosinic acid and glutathione are present.

Creatine and creatinine, the purine bases and adenine pyronucleotide and the related inosinic acid, will be dealt with respectively in Chapters XXV. and XXIV.

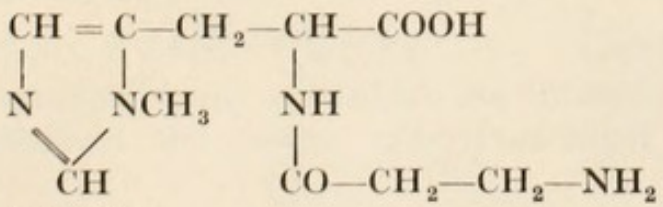
Carnosine (L. *caro, carnis*, flesh) was first isolated from muscle by Gulewitch in 1900 ; he showed that the compound, when hydrolysed, broke down to β-alanine and histidine. Its constitution was established by Bauman and Ingvaldsen, who showed that it was β-alanyl-histidine :



β-Alanine has not been found to occur naturally except when linked in the carnosine molecule. Although muscle contains an amount of this compound which must be considered far from negligible, its function is not yet known. It is soluble in water and dextro-rotatory.

Starvation lowers the carnosine content of muscle ; a meat diet restores it. A compound has recently been extracted from muscle which is possibly formed from carnosine and a carbohydrate derivative. Statements have been made that intravenous injections of carnosine stimulate secretion of gastric juice ; their truth is doubtful.

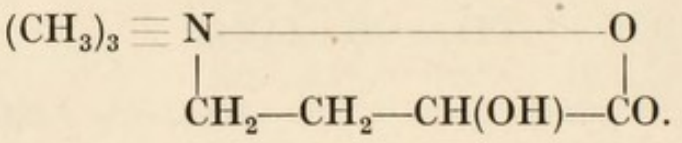
Methyl carnosine



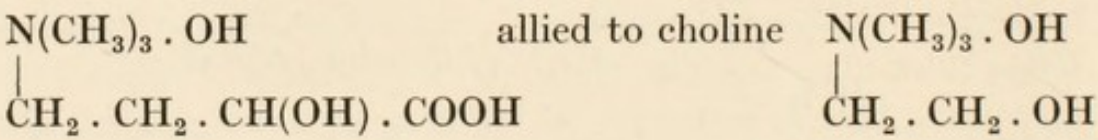
has been obtained from goose-muscle, and has therefore been named *anserin* (L. *anser*, goose). The maximum amount found was 0·12 per cent. It is absent from other tissues of the goose.

Anserin is also present in the muscle of fowl, pigeon and crow, and appears to replace carnosine in these birds. It is present along with carnosine in crocodile muscle, but is absent from the muscle of the dog-fish and beef muscle, although stated to be present in dog muscle. Carnosine is present in the muscle of pythons and the boa-constrictor.

Carnitine was isolated from beef extract by Gulewitch and Krimberg in 1905. It is a *betaïne*, and its formula has been established as :

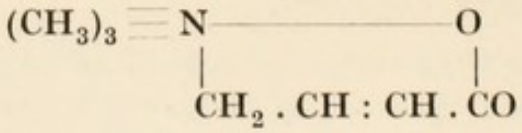


When it is hydrolysed with baryta trimethylamine is liberated. Carnitine can be regarded as the anhydride of

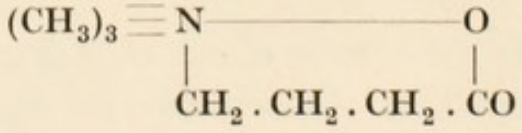


Nothing is as yet known of the function of carnitine.

The related croton betaïne



has recently been isolated from beef muscle. Feeding experiments have shown that some γ-butyrobetaïne

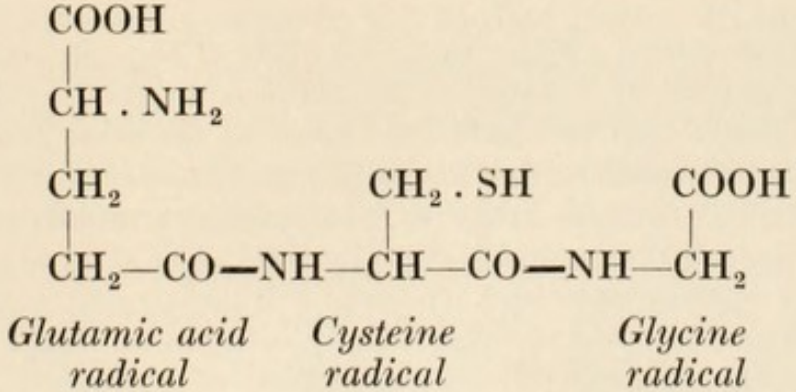


and less croton betaïne are oxidised to carnitine in the organism. These have a slight curare-like action, but carnitine is innocuous.

Glutathione was isolated from muscle in 1921 by Hopkins, and was at first believed to be the dipeptide cysteine-glutamic acid. de Rey-Pailhade had many years previously shown that tissues contained some compound, which he termed " philothion," possessing transferable hydrogen, and which could in consequence form hydrogen sulphide from sulphur compounds. Hopkins suggested the name glutathione, because " it leaves a link with the historic *philothion*, has the same termination as *peptone*, which has long served as a name for the simpler peptides, and is a sufficient reminder that the dipeptide contains glutamic acid linked to a sulphur compound."

In 1927 G. Hunter and Eagles questioned the dipeptide nature of the compound. Hopkins has in consequence re-studied the whole problem of its preparation and composition and has obtained it in crystalline form. It is a tripeptide, built up from glycine, cysteine and glutamic acid. Kendall has independently and by different procedures also obtained crystalline glutathione and has shown that it contains the same radicals.

Numerous investigators have attempted to determine the constitution of glutathione, and the balance of evidence favours the linkage of its constituent amino-acids in the order *glutamic acid—cysteine—glycine*, whilst it seems not improbable that its actual constitution is



Glutathione

Glutathione possesses important properties as an agent involved in oxidative processes. In these, "reduced glutathione" changes to "oxidised glutathione," by conversion of the cysteine radical into a cystine radical. Considering glutathione as a derivative of hydrogen sulphide, in which a hydrogen atom is replaced by a complex *G*, we can write :

$$2G . SH \rightleftharpoons GS . SG + 2H.$$

The changes in which glutathione takes part will be dealt with in the discussion on biological oxidations (see Chapter XXXII.).

Glutathione is found in many tissues. The striped muscle content is of the order 0·06 per cent., cardiac muscle 0·12, and smooth muscle 0·13. Liver contains more, up to 0·38 per cent., and it is present in similar amounts in most of the other body organs. Red blood cells contain amounts of the order 0·1 per cent. It is absent from blood plasma and from eggs, present in invertebrates and even in yeast (0·15 to 0·22 per cent.). There is evidence that it may be present in the seeds of some of the higher plants. The muscles of amphibians and of fishes contain much less than those of mammals. Some evidence suggests that it is present in relatively greater amount in the foetus, and shows a relative steady decrease with age.

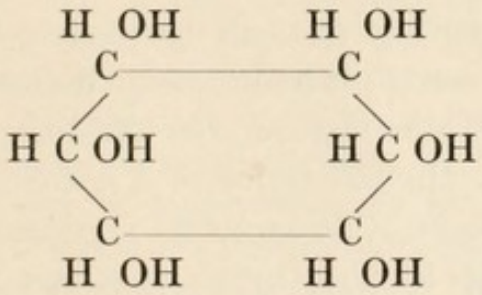
Methyl-guanidine, $NH_2 . C(: NH) . NH . CH_3$, is commonly stated to be present in muscle, but there is no good evidence for this statement. All methods that have so far been used for its isolation from muscle utilise reagents which, under the conditions employed, will convert creatine into methyl guanidine through the intermediate compound $NH_2 . C(: NH) . N(CH_3) . CO .COOH$, which has been isolated and studied by Bauman and Ingvaldsen. Dakin has also isolated an intermediate compound, $NH_2 . C(: NH). N(CH_3) . CH(OH) . COOH$, by the action of hydrogen peroxide on creatine.

Histamine (*cf.* p. 171), according to Dale and his co-workers,

can be obtained from muscle, liver, and lung by the simple procedure of mincing the tissue and extracting it with cold alcohol, thereafter subjecting the extract to usual chemical procedures. By such means 60 mg. of histamine have been isolated from 10 kg. of ox lung (a yield of 0·0006 per cent.). The actual amount present in the extract is probably much larger. Muscle contains much less histamine than lung, liver somewhat less.

Such amounts, if present in and liberated from tissues during life, would cause marked pharmacological action (*cf.* p. 172). This consideration suggests that in the living tissue histamine either cannot itself pass through the cell membrane, or else exists in loose state of combination with some other compound and so cannot pass the membrane. Best has shown that these tissues can destroy free histamine.

B. *Non-Nitrogenous Compounds. Inositol*, $C_6H_6(OH)_6$, H_2O, is not a carbohydrate (though in the anhydrous form its empirical formula is $C_6H_{12}O_6$) but a hexahydric alcohol, with the composition :



It is present in most tissues, and in traces in normal urine. It is widely distributed in plants, especially in the unripe beans of *Phaseolus vulgaris*, the kidney bean. In plants also occurs an inositol-phosphoric acid, whose calcium-magnesium salt is called *phytin*, and which is decomposed by the enzyme *phytase* present in these plants into inositol and phosphate. Growing plants contain more inositol than the full grown ; the organs of the growing animal are said to contain more of the compound than those of the adult. It has been suggested that the presence of inositol in animal tissues is merely accidental, through the decomposition in the body of phytin from the diet. There is recent evidence, however, to show

that it is actually formed from glucose by the growing animal (the egg-embryo).

Inositol from muscle crystallises well in octahedra, is moderately soluble in water, and has a slightly sweet taste. It dissolves cupric hydroxide in alkaline solution, but does not reduce it on boiling.

Various stereo-isomeric modifications of inositol are known. Muscle inositol is the inactive meso-form. *Scyllitol*, an isomer, is present in the kidneys and certain other organs of some fishes, and in a number of plants. Needham has shown that the embryo of the dog-fish *Acanthias* forms scyllitol, just as that of the chick forms inositol.

Glycogen occurs in muscle in varying quantities, according to the state of nutrition of the animal. In dog's muscle as much as 3·7 per cent. has been reported. After death it disappears very quickly.

Various statements have been made as to the presence of a *sugar* in muscle, but it has not been definitely shown that living muscle contains sugar. On the other hand, there is some evidence for the existence of a *hexose-diphosphate*, the so-called *lactacidogen* (lactic acid precursor) of Embden, formed, according to this biochemist, as an intermediate product in the transformation of glycogen to lactic acid.

Lactic acid, $CH_3 . CHOH . COOH$, is absent in detectable quantities from resting muscle, but is present in distinct amounts in fatigued muscle, and owing to its presence such muscle gives an acid reaction with litmus.

Mineral Constituents. Muscle ash contains, especially, potassium and phosphate. Smaller amounts of sodium and magnesium are present, and traces of calcium, chloride and iron. Sulphate is present in the ash, but is formed during ashing from sulphur of the proteins. Muscle of course contains carbonate, and can be regarded as saturated with carbon dioxide.

The Constituents of Bone and Connective Tissues

Bone consists of water (20 to 24 per cent.) and about equal parts of organic and inorganic material. The organic matter is chiefly *ossein*, which would seem to be the same compound as collagen from fibrous tissue, and which yields gelatin on boiling with dilute mineral acids. A mucoid and a scleroprotein are also present.

The inorganic constituents of bone can be regarded as consisting almost entirely of a mixture of calcium phosphate and carbonate, in the proportion of three to (somewhat less than) one, built together into some complex along with traces of the corresponding magnesium compounds and of chloride and fluoride. No definite clue to the constitution of this complex has as yet been obtained, though the proportions of the constituents are very constant.

Roseberry, Hastings, and Morse (1931) from X-ray examination of bone conclude that it has a crystalline structure, which is fundamentally the same as that of the apatite minerals. By such examination, and by chemical analysis, it seems to be very similar, as far as its inorganic constituents are concerned, to the mineral dahlite, and the calcium salts of both bone and the enamel of teeth can be represented by the formula $CaCO_3 \cdot n\ Ca_3(PO_4)_2$, where n is not less than 2, nor greater than 3.

Recent chemical work is not in complete agreement. Klement and Trömel consider that over 90 per cent. of the solid material of bone consists of a basic calcium phosphate, $Ca_{10}(PO_4)_6(OH)_2$, which they term *hydroxylapatite*. Morgulis holds somewhat similar views.

The *cement* and *dentine* of teeth possess practically the same inorganic chemical composition as bone. *Enamel* of teeth is the hardest substance in the body, and contains the least percentage of water (3 to 10). It contains relatively less magnesium and more fluorine than does dentine.

The shells of marine and land invertebrates, the secreted tubes of marine worms, and the eggshells of birds, consist almost entirely of calcium carbonate. But they always contain some magnesium and a trace of phosphate. There is good ground for

the belief that the presence of phosphate is absolutely essential for both shell and bone formation, and that a calcium carbonate-phosphate complex is initially formed, and laid down as such in bone, but changed after secretion by the mantle cells of invertebrates to calcium carbonate, which is deposited first in amorphous form, and which subsequently crystallises to aragonite or calcite according to the species of animal.

The skeleton of the mammal is not only a supporting structure, but is also a reservoir of calcium and phosphate. Calcium can not only easily be laid down in the skeleton, but is easily removed. Bone minerals are in a state of flux, being continuously removed, and renewed, and not once and for all laid down and fixed. During lactation the cow can lose from its body nearly 20 per cent. of its bone-calcium, and similar losses have been shown for other animals. Probably bearing on this point are observations that the bones of virgin female rats contain, for animals of equal weight, more calcium than do the corresponding bones of male rats.

We do not know why the complex of calcium, magnesium, carbonate, phosphate and fluoride is so efficient in the skeleton and, with slight changes in their ratios, in the different parts of teeth, though there is some evidence that an increase in carbonate makes for hardness, and certainly this seems to hold with an increase in fluoride; it has been stated that an increase in magnesium content makes certain marine shell structures more compact.

Robison has shown that many tissues, but especially bonic teeth and kidney, contain an enzyme, a *phosphatase*, that can hydrolyse hexose phosphates, liberating inorganic phosphate. It has no action on lecithin. It is absent from purely cartilaginous tissue. This enzyme would appear to play an important *rôle* in bringing about a local increase in phosphate ions, and so facilitating deposition of calcium phosphate in growing bone. It acts best in a distinctly alkaline medium (*p*H 8·4).

Kay had developed a method for its estimation in blood. The content in blood plasma is normally relatively small, compared with that of bone or kidney, but in cases of generalised bone disease, such as generalised osteitis fibrosa,

osteomalacia, or rickets, it may increase to twenty times above normal, due, according to Kay, to leakage of the enzyme from the diseased tissue into the blood.

Klement and Trömel suggest the following mechanism for bone deposition : Phosphatase acts on the organic phosphate of the plasma, and there results an increase in HPO_4^{--} ions. These unite with calcium ions adsorbed on the organic matrix of bone and form acid calcium phosphate to such an extent that its solubility is exceeded and it is deposited as $CaHPO_4, 2H_2O$. This rapidly changes to hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, while simultaneously a small amount of calcium carbonate is deposited. Traces of other compounds are similarly included through the presence of the corresponding ions.

Of *connective tissue* white fibrous tissue and yellow elastic tissue contain from 57 to 67 per cent. of water. The principal solid constituent of the first is *collagen* (32 per cent.), of the second *elastin* (also 32 per cent.). Both of these compounds are scleroproteins. Collagen, on boiling with water, is changed to gelatin. Collagen contains, apparently, more tyrosine radicals than does gelatin, since the former gives a distinct, the latter only a very slight, Millon's reaction.

These tissues also contain a mucoid. Only a trace of inorganic material is present.

Cartilage contains *chondromucoid*, and its derivative, *chondroitin-sulphuric acid*. The latter, $C_{26}H_{48}O_{29}N_2S_2$, is found in various tissues. It hydrolyses with acids to chondroitin, a gum-like substance, and *chondrosine*, $C_{12}H_{21}O_{11}N$, which seems to be built up from glucuronic acid and *chondrosamine*, a galactosamine isomeric with glucosamine. In chondroitin sulphuric acid the chondrosamine is acetylated.

Cartilage also contains a scleroprotein similar to elastin and keratin.

Epithelial Tissue

Epithelial tissue, such as hair, horn, hoof, feathers, nails, and the epidermal layer of the skin, is composed chiefly of *keratins*, extremely insoluble proteins, of the scleroprotein

class, which contain relatively large amounts of leucine, cystine and tyrosine radicals.

According to Marston, in animals which produce hair or wool there is an excessively great demand for cystine for keratin production, the keratin of wool fibre containing 13·1 per cent. of cystine ; the cystine content of a diet may actually limit the growth of hair. Rimington's results are in general agreement.

The epidermal layer of human skin contains only 20 per cent. of water. Of the 2 per cent. fat it is not surprising that from one-eighth to one-quarter consists of cholesterol and its esters, when the nature of the sebaceous secretion is remembered. The ash content varies from 0·6 to 1·5 per cent. It invariably contains minute traces of silica, which decrease very slowly with age.

The Constituents of Nerve Tissue

Most of the studies of such material have been so far concerned with brain tissue, and since great difficulties are encountered in the chemical separation of many of the compounds present, our knowledge of this part of the subject is relatively obscure.

Typical analyses of human brain are given in Table XVII.

TABLE XVII. COMPOSITION OF HUMAN BRAIN (AFTER KOCH)

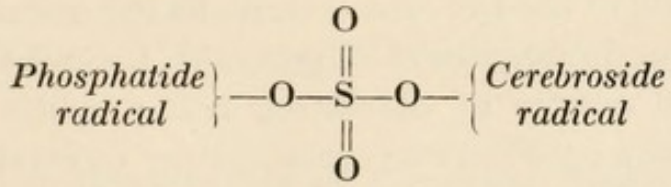
	Corpus callosum.	(Prefrontal) Cortex.
	Per cent.	Per cent.
Water	68·0	84·1
Proteins	3·2	5·0
Nucleoproteins . . .	3·7	3·0
Neurokeratin . . .	2·7	0·4
Water soluble extractives .	1·5	1·6
Lecithin	5·2	3·1
Kephalin	3·5	0·7
Phrenosin and Kerasin . .	4·6	1·6
Cholesterol	4·9	0·7
Sulphur containing compounds	1·4	1·4
Inorganic compounds . .	0·8	0·9

The mineral constituents approximate to 0·3 per cent. potassium, 0·2 sodium, 0·1 chloride, 0·02 magnesium, 0·01 calcium, and 0·006 per cent. iron.

The proteins of the white and grey matter do not differ much either in amount or in the amino-acid radicals present. Glycine radicals appear to be absent.

The *enzymes* include a catalase, a peroxidase, lipase, amylase, proteases, nucleases, and a phosphatase.

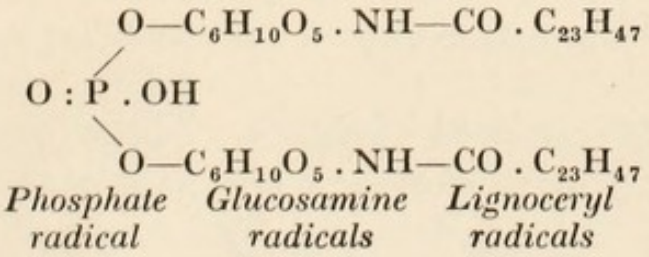
If brain is dried to remove water, and the dried residue is extracted successively with acetone and ether, and then treated with 85 per cent. alcohol at 45°, the extract, on filtering and cooling it to 0°, deposits a precipitate. If this is purified by extraction with ether, and is then recrystallised from alcohol, an apparently pure homogeneous compound is obtained that has been named *protagon*, and whose constitution has been suggested to be of the type :



The general consensus of present opinion, however, is that protagon is a mixture. It contains nitrogen and phosphorus, and on hydrolysis with baryta yields cerebrosides and the products of lecithin hydrolysis.

As Table XVII. shows, the brain contains marked amounts of lecithins and kephalins. Sphingomyelins are also present, and the four cerebrosides, phrenosin, kerasin, nervone, and hydroxynervone.

Another compound of somewhat similar type has been isolated, and has the composition $C_{60}H_{117}N_2PO_{14}$, being di-lignoceryl-di-glucosamine-monophosphate :



This is insoluble in water, slightly soluble in acetone, and soluble in hot alcohol and ether. It can be recrystallised from alcohol. Probably a number of compounds of similar complexity are present.

The water-soluble extractives of brain tissue are similar to those in muscle, though present in smaller amount. They include creatine, purine bases, inositol, choline, and lactic acid.

Spinal Cord. The human spinal cord contains 74 per cent. water, 18 per cent. lipoids, and 8 of proteins. Of the lipoids, 4 per cent. is cholesterol, 12 unsaturated phosphatides, and 1·5 saturated phosphatides, the spinal cord containing relatively more unsaturated phosphatide than any other part of the nervous system, and marked amounts of kephalin.

Neurokeratin shows considerable variation in amount in different parts of the nervous system, as the following figures show: plexus brachialis, 0·32 per cent.; cerebellar cortex, 0·31; white matter of cerebrum, 2·24; white matter of corpus callosum, 2·57–2·90; grey matter of cerebral cortex, 0·33.

TABLE XVIII

	Human cerebrospinal fluid.	Human blood plasma.
	Per cent.	Per cent.
Total solids	1·0	8·5
Protein	0·07	7·5
Non-protein-N . .	0·021	0·030
Urea	0·022	0·033
Creatinine . . .	0·001	0·001
Glucose	0·08	0·08
(Na)Cl	0·73	0·60
Inorg. P. . . .	0·0025	0·003
Na	0·323	0·316
K	0·012	0·020
Ca	0·006	0·011
Mg	0·0033	0·0026

Cerebrospinal Fluid. Since the function of the cerebrospinal fluid may be supposed to be somewhat comparable, as regards the brain, with that of the blood plasma as regards the rest of the body, it is of interest to contrast the compositions of these two fluids. These are shown, in percentages, in Table XVIII.

The plasma figures are for venous blood. Arterial blood plasma will certainly show a closer approximation for urea, and probably for certain other constituents. We may therefore reasonably regard the cerebrospinal fluid as a filtered blood plasma containing all the constituents which normally would pass through a semipermeable membrane, and in the right amounts with the exception of calcium, the plasma calcium being partly in non-diffusible organic combination. Most, if not all, of the slight differences are those of Donnan equilibria.

The *p*H of normal spinal fluid is the same as that of blood.

The Constituents of Glandular Tissue

The liver and pancreas can be taken as typical secreting glands.

Liver. The composition of the liver shows marked variations according to the diet and the condition of the individual. These variations are shown chiefly in the fat and glycogen content.

Magnus-Levy analysed the liver of a suicide and found 60·6 per cent. water and 39·4 per cent. solids, containing 21·3 per cent. fat and 16·9 per cent. protein. Hoppe-Seyler found that the liver from a normal man accidentally killed contained 70·8 per cent. water, and of the 29·2 per cent. solids there were 2·8 per cent. fat, 1·6 per cent. connective tissue, and 1·2 per cent. ash.

The glycogen content is usually between 1·2 and 4 per cent., though at least as high a figure as 18 per cent. has been recorded for dogs on a high carbohydrate diet.

The proteins consist of at least two globulins, one or more nucleoproteins, and other less soluble proteins. The brown colouring matter has not been thoroughly investigated.

The liver contains not only neutral fats, but also the different types of phosphatides that have been dealt with in Chapter VII. About half the fatty content of normal beef livers consists of phosphatides. In fatty degeneration of the liver, produced experimentally by such means as phosphorus poisoning, and occurring in various pathological conditions, the fat content is markedly increased, and along with it the water content, so that the protein content *relatively* is decreased.

The usual mineral constituents are present, with potassium in excess of sodium. Iron is present in very varying amount, averaging about 0·02 per cent. The amount in man is stated to be greater than in woman. It is increased in any condition in which there is unusual destruction of red blood corpuscles, and also by feeding inorganic iron compounds. Obviously the liver acts as a storehouse of iron.

The liver contains a great number of different enzymes, capable between them of bringing about most of the different kinds of chemical actions that take place in the body.

Pancreas. Human pancreas contains about 28 per cent. of solid content (and 72 per cent. of water). Of the solids 10 per cent. is lipide material and 15·5 per cent. protein. The pancreas is rich in nucleo-proteins, one of which is peculiar in that it is built up of protein and a nucleotide, *guanylic acid*, instead of nucleic acid, which is a tetra-nucleotide. Only traces of albumins and globulins are present, the remaining proteins being compounds that are insoluble in water and neutral salt solutions.

The pancreas contains numerous enzymes or their zymogens, as is to be expected from the composition of pancreatic juice. Inositol, purine bases, lactic acid, volatile fatty acids, and fats can be extracted, though some of these undoubtedly

are set free from more complex compounds following the death of the tissue and during extraction.

Other glandular tissues show similar composition. It would appear that the proteins present in each tissue are, or at any rate some of them are, specific to that tissue. It is evident that each tissue contains a number of enzymes. The fat content naturally shows marked variations, and, with the exceptions of the liver and areolar tissue, neutral fats are not present in marked amount. Phospho-lipides are always present. The amount of nucleo-protein present is to a large extent dependent on the relative number of cell-nuclei.

Recent References

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CHAPTER XIX

THE INTERNAL SECRETIONS

CERTAIN glands of the body manufacture specific compounds which, instead of leaving these glands by special ducts, to be poured out on to the surface—such as is the case with the digestive juices, poured into the alimentary tract, and the sweat, poured on the skin surface—are added to the blood constituents, leaving these glands by their veins, and are, hence, known as *internal secretions*. The glands are usually referred to as *endocrine glands* (Gk. *endos*, within, *krinein*, to separate), and their study, as *endocrinology*.

For various reasons other names, such as *hormones* (Gk. *hormao*, to excite), and autacoids (Gk. *autos*, self) have been coined for these compounds. Each compound has specific and *different* properties, and such class-terms tend to become misleading.

These *internal secretions* take rank with the *enzymes* and the *vitamins* in being compounds which, when present in excessively small amount, will yet produce vast chemical changes. Our chemical knowledge of them is just a little greater than that of the other two classes, though it is still only in its initial stages. Our knowledge of the chemical mechanisms by which they act is still slighter.

The demonstration of the definite existence of these compounds has involved clinical and pathological, as well as physiological, pharmacological and biochemical studies. Biological tests of various kinds have had to be employed in the preparation and concentration of their extracts. Clinical studies and post-mortem examinations have asso-

ciated a disease with a pathological condition of some one or other endocrine gland, suggesting over- or under-secretion, then the effects following extirpation of such glands in animals have been ascertained, as also those following the feeding of the glandular tissue, or the transference of fresh grafts of this tissue to other animals, or the injection of its extracts. These have all gradually led to biochemical studies of the glandular material, and in a number of instances such studies have been successful in yielding the pure chemical compound. Little more than the essential chemistry of the secretions can be given in this volume.

Glands known definitely to elaborate one or more internal secretions are the *thyroid*, the *parathyroids*, the *anterior pituitary*, the *posterior pituitary*, the *mucous membrane of the upper intestinal tract*, the *islets of Langerhans* of the pancreas, the *adrenal medulla*, the *adrenal cortex*, the *ovaries*, and the *testes*. It is possible that endocrine secretions are produced by the *pineal* and the *placenta*. The evidence regarding the *thymus* as an endocrine gland is still insufficient. Claims for the existence of several other secretions have been made, but require substantiation.

In establishing the entity of an internal secretion, it is necessary to do more than show that an extract of some gland when injected into an animal produces a demonstrable pharmacological action. Such a test in no way proves that an active principle is poured out from the gland into the general circulation.

Chemically, the endocrine compounds are of various types, and of varying degrees of complexity. The extremes are illustrated by *adrenine*, a benzene derivative and a relatively simple base (from the adrenal medulla), and *insulin*, a protein (from the pancreas).

The Thyroid Gland and its Secretion. The thyroid gland is the only gland in the body which specifically stores up the element iodine and forms definite iodine compounds. While such iodine is present in the secreting cells of the gland it is

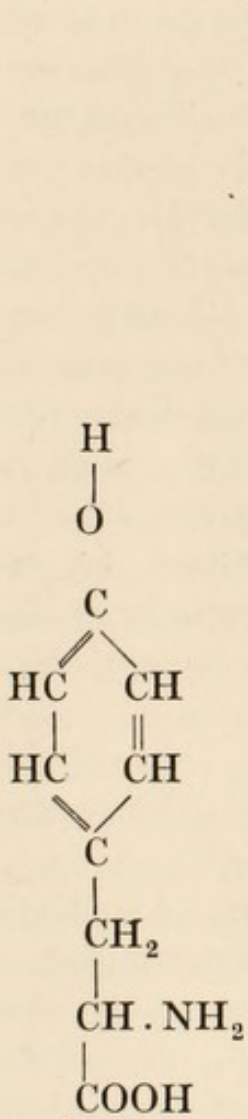
mainly to be found in the colloid material of the *acini* (which masses are bounded by these secreting cells) in the form of the pseudo-globulin *iodo-thyro-globulin*, a protein soluble under certain conditions in water, easily soluble in 1 per cent. sodium chloride solution, and precipitated from this solution by half saturation with ammonium sulphate. This protein may contain as much as 1·7 per cent. of iodine, but usually the content is between 0·3 and 0·6 per cent., while the desiccated glandular tissue usually contains from 0·1 to 0·3 per cent. Desiccated thyroid tissue usually contains over 10 per cent. of iodothyroglobulin.

Baumann, who discovered the presence of iodine in the thyroid in 1894, thought that he had isolated the iodine compound as iodothyrin, containing as much as 9 per cent. iodine. Iodothyrin is now known to be a mixture of compounds.

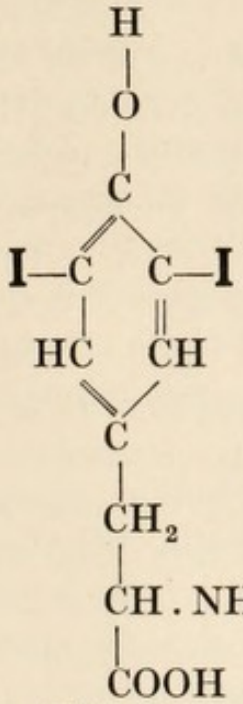
From hydrolysis of thyroid material Kendall (1912–1919) isolated crystalline *thyroxine* (so-named because he considered it to be a derivative of tryptophane and to have an oxy-indole structure—thyro-oxy-indole). Harington (1926) has shown that thyroxine is a derivative of the amino-acid tyrosine, and is closely related to di-iodo-tyrosine. Harington and Barger have synthetised thyroxine, Harington and Randall have isolated di-iodo-tyrosine from thyroid material, and Foster has obtained both compounds from the hydrolysed products of thyroglobulin. (Di-iodo-tyrosine had been previously isolated from the hydrolysed products of sponges and corals, and di-iodo-tyrosine radicals are formed in proteins that are subjected to the action of iodine.) Harington has finally subjected thyroglobulin to enzymic digestion, and has shown that the thyroxine then obtained (and therefore the thyroxine prepared by the thyroid) is the laevo-rotatory compound.

Thyroxine is the 3, 5-di-iodo-*p*-hydroxy-phenyl-ether of di-iodo-tyrosine. Both thyroxine (65·3 per cent. iodine) and di-iodo-tyrosine (58·7 per cent. iodine) give a very specific

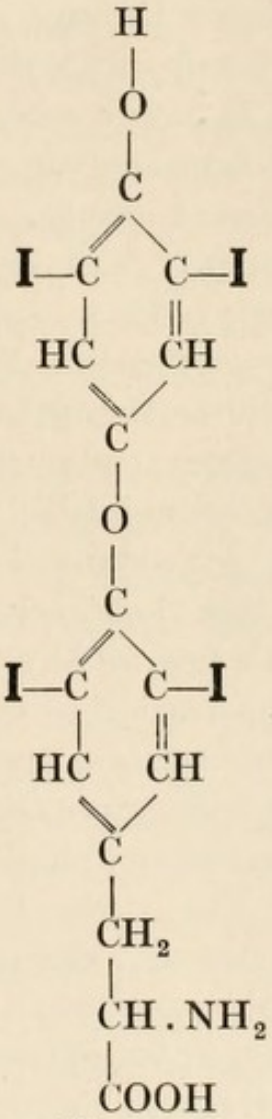
colour reaction when suspended in water or dissolved in very dilute alkali. Addition of nitrous acid turns the solution yellow, and then addition of ammonia or sodium hydroxide changes the colour to rose-red.



Tyrosine



Di-iodo-tyrosine



Thyroxine

No other crystalline compound containing iodine has yet been obtained from the living cell or its products.

Desiccated thyroid when administered to animals by mouth produces a number of definite effects. It increases the oxidation processes in the body (and consequently the heat production) and in large enough doses produces symptoms of *hyperthyroidism.* Animals which, through experimental removal of their thyroids, have developed a condition known

as *cretinism*, show markedly diminished rates of oxidation and a general slowing down of metabolic processes. Adequate and continued dosage of desiccated thyroid restores them to normal condition. Human beings suffering from thyroid deficiency through some pathological change in or surgical interference with the gland (conditions of cretinism, if the onset is before adolescence, or of myxœdema, if subsequent to adolescence) are restored and maintained in normal health by correct dosage. The administration of thyroid increases catabolism, and is followed by loss of weight, increased urinary output of nitrogen and phosphorus, denudation of glycogen from the liver, and of fat from the body generally. Administration to tadpoles and other amphibian larval forms accelerates metamorphosis. Administration to young rats leads to diminished rate of growth, and hypertrophy of the most important body organs. Administration to mice increases their resistance to poisoning by acetonitrile (Reid Hunt's test for thyroid), although, curiously enough, similar administration to rats decreases this resistance.

Furthermore, several of these effects have been shown to be at least approximately proportional to the iodine content of the thyroid employed.

Of the three known iodine compounds of biological importance iodothyroglobulin behaves very similarly to thyroid itself when tested by the production of the above effects, di-iodo-tyrosine shows no appreciable physiological activity, and thyroxine, inactive or laevo-, whether injected or fed, will bring about every kind of change that is produced by feeding thyroid itself, but, if comparison is made on the legitimate basis of iodine content (since thyroid activity is roughly proportional to its iodine content), there is some evidence that thyroxine is quantitatively somewhat less powerful. The actual compound secreted by the thyroid is not improbably a tri- or a tetra-peptide, containing one thyroxine radical, although the evidence is still inconclusive.

As indicated already, the secretion of the thyroid controls in considerable degree the oxidative processes throughout the body. When the thyroid is completely inactive (or completely removed) the oxidation of the organism is sluggish; it amounts to but 60 per cent. of the normal total, so that the thyroid secretion is responsible for inducing the remaining 40 per cent. When thyroid is fed to excess, or when through some pathological cause the human thyroid overfunctions, the total oxidation of the organism is increased; the increase bears some relation to the dosage, or the severity of the pathological state of the gland, and the total oxidation may even reach double the normal figure. The heat production, of course, parallels it.

All the manifest physiological and pathological changes which occur as a result of overdosage with thyroid, or overfunctioning of the gland, can be traced to the various grades of excess oxidation induced by such feeding, and changes secondary to these.

The normal condition of the thyroid depends upon a sufficiency of iodine in the diet. Since the function of the gland is to elaborate an iodine compound, such dependence is comprehensible. The minimum quantity of iodine required has been estimated to amount only to between 35 and 70 micrograms (millionths of a gram) per day, yet in many parts of the world diets may not contain even this minute trace, in which case a large proportion of the children and young adolescents develop a condition of *simple goitre* (enlargement of the gland, with some modification of its structure), which may persist to adult years, and subsequently develop into more serious pathological thyroid states. Hence a considerable degree of iodine prophylaxis is being practised in a number of those regions where iodine is deficient.

The secretion of the thyroid gland is one of the few that can withstand digestion. Thyroid given by mouth is effective.

The Secretion of the Parathyroid Glands. The four small parathyroid glands, situated in pairs posteriorly to the two thyroid lobes, in positions varying somewhat in different species of mammals, secrete some compound which assists in the control of calcium metabolism. When these glands are completely removed from an animal, within a few days the animal displays definite symptoms of the condition known as tetany. These symptoms are all associated with increased irritability of nervous tissue, and tetany is due to an upset of the ionic balance of blood and tissues, and especially of the nerve cells, whereby these cells become more susceptible to slight stimuli. A disturbance of this ionic balance, with tetany as a result, may be brought about in a number of ways, such as parathyroid deficiency, one type of rickets, hyper-respiration, and certain gastro-intestinal disturbances. The two latter involve an alkalosis, to which the tetany is traceable. An artificial tetany can be produced by the injection of the toxic compound guanidine, or of its methyl derivative.

Removal of the parathyroids leads to three marked abnormalities; onset of tetany, diminution of blood calcium, and retention of phosphate in the organism. The normal height of plasma calcium is 10 to 11 mg. per 100 c.c., and when it falls below 7 mg. symptoms of tetany are usually detectable. In extreme tetany due to parathyroid deficiency the figure for calcium may fall to 4 mg. per 100 c.c. plasma.

Animals in parathyroid tetany, untreated, may die within a few days, if no parathyroid tissue has been left. Repeated injection of calcium (or strontium) salts is temporarily remedial. Removal of the thyroid for hyperthyroid conditions in man is sometimes followed by tetany through removal of or damage to too great an amount of parathyroid tissue.

The parathyroidectomised animal affords a biological medium for the testing of parathyroid preparations. Desiccated parathyroid, and parathyroid extracts generally,

are ineffectual when given by mouth ; they do not improve the condition of such an animal.

Collip, in 1924, hydrolysed beef parathyroid glands with hydrochloric acid, and, fractionating the products, obtained a concentrated extract which, when injected into a parathyroidectomised dog in tetany, relieved the tetany within two or three hours, and restored the plasma calcium to normal level. The effect is temporary, as is to be expected in such a replacement therapy. Nevertheless, Collip succeeded in keeping completely parathyroidectomised dogs alive for many months by daily injections of this extract ; if at any period the injection was withheld, the dog developed acute tetany ; subsequent injection rapidly restored its normal condition. His extract has also been used clinically with marked success.

Collip showed further that when the extract was injected into a dog repeatedly, at three-hour intervals, whether the dog was normal or parathyroidectomised, the blood calcium rose steadily, until after twenty-four to forty-eight hours, when it had reached a height of 22 to 24 mg. per 100 c.c. plasma (about twice the normal value), other symptoms intervened, including marked concentration of the blood, and such animals then died within a few hours.

The internal secretion of the parathyroid has not yet been obtained in pure form, but appears to be a protein. It is decomposed by trypsin. On this account it is ineffectual by oral administration, unless such an excessive dose is given that a little escapes proteolytic digestion and becomes absorbed from the intestine.

The height of plasma calcium is an important factor in the correct deposition of the calcium salts of bone. Low plasma calcium is associated with rickets, a disease mainly traceable to deficiency in vitamin D. Evidently both vitamin D and the parathyroid secretion are controllers of calcium metabolism, and it has been suggested that the vitamin acts through the parathyroids, although there

is as yet insufficient experimental evidence to settle this point.

The relationship between bone disease and the parathyroids, the intermediary being the height of plasma calcium, is further exemplified by the condition of *generalised osteitis fibrosa* (a chronic inflammation of many of the bones of the body, with marked softening and bending, thickening of the affected bones, and occurrence of fibrous nodules), a condition that has recently been recognised as due to a parathyroid tumour of the nature of an adenoma, and producing a hypersecretion. In such condition therefore, there exists a hyperparathyroidism, as a result of which the plasma calcium may be raised to from 12 to even 17 mg. per 100 c.c. Removal of such tumours leads to marked improvement in the patient, coincident with restoration of a normal blood calcium. It has recently been suggested that " marble bone disease " is also a manifestation of chronic hyperparathyroidism.

The Secretion of the Islets of Langerhans. The *pancreas* possesses an internal secretion, as well as an external secretion, the pancreatic juice. The internal secretion is almost certainly manufactured by specific cells forming the so-called *Islets of Langerhans.*

It had long been suspected that the internal secretion of the pancreas was concerned with the catabolism of glucose, and that the disease *diabetes mellitus*, whose main anomaly appears to be a deficient power of oxidising glucose, was attributable to pancreatic deficiency. Removal of the pancreas of the dog rapidly leads to a condition in every way resembling human diabetes.

In 1921–22 Banting and his collaborators, working in J. J. R. Macleod's laboratory, succeeded in proving this definitely, and in isolating a very concentrated pancreatic extract, whose essential active constituent they termed *insulin* (L. *insula*, island). This extract, when injected into the blood stream of an animal, immediately causes a lowering

of blood glucose, and large doses cause such a lowering as leads to convulsions and death, these being actually due to the glucose deficiency, and preventable by glucose injections. By aid of the extract diabetes mellitus has now become a disease which, not yielding to the treatment, is yet capable of correction by continued treatment.

Just prior to Banting's work, Mann and Magath had shown that extirpation of the liver in an animal led to convulsions associated with a marked lowering of blood sugar, and palliated by intravenous injection of glucose. The association is easily comprehensible since the liver is the carbohydrate storehouse of the organism, replenishing from its glycogen the glucose steadily drained from the circulation by the muscles. In absence of the liver therefore hypoglycaemia must result. The work of Mann and Magath was timely in giving an explanation of the convulsions following overdosage of insulin and a clue to the rational treatment of these.

Various methods have been employed for obtaining highly concentrated insulin preparations. One, giving an excellent yield, is that of Dodds and Dickens, modified from Dudley's. Picric acid is mixed directly with crushed pancreas, the insulin picrate leached out with 70 per cent. acetone, the acetone evaporated, and the potent residue extracted with ether and then converted into the hydrochloride.

Abel succeeded in crystallising insulin in 1926, using pyridine as an essential agent to precipitate impurities from concentrated solutions of insulin. He was able to obtain 0·53 gm. of rhombohedric crystals from 2 gms. of a powerful commercial (solid) preparation. One-fortieth of a milligramme of these crystals is sufficient to produce hypoglycæmic convulsions in a rabbit.

Abel's co-workers have hydrolysed insulin crystals and have shown that the hydrolysate contains 12 per cent. of tyrosine, 12 per cent. of cystine, 21 per cent. of glutamic acid, 30 per cent. of leucine, 3 per cent. of arginine, 8 per cent. of histidine, and 2 per cent. of lysine. They consider

that other amino-acids cannot be present in more than traces. Insulin crystals are actually crystals of the zinc salt.

Svedberg has shown that insulin is a protein, with a molecular weight of about 35,000. It has the same molecular size as egg-albumin.

The precise mechanism whereby insulin controls the metabolism of glucose is still undetermined. There is strong evidence that its main action is in bringing about the conversion of glucose into glycogen, both in the liver and in muscle. There is no definite evidence that it has any other direct action than this, although this limitation of its action to causation of glycogen formation cannot be regarded as proved. It seems probable that the cystine radicals in insulin play an important *rôle* in its functional activity.

Secretin. The internal secretion of the *intestinal mucous membrane* has already been referred to in Chapter XI. Bayliss and Starling showed in 1902 that if the nerve supply to an isolated loop of intestine were cut, and then acid was injected into the loop, a well-marked flow of pancreatic juice followed. Then they demonstrated that if the mucous membrane was scraped off a loop of jejunum, and rubbed up with sand and 0·4 per cent. hydrochloric acid, and then boiled to coagulate the protein content and filtered from this, the filtrate, when injected into a vein of this or another animal, produced within twenty seconds a copious flow of pancreatic juice. They named the essential constituent *secretin*, and assumed that a precursor, *pro-secretin*, is present in the cells of the mucus, a change of *p*H in the direction of acidity leading to the formation of the active compound. The presence of secretin has actually been demonstrated in the blood leaving a loop of intestine into which acid has been introduced. Its action is not limited to the pancreas ; the flow of bile is increased. The mode of action is not known.

J. Mellanby has recently contributed greatly to our knowledge of secretin and has obtained it in approximately pure condition. His original procedure consisted essentially in

extracting the duodenal mucosa with absolute alcohol, adding to the extract bile salts and precipitating secretin along with bile acids by addition of dilute acetic acid, extracting secretin from the precipitate by absolute alcohol and precipitating it with excess of acetone, and finally purifying by dissolving it in water and precipitating with dilute acetic acid.

He has recently modified his procedure, omitting the use of bile salts, and obtained finally a white amorphous powder. One kilogram of fresh mucous membrane yielded 20 mg. of this product. It is soluble in water, dilute alkali, and 80 per cent. alcohol, and insoluble in ether, chloroform, and acetone. It appears to be a polypeptide, and contains carbon, hydrogen, oxygen, nitrogen, and sulphur. Phosphorus is absent.

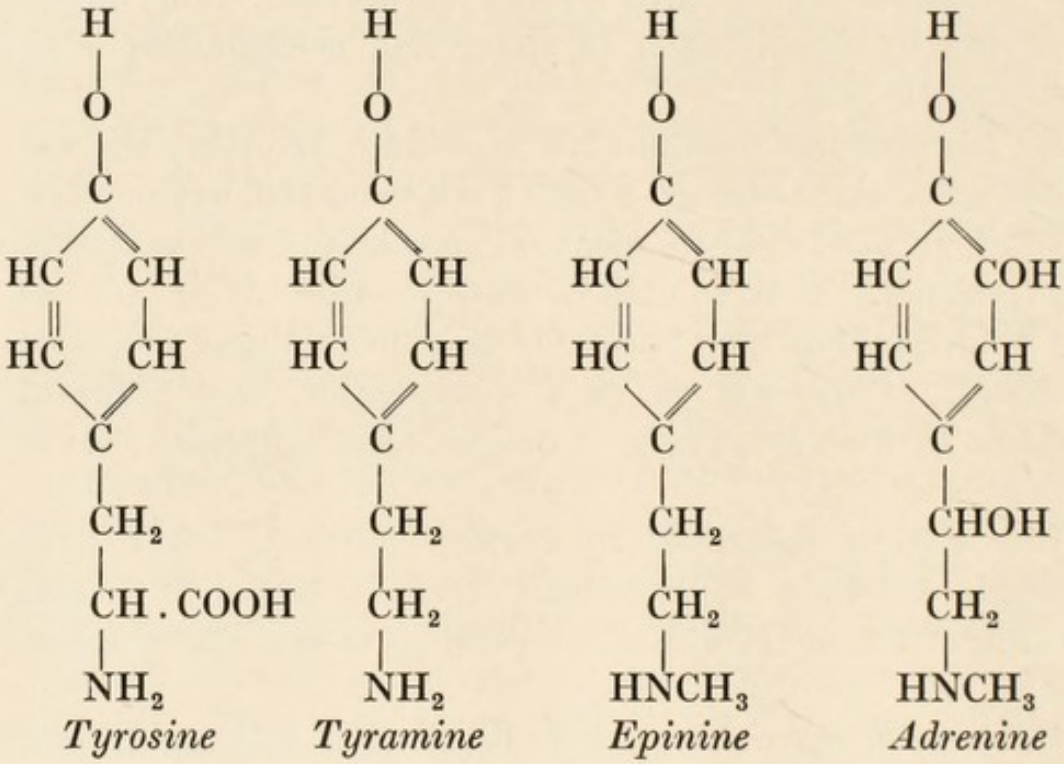
It is rapidly destroyed by trypsin and does not dialyse through collodion membranes. When injected it causes considerable liberation of pancreatic juice and slight secretion of bile.

Mellanby's researches also indicate that secretin is present in a preformed condition in the intestinal mucosa, and that in the living animal the secretion of pancreatic juice is determined by the entrance of bile into the duodenum. Bile salts, absorbed from the intestine, carry secretin from the cells of the duodenal mucosa into the portal blood and so to the pancreas. This theory does not need the existence of a pro-secretin.

Ågren and Wilander have obtained a crystalline preparation and believe that secretin is a polypeptide with a molecular weight of about 5,000.

The Secretion of the Adrenal Medulla. The *adrenal medulla* is the largest mass of *chromaphil* tissue present in mammals. In all probability its secretion is identical with that of other mammalian chromaphil tissue, such as the *abdominal chromaphil body*, and the *chromaphil bodies* found throughout vertebrates in close proximity to ganglia of the

sympathetic nervous system. The human adrenal glands contain between 8 and 9 mg. of a very powerful compound, *adrenine*, or *adrenaline*, also referred to as *epinephrine* (L. *ad*, to ; *renes*, kidneys ; Gk. *epi*, upon ; *nephros*, kidney).



This was isolated from the glands independently by Aldrich and by Takamine in 1901, and its chemical constitution has been completely determined. It is another derivative of tyrosine, and its formula, along with that of some analogous compounds, is given above.

Natural adrenine is a white crystalline substance melting with decomposition at 211° C. It is very slightly soluble in water and almost insoluble in the common organic solvents. It is lævo-rotatory, with a specific rotation of — 53·4°. It yields a crystalline hydrochloride and tartrate. It reduces Fehling's solution, and is itself very readily oxidised, especially in presence of minute traces of ferric salts. It gives a green colour changing to violet, with ferric chloride, a reaction due to the fact that it is a derivative of catechol. It oxidises to a pink compound. Application of heat, in pre-

sence of potassium persulphate, gives a rose-red colour which permits its detection in dilutions of one part in five millions.

Adrenine has been synthesised ; the synthetic product is optically inactive ; it has been split into its dextro- and laevo-forms. Dextro-adrenine is stated to possess about one-third the physiological activity of the laevo-compound.

When injected into the body adrenine produces a series of effects which can all be paralleled by stimulation of various sympathetic nerves, whence adrenine and similar compounds such as tyramine and epinine have been termed *sympathomimetic*. The action is believed to take place either on the sympathetic nerve endings in muscle, or on the myoneural junctions themselves. Two of the most marked effects thus produced by the intravenous injection of adrenine solutions are an increase of blood-pressure, due to constriction of the smooth muscles of the arterioles, and a mobilisation of liver glycogen to blood sugar. In muscle, glycogen is broken down to lactic acid in increased amount.

Adrenine produces none of these effects when given by mouth.

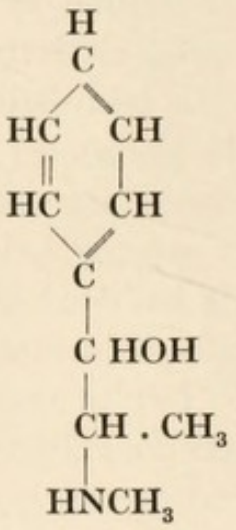
Cannon's emergency theory is usually accepted as describing the function of the adrenal medulla. Any stimulation, emotional or otherwise, which affects the adrenal glands through the sphlanchnic nerves leads to output or increased output of adrenine from the medulla. This outpoured adrenine produces its two-fold effect, a general " stringing up " of the organism through the increase in blood-pressure, and mobilisation of liver glycogen to produce a heightened level of blood sugar. The animal is thus conditioned to meet an emergency.

As yet we know nothing of the mechanism by which adrenine is produced in the gland.

Adrenine is present in the poison glands of a tropical toad, though these contain no true chromaphil tissue. On the other hand, the posterior salivary glands (venom glands) of the octopus contain chromaphil granules, analogous to those of the adrenal

medulla, but these secrete tyramine. Tyramine has also been found as the active principle of milk thistle seeds.

The Chinese plant *Ma huang* (*Ephedra vulgaris*), which has been used in their pharmacy for over 5,000 years, derives its potency from the presence of *ephedrine :*



closely related to adrenine, and capable of producing similar qualitative effects on blood-pressure, etc., through its action on the sympathetic nervous system. It produces the same effects whether injected or given by mouth.

Treatment with a strong oxidising agent, such as chlorine water, converts adrenine into brown and black coloured substances which resemble the naturally occurring melanins in chemical properties.

The Secretion of the Adrenal Cortex. The *adrenal cortex* is not embryologically related to the medulla, and the juxtaposition of these two tissues in mammals has not yet received an explanation. *Accessory cortical bodies* are found in various positions. When the adrenal cortex is completely extirpated from an animal death invariably follows within a few days. The most characteristic symptom, prior to death, is muscular weakness. Stewart and Rogoff developed the technique by which prolongation of the life of doubly adrenalectomised animals could be used as a suitable biological test for the potency of cortical extracts. With skilled surgery untreated cats and dogs survive the operation eight to twelve days.

Hartman and his co-workers, and Swingle and Pfiffner,

have made great strides in the preparation of highly potent concentrates of the cortical principle, and its ultimate isolation in pure form may be expected in the very near future.

Swingle and Pfiffner extract beef adrenal cortex with alcohol, and the extract is subsequently fractionated by successive treatment with benzene, acetone, etc., the last traces of adrenine being removed by filtration through permutit. They finally obtain a water-soluble product, which seems to be of a relatively simple nature and to resemble lipides in its properties.

Daily injection of a few milligrams of this product has kept dogs and cats from whom both adrenals have been removed alive for periods of over a year, and in perfectly normal condition. If at any period the injections were stopped, symptoms of acute adrenal insufficiency developed within a few days; if injections were not recommenced the animals died within ten days.

The cortical principle is effective when administered by mouth. Somewhat larger dosage is necessary.

The condition described by Addison in 1855 and named after him as "Addison's disease," is associated with a diseased condition of the adrenals. Muscular weakness is an outstanding feature, and disease of the cortex is probably the conditioning factor. The most satisfactory treatment so far instituted is the Muirhead *régime*, adrenine by injection, and adrenal cortex (whole gland) by mouth. Both Swingle's and Hartman's preparations have recently been used with marked success in controlling acute exacerbations of the disease.

Szent-Gyorgyi has recently isolated from the adrenal cortex, and also from cabbage leaves, an isomer of glycuronic acid, which is termed *ascorbic acid*, and is identical with vitamin C (*cf.* Chapter XXVIII.).

The Secretions of the Pituitary Body. There are a large number of compounds elaborated by the pituitary (hypo-

physis), two associated with the posterior, and possibly eight with the anterior lobe. These two parts of the pituitary are of unrelated embryological origin. The anterior portion is typically glandular in structure, while the posterior, on the other hand, shows distinct resemblance to nervous tissue. It seems reasonable to consider the two lobes as separate glands.

The *anterior lobe* has three distinct types of cells. One of these does not stain easily, and is in consequence termed *chromophobe* (Gk. *chroma*, colour ; *phobos*, fear) ; the other two types stain readily and are termed *chromophile* (Gk. *phileo*, I love). One type of these chromophile cells stains more readily with acid, and the other with basic, dyes. Hence they are respectively termed *acidophile* and *basophile*. The function of the chromophobe cells is not known. Both the acidophile and basophile cells secrete endocrine compounds, and most of these compounds appear to be proteins, although none has yet been obtained in a state of purity. The name *phyone* (Gk. *phyo*, I cause to grow) has been suggested for that compound of the acidophile cells, which controls growth, and *hebin* (Gk. *hebe*, puberty) for that of the basophile cells which controls the development of the gonads.

Our present knowledge of the functions of these compounds is due to extirpation experiments, experiments in which small pieces of pituitary tissue have been " implanted " into animals daily for considerable periods, and experiments in which daily injections of potent extracts have been made, combined with study of the effects which pituitary and neighbouring tumours produce in man. With this work the names of Evans, Smith, Allen, Zondek, Houssay, Collip, and especially of Cushing, are associated.

While complete extirpation of the pituitary in animals usually leads rapidly to death, partial extirpation in young animals leads to stunted growth and retardation of sexual maturity. Daily implants or injections of potent extracts into young animals lead to gigantism. Evans and Cushing and their co-workers have produced giant rats and giant dogs.

The gonads are stimulated, and as a result the secondary sex organs hypertrophy.

In human beings pathological conditions due to under-function may arise from atrophy of the anterior pituitary, but they are probably more commonly due to "neighbourhood pressure effects." Tumours of the chromophobe cells compress and ultimately destroy the acidophile and basophile cells, so that there results a gradual depression in the production of their endocrine compounds. On the other hand, while a simple hyperplasia of the cells is possible, leading to increased production of their specific compounds, probably in the majority of cases such overproduction is due to tumours of these cells, of adenoma type. Thus a number of diseased conditions are associated with various abnormal conditions of the pituitary.

Tumours of the acidophile cells (and sometimes perhaps mere hyperplasia of these cells) produce, when they occur in youth and adolescence, the condition of *gigantism*. These giants mature early, may reach over 8 feet in height, and are correspondingly strong. In most cases they gradually grow feeble before they reach the age of thirty, and the enfeebled giant dies as a rule before middle age.

When such tumours occur after adolescence, after normal growth is complete, then the condition of *acromegaly* results, in which growth occurs where it can. There may be a little increase in height, or none at all, depending on the age of onset. There is over-growth of certain bones, such as the jaw, the ribs, and the bones of the hands and feet. As a result the face and hands of the acromegalic present a characteristic appearance. If the tumours are large enough to exercise neighbourhood pressure effects on the basophile cells, then depressed sex function may complicate the picture.

When atrophy, or chromophobe-tumour-pressure effects, occur in youth the *pituitary dwarf* results, and since acidophile and basophile cells are both usually affected the dwarfism is generally accompanied by sexual infantilism.

When obesity is not present, the combination is termed the Loraine-Levi syndrome. The majority of the cases are obese, however, The obesity is associated with disturbance of the function of the posterior pituitary (also through neighbourhood pressure), and the combination of obesity, sexual infantilism, and dwarfism (if the onset is sufficiently early) constitutes Fröhlich's syndrome.

Tumours of the basophile cells set up a syndrome which has recently been described by Cushing. Marked obesity is accompanied by depressed gonadal function, and, in women, by hirsutism.

Evidence now available from all sources suggests that the endocrine compounds of the anterior lobe include (i.) *phyone*, controlling growth, (ii.) *hebin*, controlling the gonads, and through them the secondary sex glands, (iii.) a thyrotropic principle controlling thyroid function, (iv.) an adrenotropic principle controlling the adrenal cortex, (v.) *prolactin*, controlling secretion of milk, (vi.) a diabetogenic principle acting antagonistically to insulin, (vii.) a principle controlling fat metabolism, (viii.) a principle controlling the parathyroids (which may or may not be identical with one of the others), and (ix.) a synergistic principle which acts along with the A-P-L. principle of the placenta.

From the *posterior lobe* of the pituitary very powerful concentrated extracts have been prepared, especially by Abel. Such extracts have recently been fractionated into two separate portions by Kamm and his co-workers. The active principles of these fractions have not yet been obtained in pure form, but appear to be relatively simple basic compounds. They were at first termed *α-* and *β-hypophamine ;* they are now frequently referred to as *pitocin* and *pitressin*.

Pitocin (*α*-hypophamine) stimulates contraction of uterine muscle, while pitressin (*β*-hypophamine), when injected intravenously, increases blood pressure. It also controls (depresses) urinary secretion.

In the diseased condition known as *diabetes insipidus* the

volume of urine excreted is greatly increased; sugar is absent from it, and it is more dilute than usual. This "polyuria" is in most cases transiently decreased by injections of posterior pituitary extract, or, more specifically, of pitressin. The precise nature of the action is not completely understood, since centres in the neighbouring brain tissue also seem to be involved.

The Secretion of the Ovaries. The isolation of the internal secretion of the ovaries has resulted from the long known effect of castration in the female in producing atrophy of the uterus and tubes, and, in young animals, non-development of the mammæ and secondary sex characters, and from long continued efforts to prepare ovarian extracts which would prevent the onset of these sequelæ.

The immediate stimulus to the ultimate success of such endeavours was the demonstration by Stockard and Papanicolaou, in 1917, that œstrus in the guinea-pig produced a definite effect on the types of cells present in a vaginal smear, so that microscopic examination of such smears could be used as a test for œstrus. Long and Evans, in 1921, showed that the same series of changes occurred in the rat, and Allen and Doisy applied the test in 1923 to measure the potency of the ovarian internal secretion which controls œstrus.

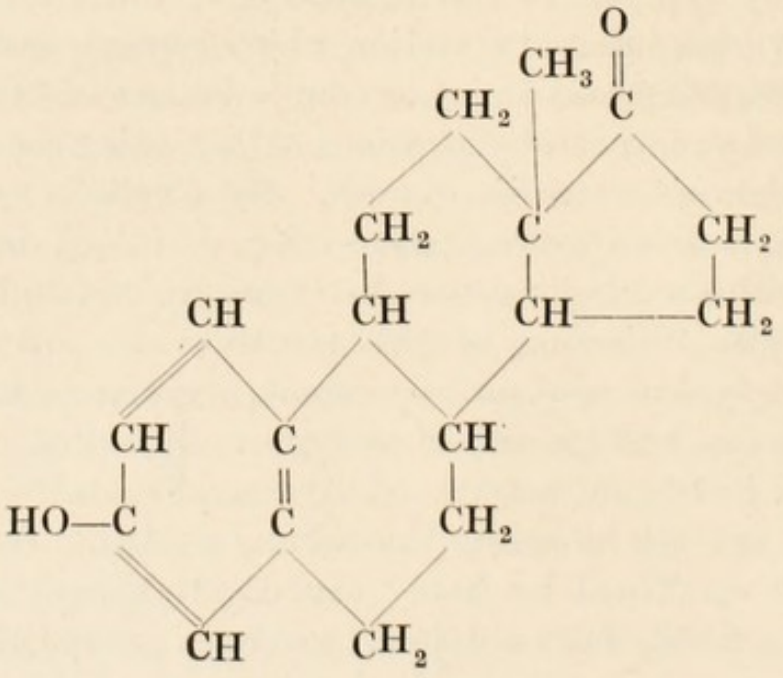
In 1923, Allen and Doisy announced a successful attempt to prepare a concentrated ovarian extract, using the follicular liquor of hog's or cow's ovaries. By alcoholic extraction and fractionation with acetone they obtained a solution which withstood boiling, was free from lipides, and gave no biuret test. Injection of this solution into spayed mice produced typical œstural hyperæmia, hypersecretion of the genital tract, and growth of mammæ. Injection into mice that had just been weaned caused sexual maturity in from three to five weeks before the normal period. Their work has been confirmed by many investigators, and the compound has finally been obtained by several groups of workers

in crystalline condition. It has been termed *theelin* by Doisy (Gk. *thelus*, feminine); *thelykinin* by Loewe (Gk. *thelus*, and *kineo*, I set going), and *oestrin* and *oestrone* by Marrian and others. Numerous other scientific and commercial names are being used for it.

It is present in the follicular liquid, in urine, especially that of pregnant women, and in the placenta. It is present in traces in female blood. It has been isolated from plant material.

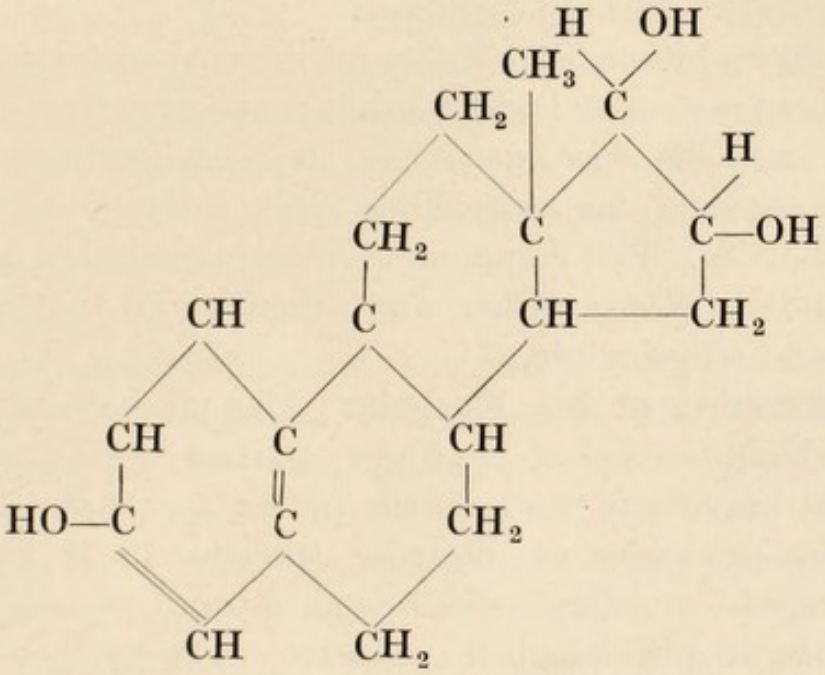
At puberty, through the action of one of the secretions of the anterior pituitary, the ovaries become functional and secrete œstrin. This stimulates growth of the uterus, vagina, and breasts, and leads to the appearance of the first œstrus or menstruation, thereafter acting as a regulator of the sexual cycle.

Doisy, Butenandt, and others have obtained the crystalline compound from the urine of pregnant women, a relatively very rich source. It has the formula $C_{18}H_{22}O_2$, and possesses one keto- and one hydroxy-group. Largely through the work of Butenandt its constitution has been almost completely elucidated, so that the following is at least a very close approximation :



The endocrine compound secreted by the ovaries thus turns out to be closely related to cholesterol and cholic acid (*cf.* p. 93) and to be one of the simpler derivatives of the four-ring skeleton which is the basis of all this group of important compounds.

Marrian, and somewhat later Doisy, isolated crystals from another fraction of the urine of pregnant women, which have the formula $C_{18}H_{24}O_3$, and, according to Butenandt, the constitution :



This compound Marrian has termed *trihydroxyoestrin*, on account of its three hydroxy groups. The ovarian principle, using similar terminology, becomes *ketohydroxyoestrin*. Doisy names them *theelol* and *theelin* respectively, and the English workers *oestriol* and *oestrone*. They differ by only one molecule of water, and oestriol can be converted to oestrone by distillation in a high vacuum with potassium hydrogen sulphate.

The activity of oestrone (theelin) is such that 1/3,000 milligram is sufficient to produce onset of oestrus in a spayed rat. That of oestriol (theelol) seems to be much less, but its precise degree of activity is not yet determined. It seems capable of existence in more than one form. The

functional reason for its formation is not definitely known, but it has been suggested that since it is more soluble than the keto-compound it is produced to aid excretion of excess of the latter.

The Secretion of the Corpus Luteum. The corpus luteum is formed from a mature (Graafian) follicle of the ovary, after rupture and discharge of its ovum. In absence of pregnancy involution of the corpus luteum commences in about two weeks. If the ovum is fertilised the corpus luteum persists during pregnancy.

The corpus luteum elaborates an internal secretion which is soluble in water, and which inhibits the growth of ovarian follicles, and likewise produces the sensitisation of the uterus necessary for embedding and early growth of a fertilised ovum. This compound is closely related to oestrone (Butenandt). Hisaw, Allen, and others claim that there is more than one principle.

The Secretion of the Placenta. The placenta stores or forms (which, we do not yet know) at least three principles. The most important work on the placenta is that of Collip. The three principles are oestrone (theelin) itself, *emmenin* (Gk. *emmenos*, monthly), which is an ester of oestriol, and a protein with physiological properties closely resembling those of "hebin" of the pituitary gland, and which Collip terms the "Anterior-Pituitary-Like" (A.P.L.) principle.

Zondek and Aschheim isolated from the urine of pregnant women a concentrate of what they termed *prolan*, which they considered to be identical with the principle of the basophile cells of the pituitary (hebin). It seems rather probable that "prolan" and Collip's A.P.L. principle are identical, and that the compound acts in conjunction with a pituitary principle which is not hebin.

Emmenin and the A.P.L. principle have been used therapeutically in various menstrual disorders in women, and good results have been obtained.

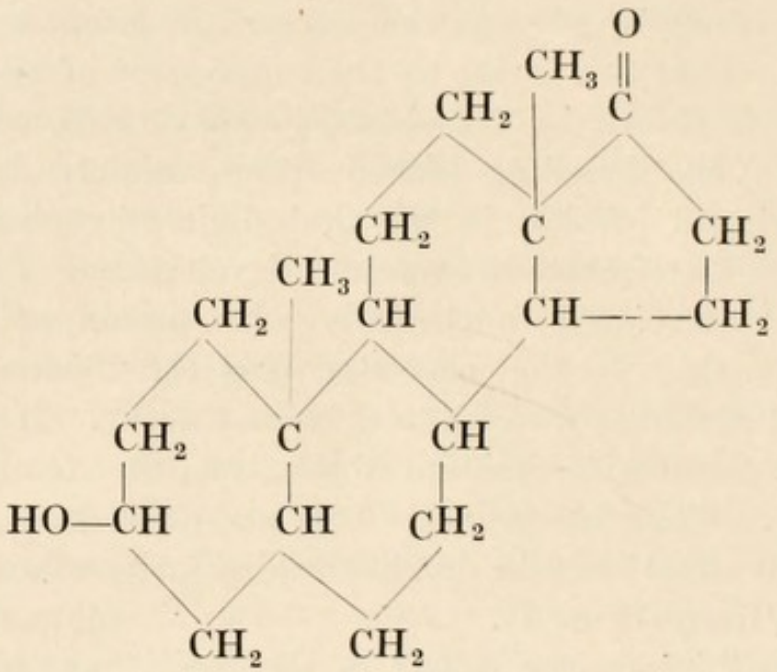
The Secretion of the Testes. Castration in the young male prevents the development of the secondary sex characters, leading to preservation of certain infantile characteristics. That this is due to the suppression of an internal secretion is shown by Nussbaum's experiments with male frogs. In the breeding season these animals develop a thickened pad of skin in the first digit of each forearm, associated with increased muscular development of the forearm, and associated functionally with prolonged clasping of the female. In the castrated frog the thickening and increased muscular development do not occur. If pieces of testis are grafted into the dorsal sac, then the changes occur normally. Since there is no nervous connection to such grafts, the effect must be due to the absorption of an internal secretion from the graft.

Certain " interstitial cells " of the testis have a distinct glandular appearance, and it is generally believed that they elaborate the secretion ; the proof is not yet final. The ingrafting of testicular grafts has been very frequently carried out in ageing men, and beneficial results have been claimed, but it is so difficult to eliminate the psychic element that these effects are largely discounted.

Within the past few years four groups of investigators, Koch, Moore and Gallagher, Martins and Silva, Loewe and Voss, and Funk, Harrow and Lejwa, have extracted from testicular material or from male human urine fractions, which, in solubility and certain chemical properties, show marked resemblances to the ovarian secretion, and which seem to possess definite specific activity. These extracts will prevent atrophy of the prostate and seminal vesicles following castration in the rat, and will induce comb growth in capons (castrated cockerels). Such names as *androkinin*, and *androtin* (Gk. *andreios*, masculine) have been suggested for the principle.

Butenandt has obtained minute amounts of the principle in crystalline form. Its formula is either $C_{19}H_{30}O_2$ or

$C_{18}H_{28}O_2$ and Butenandt believes that its constitution is probably :



Testicular principle, $C_{19}H_{30}O_2$

It is thus closely related to the oestrin compounds and to cholesterol and cholic acid.

Other Presumptive Internal Secretions. Evidence has been put forward that the gastric mucosa elaborates a secretion, *gastrin*, which, when injected intravenously, provokes the secretion of gastric juice. The existence of gastrin has not been completely substantiated.

Le Heux isolated *choline* from the intestinal mucosa, and suggested that it is the " hormone " producing intestinal peristalsis. This work has not been substantiated.

Ivy claims to have extracted from the duodenal mucosa of dogs, by treatment with dilute hydrochloric acid, material which after fractionation is capable, when injected intravenously into animals, of causing contraction of the gall-bladder with outpouring of its contents. He considers that the compound is not secretin, but is closely allied to it. He terms it *cholecystokinin*.

Laughton and Macallum have put forward evidence that the duodenal mucosa elaborates a secretion which stimulates

the islets of Langerhans to produce insulin, and which is not secretin.

There is some evidence that the *pineal* gland, a tiny organ situated in mid-brain, elaborates a secretion associated with growth. Young boys, who at autopsy show a teratoma of this organ, exhibit abnormal tallness, unwonted growth of hair, premature sexual and genital development, and earlier maturity. Feeding experiments with desiccated pineal have led to only one definite (and peculiar) result. If young tadpoles are fed pineal along with plant food from the beginning of larval life they become translucent thirty minutes after each feeding, the translucency persisting about three hours. This phenomenon lasts until metamorphosis.

The *thymus* gland grows until puberty, then gradually atrophies, but still appears to function to some extent even till old age. Histologically it strongly resembles lymphoid tissue, and its chief physiological significance is probably related to its lymphoid character. Claims for an internal secretion of the thymus have been made, associating it with growth. These are not generally accepted. Within the last few years Asher and his pupils have obtained what appears to be an active thymus extract by the following procedure : Fresh thymus was extracted successively with acetone and with ether, and the residue then extracted with water ; this aqueous extract was precipitated with 90 per cent. alcohol and the precipitate extracted with 70 per cent. alcohol. The residue from this extract was dissolved in a little water and precipitated by ammonium sulphate. By repeating this final fractionation they ultimately obtained a powder free from protein and lipide. It appears to be a polypeptide containing sulphur. When injected into rats it increases general growth, growth of the skeleton, and growth of the gonads. Asher terms it *thymocrescin*. Extracts from lymph glands do not exhibit such activity.

Various groups of workers have recently prepared extracts from the pancreas which are stated to be free from insulin,

and to have a definite effect on the circulation, lowering the blood pressure. The hypothetical principle is referred to as *angioxyl, kallikrein, vagotonine*, etc.

The remarkable results obtained within recent years by Minot and Murphy, and confirmed by many others, by feeding liver or stomach extract in cases of pernicious anaemia, do not prove that there is an endocrine secretion with " blood-building " function, though they do suggest the possibility.

Cannon believes that an adrenine-like compound is passed into the circulation from the sympathetic ganglia when the smooth muscle of the abdominal viscera receives sympathetic impulses. This he terms *sympathin*.

Anti-endocrine Compounds. The body appears to react to excess of at least some of the endocrine compounds by developing compounds which counteract their action and so help to maintain endocrine equilibrium. These appear to include an anti-thyroid, an anti-thyrotropic and an anti-gonadotropic compound.

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CHAPTER XX

AUTOLYSIS, INTRACELLULAR ENZYMES, AND ENZYMIC SYNTHESIS

In 1871 Hoppe-Seyler drew attention to the "liquefaction" of dead tissues within the body which occurred even though no putrefaction took place, and which resembled the effects produced by digestive enzymes. This change in dead tissues was shown by Salkowski in 1890 to be a true digestion by intracellular enzymes, in which were produced the amino-acids leucine and tyrosine, products which at that time were considered characteristic of tryptic digestion. Salkowski named the process "autodigestion"; Jacoby, in 1900, termed it *autolysis* (Gk. *autos*, self; *lysis*, a loosing).

Autolysis processes are generally studied by Salkowski's method, which takes advantage of the different susceptibility of enzymes and bacteria to antiseptics, bacteria being more easily put out of action. Obviously bacterial action must be excluded. Organs are ground to a pulp, placed in flasks with or without addition of water, and bacterial action prevented by addition of antiseptics which have little action on enzymes; toluene and chloroform are usually used.

It is, of course, theoretically possible to remove organs under aseptic conditions, and to allow autolysis to proceed without addition of antiseptics, but the practical difficulties of preserving asepsis—complete absence of bacterial action—are great, and "aseptic autolysis" is little studied.

It is found that digestive changes take place fairly rapidly. To quote the results of a typical experiment, in which a given specimen of emulsionised liver was allowed to digest itself for twenty-two days at body temperature, 37° C., at the end

of this time 39·4 per cent. of the nitrogen was present in insoluble form, and 60·6 per cent. in soluble form. A control, initially boiled to destroy enzymes, and then kept for the twenty-two days under the same conditions, contained 90·4 per cent. of insoluble nitrogen compounds, and only 9·6 per cent. of nitrogen in soluble form, so that 51 per cent. of the nitrogen compounds had been changed from insoluble to soluble form, that is, had undergone digestion to some degree. In another experiment it was determined by hydrolysis with mineral acids that the liver employed would yield, in the amount used, 45·8 gm. of amino-acids. Autolysis set free in ten days 1·85 gm. ; in thirty days 10·1 gm. and in fifty days 29·1 gm. Complete disintegration of proteins with liberation of all the amino-acids is probably never attained *in vitro*.

Practically every tissue of the body has been examined, and all those that have been examined were found to possess the power of self-digestion, so that *every cell in the body contains proteases*. Different tissues self-digest at different rates. The liver digests itself fairly rapidly, brain and muscle tissue much more slowly. The activity of the enzymes varies under different conditions. Fever causes marked increase in the proteolytic activity of muscle. The character of the antiseptic used greatly modifies the rate. Salicylic and benzoic acids permit the most rapid action ; of non-acid antiseptics toluene allows the fastest action. These results are due to variations of hydrogen-ion concentration. Sufficient acidity inhibits the action altogether. At an alkalinity corresponding to that of blood autolytic action is reduced to a minimum. The optimum *p*H for autolysis is between 6·6 and 6·8 ; such a *p*H is developed in tissues during asphyxiation. The rate of action in laboratory experiments in glass vessels is slow at first, and then accelerates. This corresponds to an increasing though slight acidity, developed during the process.

The intracellular proteases differ from trypsin, since they act best in a very slightly acid medium, and, furthermore,

produce more ammonia than does trypsin. *They differ from pepsin*, not only in the much less degree of acidity requisite for their action, but also because they carry hydrolysis to the amino-acid stage. *They differ from erepsin* in their ability to attack the cell-proteins. They cannot therefore be considered to be digestive enzymes which have passed to the various tissues by way of the blood. The products from their action most closely resemble those from tryptic digestion. Thus Dakin has detected in these products (from kidney autolysis) ammonia, alanine, cystine, α-aminovalerianic acid, leucine, α-pyrrolidine carboxylic acid, phenylalanine, tyrosine, lysine, histidine, hypoxanthine, and indole compounds (probably including tryptophane).

Other enzymes are, of course, also present in the cell. Glycogen disappears very rapidly from autolysing liver and muscle ; fats are split by a lipase, and the fatty acids that are set free are found in autolysed organs. Lactic acid is formed (and is one factor in the change of *p*H). The total fat (including its products) appears to increase ; this is probably due to the decomposition of lecitho-proteins, and perhaps of the phosphatides themselves (esterases are present). Nucleo-proteins are broken down and purines set free.

Not only do such processes go on in the dead animal, but it has been shown that they proceed in any dead (or damaged) tissue in the living animal. Thus Jacoby found that on ligaturing off a portion of the liver in an animal, and allowing it to remain *in situ*, after some time the necrosed tissue showed an accumulation of leucine, tyrosine, and other hydrolytic products. Whenever tissues are disintegrated in any considerable quantity, as after extensive burns, then proteolytic enzymes become demonstrable in blood and urine ; these are presumably derived from the damaged tissue.

It might be thought at first sight that the tissues would continually undergo digestion by the enzymes they contain. But it must be remembered that normally the tissues are

slightly alkaline, and that at this degree of alkalinity autodigestion scarcely occurs. Further, as we shall see shortly, in life the tissues are continually being provided with fresh supplies of amino-acids, and since these are the end products of autolytic protease digestion, if there is anything in the nature of a balanced reaction, the presence of these will also lessen any autolytic activity. All dead and dying cells contain acids, largely lactic acid, which has escaped further oxidation through lack of oxygen. With death, therefore, the conditions rapidly become optimal for autolysis, and autolysis takes place.

To a certain extent the autolytic *proteases* of each organ are specific in their action. *They can only attack proteins that are present in that organ.* Their specificity is limited to the initial stage of the hydrolyses. Thus, liver extract will not hydrolyse lung tissue, but it will hydrolyse the proteoses derived from lung tissue.

Intracellular enzymes play a relatively greater part in the lives of the simpler-constituted animals, and in plants. Some of the enzymic actions of yeast, as typifying those of the simplest plants, will be discussed in the next chapter.

All the protozoa, and many somewhat more developed animals, obtain their nourishment by the actions of intracellular enzymes. In sponges, coelenterates, and some of the lowest forms of worms such as turbellaria, cells lining the digestive cavity *absorb* particles of food material, and dissolve them usually within vacuoles in their cytoplasm. Extracellular digestion by enzymes secreted into the digestive tract seems first to occur in the higher worms, such as nematodes and earthworms.

Proteolytic enzymes have been extracted from the bodies of amœbæ and from the " phagocytic " cells of coelenterates such as the sea-anemone. These enzymes digest proteins both in very weakly acid and very weakly alkaline solutions.

Enzymes present in the White Corpuscles of the Blood. The *polynuclear leucocytes* contain a protease, *leucoprotease*, which can be extracted from purulent sputum or fresh pus by glycerol, or can be obtained from the leucocytes of a sterile inflammatory

exudate produced by injection of aleuronal into the pleural cavity of a dog, by treating the washed cells with absolute alcohol in sufficient amount to cause dehydration, then washing with ether, air-drying, and powdering. Such preparations actively digest the serum-proteins, either in a neutral or a very slightly alkaline medium. Acidity corresponding to 0·2 per cent. acetic acid inactivates the enzyme. Its activity is impaired at 65° C., and it is destroyed between 70° and 75°. Leucoprotease is present in bone-marrow, and is formed within the polynuclear leucocytes before they leave the bone-marrow.

The expressed juice of the spleen contains two proteases, one of which will act only in acid medium, but the other strongly resembles leucoprotease, digesting in a slightly alkaline medium not only spleen cells, but also fibrin, casein and coagulated serum proteins.

Rabbit leucocytes do not contain leucoprotease, but an ereptase. Leucoprotease is absent from the leucocytes of the fowl.

Leucoprotease is much less active than trypsin, and seems to produce relatively more proteose and less amino-acids.

The resistance of living bacteria to such enzymes seems due to anti-enzymes (the spleen also contains an anti-enzyme). On the other hand, their inactivity with regard to blood proteins under normal conditions seems due to a pseudo-anti-enzymic activity of, probably, lipide substances present in the blood.

The *mononuclear phagocytes* (*macrophages*) which, during the later stages of acute inflammation increase, and engulf and digest the polynuclears, red cells, and other cellular constituents, contain a different protease which digests best in a slightly acid medium, such as that of 0·2 per cent. acetic acid. It is almost entirely inactive in neutral and slightly alkaline media, and is more susceptible to heat than is leucoprotease, resembling very closely in its properties the autolytic enzyme of parenchymatous tissue.

Leucocytes are also stated to contain an ereptase.

The Synthetic Activity of Enzymes

Since all tissues contain autolytic enzymes which, following the slight acidity which develops during the asphyxiation of those tissues through lack of oxygen, digest the tissues, breaking down proteins, complex fat-derivatives and carbohydrates to their simplest derivatives, and since these

enzymes are practically inactive in producing such hydrolyses at the normal alkalinity of the tissues, one at once suspects that possibly at this slight alkalinity these enzymes, or some of them, may possess synthetic activity, especially since a number of instances are definitely known in which with changed conditions enzymes can produce synthetic products from the compounds they have themselves produced by hydrolysis.

The recent work of Wasteneys renders such a supposition very probable. He has shown that if pepsin is added to the concentrated solution of the products of peptic digestion of egg-albumin a protein *plastein* is synthetised of the same order of complexity as the original egg-albumin (though less soluble) to such an extent that it contains as much as 39 per cent. of the nitrogen present in the digest. The synthesis takes place within a few minutes, and the essence of the production of synthesis rather than of hydrolysis seems in this, as in other enzymic syntheses, to depend on the concentration of the hydrolysate. The optimum temperature is somewhat higher than for digestion. Feeding experiments on mice have shown that this *plastein* satisfies the protein requirements of the organism. Trypsin synthesis has also been produced.

It can be shown on theoretical grounds, based on the law of mass action, that in considering the decomposition of proteins and their possible synthesis from their hydrolysed products, increasing the concentration of the protein lessens the degree of its decomposition, and so facilitates synthesis, while increase of temperature also facilitates synthesis.

Numerous observers, commencing with Danilewski in 1886, noted that the addition of pepsin to the concentrated products of pepsin hydrolysis resulted in the formation of precipitates, which were assumed to be of protein nature, and which Sawjalow, in 1901, termed *plasteins*. Henriques and Gjaldbäkin (1911) showed definitely that synthesis occurs, by demonstrating that following plastein formation there is a diminution of free amino-nitrogen. Plastein is rapidly hydrolysed by pepsin, this establishing its protein nature. Wasteneys and Borsook have shown

that it is digested at the same rate as native proteins, and like them yields proteoses and peptones, and have demonstrated a simple procedure for its production.

Taylor (1907) digested protamine sulphate with a glycerin extract of clam liver, complete hydrolysis resulting. The digest was concentrated, and then more of the glycerin extract added ; a precipitate formed (during a period of five months) which was identical chemically with the original protamine sulphate.

There is evidence that the proteins of plastein type are soluble as synthetised, but are rendered insoluble by some process of denaturation brought about through the presence of the hydrolysed product. For example, when a solution of albumin is added to a concentrated peptic digest at *p*H 4·0, it is precipitated rapidly and completely within one hour.

The constitution of the plasteins tends to be more variable than that of naturally occurring proteins. The higher the temperature at which they are formed, the higher is the yield. With pepsin, 72° C. gives the maximum yield (pepsin in presence of substrate will resist that temperature for some time). The optimum *p*H, according to Wasteneys and Borsook is 4·0, but definite amounts are formed even at *p*H 5 to 6. Wasteneys states " Contrary to classical conceptions of enzyme action it is found that the equilibrium position is definitely affected by the concentration of enzyme " ; the more enzyme present, the greater is the synthesis. Different substrates give different yields ; from albumin a 50 per cent. yield is obtainable, but from gliadin only 10 per cent.

Since digestive enzymes which can break down protein can also build up protein, we may suppose that there is great probability that the intracellular enzymes possess similar synthetic powers when the necessary conditions exist.

Abderhalden (1916) autolysed various macerated organs, liver, thyroid, lung, kidney, until no biuret test was given, then concentrated the digests and added extracts of various tissues. After five months he obtained definite evidence of synthesis of heat-coagulable material of protein nature, provided the extract added was of the same organ.

Further, since, as has already been mentioned, these intracellular proteases are specific in their initial action, acting only on proteins in the tissues that contain them, though

they are not specific in their action on proteoses, it is not surprising that if they do synthetise protein, the protein that is formed will also be specific : the specific protein present in their tissues.

If this be the case *the activities of cells will consist of a series of balanced reactions between complex compounds and their enzymic products of hydrolysis, the equilibria attained depending on the actual conditions existing in the cells from time to time.*

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CHAPTER XXI

SOME ACTIONS OF THE YEAST CELL ILLUSTRATIVE OF INTERMEDIATE METABOLISM

Intermediate Metabolism

A STUDY of the diet and of digestion tells us what compounds are absorbed into the body and brought into contact with the individual cells through the circulation of the blood. A further study of compounds present in the blood and in the excreta tells us something of the nature of the end products, the final oxidised (or partly oxidised) degradation products formed in the cells.

We have now to consider what has been ascertained about the chemical processes by which the first series of compounds have been changed into the second. We have to deal with what the Germans call " Stoffwechsel," change in substance, a perhaps more accurate expression of the processes than " Metabolism " (Gk. *metabole*, change). This we have to consider from two points of view, the qualitative and the quantitative. The first deals with the chemical nature of the changes, the second, not only with the amounts of substance so changed, but also with the energy transformations involved in these changes.

These series of chemical changes are inextricably involved with each other, and we shall not commence to know the whole truth of intermediate metabolism until we know much more about these interlocked reactions than we do at present. We may consider one such example of interlocked reactions. The body oxidises fatty acids to butyric acid, and this to β-hydroxybutyric and acetoacetic acids. While

glucose is normally undergoing oxidation the organism oxidises these acids to acetic acid, and, finally, to carbon dioxide and water. But in the absence of correct glucose oxidation the organism can only form acetone from the partly oxidised fatty acids. Again, as long as carbohydrate metabolism is proceeding normally, a certain amount of creatine is steadily converted by muscular tissue into creatinine ; if the glucose supply is cut off so that the glycogen store in muscle is depleted the dehydration of the creatine is lessened, and some proportion of it is unconverted and excreted unchanged.

Further examples of such interlocked reactions will become evident in the next few chapters. The simplest method of attacking the study of intermediate metabolism is to deal with the different classes of compounds that have already been enumerated, and to follow them as far on their path of change as possible, filling in as many of the gaps as present knowledge allows.

It must be remembered always that *the substances taken within the organism serve one or both of two purposes, to provide the organism with energy, and to provide material for the normal upkeep of the activities of all the cells of the organism.*

Before proceeding to deal with the complex mammalian organism some advantage is to be gained by studying certain series of reactions which take place in such a single-celled organism as yeast, whose most spectacular activity is the production of carbon dioxide and alcohol from glucose, a process whereby the cell itself gains energy and certain material that it requires.

Some Phases of Metabolism in the Yeast Cell

If yeast is added to a solution of glucose, or fructose, or mannose, the sugar is decomposed and the chief products are carbon dioxide and ethyl alcohol :

$$C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2.$$

If yeast cells are ground up with sand, mixed with kieselguhr (a silicate), and subjected to great pressure, a somewhat viscous opalescent brownish-yellow liquid, *yeast-juice*, is expressed, which retains this fermentative property. It is usually faintly acid and almost optically inactive. This juice contains a number of enzymes :

(i.) The enzyme *zymase* which is usually considered chiefly responsible for the fermentation of sugar.

(ii.) A powerful protease, *endotrypsin*, which not only digests ordinary proteins, but also digests zymase, showing that this enzyme is itself of protein nature (zymase is also digested by pancreatic trypsin).

(iii.) A *hexose-phosphatase* which splits hexose-phosphates into hexose and phosphate.

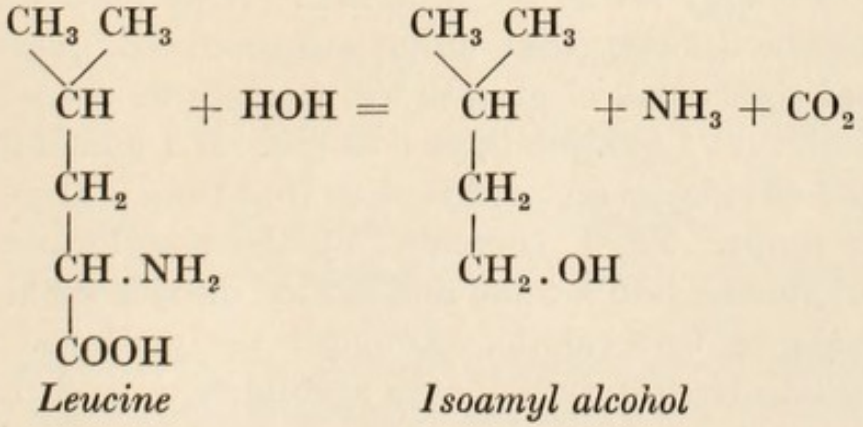
(iv.) A *carboxylase* which splits off carbon dioxide from *keto-acids*, producing aldehydes :

$$R.CO.COOH = R.CHO + CO_2$$

(v.) Apparently a reducing enzyme, but actually in all probability *glutathione*. Its action can be illustrated by the formation of the colourless base of methylene blue in presence of acetaldehyde by yeast juice, there being involved a *simultaneous* oxidation and reduction :

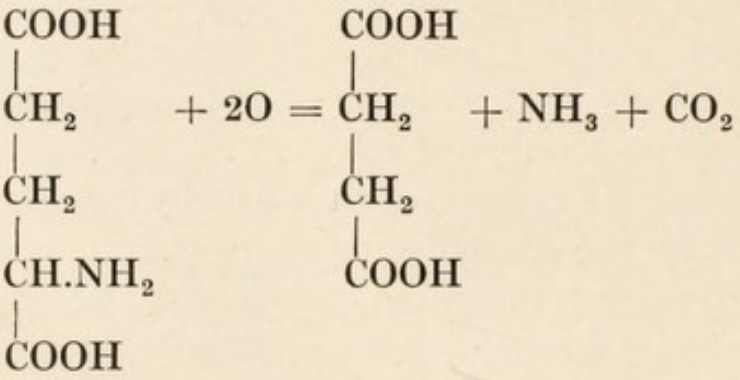
$$CH_3.CHO + \text{Methylene blue} + HOH = CH_3.COOH + (\text{Methylene blue} + 2H = \text{leuko-base}).$$

(vi.) A specific enzyme capable of acting on certain amino-acids and converting them into higher alcohols :



Leucine *Isoamyl alcohol*

Either this enzyme acting as an oxidase, or another specific enzyme, can convert glutamic acid into succinic acid :



Glutamic acid *Succinic acid*

In addition a *co-enzyme* is present. If yeast juice is dialysed under pressure the zymase left behind is inactive, and the dialysed juice is inactive. On mixing the residue and the dialysed juice the activity is at once regained. The nature of this co-enzyme is not yet definitely ascertained. It is, however, known to be an organic compound, and there is some reason to believe that it is potassium pyruvate, $CH_3 . CO . COOH$, but von Euler and his colleagues conclude from diffusion experiments that it is a much more complex organic compound. They consider that it is a normal constituent of all cells concerned with carbohydrate metabolism.

How is the life of the yeast cell bound up with the alcoholic fermentation, and what do these various enzymes do ?

The alcoholic fermentation is used by the cell to gain a supply of energy for its life processes. If we burn 1 gm. of glucose 3·74 calories (heat units) are produced. One hundred and eighty gm. of glucose will produce 92 gm. of ethyl alcohol, so that 1 gm. produces 0·51 gm. If 1 gm. of alcohol is burnt 7·09 calories are produced, so that from 0·51 gm. 3·61 calories result. Yeast, therefore, by the transformation of 1 gm. of glucose into alcohol and carbon dioxide obtains the slight balance, 0·13 calorie. Amongst the products of the intermediate transformations are acetaldehyde and pyruvic

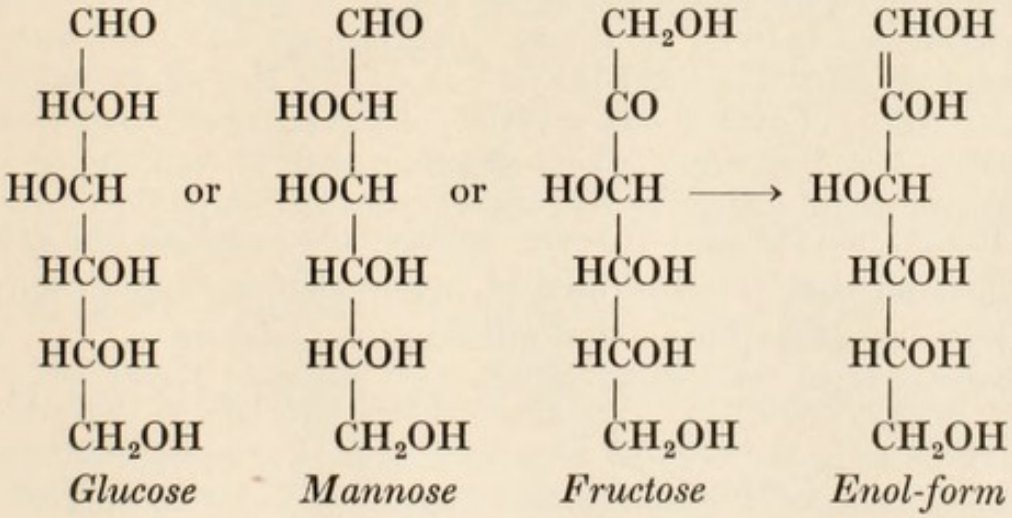
acid, and since small amounts of these escape the final transformation to alcohol it must be concluded that the yeast uses these small amounts for its own purposes, the alcohol being merely an excretory product.

When yeast is allowed to act on a crude sugar solution like molasses, which contains a certain amount of protein, the yeast rapidly grows, *i.e.*, the number of yeast cells rapidly increases, and at the same time, in addition to alcohol and carbon dioxide, there are produced as other bye-products small amounts of *fusel oil* (chiefly isoamyl alcohol), of glycerol, and of succinic acid.

The isoamyl alcohol is formed, as we have seen, from leucine obtained from protein, and the succinic acid from glutamic acid. The proteins of the molasses will furnish the necessary supply of these amino-acids. From the breakdown products of protein the yeast can build up its own protein, perhaps in part through the agency of *endotrypsin*.

How are the alcohol, carbon dioxide and glycerol formed from sugar ?

Since either glucose or fructose, or mannose can be acted on by yeast the first change is believed to be the formation of an enolic hexose.



It has been shown that the presence of phosphate materially accelerates the reaction and that a hexose-diphosphate

is formed. According to Harden and Young, the next stage is therefore :

$$\underset{\textit{Enol-hexose}}{2C_6H_{12}O_6} + 2PO_4HR_2 = 2CO_2 + 2C_2H_6O + 2HOH + \underset{\textit{Hexose-diphosphate}}{C_6H_{10}O_4(PO_4R_2)_2} \quad (1)$$

The hexose-phosphatase immediately breaks down the hexose-phosphate to hexose and phosphate :

$$C_6H_{10}O_4(PO_4R_2)_2 + 2HOH = C_6H_{12}O_6 + 2PO_4HR_2 \quad (2)$$

Equation (1) is, however, not brought about in a single stage. It seems to involve the breakdown of the enol-hexose into some compound $C_3H_6O_3$, whose nature is not yet known, and which is immediately further changed. This breaking up of the carbon chain is the part presumably attributable to zymase with the aid of the co-enzyme (potassium pyruvate ?). This equation should therefore be written :

$$2C_6H_{12}O_6 + 2PO_4HR_2 = 2C_3H_6O_3 + C_6H_{10}O_4(PO_4R_2)_2 + 2HOH \quad (3)$$

The final stages are :

$$2C_3H_6O_3 = \underset{\textit{Pyruvic acid}}{2CH_3 . CO . COOH} + 4H \quad (4)$$

$$2CH_3 . CO . COOH = \underset{\textit{Acetaldehyde}}{2CH_3 . CHO} + 2CO_2 \quad (5)$$

$$2CH_3 . CHO + 4H = \underset{\textit{Ethyl alcohol}}{2CH_3 . CH_2OH} \quad (6)$$

Reactions (4) and (6) are interlocked, and it has been suggested that the intermediate agent, the *hydrogen-carrier*, is possibly glutathione, and not a special enzyme—

$$2C_3H_6O_3 + \underset{\textit{Oxidised Glutathione}}{2G—S—S—G} = 2CH_3 . CO . COOH + \underset{\textit{Reduced Glutathione}}{4G—SH} \quad (4A)$$

$$2CH_3 . CHO + 4G—SH = 2C_2H_6O + 2G—S—S—G \quad (6A)$$

Reaction (5) is brought about by the carboxylase.

The glycerol has still to be accounted for. It has been found that if sodium sulphite is added to the solution in which yeast is acting, so that acetaldehyde forms an insoluble acetaldehyde-bisulphite, and is thus removed from the field of reaction, glycerol is formed, and for every molecule of acetaldehyde that is so precipitated one molecule of glycerol appears and one molecule less of alcohol is produced. Such a change is easily understood from the above reactions. If the aldehyde in reaction (5) is not available for reaction (6A), then the glutathione reacts with a corresponding amount of the unknown compound, $C_3H_6O_3$, causing it to take up hydrogen and form glycerol :

$$2C_3H_6O_3 + 4G{-}SH = 2C_3H_5(OH)_3 + 2G{-}S{-}S{-}G \quad . \quad (7)$$

Glycerol

This reaction shows that for every molecule of aldehyde removed one molecule of glycerol will be formed. Under normal conditions 3·8 per cent. of sugar is converted to glycerol, and this strongly suggests that the aldehyde is removed by the yeast and converted into something for its own use.

Under abnormal conditions, as in war, this bye-reaction can be accentuated by addition of sulphite for the production of glycerol commercially, and the Germans used it to produce one million kilograms of glycerol per month, the yield being 15 to 20 per cent. of the sugar fermented.

In an alkaline solution a certain amount of acetic acid is produced. This is due directly to the action of the alkali in promoting the reaction :

$$2CH_3 . CHO + HOH = CH_3 . COOH + CH_3 . CH_2OH \quad . \quad (8)$$

To the extent to which reaction (8) takes place hydrogen will not be used up in (6A) and (7) will also take place. Half the amount of alcohol is formed in (8) that would be in (6A), so that for every molecule of acetic acid formed the yield of alcohol will be diminished by one molecule, and two molecules of glycerol will be produced. These quantitative changes have been shown to take place.

Meyerhof believes that Harden and Young's diphosphate is not initially produced in yeast fermentation, but that the essential compound is a hexose monophosphate, part of which is subsequently transformed to the diphosphate.

Neuberg represents the series of changes somewhat differently. He considers that the sugar first loses water to form methyl glyoxal aldol, which yields methyl glyoxal.

$$C_6H_{12}O_6 - 2H_2O \rightarrow C_6H_8O_4$$
$$\rightarrow 2CH_2 : C(OH) . CHO$$
Enol-form of methyl glyoxal

The methyl glyoxal reacts with water to produce glycerol and pyruvic acid :

$$CH_2 : C(OH) . CHO + H_2O + H_2 \rightarrow CH_2OH . CHOH . CH_2OH$$
Glycerol

$$CH_2 : C(OH) . CHO + O \rightarrow CH_3 . CO . COOH$$
Pyruvic acid

Carboxylase decomposes the pyruvic acid to acetaldehyde, which reacts with more methyl glyoxal and water to produce pyruvic acid and ethyl alcohol :

$$CH_3 . CO . COOH \rightarrow CH_3 . CHO + CO_2$$
$$CH_2 : C(OH) . CHO + O \rightarrow CH_3 . CO . COOH$$
Pyruvic acid

$$CH_3 . CHO + H_2 \rightarrow CH_3 . CH_2 . OH.$$
Ethyl alcohol

Theoretically large amounts of alcohol can be formed without the production of more than one molecule of glycerol.

In the presence of sodium hydrogen sulphite, the acetaldehyde can no longer form alcohol, and the last double reaction is altered to

$$CH_2 : C(OH) . CHO + O \rightarrow CH_3 . CO . COOH$$
$$CH_2 : C(OH) . CHO + H_2O + H_2 \rightarrow CH_2OH . CHOH . CH_2OH$$
Glycerol

The most favourable *p*H for the normal series of reactions is 3·7.

Sufficient has been stated to give some idea of the extreme complexity of the actual changes which are summed up in the apparent reaction :

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

Such an illustration may enable us to understand a little more clearly some of the involved procedures which bring about apparently simple reactions occurring in mammalian tissue.

This account, of course, only deals with a few of the activities of the yeast cell. Under favourable conditions it stores glycogen, so that an amylase must be present for its formation, and for its breakdown to glucose. Many other enzymes must be present for the complex processes involved in the reproduction of the cell.

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CHAPTER XXII

INTERMEDIATE METABOLISM OF THE CARBOHYDRATES

Carbohydrates are formed in the green leaves of plants through the action of certain rays in sunlight—chlorophyll acting as a light filter—on carbon dioxide in presence of water, formaldehyde being first formed, and oxygen liberated .

$$CO_2 + HOH = HCHO + O_2$$

The formaldehyde polymerises to hexoses, probably a mixture of glucose and fructose, and from these the plant forms all its starch, its cane-sugar and its cellulose.

It has been shown that the animal ingests a mixture of carbohydrates. Excluding cellulose, whose decomposition by bacteria in the intestines with formation of utilisable fatty acids is merely incidental in man, and excluding such non-utilisable compounds as inulin and raffinose, in the human organism as a result of digestion there are absorbed through the wall of the small intestine (little utilisable carbohydrate reaches the ileo-cæcal valve) into the capillaries of the portal circulation glucose, and much less fructose and galactose.

In infants on human milk diet glucose and galactose only will be absorbed.

The adult intestinal wall is impermeable to sucrose and lactose. Pentoses are absorbed, but the bulk of them is excreted in the urine unaltered.

Of the three hexoses only glucose is found in the blood of the general circulation. Fructose and galactose are changed in the liver. If the blood glucose be estimated before a meal,

and at half-hour intervals afterwards it is found that the initial value is between 0·08 and 0·09 per cent., and that this increases, according to the carbohydrate content of the meal, up to between 0·12 and 0·15 per cent., then decreasing between the first and third hour to the original level. The initial rise is rapid. Following a meal of 110 gm. of glucose in solution (admittedly a large dose) an increase of blood sugar has been observed within three minutes. It frequently happens that the curve actually shows a transitory fall to somewhat less than 0·08 per cent., the " fasting " value, so that the typical daily curve of the blood glucose can be represented as in Fig. 8.

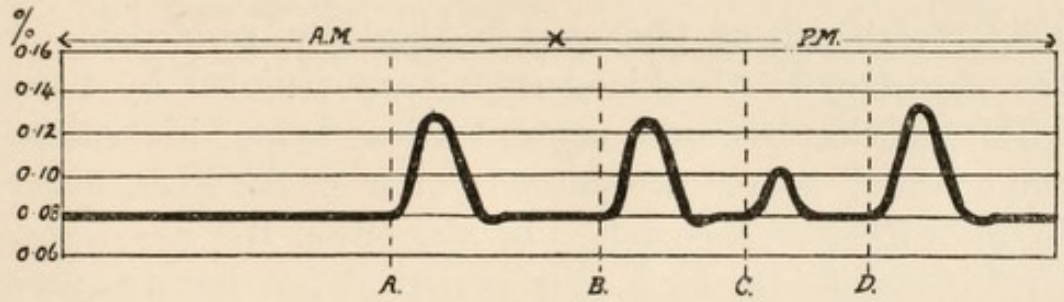


FIG. 8.—Typical twenty-four-hours' blood-glucose chart. *A*, breakfast ; *B*, lunch ; *C*, tea ; *D*, dinner.

The blood glucose remains, therefore, approximately constant for the greater part of the twenty-four hours, with a slight rise after meals. Seventy to 80 per cent. of this glucose is present in the plasma, as an ordinary equilibrium mixture of α- and β-*d*-glucose.

The carbohydrate ingested in a single meal may easily exceed 100 gm. If this were converted into glucose, and the glucose injected into the general circulation, then in the blood volume of a 70-kilogram man (8·8 per cent., or about 6 litres) there should be an increase in glucose concentration of more than 1·5 per cent. This increase does not occur. The liver removes most of the glucose and stores it as glycogen, and the increased amount of glycogen can be demonstrated.

There are limits to the rate at which the liver can convert glucose to glycogen. If a meal is very rich in cane-sugar, which is quickly hydrolysed and absorbed, or in glucose itself, so that there results a high concentration of glucose in the portal circulation, then the amount in the general circulation may exceed 0·16 per cent., and part may pass the kidney-gate to the urine, producing a condition known as *alimentary glycosuria*.

There appears to be an equilibrium between glycogen and glucose in the liver, and we may presume that an amylase can produce the change in either direction, according to the concentration of glucose in the blood passing through the liver from hepatic artery to hepatic vein. This storehouse of glycogen is therefore responsible for the constancy of the blood sugar, any removal by tissues with resulting lowered concentration being immediately compensated by glucose poured out from the liver, formed by hydrolysis of glycogen. In starvation the blood sugar level is maintained for a long period at not less than 0·07 per cent.

Glycogen is found in most of the tissues of the body, but chiefly in the liver and muscles. Schöndorff fed dogs on a rich carbohydrate and meat diet for some time and then killed them and analysed their tissues for glycogen. He found that its average distribution in percentages was: Liver 38, muscle 44, bone-marrow 9, skin 4·5, viscera 3·8, heart-muscle 0·17, brain 0·09, and the white corpuscles of blood 0·015. In dog's liver as much as 18·7 per cent. of the weight of the fresh liver tissue may be glycogen, and similar high figures have been obtained for rabbits, of course after a rich carbohydrate diet. It is estimated that the liver of a man weighing 70 kilograms can hold as much as 300 gm. of glycogen, which is far more than the carbohydrate content of an ordinary meal. If this 300 gm. were oxidised in such a fashion that 20 per cent. of the energy was converted to mechanical work, and the remaining 80 per cent. to heat,

the work done would be the same in amount as that required to lift 10 tons 30 feet.

Usually the amount of glycogen present in the human liver is of the order of 100 gm. ; in starvation it falls to a much lower level.

Schönheimer reported in 1929 the results of autopsy of an eight year old girl, dead following influenza. Liver and kidneys were enormously enlarged, and these tissues contained respectively 33·7 and 36·8 per cent. of glycogen as referred to desiccated tissue. Such a figure for kidney tissue is without precedent. Six days' autolysis of the liver tissue produced no diminution in its glycogen content, although glycogen prepared from this liver was hydrolysed rapidly by extracts of liver from other cadavers. Hence, it would appear that there had existed in this patient a disturbance preventing enzymic glycogenolysis.

In invertebrates the organs which correspond to the liver in function are the chief storehouse of glycogen ; it is present in the protoplasm of unicellular organisms, and in large amount in yeast.

In the tissues that contain it glycogen is present free in an amorphous granular state, so that the equilibrium in such tissues is represented by :

Solid glycogen $\rightleftharpoons$ *Glycogen in solution* $\rightleftharpoons$ *Glucose in solution.*

The percentage of glycogen present in muscle tissue is much less than that in liver tissue, the greater amount extractable from all the muscular tissue of the body being due to the much greater muscle mass. The amount varies greatly in different animals and in different conditions. Actually, determined values for striped muscle vary from less than 0·1 to over 3·0 per cent.

The heart muscle, in continual activity, contains very little of this reserve store of carbohydrate.

Experiments, carried out especially with toads, in which the liver was perfused with solutions containing the compound under test, and then the glycogen content of the liver estimated, have shown that the *liver tissue can form glycogen*

from glucose, galactose, maltose, glycerol, and formaldehyde, but not from sucrose, lactose, pentoses, and proteins. The main channel of glucose movement in the organism seems to be :

Glucose from the food ⟶ Liver glycogen ⟶ Glucose in the general circulation ⟶ Muscle glycogen.

Muscle glycogen does not appear to be again broken down to glucose which can pass into the circulation.

It can easily be demonstrated that muscle glycogen is utilised during the production of muscular work. If, for example, the two corresponding leg muscles of a frog are dissected out as soon as possible after pithing the animal, and the glycogen content of one be measured to serve as a standard for resting frog muscle while the other is stimulated with a tetanising current until it is so fatigued that it no longer responds, and then this fatigued muscle is immediately analysed for glycogen, it is usually found that 50 per cent. of the glycogen has disappeared.

If the total glycogen content of two animals is determined, in the one after a period of rest and in the other after a period of intense muscular exertion, the same sort of result is obtained. Always, with the performance of muscular work, there is a diminution of glycogen content. Külz forced a large and well-fed dog to draw a heavy cart for nine hours and forty minutes. Initially the dog weighed 45·5 kilograms. After the period of work the animal was immediately killed. From all its tissues only 52 gm. of glycogen were obtained, *i.e.*, 1·16 gm. per kilogram of the initial weight. A normal, well-fed dog, of similar size, contained 3·8 gm. per kilogram. Even a starved dog, similar in other respects, after starvation for four weeks, contained 1·5 gm. per kilogram body-weight, so that less than ten hours of severe muscular exertion reduced the glycogen content of the body to a greater extent than did four weeks of sheer starvation.

By what processes does the glycogen of the muscle provide energy ? Obviously by processes involving oxidation. The series of changes are at least as complex as, and bear some resemblance to, those by which the yeast cell ferments

sugar (*cf.* Chapter XXXII.). Initially glycogen is broken down to lactic acid through a hexose-phosphate stage. This involves no oxidation :

$$\underset{\textit{Glycogen}}{(C_6H_{10}O_5)_n} + nH_2O = 2n\underset{\textit{Lactic Acid}}{CH_3 . CHOH . COOH}$$

In the presence of oxygen one-fifth of the lactic acid is oxidised to carbon dioxide and water, while the remaining four-fifths is reconverted into glycogen. The energy is provided from this oxidation :

$$3O_2 + CH_3.CHOH.COOH \rightleftharpoons 3CO_2 + 3HOH.$$

Almost certainly, in this as in similar processes, pyruvic acid is an intermediate product :

$$O + CH_3.CHOH.COOH = CH_3.CO.COOH + HOH.$$

Such a change, even to a slight extent, will explain the slight formation of alcohol which has been demonstrated in muscle (*cf.* Chapter XXI).

A. E. Taylor starved a dog for one day prior to operation, then removed the whole of its alimentary tract to avoid any possibility of bacterial production of alcohol, and eighteen hours later killed the animal, examined the muscles for alcohol, and definitely identified the presence of ethyl alcohol, though only a small amount was present. A Japanese investigator has recently found that in fowls, asphyxiated in various ways (so that incorrect or deficient oxidation is established), not only is the tissue content of alcohol increased, but alcohol is definitely present in the blood. Presumably, therefore, alcohol is formed in the tissues from glycogen, but merely as an unimportant bye-product. It is also interesting to note that a slight amount of succinic acid is present in muscle, making still closer the analogy between the yeast actions and muscle metabolism ; presumably muscle also contains an enzyme capable of oxidising glutamic acid.

In absence of sufficient oxygen, as during continued severe muscular exertion with partial tissue asphyxiation, lactic acid accumulates, until a distinct muscle acidosis exists. This can be demonstrated in the fatigued muscle of the frog ; litmus paper applied to its cut surface is turned red. The lactic acid passes in part to the circulation, and subse-

quently, during rest, is in part re-absorbed ; part is absorbed by the liver and converted to glycogen there.

The actual mechanism causing the contraction of muscle still remains to be determined. It is not directly connected with the glycogen-lactic acid changes (*cf.* Chapter XXXII.).

What other Compounds can the Body Form from Glucose ?

When the body continuously ingests excess carbohydrate, the excess tends to be laid down as fat. Such a change seems obvious from the ordinary practice followed by farmers in fattening animals. It was first demonstrated scientifically by Lawes and Gilbert, the founders of the celebrated Rothamsted experimental farm. In 1852 they published the results of experiments in which they fed young pigs on barley, and showed that, since barley contains but a trace of fat, and the amount of fat laid on was considerably greater than could possibly have come from the proteins of the diet after deducting that used up for tissue formation, some must certainly have come from carbohydrate. (Details of their experiment are given in Chapter XXVI.)

Such changes must obviously proceed through the stage of glucose, and in order that a neutral fat may be formed it is necessary that both glycerol and fatty acids be produced from glucose.

There is evidence that glucose is oxidised in other tissues than muscle. This also probably occurs through the intermediate stage of hexose-phosphate, since whenever by some drastic procedure, as by injection of insulin, the blood glucose concentration is rapidly lowered, there is coincidently a depletion of inorganic phosphate from the blood plasma.

Some idea of the series of reactions involved in the conversion of glucose into fat, and in the direct oxidation of glucose, is gained by study of the changes that can be brought

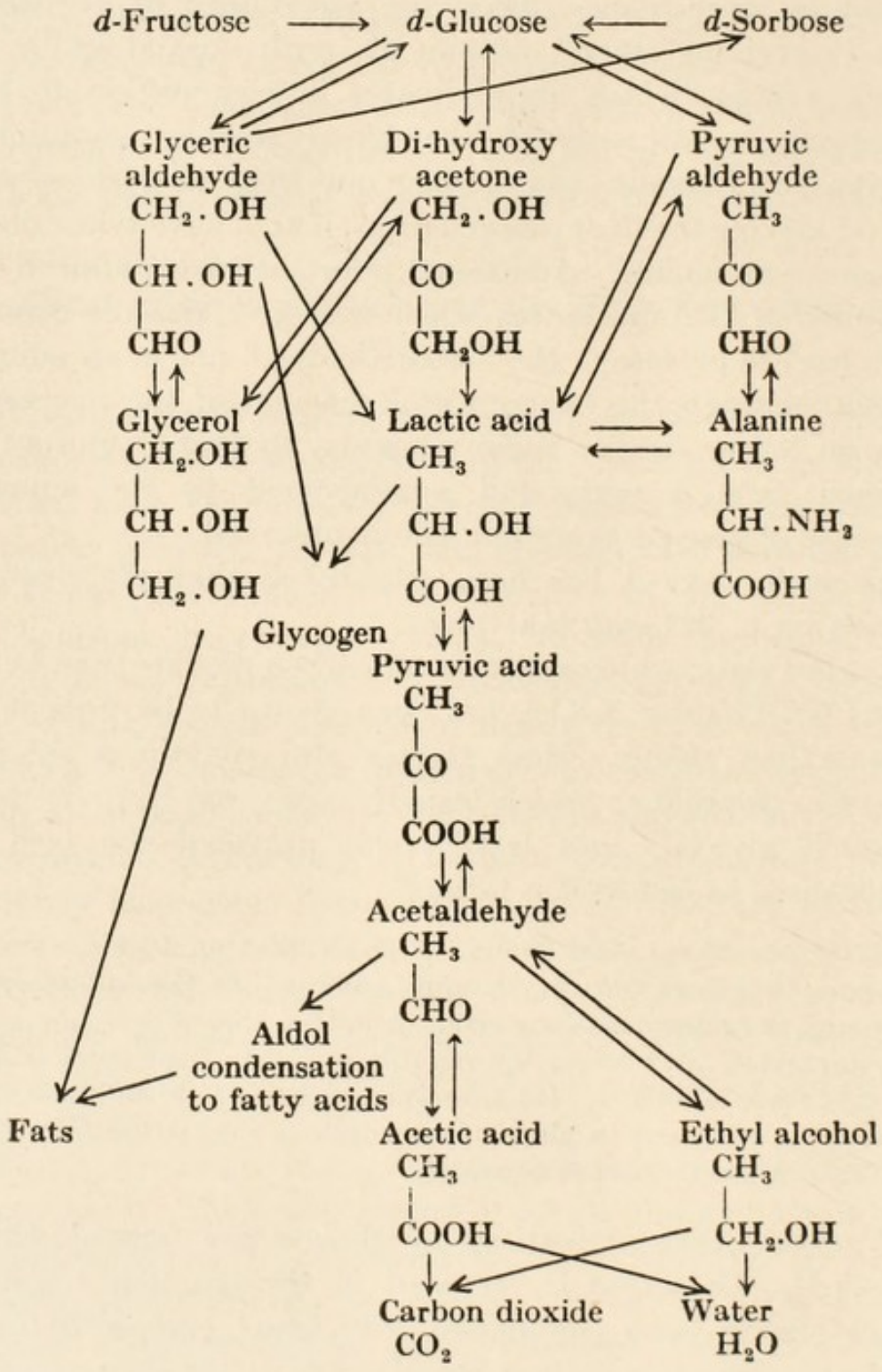
about by liver tissue. These are ascertained by perfusing the "surviving liver" of animals with normal saline or Locke's fluid (which approximates in composition to the inorganic constituents of the blood plasma) containing various compounds, and finding out from analysis of the liquid leaving the liver what changes, if any, have taken place in such compounds. Additional information is obtained by a study of the substances which can give rise (or cannot give rise) to glucose in the "diabetic dog," *i.e.*, in an animal which, through the experimental removal of the pancreas, can no longer oxidise sugar properly, so that if glucose is formed from a compound administered to the animal, increase of glucose excretion will be observed.

In such ways it has been established that the changes shown on p. 324 *may* take place.

Carboxylase, which will split off carbon dioxide from keto-acids (*cf.* Chapter XXI.), has been shown to be present in mammalian tissue. Most tissues also contain a specific enzyme *glyoxalase*, which rapidly converts pyruvic acid (methyl glyoxal) into lactic acid, provided the acid is neutralised as fast as it is formed.

Attempts have been made to show that glucose probably changes to a very active "gamma-glucose" in the body before subsequent transformations occur, but this theory has not been substantiated, and seems improbable in light of the most recent studies made to test it. Gamma-glucose does exist, and probably contains a butylene in place of an amylene ring structure. It is doubtful if it ever occurs naturally.

Various other derivatives of glucose are formed where required. Galactose is prepared in the mammary glands from glucose, and conjugated with more glucose to form lactose. Glucosamine and chondrosamine and its derivative chondroitin sulphuric acid must be built up from glucose. It is not certain from what the pentose of certain nucleotides is formed, but glucose may well be the precursor. Glycuronic acid is formed from glucose in the liver whenever certain



toxic compounds require to be rendered harmless. Once formed, the body cannot easily destroy this acid ; its formation is abnormal and not the usual channel of glucose catabolism.

Can Glucose be Formed from Other Sources than Carbohydrate of the Diet?

This can very conveniently be determined by experiments on the diabetic dog. As has been stated, since this animal can no longer oxidise glucose, any substance which can give rise to glucose will, when fed, be converted to glucose (in whole or part) and excreted as glucose. Experiments of this kind have shown that 58 per cent. of proteins, 10 per cent. of fats (the glycerol portion), and 100 per cent. of carbohydrates (excluding cellulose and other non-utilisable

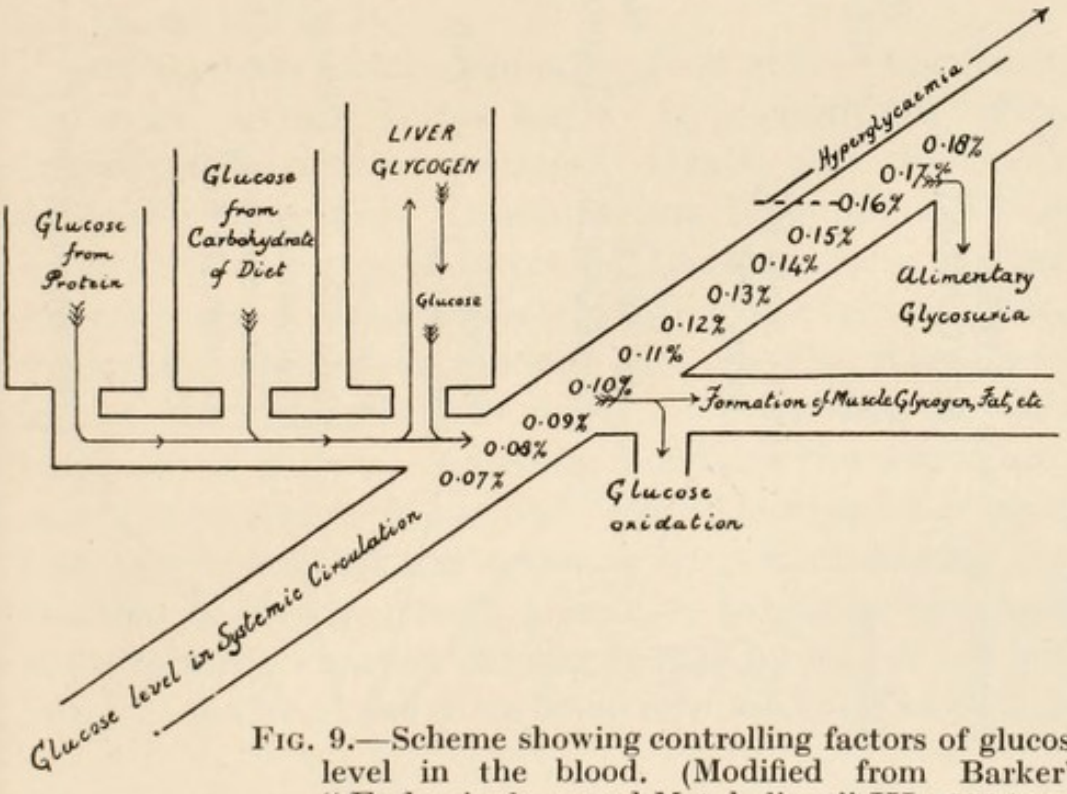
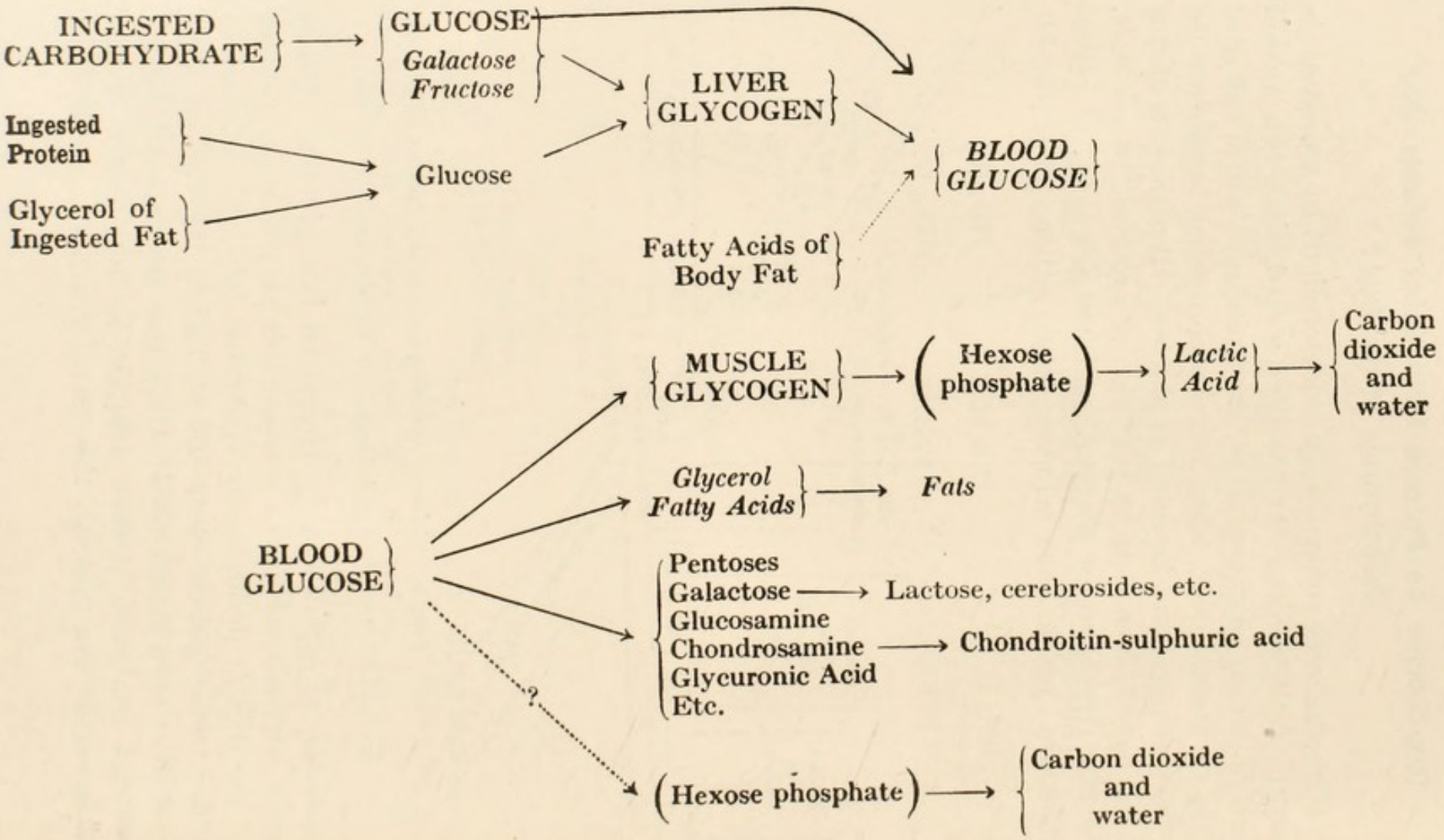


FIG. 9.—Scheme showing controlling factors of glucose level in the blood. (Modified from Barker's "Endocrinology and Metabolism," III., 252.)

material) of a diet can be converted into glucose. Hence *under normal conditions* a considerable proportion of protein of the diet is undoubtedly converted into glucose, and so added to the glucose store and utilised in the various ways that have been enumerated. Obviously the extent to which proteins can form glucose depends on how many of the amino-acids can undergo this transformation. It has been


INGESTED CARBOHYDRATE
GLUCOSE
Galactose
Fructose
LIVER GLYCOGEN
Ingested Protein
Glycerol of Ingested Fat
Glucose
BLOOD GLUCOSE
Fatty Acids of Body Fat
MUSCLE GLYCOGEN
Hexose phosphate
Lactic Acid
Carbon dioxide and water
Glycerol
Fatty Acids
Fats
BLOOD GLUCOSE
Pentoses
Galactose
Lactose, cerebrosides, etc.
Glucosamine
Chondrosamine
Chondroitin-sulphuric acid
Glycuronic Acid
Etc.
?
Hexose phosphate
Carbon dioxide and water

shown that at least ten are capable of the change (see Chapter XXIII.).

Certain recent evidence adduced by J. J. R. Macleod and others suggests that very probably fatty acids are also transformed to glucose within the organism. Though many investigators consider that this change does not take place, the evidence favouring such transformation seems to be increasing.

The metabolism of the carbohydrates can be summed up in the scheme on p. 326, in which the dotted lines indicate possible but still unproved channels of change.

The factors determining the constancy of the blood glucose are shown in Fig. 9.

Carbohydrate metabolism is involved with all the principal metabolic changes in the body. It is controlled by both enzyme and endocrine actions. Insulin and adrenine are especially concerned. Insulin action is an important and apparently an essential factor for the conversion of glucose into glycogen in all tissues in which this takes place. Glucose oxidation either must proceed through this stage or else insulin has the additional power of promoting the direct oxidation of glucose. Adrenine facilitates the conversion of glycogen to glucose in the liver, and to lactic acid in muscle. Cori has shown that this action of adrenine can bring about transfer of glycogen from muscle to liver, the lactic acid, when formed in excess, passing in part by way of the circulation to the liver, and there being retransformed to glycogen.

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CHAPTER XXIII

INTERMEDIATE METABOLISM OF THE PROTEINS

Synthesis of Proteins in Plants. The animal depends on the plant not only for its carbohydrate, but also for the formation of several of the amino-acids from which it builds its proteins.

In the plant, protein, as well as carbohydrate, is essentially synthetised by the leaves. The probable stages of amino-acid formation in the plant are revealed by the studies of Baudisch and of Baly.

When carbon dioxide is passed through an aqueous solution of potassium nitrite that is exposed to ultra-violet light *activated* formaldehyde, H . C . OH, in which carbon is divalent, is produced, and reacts with the nitrite to form potassium formhydroxamate (with liberation of oxygen, which oxidises more of the formaldehyde to formic acid) :

$$\mathrm{H.C.OH + O{:}N.OK \longrightarrow H{-}C(=N{-}OK){-}OH \longrightarrow H{-}C(=N{-}OK){-}OH + O}$$

The free formhydroxamic acid readily loses oxygen :

$$\mathrm{H{-}C(=N{-}OH){-}OH \longrightarrow H{-}C(=N{-}H){-}OH}$$

The resulting compound by combination with more formaldehyde yields compounds which by rearrangement give amino-acids, as for example :

$$\mathrm{H{-}C(=NH){-}OH + H{-}C{-}OH \longrightarrow \underset{\displaystyle N\,H}{\overset{\displaystyle H\quad OH}{C}}\!\!-\!\!\underset{\displaystyle H}{\overset{\displaystyle H\quad OH}{C}} \longrightarrow CH_2{-}COOH \text{ (}NH_2\text{)}}$$

Baly has actually obtained α-amino-acids by such procedures.

There is, however, evidence that proteins can be formed by the plant in darkness, whence it would appear that the actual assimilation of nitrogen by the plant is not *necessarily* a photochemical process.

The digestive processes of the animal convert most of ingested protein into a mixture of amino-acids ; these are absorbed from the intestine into the blood vessels of the portal circulation, and so pass to the liver and the general circulation.

There is some experimental evidence that under unusual conditions certain proteins, such as those in egg-white, can be absorbed through the intestinal mucosa unchanged. During the first few days of life the cellular lining of the alimentary canal seems more permeable to " foreign " proteins than it is later. But under normal conditions in the adult any protein, proteose, or peptone that is taken up unchanged from the gut is broken down to amino-acids before it can reach the blood stream. Certain di- and tri-peptides are possibly absorbed from the intestine in slight amount. The large intestine has practically no power of protein absorption, and nutrient enemata are valueless as far as proteins are concerned.

Shortly after a meal there is a marked rise in the amino-acid content of the blood.

Van Slyke has greatly extended our accurate knowledge of such changes by devising an accurate instrument for the measurement of the nitrogen that is evolved when nitrous acid reacts with amino-acids, with the simultaneous formation of corresponding hydroxy-acids :

$$R.CH(NH_2).COOH + HONO = R.CH(OH).COOH + N_2 + HOH$$

Since this procedure only measures *nitrogen*, and the different amino-acids contain different percentages of nitrogen, it is necessary to express results based on this method in terms of *amino-acid nitrogen ;* they cannot be expressed in terms of the amino-acids themselves.

In 1912 Van Slyke and Meyer showed that after twenty-

four hours' starvation the blood of a dog contained amino-acids equivalent to 4 milligrams of nitrogen per 100 c.c. The dog was then fed meat, and during digestion the amino-acid nitrogen rose to 10 milligrams per 100 c.c. This excess of amino-acids very rapidly disappears from the blood. In another experiment these investigators injected 12 gm. of alanine (in solution) into the vein of a dog, the injection requiring ten minutes for completion. Five minutes later a sample of blood was withdrawn and analysed ; its content of amino-acid nitrogen indicated that only 1·5 gm. of alanine remained in the circulating blood. During this time 1·5 gm. had been excreted through the kidneys, so that the remaining 9 gm. must have been taken up by the tissues.

In a third experiment they injected intravenously into a dog a casein digest which contained 1·9 gm. of amino-acid nitrogen. Before injection the amino-acid nitrogen content of the blood was 4·05 milligrams per 100 c.c. ; one-half hour later it had risen to 19·7 milligrams, and three and one-half hours later had decreased to 7·85 milligrams, while at the same time *there was a rise of blood urea.* Their experiments also show that, unlike the passage of glucose to the general circulation, that of amino-acids is but little affected by passage through the liver. Thus in one experiment, before a meal, the blood of the femoral artery contained 3·7 milligrams of amino-acid nitrogen per 100 c.c., while after feeding the figure rose to 8·6, and, at the same time, the value for blood from the portal vein was 9·5. Thus the concentration had decreased by passage through the liver only by 0·9 milligram per 100 c.c., which is $0{\cdot}9/(9{\cdot}5 - 3{\cdot}7) \times 100$, or 15·5 per cent., an amount of the order to be expected if the liver removed these acids in proportion to the relative size of that organ.

Some idea of the amounts taken up by the different tissues is given by still another experiment of Van Slyke and Meyer. Amino-acids corresponding to 4·06 gm. of amino-acid nitrogen were injected intravenously into a dog. One-half hour

later the dog was killed and the blood and various tissues analysed.

Contents of—	Before injection.	After injection.
blood	3·9 mg. per 100 gm.	45·2 mg. per 100 gm.
liver	31·5 „ „ „ „	93·5 „ „ „ „
muscle	43 „ „ „ „	70 „ „ „ „
kidneys	45 „ „ „ „	106 „ „ „ „

Such experiments, therefore, show that there is rapid abstraction of amino-acids from the blood, so that their concentration in the blood tends to be kept at a fairly low and fairly constant level. Different tissues absorb them to different extents. The tissues do not immediately bring about marked changes in the amino-acids that they take up, but for some time hold them by mechanical absorption, or, more probably, in loose chemical combination with protein.

By a vividiffusion experiment Abel has succeeded in identifying a large number of the amino-acids that are present in blood.

This method consists essentially in passing the blood from an artery through a collodion tube, and so back to a vein. On the outside of the collodion tube is a normal saline solution, and substances which can diffuse easily (those having small molecules) pass into this solution until their concentration is the same on both sides of the collodion membrane.

Abderhalden, working on large quantities of blood, and using ordinary chemical procedures, has succeeded in identifying in the blood glycine, alanine, valine, leucine, aspartic and glutamic acids, proline, lysine, arginine, histidine, and tryptophane. Evidently, as is to be expected, all the amino-acids are normally present in traces. The corpuscles contain relatively larger amounts than does the plasma.

György and Zunz found in the blood of fasting dogs from 4 to 5 mg. of amino-acid nitrogen per 100 c.c. The plasma contained 1·8 to 3·9 mg., the corpuscles 7·2 to 8 mg. per 100 c.c. respectively. Cary and Meigs (1928) find the average tryptophane content of both cow's whole blood and plasma is 1·1 mg. per 100 c.c.

What Happens to the Amino-acids in the Tissues?

Some proportion of the amino-acids withdrawn from the blood will be built up in the tissue cells to specific proteins required to repair tissue waste. Frequently these specific tissue proteins exhibit marked chemical differences, those of fibrous tissue being especially rich in glycine, those of elastic tissue in glycine and glutamic acid, while the keratin of horny tissues is exceptionally rich in cystine; protamines and histones are especially rich in *di*-amino-acid radicals.

The blood brings a mixture of all the acids to the tissue cells. These may exhibit selective action during the initial absorption from the blood, or more probably subsequently in transforming the amino-acids to protein. The actual synthesis is probably brought about by the specific proteases of the individual tissues, the increased amino-acid concentration producing the necessary conditions for synthesis, provided a protein lack exists in individual cells. We know little as yet about such processes, but Wasteneys' work shows that protein formation can take place with great rapidity. The specificity of the proteases determines the specificity of the proteins whose formation they govern.

One or more of the amino-acids are in part used up in forming the internal secretions. Both thyroxine and adrenine are derivatives of tyrosine. Many of the others are almost certainly amino-acid derivatives.

It has been demonstrated by experimental work, which will be dealt with in connection with diet (Chapter XXXI.), that the ordinary diet of most people provides at least twice, if not three times, the amount of amino-acids required by the tissues. What happens to the rest?

Experiments on the diabetic dog have shown that the organism has the power of transforming glycine, alanine, aspartic, glutamic and β-hydroxyglutamic acids, serine, cystine, arginine, proline and hydroxyproline into glucose. (Doubtful results have been obtained with valine, isoleucine,

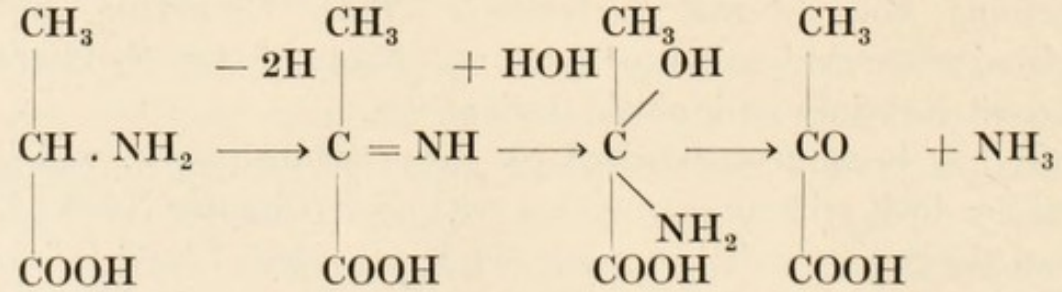
and caprine.) Similar experiments have shown that phenylalanine, tyrosine and leucine, and possibly histidine, can give rise to acetoacetic acid. How are these and similar changes brought about? Obviously they involve the loss of the amino-group.

From the amino-group urea is formed. The residue is either converted into glucose, or immediately oxidised. These processes are probably interdependent, but will be dealt with separately.

The Production of Urea from Amino-acids

Many tissues possess the power of splitting off ammonia from amino-acids. If pulp of liver or of various other organs is mixed with solutions of such amino-acids as leucine, glycine, tyrosine or cystine, the ammonia content of the mixture increases. All amino-acids are not so changed; *e.g.*, phenylalanine is not acted upon. The liberation of ammonia probably involves an oxidation, with the formation of a keto-acid through a complex series of changes.

The possible series of linked reactions may be exemplified for alanine—



Alanine *Pyruvic acid*

The pyruvic acid is then possibly changed to glucose, or through the action of carboxylase is converted to acetaldehyde, which is easily oxidised to carbon dioxide and water.

Dakin and Dudley have shown that a somewhat similar

change occurs spontaneously in solutions of alanine, pyruvic aldehyde being formed—

$$CH_3 . CH(NH_2) . COOH \longrightarrow CH_3 . CO . CHO + NH_3$$

While such changes indicate a mechanism for the tissue formation of ammonia, the experiments of Bollmann, Mann and Magath demonstrate that, at least in the dog, the liver is the chief site of deaminisation, while it is the sole site of urea formation. With these facts must be contrasted the results of Van Slyke's experiments, that the disappearance of amino-acids from the blood after a meal is accompanied by an increase in its urea content, while many tissues take up these amino-acids, and also the now universally accepted finding that under normal conditions the ammonia content of the blood is negligibly small.

Rabinowitch reported in 1929 some extraordinary but carefully checked findings in a case of acute yellow (idiopathic) atrophy of the liver, which at post-mortem showed complete atrophy of that organ. Just prior to death the urine contained only traces of urea and ammonia, and there was no detectable urea in the blood at all. On the other hand, the blood contained 0·2 per cent. of amino-acid nitrogen, a very high figure. The results support the view that urea is formed exclusively in the liver.

Evidently, if tissues liberate ammonia to the blood, the liver possesses an extremely efficient mechanism for removing it rapidly and completely. There remains to be considered the mechanism for conversion of ammonia to urea.

There is ample evidence that moderately strong oxidising agents will produce urea from proteins and amino-acids *in vitro*. If egg- or serum-albumin, or fibrin, or gluten, are oxidised with potassium permanganate or persulphate, urea is present in the products. Similar treatment produces urea from amino-acids, especially in the presence of ammonia. In 1896 Hofmeister showed that if 10 gm. of glycine in ammoniacal solution were oxidised with permanganate 3 gm.

of urea nitrate were produced, and he obtained similar results with asparagine, leucine, acetamide, etc.

The formation of urea is even more general. Fosse, in 1912, showed that considerable amounts are produced if carbohydrates such as glucose, sucrose, starch, glycerol, or even formaldehyde, are oxidised by permanganate in presence of ammonium salts. The ease of urea formation is illustrated by the fact that when such proteins as serum-albumin or casein are refluxed for twenty minutes with 10 per cent. alkali, or longer with more dilute alkali, an appreciable amount of urea is produced.

Such transformations, according to E. A. Werner, all take place through the intermediate formation of *cyanic acid.*

Werner's co-workers, Fearon and Montgomery, have recently (1924) put forward evidence that cyanic acid is formed during the oxidative deamination of glycine, sarcosine and alanine by hydrogen peroxide. So far the presence of cyanic acid has not been conclusively demonstrated in body tissue (Montgomery claims to have shown that a trace—of the order of 1 milligram per 100 c.c.—is present in the blood plasma of cats and rabbits after a protein meal), but the procedure is obviously difficult, since it is to be expected that the acid will be transformed to urea as fast as formed.

Numerous experiments associate the presence of ammonia with the production of urea in the body. According to the older theory of urea production, ammonia, produced by deaminisation of the amino-acids, in the presence of water united with carbon dioxide (always present in tissues and blood) to form ammonium carbonate and this, by successive loss of two molecules of water, gave in turn ammonium carbamate and urea :

$$2NH_3 + HOH + CO_2 = O:C\begin{matrix}O{-}NH_4\\O{-}NH_4\end{matrix} = O:C\begin{matrix}O{-}NH_4\\NH_2\end{matrix} + HOH$$

$$= O:C\begin{matrix}NH_2\\NH_2\end{matrix} + 2HOH$$

At first sight experimental evidence supports this view. Schröder, in a celebrated experiment in 1882, passed defibrinated

blood mixed with ammonium carbonate through a surviving liver. After a while the ammonium carbonate had disappeared and its place was taken by urea. Nencki and Pawlow showed that the percentage of ammonia contained in the blood from the portal vein was considerably higher than that from the hepatic vein, indicating that ammonia is retained by the liver as the portal blood passes through it, and is presumably converted into urea. When ammonium carbonate is administered to animals there is an increased output of urea.

The doubling of the yield of urea from cyanic acid through interaction with ammonia could explain all these results.

It has been stated that ammonium carbamate actually occurs in the urine, and that in dogs with Eck's fistula (portal vein joined to vena cava, so that portal blood passes directly into the general circulation, and the blood flow through the liver is materially decreased) the blood ammonia increases and the ammonium carbamate in the urine increases, while urine-urea decreases. Werner has pointed out that the tests employed for carbamate in such experiments will give equally positive results with cyanate. Finally, it is very difficult to prepare urea from ammonium carbamate experimentally, and the yield is poor.

S. R. Benedict believes that kidney tissue can form ammonia from urea, the ammonium salts present in urine being produced during the filtration of blood in the kidney and not derived from ammonia of the blood. The trace of ammonia present in blood is, of course, far too small to account for the amount present in urine. Bornstein and Budelmann (1930) have actually demonstrated the formation of ammonia by the surviving kidney. A kidney removed from a dog and kept in good condition formed ammonia at the rate of 0·189 mg. per gm. kidney per hour. It seems just possible, according to recent work of Bliss, that blood contains some complex amide, some R . $CONH_2$ compound which liberates ammonia under the action of a special enzyme present in kidney tissue. Such a process would be obviously of service in maintaining blood neutrality by neutralising varying amounts of acid radicals submitted to the kidney for excretion and thereby sparing the removal of corresponding amounts of blood bases.

Formation of Urea from Arginine. The liver, and to a much less extent the kidney (and in fishes, and possibly in mammals, other tissues such as the spleen and intestinal mucosa), contain an enzyme *arginase*, which specifically acts on arginine, hydrolysing it to ornithine and urea.

$$HN : C\begin{matrix}NH_2\\ \diagdown\\ \diagup\\ NH\,.\,(CH_2)_3\,.\,CH(NH_2)\,.\,COOH\end{matrix} + HOH =$$

Arginine

$$HN : C\begin{matrix}NH_2\\ \diagup\\ \diagdown\\ OH\end{matrix} + HNH\,.\,(CH_2)_3\,.\,CH(NH_2)\,.\,COOH$$

Urea *Ornithine*

Formation of Urea in the Animal Organism. Wada's discovery of citrulline (*cf.* p. 120) has led to a theory, put forward by Krebs and Henseleit, according to which citrulline acts as the agent for the conversion of ammonia into urea :—

NH		NH		NH_2		NH
|		|		|		|
CO		C : NH		HO . C : NH		CO
|		|		|		|
NH	$+ NH_3$	NH		NH_2	$+ NH_3$	NH
|	$\longrightarrow$	|	$\longrightarrow$	|	$\longrightarrow$	|
$(CH_2)_3$		$(CH_2)_3$		$(CH_2)_3$	$+ CO_2$	$(CH_2)_3$
|		|		|		|
$CHNH_2$		$CHNH_2$		$CHNH_2$		$CHNH_2$
|		|		|		|
COOH		COOH		COOH		COOH
Citrulline		*Arginine*		*Urea* plus *Ornithine*		*Citrulline*

Evidence is accumulating that this is the true mechanism of urea formation.

Formation of Ammonia from Asparagine. An example of specific enzyme action on the amide group $—CONH_2$ is furnished by *asparaginase*, present in yeast and in calf liver. This acts on the amide *asparagine*, liberating ammonia quantitatively, but has no action on the amino-group :

$$COOH\,.\,CH_2\,.\,CHNH_2\,.\,CONH_2 + HOH \longrightarrow COOH\,.\,CH_2\,.\,CHNH_2\,.\,COOH + NH_3.$$

It will act similarly on the corresponding amide derivative of glutamic acid, but does not appear to act on other amides.

The Fate of the Amino-acid Residues

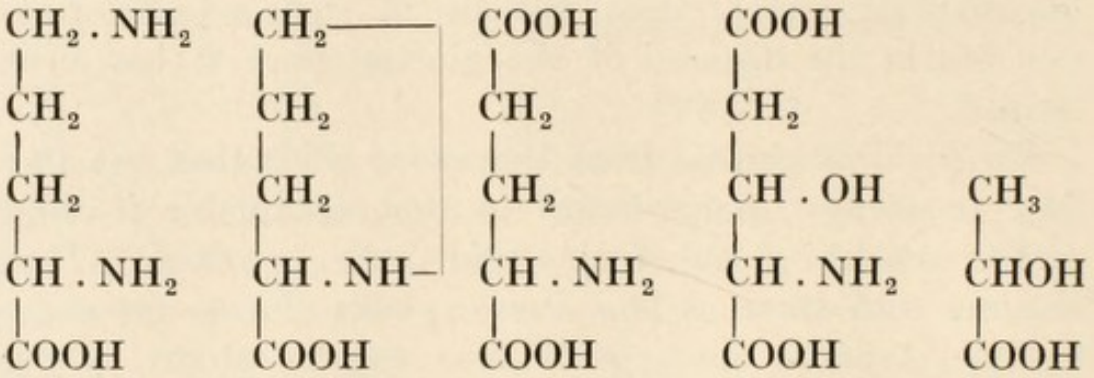
We have seen that the diabetic animal can transform 58 per cent. of a protein intake into glucose ; glycine, alanine,

serine, cysteine, aspartic acid, glutamic acid, hydroxy-glutamic acid, arginine, proline and hydroxy-proline can take part in this transformation. These changes are therefore possible also in the normal animal, since the fault in the diabetic animal does not lie in such a transformation but in the disposal of the glucose once it has been formed.

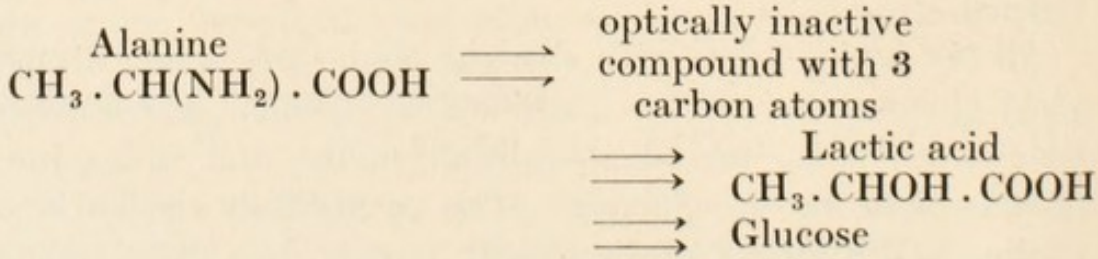
The yield of glucose from the amino-acids that can produce it always approximates to that obtainable if three carbon atoms of the amino-acid were involved. Thus alanine, with three carbon atoms, yields glucose quantitatively. Aspartic acid, with four carbon atoms, yields glucose corresponding in amount to three-fourths of its carbon atoms. Glutamic and hydroxyglutamic acids, proline and ornithine, all with five carbon atoms, give close to three-fifths of the theoretical yield based on carbon content. Only those amino-acids with three, four and five carbon atoms will yield glucose with the exception of glycine and arginine. Arginine gives rise to glucose because arginase liberates ornithine, with five carbon atoms. Glycine yields glucose quantitatively in the diabetic dog, but the change by which this is brought about is not yet understood. *All* the amino-acids with three, four and five carbon atoms will yield glucose except valine. Since in all such cases the glucose appears to result from three of the carbon atoms present, one may strongly suspect that lactic or pyruvic acid is the intermediate stage in the production of this sugar. This theory is strengthened by the fact that if either lactic or pyruvic acid is fed to the diabetic dog glucose is formed.

All the amino-acids with straight chains of carbon atoms yield glucose except the diamino-acid lysine. Those with branched chains, including valine, leucine and isoleucine, furnish little or no glucose. The pyrrolidine derivatives proline and hydroxy-proline yield glucose, but amino-acids with a benzene nucleus do not do so. One may stress the close

structural relationship between ornithine, proline, glutamic, and hydroxyglutamic acid, all of which contain five carbon atoms and yield glucose corresponding to three, as evidencing a common catabolic path, probably through lactic acid :



The only hydroxy acids known to yield glucose in the diabetic animal are lactic and malic acid. The latter, with four carbon atoms, certainly plays no normal part in mammalian metabolism. Since in the diabetic organism *d*- and *l*-lactic acids, and *d*- and *l*-alanine are quantitatively transformed into *d*-glucose, some optically inactive intermediate product common to all these transformations seems probable, and pyruvic acid seems most likely. If this is fed to the diabetic dog lactic acid and some glucose result, along with some acetoacetic acid, so that since acetoacetic acid is not formed by feeding alanine and the other amino-acids under discussion this stage of the transformations is uncertain. The probable course of transformation of alanine, selected as an example of these amino-acids, can be written :



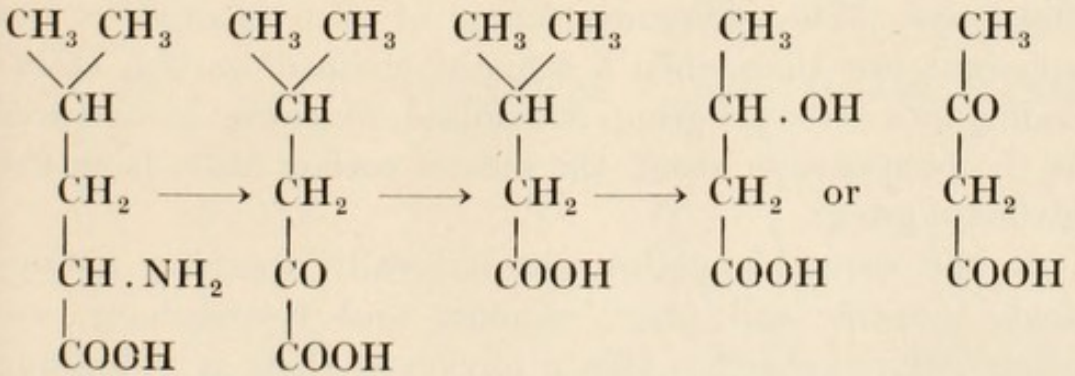
It has been shown that valine, leucine, isoleucine, lysine,

histidine, phenylalanine, tyrosine and tryptophane do not give rise to glucose in the diabetic animal, and therefore it may be assumed that they cannot do so in the normal organism.

Fate of Individual Amino-acid Residues. *Cystine* gives rise to glucose. It is partly converted to taurine, utilised in the formation of taurocholic acid (see p. 156). The sulphur of cystine is partly excreted as inorganic sulphate, and is partly responsible for the "neutral sulphur" of the urine.

In the condition known as *cystinuria* the organism cannot properly dispose of the cystine portion of the protein molecule, and cystine itself is excreted.

Using the liver perfusion method Embden has shown that the general fate of the amino-acids with side-chains is similar to that of their derived fatty acids with one less carbon atom. Such fatty acids, undergoing oxidation in the body, tend to lose their side-chains first, the fatty acids, or derivatives of fatty acids, that result, being then oxidised in normal fashion. Hence we may suppose the changes undergone by *leucine* to be of the nature :



Leucine — *Isovalerianic acid* — *β-hydroxybutyric acid* — *Acetoacetic acid*

Phenylalanine and tyrosine also give rise to acetoacetic acid in the diabetic animal, but by a different route. Histidine is also a possible precursor of this acid.

Leiter, in 1925, showed that the animal organism has a marked capacity for destroying the iminazole ring, being capable of oxidising histidine, iminazol-lactic acid, and methyl-iminazole, but seems unable to change iminazole itself (glyoxaline) in absence of a side-chain from this ring.

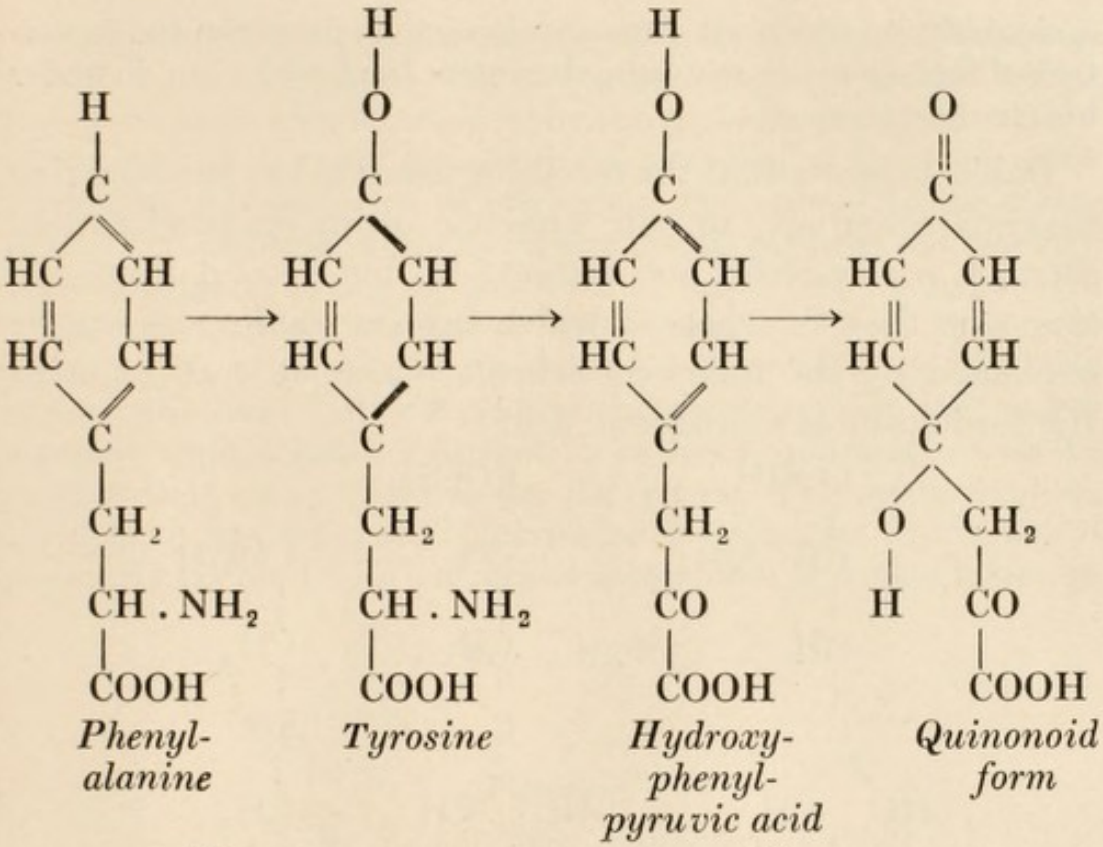
Most compounds with a *benzene nucleus* are not completely oxidised in the body. This benzene nucleus is not easily broken, and usually only the side-chain is oxidised, and then only completely if the *first* carbon atom adjacent to the ring can be oxidised. When benzoic acid is fed, hippuric acid is excreted (*cf.* p. 248). When phenyl propionic acid is fed, hippuric acid is excreted, and the probable series of changes is :

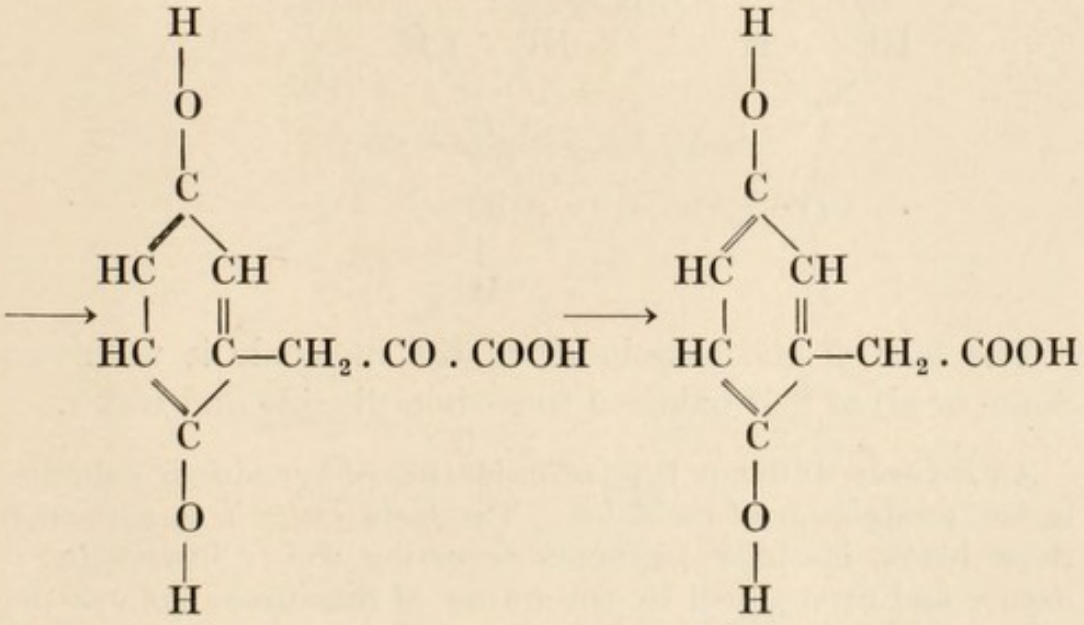
$$C_6H_5 . CH_2 . CH_2 . COOH \rightarrow C_6H_5 . CO . CH_2 . COOH$$
$$\rightarrow C_6H_5 . COOH$$
$$\rightarrow C_6H_5 . CO . NH . CH_2 . COOH$$

When phenylacetic acid is fed, on the other hand, it is not oxidised. In dogs it is conjugated directly with glycine and excreted as phenylaceturic acid. In man it is conjugated with glutamine and excreted as phenylacetylglutamine. The governing factor of such changes is the apparent law that when a straight chain of carbon atoms ending in a carboxyl group is oxidised, oxidation commences at the beta-carbon atom, the second carbon atom from the carboxyl group.

In the normal organism the naturally occurring amino-acids tyrosine and phenylalanine, and tryptophane, are completely oxidised. Hence obviously there is something in their configurations which facilitates rupture of the ring nuclei.

In the condition known as *alkaptonuria* the human organism cannot completely oxidise tyrosine and phenyl alanine, but forms from them *homogentisic acid* through a series of changes which are believed to be :

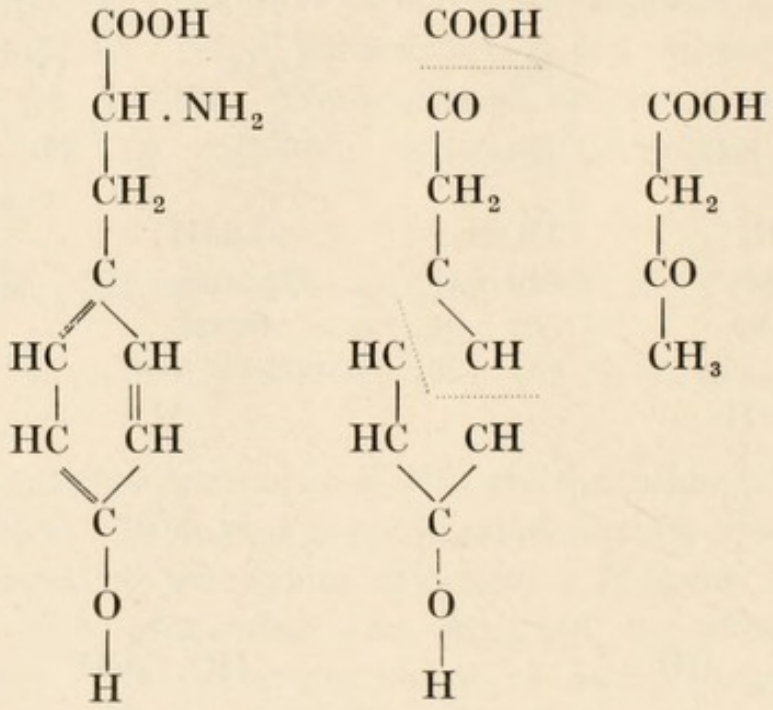




Many investigators believe that the formation of homogentisic acid in the alcaptonuric indicates that this acid is *normally* formed from tyrosine and phenylalanine when these compounds are oxidised in the body. However, as Dakin has pointed out, it is equally or more probable that *the fault in the alcaptonuric* lies in

an inability to carry out some previous stage properly, the formation of homogentisic acid being therefore the best he can do under his circumstances.

Dakin suggests that the oxidising away of the benzene ring depends essentially on the presence of an oxidised carbon atom in a side-chain not *adjacent* to, but second from, the ring, and that the changes which tyrosine undergoes can be explained by the following scheme which at once explains the formation of acetoacetic acid :

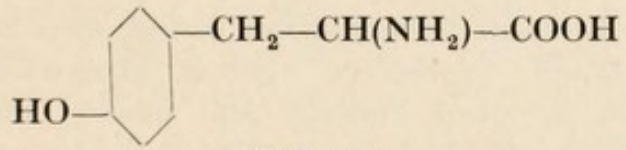


The fate of the remainder of the molecule is unknown. Some or all of it is oxidised to carbon dioxide and water.

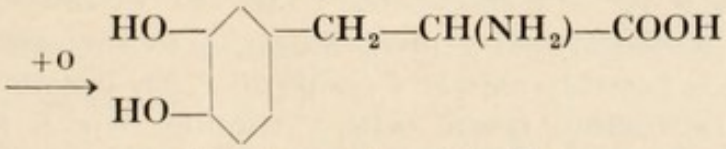
An entirely different type of oxidation of tyrosine is exhibited in the production of melanins. The term *melanin* is applied to those black, insoluble pigments occurring widely distributed in nature and exemplified by the retinæ of mammals, the coloured material of hair, horn, and feathers, of the skin of the negroid races and of the skin of many lower animals and insects (and the protective pigment of the " sunburn " caused by ultra-violet light). Into this category also come the " ink " ejected by squids, the pigment of melanotic tumours in man and animals, especially white and grey horses, and that producing the brownish discoloration when fresh surfaces of many fruits and tubers, such as apples and potatoes, are exposed to air.

Whether these pigments are one or more than one compound is still to be ascertained, but it is reasonably certain that they are all at least closely allied with the melanin produced by the action of the enzyme *tyrosinase* on tyrosine, a black pigment containing 8·5 per cent. of nitrogen. The nature of the series of changes resulting in the formation of this pigment has recently been elucidated, chiefly by the work of Raper and his associates.

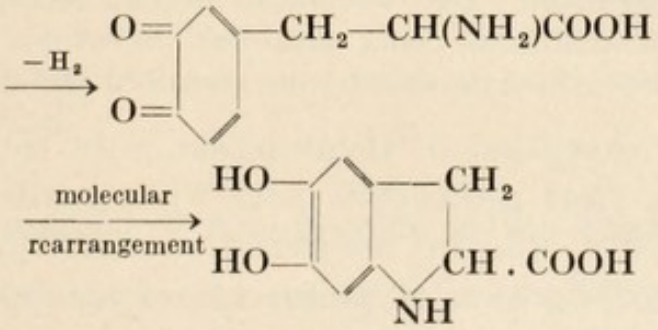
Enzymes having the action of tyrosinase are widely distributed. The same compound exists in the mealworm *Tenebrio molitor* (the black beetle larva), in the potato, and in the fungus *Agaricus dryophilus*, a distribution in itself sufficiently wide to suggest that all tyrosinases are the same. Tyrosinase oxidises tyrosine to produce a red-coloured *indole-derivative*, which is subsequently reduced to a colourless compound, and this takes up

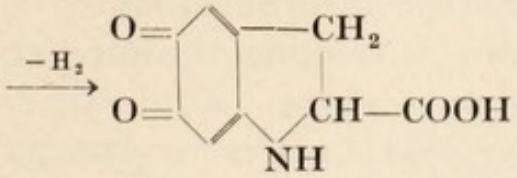


Tyrosine

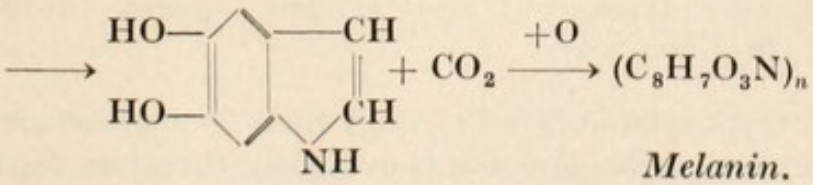


Dihydroxyphenylalanine.





Red compound.



Melanin.

oxygen to form the black melanin. The enzyme only acts in producing the first of these changes, although even this involves several steps, as the scheme on p. 345 indicates.

Very important also is the work of Bloch, who has studied the distribution of an oxidase, which, since it oxidises *di*-(hydr)*oxy*-*p*henyl-*a*lanine, with melanin as a result, he has termed *dopa-oxidase*. This oxidase is very specific in its action, having no effect on tyrosine. It is widely distributed in the skin (in the epithelial cells in the basal layers of the epidermis and in the hair-producing cells), but is absent from the skin of albinos.

The urine of patients suffering from melanotic tumours turns dark on exposure to the atmosphere or treatment with oxidising agents, and the melanin so produced can be prepared from such urines in unusually pure condition. Analyses suggest a very close chemical resemblance to Raper's melanin from tyrosine.

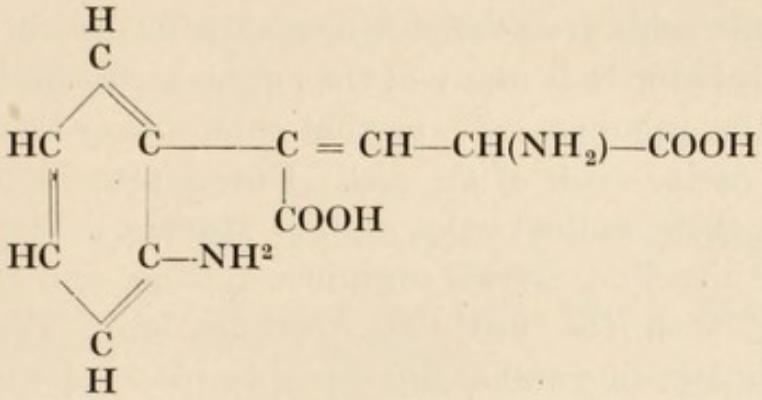
The purpose of melanin formation would seem to be, at least in many instances, protective against ultra-violet radiation. Its distribution in lower animals and insects such as moths, suggests another protective adaptation, mimicry or else possibly an adaptation for sex attraction. The ink of the squid is still another type of protective mechanism. The melanin formation in tumours is possibly merely a result of hyper-function or hyper-formation of certain tissue cells, "running wild," an incident which in itself may not be harmful, though signalling a most dangerous condition. The formation of the red compound by tyrosinase illustrates at least one way in which indole compounds may be formed in nature from simpler benzene derivatives.

With the exception of tryptophane only those benzene acids which yield acetoacetic acid when perfused through a surviving liver can be *completely* oxidised in the body, so that the formation of acetoacetic acid appears to be a necessary condition of their oxidation.

Little is known concerning the mechanism involved in the oxidation of *tryptophane*. Evidently the correct initial oxidation of the side-chain is necessary, since the body cannot oxidise indole and skatole beyond the stages of indoxyl and skatoxyl, and cannot oxidise indole-acetic acid at all (*cf.* p. 250).

When tryptophane is fed to dogs there is an increased excretion of kynurenic acid, which is evidently therefore formed from

it (*cf.* Chapter XVII., p. 252). Kotake has shown (1931) that a compound



which he terms *kynurenin*, is an intermediate step in this formation. Most, if not all, of the change takes place in the liver. The bile of dogs contains kynurenic acid often to a greater extent than their urine. When injected, it is recovered quantitatively, suggesting that it is one end product of tryptophane catabolism (in the dog). There is also some evidence that kynurenin is utilised in the formation of the urinary pigment urochrome.

The mode of oxidation of the *di*-amino-acids, other than arginine, is obscure.

According to Edlbacher (1926, 1930) the liver of most animals contains a specific enzyme, *histidase*, which hydrolyses histidine in such a way that two-thirds of the nitrogen is converted into ammonia. Glutamic acid is produced and accounts for the remaining third.

Formation of Amino-acids in the Organism

Alanine, phenylalanine and tyrosine can be synthetised by perfusing the surviving liver with the ammonium salts of the corresponding ketonic acids :

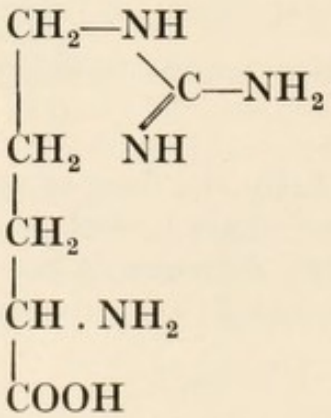
$$R \cdot CH_2 \cdot CO \cdot COONH_4 \longrightarrow R \cdot CH_2 \cdot C\begin{matrix} OH \\ COOH \\ NH_2 \end{matrix}$$

$$\xrightarrow{\text{reduction}} R \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

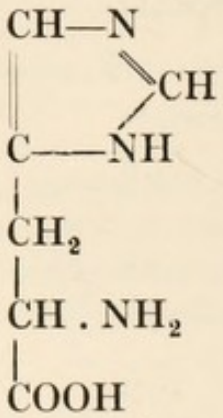
The alanine and tyrosine so produced are optically identical with the naturally occurring acids. Similarly α-amino-

butyric acid and caprine have been synthetised. This suggests what may be the normal mode of synthesis, provided the ketonic acids are available or can be formed in the body.

It is probable that many of the amino-acids can be synthetised in the organism. Those that cannot may be termed *the essential amino-acids of the diet.* Unless protein of the diet contains their radicals the animal starves. Tryptophane, tyrosine, histidine, lysine, arginine, cystine, and proline are essential, with the following qualification. Partial deficiencies in tyrosine and arginine can be made up by excess of phenylalanine and histidine respectively. Methionine can replace cystine to some extent. Hydroxyproline can replace proline. The close relationship of most of these pairs is obvious. That which exists between arginine and histidine is displayed by writing their formulae :



Arginine



Histidine

The body appears to be able to break the iminazole ring, converting histidine into arginine, but cannot form the iminazole ring, converting arginine into histidine. It can only bring about such minor changes as the conversion of iminazol-pyruvic or lactic acid into histidine.

It must be remembered that *under normal conditions the body does not convert* 58 *per cent. of the protein intake into glucose, but that part of the amino-acids not required for specific purposes, such as the construction of body proteins, formation of certain internal secretions, of compounds like glutathione,*

carnosine, creatine, etc., is converted into glucose, or oxidised directly through intermediate stages, some idea of which has been given in the foregoing pages, the amino-group being transformed to urea.

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CHAPTER XXIV

INTERMEDIATE METABOLISM OF THE NUCLEOPROTEINS AND NUCLEIC ACIDS

NUCLEOPROTEINS consist essentially of a radical of nucleic acid, a derived phosphoric acid, united, in virtue of its acid properties, to one or more molecules of protein, in virtue of their basic properties. The union is of such a type that it can be broken during proteolytic digestion.

The nucleoproteins are especially abundant constituents of most cells and are present in the cell-nuclei. They are present in relatively large amounts in glandular tissue, such as the thymus, pancreas and spleen, and occur in all animal foods with the exception of milk and eggs. The amount of nucleic acid obtainable from a tissue is proportional to the richness of the tissue in cell-nuclei, whence the name of the acid.

Nucleo-proteins are acid in character, insoluble in water, readily soluble in dilute alkali and in dilute mineral acids. They are precipitated from weak alkaline solution by acetic acid, and are only soluble with difficulty in excess of acetic acid. Their composition is variable, the variation depending essentially on the type of protein radical present.

According to Kossel when nucleo-proteins are subjected to gastric digestion they are hydrolysed, and a molecule of protein is split off leaving a molecule of *nuclein*—a mono-protein nucleate—whose protein radical is either that of a protamine or of a histone. Tryptic digestion splits nuclein into its protein and nucleic acid.

Jones has put forward a somewhat different view of the nature of the protein-nucleic acid combination in nucleoproteins suggesting that the relationship between nucleoprotein, " nuclein," and nucleic acid may be aptly compared with that between basic lead acetate, lead acetate, and acetic acid.

The metabolism of the proteins set free during such digestion is subsequently similar to that of other proteins.

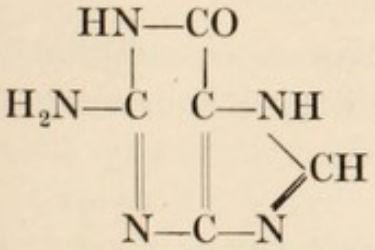
Nucleic Acid. There appear to be only two nucleic acids which have been named, after the material from which they are usually prepared, *thymus nucleic acid* and *yeast nucleic acid*, or, alternately, *the animal and plant nucleic acids*. Yeast nucleoprotein can be extracted from yeast by grinding it up with a little water and ether (the latter to kill the yeast cells), extracting with 0·4 per cent. sodium hydroxide, filtering, and adding to the filtrate dilute hydrochloric acid until it is just acid. The nucleoprotein is precipitated. If, instead of acidifying, the alkaline solution is heated for a little while, and to the cooled filtrate acetic acid is added until the reaction is just acid to litmus, and then after filtration the filtrate is added to excess of 95 per cent. alcohol, the nucleic acid is precipitated. Similar treatment of minced thymus gives the corresponding nucleoprotein and nucleic acid.

When yeast nucleic acid is hydrolysed with mineral acids six compounds are present in the hydrolysate :

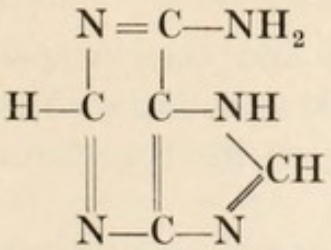
(i.) Phosphoric acid, H_3PO_4.

(ii.) A pentose, *d*-ribose, $CH_2OH . HCOH . HCOH . HCOH . CHO$.*

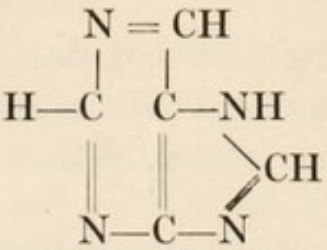
(iii.) and (iv.) *Two purine bases*, *guanine* and *adenine*, amino-derivatives of *purine*.



Guanine, $C_5H_5N_5O$



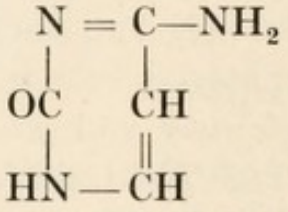
Adenine, $C_5H_5N_5$



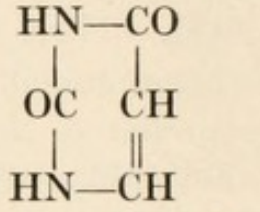
Purine, $C_5H_4N_4$

* Professor Robinson (*Nature*, July 9th, 1927) suggests that yeast nucleic acid contains *d*-xylose radicals, and that during hydrolysis *d*-xylose phosphoric acid, formed as an intermediate product, is changed to *d*-ribose (by a Walden inversion) and phosphoric acid. Xylose is widely distributed in nature ; the presence of ribose radicals has always been difficult to explain. Levene does not support this suggestion.

(v). and (vi.) Two *pyrimidine bases, cytosine* and *uracil.*



Cytosine, $C_4H_5N_3O$



Uracil, $C_4H_4N_2O_2$.

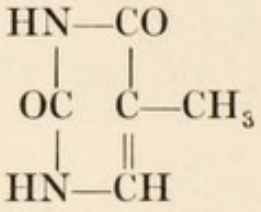
There is similar evidence that thymus nucleic acid is built up from :

(i.) Phosphoric acid.

(ii.) A sugar, long believed to be a hexose, but very recently shown by Levene, London, and Mori to be a desoxypentose, *d*-ribodesose (which they have named *thyminose*), $CH_2OH.HCOH.HCOH.CH_2.CHO$ (closely related to *d*-ribose).

(iii.) and (iv.) The two purine bases guanine and adenine.

(v.) and (vi.) Two pyrimidine bases, cytosine and *thymine* (methyl uracil).

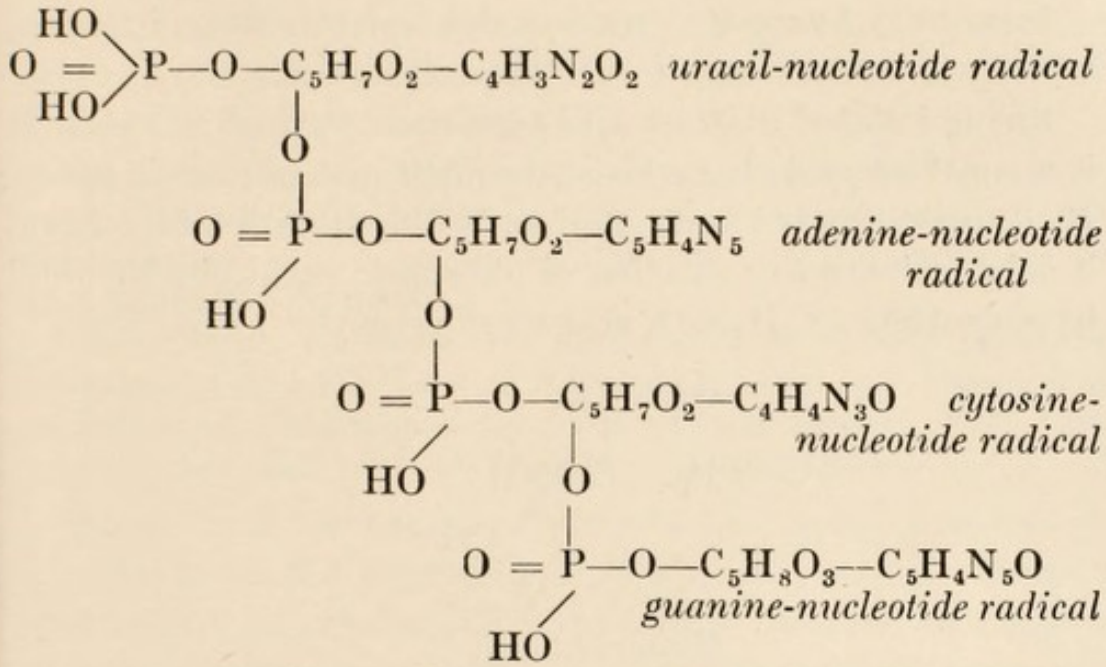


Thymine, $C_5H_6N_2O_2$.

Levene and Jones and their respective associates have shown that the nucleic acids are each built up of four *nucleotides*. Each of these has the type-formula :

Phosphate radical—sugar radical—purine or *pyrimidine radical.*

The way in which these nucleotides are linked together in the nucleic acids is still not completely determined, and the linkages probably differ in the two acids. Levene's latest formula for yeast nucleic acid is :



Levene suggests a similar structure for thymus nucleic acid, the nucleotides being linked in the order ; thymine nucleotide, adenine nucleotide, cytosine nucleotide, guanine nucleotide.

The nucleotides are crystalline compounds and dibasic acids. They closely resemble phosphoric acid in their acid behaviour. They can be separated from each other by preparing their di-brucine salts, which have different degrees of solubility. When heated with ammonia to 150° C. they all yield *nucleosides* of the type :

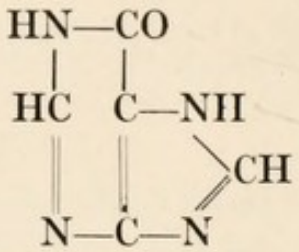
sugar radical——guanine radical

Hydrolysis of the purine nucleosides with acids splits them to their constituents. The pyrimidine nucleosides are more stable and are not affected by heating with dilute acids.

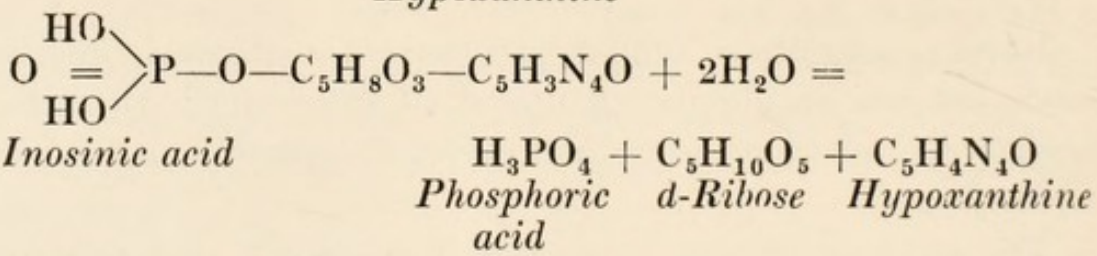
Neutral hydrolysis of yeast nucleic acid at 175° under reduced pressure results in a mixture of the nucleosides *adenosine*, *guanosine*, *cytidine* and *uridine*. These do not reduce Fehling's solution, indicating that the sugar radical is united by a glucosidic linkage. The phosphate and sugar in the nucleotides are probably combined through an ester linkage between the phosphoric acid and the primary alcohol group of the sugar molecule.

Some very interesting nucleotides occur in animal tissues, which are not associated with the cell nuclei.

Liebig isolated *inosinic acid* from meat extract in 1847. It is a constant and characteristic constituent of muscle tissue. Its composition is known, and on boiling it with mineral acid it is hydrolysed to a mixture of phosphoric acid, *d*-ribose and hypoxanthine, $C_5H_4N_4O$, still another purine :



Hypoxanthine



Inosinic acid

$$H_3PO_4 + C_5H_{10}O_5 + C_5H_4N_4O$$

Phosphoric acid *d-Ribose* *Hypoxanthine*

When inosinic acid is heated with ammonia under pressure *inosine*, a nucleoside, $C_5H_9O_4—C_5H_3N_4O$, is formed.

This inosinic acid of muscle has been shown by Embden and his co-workers to be produced (along with ammonia) by the deaminisation of " yeast " adenylic acid (adenine nucleotide) during muscular activity. The process appears to be reversible.

The adenine nucleotide had previously been prepared in crystalline form from pig's blood. It appears to be present in normal whole blood to the extent of from 25 to 35 mg. per 100 c.c. Human blood contains amounts of the same order. That of the cat, dog, guinea-pig, and rat, varies from 7 to 12 mg., duck blood contains over 30 mg., and pigeon blood from 60 to 80 mg. per 100 c.c. It is evidently a compound of metabolic importance.

In 1894 Hammarsten prepared two " nucleoproteins " from pancreatic tissue, and from one of these he separated

guanylic acid. This has also been prepared from various other glandular tissues, and is identical with the guanine nucleotide from *yeast* nucleic acid.

Jones and Perkins (1924) claim that Hammarsten's β-nucleoprotein from pancreas really contains plant nucleic acid, and that they have isolated from it not only guanylic acid, but also cytosine and adenine nucleotides.

The existence of these two nucleotides in various tissues is doubtless responsible for the fact that pentose has been found present in the hydrolysed products of such tissues. It is very unlikely that such pentose exists free in these tissues.

Levene and his associates are responsible for most of our knowledge of the digestion of the nucleic acids. Neither gastric nor pancreatic juice appears to possess an enzyme capable of acting on them. Three groups of enzymes are concerned. Intestinal juice contains (i.) a *nucleinase* which hydrolyses the tetranucleotides (*i.e.*, the nucleic acids) to mononucleotides, and (ii.) *nucleotidases* which convert *purine nucleotides* to phosphoric acid and nucleosides, but which do not affect pyrimidine nucleotides. Extracts of the intestinal mucosa contain nucleinase, nucleotidases which hydrolyse *both* purine and pyrimidine nucleotides, and (iii.) *nucleosidases* which hydrolyse purine nucleosides.

Pyrimidine nucleosides are apparently not hydrolysed in the intestinal wall nor by extracts of any tissue, although intestinal bacteria can rupture the purine ring, producing ammonia.

As a result of these consecutive fermentations a mixture of purines, nucleosides and nucleotides is absorbed into the blood from the alimentary canal, in all probability *viâ* the portal circulation and not through the lymphatics.

To the extent that purines are liberated during digestion and absorption ribose and thyminose must also be set free. It will be shown subsequently that part of the purines guanine and adenine is deaminised and oxidised to uric acid, prior to excretion (*cf.* p. 357). The nucleosides can also be oxidised as such (*cf.* p. 362).

Since at least one nucleotide (yeast adenylic acid) is utilised functionally (in muscle, *cf.* p. 354 and Chapter XXXII.), while a second (yeast guanylic acid) is present combined with protein in the pancreas, evidently the nucleotides are in part utilised in that complex form.

Body Synthesis of Purines and Nucleoproteins

The occurrence of nucleoprotein in all cell-nuclei obviously suggests utilisation for synthetic purposes of the split products of the digested nucleoproteins, but little is known of the processes by which the body builds up its own nucleoproteins and to what extent, if any, the split products of digestion are utilised.

Apparently the animal organism is not dependent upon pre-formed purines from the diet for the formation of its own supply of nucleic acid. Thus Miescher showed that the migrating salmon forms relatively enormous amounts of nuclein for the generation of spermatozoa at the expense of its own muscular tissue; it fasts during the whole of this process. Kossel has shown that fresh hens' eggs contain no purines. After fifteen days' incubation, during which time there is tissue formation with a rapid increase in the number of nuclei, he was able to isolate 0·94 per cent. of purine from the dried substance of the embryo, chiefly guanine and hypoxanthine, with some adenine. Phosphoric acid can apparently be supplied from the vitellin of egg-yolk.

Burian and Schur have obtained similar results for growing mammals. The Dalmatian dog normally excretes uric acid from oxidation of purines (most breeds of dogs excrete but little). Such a dog, kept for nearly a year on a purine-free diet, in that period excreted more than 100 gm. of uric acid, of which not more than 10 per cent. could have come from pre-formed purines of the tissues. Kollman kept a healthy young woman on a constant diet containing only a very little purine for a period of fifty days. She gained 4 kilograms in body-weight, indicating that there was no undue

degree of tissue destruction. Her uric acid output exceeded the total purine taken by 15 gm.

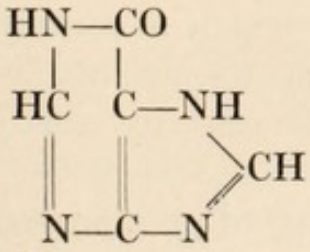
Numerous growth experiments with purified foodstuffs, carried out by Osborne and Mendel, Abderhalden and others, have repeatedly demonstrated that purines are not an essential constituent of the diet.

But while we can therefore definitely conclude that the body has the power to synthetise such purines as it needs, we do not yet know definitely the process employed, nor the materials used.

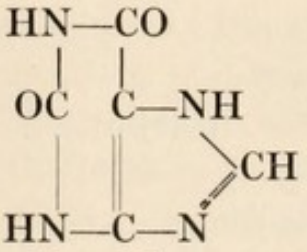
There is a certain amount of experimental evidence that the body can use as precursors of purines the amino-acids arginine and histidine. Histidine seems to be the more probable precursor. Thus Calvery has shown that in the developing hen's egg (in which, of course, purines are being formed) the content of arginine remains stationary, whilst that of histidine markedly decreases.

Catabolism of Purines

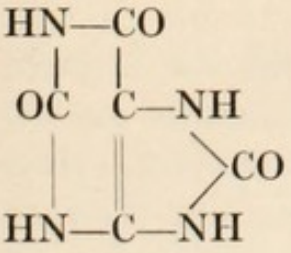
Purines undergo a gradual series of oxidations in the body, through the stages hypoxanthine, xanthine, uric acid and allantoine. For the significance of the chemical names shown below the formulæ, see the purine-key on p. 365.



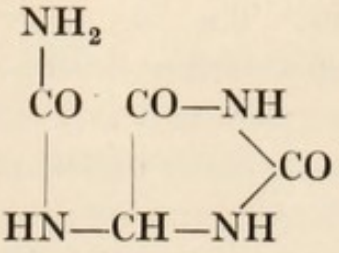
Hypoxanthine
6-oxypurine



Xanthine
2, 6-dioxypurine



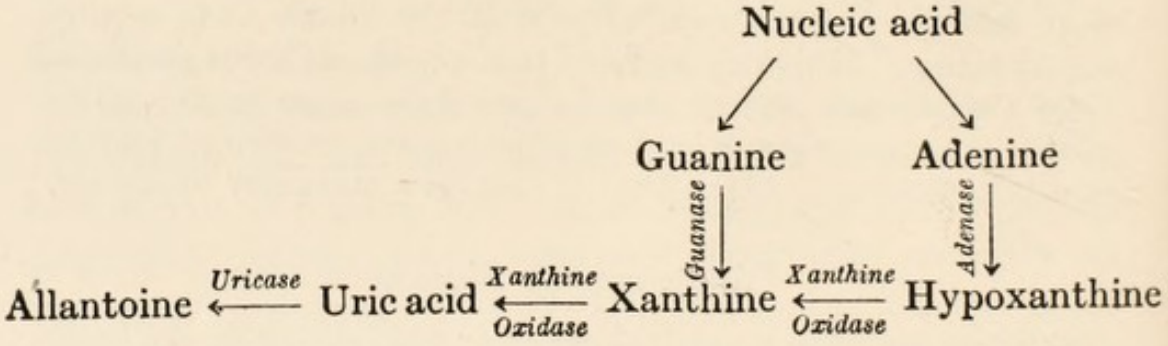
Uric acid
2, 6, 8-trioxypurine



Allantoine

The intermediate products hypoxanthine and xanthine are fairly widely distributed in the tissues. Xanthine is found in muscle, brain, spleen, pancreas, kidneys, testes and liver, and in urine. Hypoxanthine, in lesser amount, is found in muscle, in bone marrow and in milk. The chief excretory product in man is uric acid ; in most of the lower mammals it is allantoine.

Deaminisation and oxidation of guanine and adenine are brought about by the enzymes adenase, guanase, xanthine oxidase, and uricase. The following scheme represents the various stages :



With rare exceptions the four enzymes are not present in any one tissue. Their distribution is characteristic of the tissue and of the species. Using Jones' abbreviation of the above schematic arrangement, in which a continuous line indicates presence, and a dotted line absence of the enzyme, this variable distribution is illustrated by the examples on p. 359.

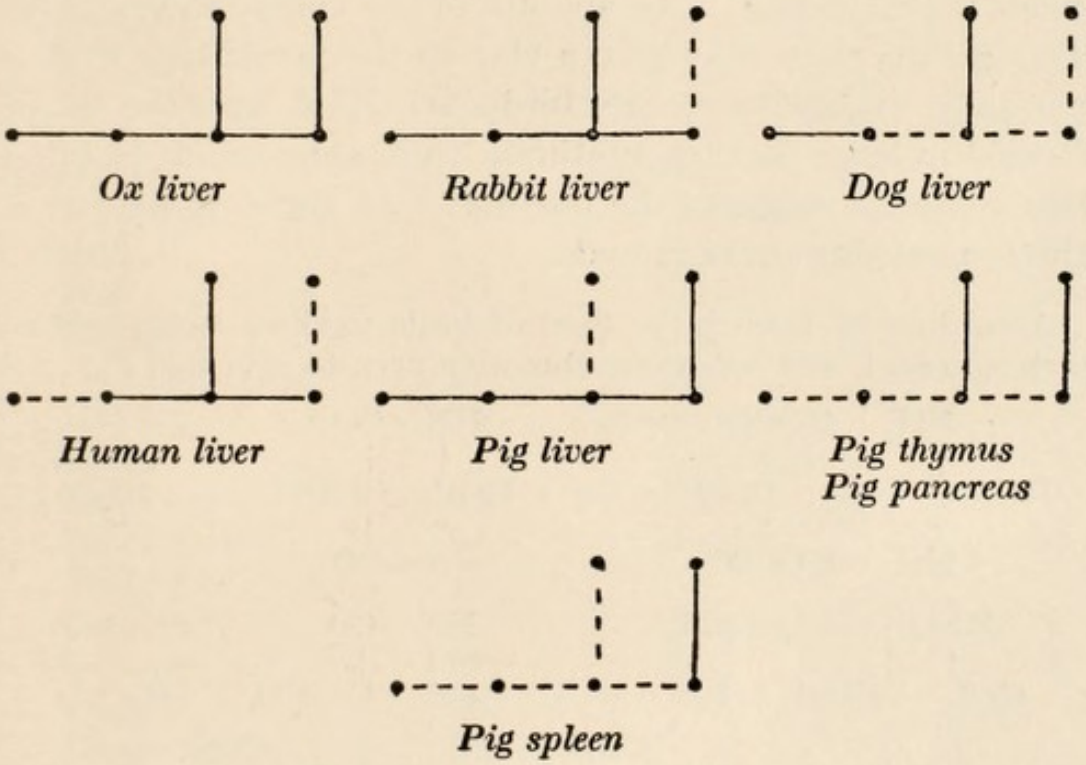
Uricase is found in the liver tissue of all mammals except man and the ape.

Xanthine oxidase is usually, but not so invariably, found in the liver. The dog and rat liver tissues contain none, so that in these animals uric acid must be formed in other tissues and pass to the liver for its further oxidation. The monkey is specially deficient in this enzyme, and as a result excretes more xanthine and hypoxanthine than uric acid.

Guanase is widely distributed, and in many mammals is to be found in all the principal tissues. The pig is, however,

peculiarly deficient in this enzyme, and the muscles of the pig frequently contain deposits of guanine—a " guanine gout." Adenase is found in but few organs. It cannot be demonstrated in rat, rabbit or man. In consequence, adenine is a normal constituent of human urine.

These enzymes are formed successively as the embryo develops. None are found present in the livers of pig embryos less than 90 mm. long. In such embryos of length between 90 and 200 mm. adenase is present, while xanthine oxidase only appears at birth.



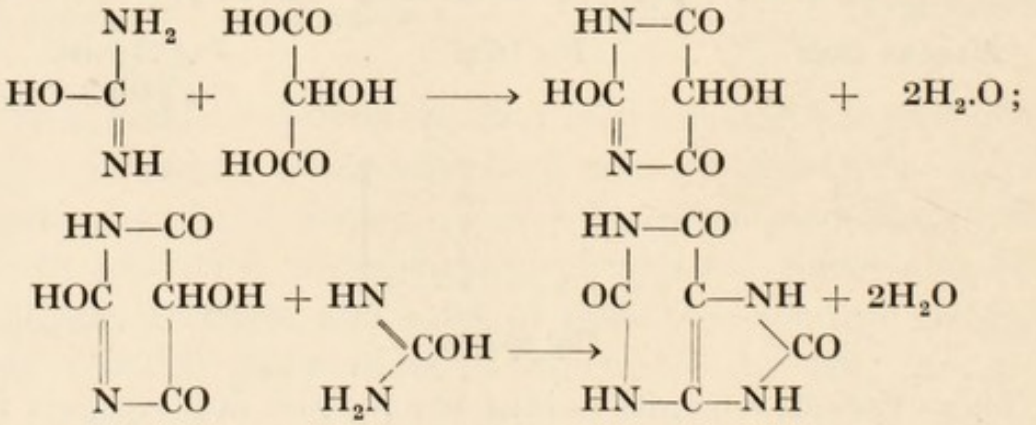
These various enzymes oxidise the purines in great part to the stage of uric acid or further. Thus in man, on a mixed diet, the average daily excretion of uric acid is about 0·7 gm., while that of the less oxidised purines is only from 0·016 to 0·06 gm.

Distribution of the Enzymes in Man. Adenase is absent from all human tissue. Guanase is present in the kidney, liver and lung, but not in spleen nor pancreas (human urine contains no guanine). Xanthine oxidase is present in relatively large amount in the liver, but in no other organ. Uricase is entirely absent.

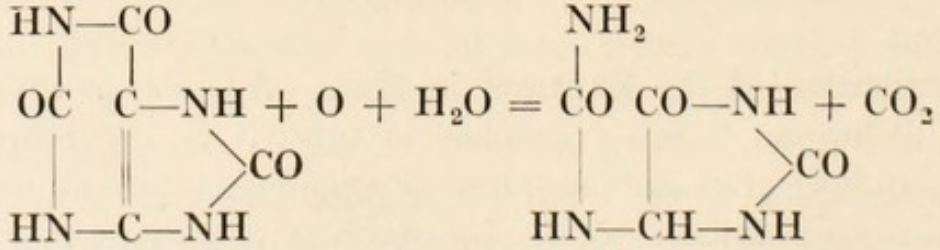
Hence in man xanthine may be formed from guanine in various tissues, but is only oxidised to uric acid in the liver.

Endogenous and Exogenous Uric Acid. Such part of the purines as is set free during digestion and absorption and escapes utilisation (if such occur) in rebuilding of nucleic acid in the body is oxidised to exogenous (Gk. *exo*, without ; *-genes*, born) uric acid. Any tissue destruction or natural " wear " will also give rise to purines autolytically ; these will also be oxidised to uric acid—of " endogenous " (Gk. *endos*, within) origin. The amount of this compound excreted during starvation may give a clue to the percentage that is normally endogenous. In birds uric acid appears to be formed in large part by synthesis from ammonium lactate ; this synthesis suggests the possibility of some similar synthetic procedure in mammals.

According to Wiener the liver of birds oxidises lactic acid to tartronic acid, and condenses this with urea to uric acid :



The Uricolytic Index. Uricase (uricacidase) brings about the following reaction :



Uric acid *Allantoine*

The transformation takes place almost quantitatively in the

dog, and can be brought about almost quantitatively *in vitro* by the action of extract of dog's liver on uric acid.

Hunter and his co-workers have determined the extent to which uric acid is converted into allantoine in a large number of species. This is ascertained by determining the uric acid, and the allantoine (expressed as uric acid) content of urine. Their sum represents the total uric acid excreted, and the percentage ratio of allantoine-uric acid to total uric acid Hunter has called the *uricolytic index*. His results are shown in Table XIX.

TABLE XIX. THE URICOLYTIC INDEX

Marsupialia :		Ungulata :	
Opossum	79	Cow	93
Rodentia :		Horse	88
Rat	96	Sheep	80
Mouse	98	Goat	92
Guinea-pig	94	Pig	98
Rabbit	95	Proboscidea :	
Carnivora :		Elephant	72
Raccoon	95	Primates :	
Black bear	94	Monkey	89
Badger	98	. . . .	
Cat	97	Chimpanzee	0
Coyote	97	Man	2
Dog	98		
Dingo dog	96		
Dalmatian coach-dog	32		

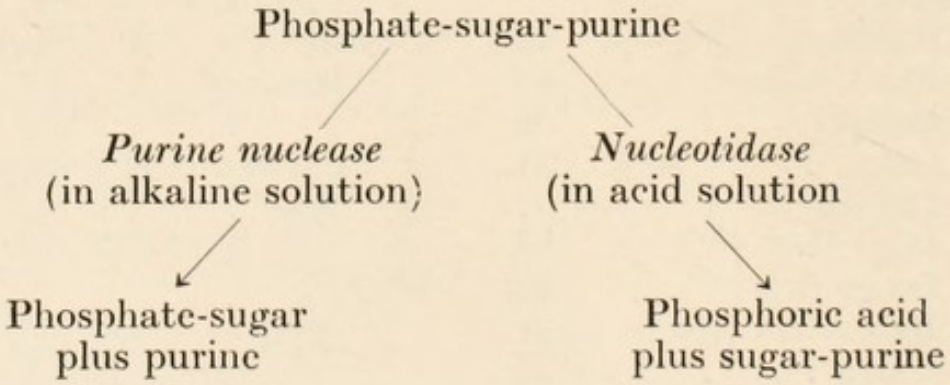
The table shows clearly that as far as the presence of uricase is concerned man and the higher apes are in a separate class, the monkey being more closely related to the lower mammals. The small amount of allantoine present in human urine comes from allantoine ingested as such in the diet.

While the enzyme responsible for uric acid formation, xanthine oxidase, is absent from yeast, and uric acid has not been found in plants, allantoine (along with its derivative

allantoic acid) has been isolated from the haricot bean ; its formation must obviously have resulted in a quite different way.

Schittenhelm (1926) states that at the definitely alkaline reaction of *p*H 9·4 ammonia in presence of oxygen slowly decomposes uric acid. It is doubtful if this reaction accounts for much uric acid destruction in tissues. The same author states that, while allantoine fed to man is largely decomposed, with an accompanying increased excretion of urea, this does not hold for the dog.

Catabolism of Nucleic Acid within the Body. The tissue cells must obviously possess the power to liberate nucleic acid from nucleoproteins (of the tissues) in some way similar to that occurring in digestion. Practically all the body-organs contain nucleinases, nucleotidases, and nucleosidases. Jones has shown that, in addition, tissues contain *purine-nucleases* which produce sugar-phosphate from nucleotides :



He has also shown that deaminisation of the purines may occur before they have been liberated from the nucleosides. For example, guanosine may be oxidised to xanthosine (*i.e.*, guanine-ribose to xanthine-ribose). Such oxidations account for the fact that in the absence of adenase man excretes only *a very little* adenine. And evidently such oxidations may proceed still further, since Benedict has found large amounts of a *combined uric acid*, the corresponding ribose-uric acid, in beef blood corpuscles.

This oxidised nucleoside is present in the red corpuscles of the bloods of most species, in descending order of amount in ox, man, and (in traces) horse, sheep, pig, dog and chicken.

Maillard considers that uric acid in the urine is in combination or part combination in ureide linkage with an amino-acid or some other amphoteric compound.

Although man possesses no uricase, nevertheless he can destroy uric acid to some extent. In 1924 Koehler showed that when uric acid is given by mouth it does not appreciably raise the blood uric acid, nor increase the amount excreted in the urine. When uric acid is injected intravenously only about one-half of it can be recovered in the urine. It seems possible that the body can break up the purine nucleus to some extent. However, if large amounts of uric acid are fed to dogs by mouth part is excreted in the fæces (*cf.* also p. 364, paragraphs in small type).

Catabolism of Pyrimidines. There is no evidence that pyrimidines occur free in tissues.

Deuel has shown that when relatively large amounts of thymine or uracil (1 to 3 gm.) are given dogs in a single dose a considerable proportion of this dose can be isolated from the urine, but when the same amount is given in divided doses over several days none can be detected in the combined urines, while there is a considerable increase in the output of urea. When 50 gm. of thymus nucleic acid were given a dog in one dose free pyrimidine could be detected in the urine. Cerecedo has confirmed the conversion of uracil and thymine to urea. Cytosine is apparently oxidised less rapidly, since when small amounts are fed to dogs, part is excreted unchanged.

In man, on a normal diet, it was not found possible to isolate a trace of pyrimidine from 150 litres of urine.

Hence it must be assumed that the body can split the pyrimidine ring and form urea, and that this is the normal fate of pyrimidine in the body, excretion of pyrimidine only occurring when the body has an unusually large amount to handle at one time.

The Uric Acid Content of Blood. Normal human blood contains between 0·7 and 3·8 milligrams of uric acid per 100 c.c., though the normal limits usually fall between 2 and 3 milligrams. The amount is smaller in other mammals, as is to be expected from the formation of allantoine. In the rabbit, sheep, pig, horse, monkey, ox and cat the figure varies between 0·05 and 0·2 milligram. In birds higher figures are found, since in them uric acid largely takes the place of urea as the end product of nitrogenous catabolism. Figures of the order 4 to 5 milligrams have been reported for chicken, duck and geese.

Folin, Berglund and Derick, in 1924, stated that when uric acid is injected into the circulation of dogs, cats or rabbits, the kidneys immediately take up much of it, becoming œdematous and greatly enlarged. That which is not so stored remains chiefly in the circulating blood until it is destroyed. All tissues other than kidney seem to be impermeable to uric acid. This kidney storage does not involve excretion ; only an insignificant fraction is excreted, the rest passing back to the blood for destruction. Destruction takes place in the circulating blood (and perhaps in part in the kidneys), the action proceeding rapidly, so that in the dog after an injection of 100 mg. per kilogram body-weight 70 per cent. is destroyed within ten minutes. Folin thinks that the oxidising agency is not enzymic. Herbivorous animals destroy uric acid much more slowly than does the dog.

Intravenous injection of 20 mg. of lithium urate into man is followed by excretion of from 30 to 90 per cent., the average destruction being 50 per cent. The excretion lasts from one to four days, the duration being determined by the speed of destruction. The unique and characteristic high levels of uric acid in normal human blood are due, according to Folin, to lack of responsiveness on the part of the human kidney in the actual absorption of the acid. This lack of responsiveness is exaggerated in gout.

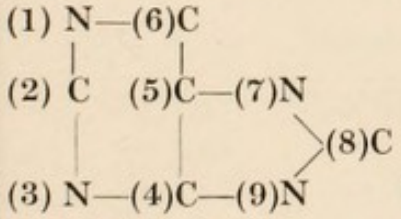
Other Purines Present in Human Urine. Human urine contains traces of adenine, hypoxanthine and xanthine, and, in addition, *small* amounts of 1·7-dimethyl-xanthine, 1-methyl-xanthine, 7-methyl-xanthine and 7-methyl guanine. The methyl-purines are derived from caffeine, theobromine

and theophylline, ingested with coffee, tea, etc., though these compounds seem to be largely destroyed. They are not oxidised to uric acid.

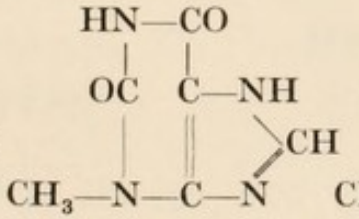
Nucleic Acid Derivatives Present in Plants. The distribution of " yeast nucleic acid " in plants is obviously as wide as that of " thymus nucleic acid " in animals ; its amount is governed by the relative distribution of the cell-nuclei. Much less is known of the catabolism of nucleic acid in plants, but it is evidently not comparable with that in animals, and uric acid does not appear to be formed. Evidence is accumulating, however, that the two metabolisms must have many points of liaison.

Calvery has obtained crystalline adenine-nucleotide from tea-leaves identical with that derived from yeast nucleic acid (1926), and Camargo (1924) has obtained a guanine-pentoside, probably guanosine, from the green leaves and berries of the coffee plant, and considers that it is a precursor of caffeine.

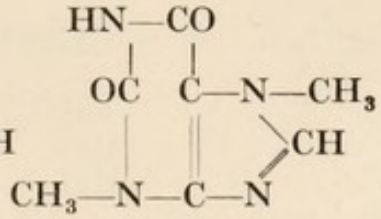
The purines of plants are found, as is to be expected, in such parts of the plants as are richest in nucleoprotein. The three most important are methyl-xanthine, caffeine and theobromine. Assistance in the nomenclature of these purines is obtained by the use of Fischer's numbered purine-nucleus :



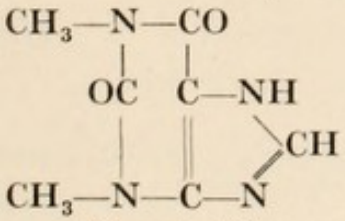
Purine-key



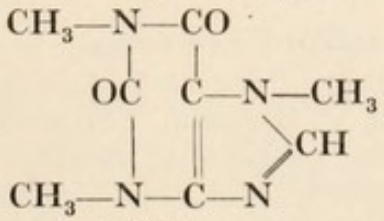
Methyl-xanthine
(3-methyl-
2, 6-dioxypurine)



Theobromine
(3, 7-dimethyl-
2, 6-dioxypurine)



Theophylline
(1, 3-dimethyl-
2, 6-dioxypurine)



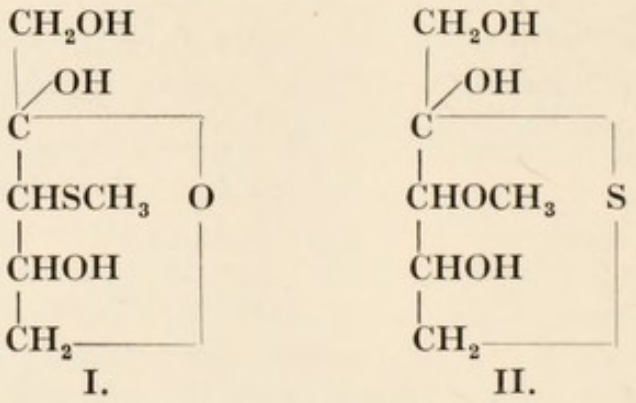
Caffeine
(1, 3, 7-trimethyl-
2, 6-dioxypurine)

Obviously these purines can all be regarded as xanthine derivatives. Traces of xanthine, adenine and hypoxanthine have also been found. These compounds are widely distributed in the phanerograms. There is some evidence that to some extent they

are present in glucosidic combination (*cf.* the nucleosides), and that they may be to some extent also combined with radicals of benzene derivatives.

Caffeine is present in the leaves of the tea-plant, and in the leaves and beans of the coffee-plant in amounts of from 1 to 5 per cent. It is also present in the seeds of *Paullinia sorbilis* (5 per cent.) and in cola beans. Theobromine is not so widely distributed; from 1 to 3 per cent. is found in cacao beans, and traces in cola beans. Theophylline has been obtained from the leaves of *Thea sinensis*. Methyl-xanthine is stated to be present in all plant tissues that contain caffeine, from which it is probably formed. Xanthine itself has been prepared from extracts of the tea-plant.

An adenine nucleoside has been prepared from yeast which Suzuki and his collaborators have shown contains sulphur in the sugar radical, the sugar being a *methyl-thio-pentose*. Levene believes that its constitution should be represented by one of the two forms:



I. II.

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Jones. "Nucleic Acids," in Barker's "Endocrinology and Metabolism," Vol. III., 135–60 (New York and London, D. Appleton & Co., 1922).

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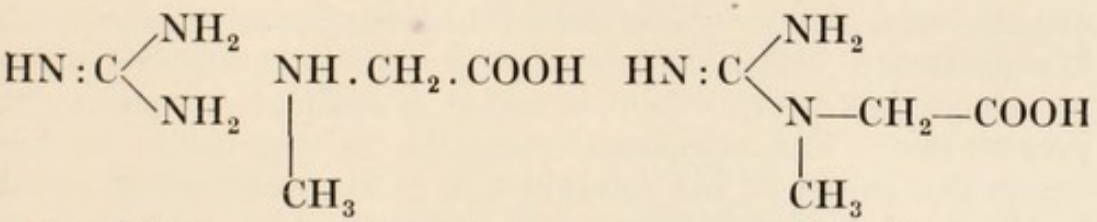
CHAPTER XXV

THE INTERMEDIATE METABOLISM OF PROTEIN DERIVATIVES

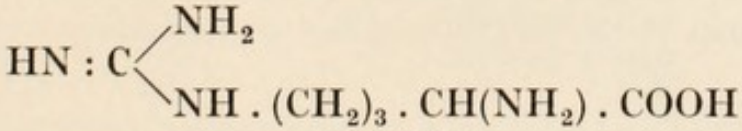
We do not know how the body forms its supply of glutathione, carnosine, carnitine and similar compounds, but they are obviously formed from amino-acids. There is strong evidence that creatine has a similar origin. Our present knowledge permits us to deal in any detail only with the metabolism of creatine and creatinine.

The Metabolism of Creatine and Creatinine

Creatine (Gk. *kreatos*, of meat), methyl-guanidine-acetic acid, can be regarded as a derivative of guanidine, and of methyl-glycine or *sarcosine* (Gk. *sarkos*, of flesh), and as closely related to arginine.



Guanidine *Sarcosine* *Creatine*

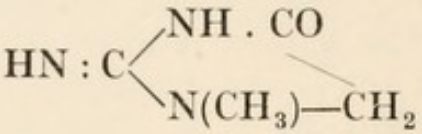


Arginine

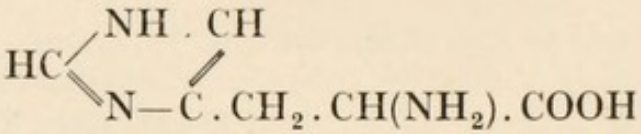
Plimmer has suggested formulæ for guanidine and creatine in neutral solution analogous to the ring formula of Werner for urea.

Creatine was first isolated from beef extract by Chevreuil in 1835. Later on Liebig, Gregory and others showed that it is a constant constituent of muscle tissue.

By loss of water creatine yields its anhydride *creatinine*, whose ring corresponds to that in histidine :



Creatinine

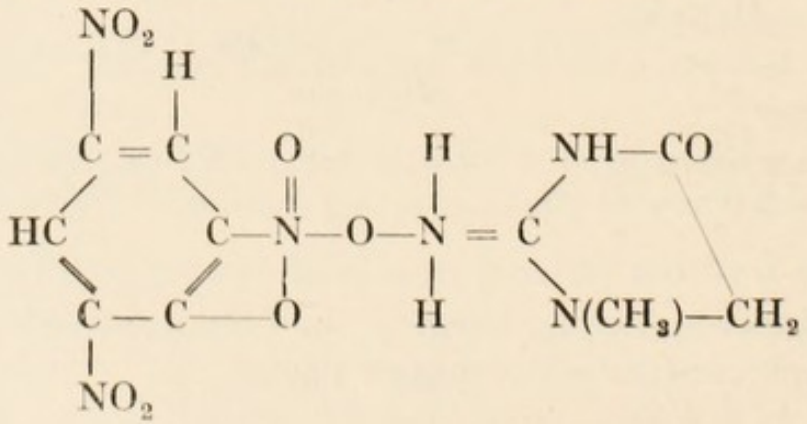


Histidine

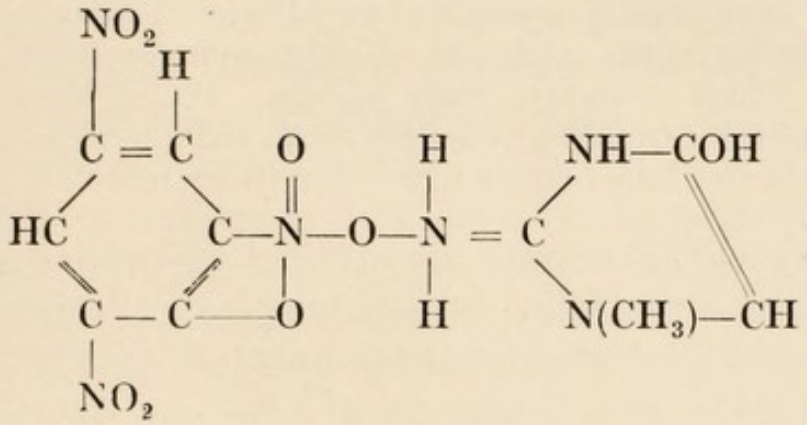
This transformation gradually takes place in acid solution at ordinary temperatures, and is brought about quantitatively in from fifteen to twenty minutes at 120° C. under pressure.

Creatinine is not converted into creatine in acid solution. In alkaline solution creatine is converted into creatinine more rapidly than in acid solution of corresponding strength, but the change is masked by the still more rapid change of creatinine into creatine. Both compounds are decomposed more slowly to urea and sarcosine, and thereafter to ammonia and methyl-hydantoic acid.

Our knowledge of the metabolism of creatine and creatinine has developed almost entirely since, in 1905, Folin based a method for the quantitative determination of creatinine on Jaffé's reaction with picrate, first described in 1886. Greenwald has recently investigated the nature of this reaction. When a solution containing creatinine is added to picric acid, and then the mixture made alkaline with sodium hydroxide, an orange-red colour is produced. If concentrated solutions are used, and after the production of the red colour hydrochloric acid is added, a brilliant red powder is precipitated. This compound seems to be responsible for the orange-red colour of the solution and to be composed of equal molecular proportions of picric acid and creatinine. When heated to 139° it is changed to ordinary creatinine picrate. Greenwald considers that the latter (yellow) has the formula :



and that the red tautomer is :



Greenwald has further shown that the actual compound responsible for the red colour of the reaction is to be regarded as a compound of one molecule of the red tautomer with two molecules of sodium hydroxide.

Creatine is estimated quantitatively by converting it into creatinine.

Creatine is a constant constituent of muscle tissue in all vertebrates and is present in many other tissues. Some idea of its distribution is given by the figures :

Striped muscle of mammals	0·37 -0·60	per cent.
Striped muscle of birds .	0·35 -0·57	,,
Striped muscle of fish .	0·48 -0·74	,,
Heart muscle of mammals	0·20 -0·34	,,
Smooth muscle of mammals	0·09 - 0·13	,,
Testes (cattle) . . .	0·09 -0·21	,,
Cerebrum . . .	0·10 -0·13	,,
Cerebellum . . .	0·12 -0·16	,,
Liver	0·010–0·035	,,
Kidneys	0·012–0·018	,,
Pancreas. . . .	0·012–0·019	,,
Blood	0·004–0·010	,,

There is thus in most tissues a content of from 0·01 to 0·04 per cent., with somewhat greater concentration in testes and brain, and especially large concentration in muscle. Bürger has estimated that 98 per cent. of the creatine present in the human organism is in muscle, and three-fourths of the remainder is in the brain. These figures obviously suggest that in some way creatine is concerned with muscle function.

Emphasis is placed upon such a relationship by the fact that rapidity of contraction seems to be correlated with the content. Pale muscle, which contracts quickly, contains more than red muscle, which contracts more slowly. White muscle of the rabbit contains from 0·4 to 0·6 per cent., red muscle 0·25 to 0·38 ; breast muscle of the hen 0·4 to 0·5, and its leg muscle 0·35 to 0·37.

Embryonic tissue contains much less creatine than the same tissue after birth. Striped muscle of cattle embryos of two months has been found to contain 0·022 per cent., of five months 0·116, and of nine months 0·25, as compared with the average 0·4 for adult cattle. Similar results have been obtained for pig and rabbit fœtuses and for young chicks. After birth there is at first a rapid and then a slower increase.

A probable similar variation occurs in the creatine content of the uterus muscle during pregnancy. In cattle there is an increase from about 0·04 per cent. in the first month to 0·09 in the ninth. The post-partum return to normal is to be associated with the increased post-partum creatinuria. Similar changes have been recorded for rabbits.

Creatine is stated to be absent from the muscle tissue of certain invertebrates, its place being taken by arginine.

Creatine is a normal constituent of the *urine* of infants and young children, and of most, if not all, young animals ; its presence in the urine is independent of the presence of preformed creatine in the diet. It occurs normally in the urine of girls till puberty, and occasionally in that of human adult females even on a creatine-free (meat-free) diet, of bitches and of female rabbits. The creatinuria bears no clearly defined relation to the sexual cycle in women, but it is constant in pregnancy, and is a concomitant of lactation. It is at its maximum in bitches five days after parturition (the creatinuria being then related to involution of the uterus muscle).

On the other hand, normal male human urine contains no creatine, though traces may be present in some individuals

on a normal (not creatine-free) diet. It is not present in the urine of adult male monkeys, rabbits or guinea-pigs.

Creatinuria (the occurrence of creatine in the urine) is present in a number of abnormal conditions, such as starvation, and in various pathological conditions, but obviously the presence of creatine in the urine is an almost usual occurrence. It is an important constituent in the urine of birds.

Creatinine was first discovered by Pettenkofer, in 1844, in urine. It is essentially an end product of metabolism. Its distribution in tissues is in much minuter amounts than those of creatine. Muscle contains more—about 10 milligrams per 100 gm.—than other tissues and than blood—1 to 2 milligrams. The urine of all vertebrates constantly contains creatinine. In man the daily excretion is from 1 to $1\frac{1}{2}$ gm.

Various criticisms have been advanced concerning figures for creatinine and creatine of blood based on the picrate method of estimation, criticisms which have even led to the unlikely hypothesis that blood contains no appreciable quantity of creatinine, the kidney being responsible for the transformation of creatine into (urinary) creatinine. Such criticisms seemed finally disposed of by the results of Gaebler and Keltch (1928), who have *isolated* creatinine as its compound potassium creatinine picrate from normal cattle blood in amount corresponding to 0·9 mg. creatinine per 100 c.c. blood, and have shown by similar isolation procedures that creatinine is present in large amounts in blood during experimental and nephritic retention. Still more recently, however, Gaebler (1930) has stated that this creatinine is not present as such in blood, but as some precursor, which is not creatine.

Effect of Diet on Creatine Metabolism. When creatine is fed by mouth it is possible that a small amount is stored in muscle. There is no evidence that any is destroyed by bacterial action in the intestine, but there is no quantitative recovery of the amount fed. If adult man be given a dose less than 1 gm. no creatinuria occurs, though the excretion of creatinine may be slightly increased. With increased doses creatinuria occurs to an increasing extent : 20 per cent. of a 5-gm. dose and over 60 per cent. of a 20-gm. dose is

excreted in the urine. The accompanying increase in creatinine output is small and constant. Similar results are obtained when creatine solution is injected subcutaneously (excluding possibility of bacterial destruction).

Similar but more marked results occur when creatine is fed to children. Smaller doses induce creatinuria or increase its degree. The younger the child the more of the creatine so fed is excreted, and in infants a dose of 0·1 gm. is largely recovered in the urine.

But while the feeding or injection of creatine leads to a small increase of creatinine excretion the reverse never occurs. Creatinuria never results from feeding or injecting creatinine, but most of this creatinine is immediately excreted. *The body can apparently form creatinine from creatine, but certainly cannot form creatine from creatinine.*

There is no evidence that the body can decompose either of these compounds with the production of urea or ammonia, nor, indeed, that any alteration of creatine can be brought about other than that to creatinine.

When a diet containing meat is fed to adult man there may be increased excretion of creatinine, and sometimes some degree of creatinuria ; this is due to the fact that meat (muscle) contains distinct amounts of creatine, which during cooking is partly transformed to creatinine.

A high protein non-meat diet does not produce creatinuria in man, but may do so in women, and invariably does so in children. This, of course, suggests creatine formation from proteins, that is, from amino-acids. Such corn products as starch, linseed meal and gluten meal, which, as sold commercially, contain certain specific proteins, produce marked creatinuria when fed in large quantities to pigs, but cornmeal, equally rich in (different) proteins, produces only a trace. Evidently specific amino-acids are required for creatine production.

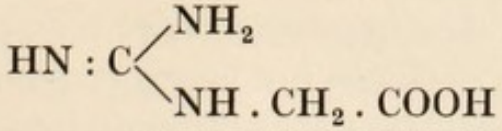
We may conclude that, although on a normal mixed diet much of the creatine excreted is derived directly from food,

yet in young children and other young animals, to some extent in women, and continuously in certain other mammals, creatinuria occurs which cannot be entirely traced to preformed creatine of the food, but seems due to creatine derived from protein sources, that is, from certain amino-acids. It is obviously reasonable to suppose that the creatine existing in the tissues is derived from similar sources.

The Source of Creatine in the Body. Most of the attempts that have been made to ascertain possible precursors of creatine in the organism have been governed by the idea that muscle manufactures its creatine, and that, therefore, if extra material of the right sort be provided, muscle should manufacture more creatine and should show a higher content. Such attempts show at best only slight increases in muscle creatine.

But, as will be seen presently, in all probability muscle does not manufacture creatine, but merely withdraws it from circulating blood until under normal conditions this tissue can be regarded as nearly saturated with the compound. That being the case, obviously if the body becomes possessed with excess of the requisite material for creatine formation, muscle tissue can take up but very little more, even if more be formed. If extra creatine were produced the main result should be occurrence or increase of creatinuria.

After feeding arginine or cystine to pigs and pups respectively, an increased creatinuria has been observed. There is some similar evidence that the closely related glycocyamine may be a precursor.



Glycocyamine

Some not very successful efforts have been made to show that creatine may be formed from guanidine.

At present we can only conclude that some one or more of

the amino-acids can be transformed to creatine within the organism, and that arginine would seem to be theoretically the most likely to be so utilised.

Folin's Creatinine Coefficient and Harding's Creatine Coefficient. Creatinine is an end product of metabolism. On a diet free from creatine and creatinine the daily output of creatinine is, within 10 per cent. variations, constant for each individual, being independent of the total volume of urine, of the total nitrogen excretion, and of the protein of the diet, provided that this does not fall below a certain minimum. This constant figure is a function of the weight of the individual, but shows considerable variation for a number of individuals. For different normal men it varies between 18 and 32 milligrams per kilogram body-weight per twenty-four hours, averaging about 24 to 25 milligrams. For women the corresponding figures are 9 to 26, average 16. Female gymnasts show figures more comparable with those for man. For children the figures are lower, at ten to fourteen days 7 to 10 milligrams, and from five to thirteen years 9 to 13 milligrams per kilogram.

The rate of creatinine excretion is definitely lower during sleep.

Folin regards the creatinine output as "an index or measure of the total normal tissue metabolism." Shaffer relates it to one special catabolic process which takes place largely, if not wholly, in muscle tissue, so that it can be regarded as proportional to the muscular efficiency of the individual.

Harding and Gaebler have shown that if children are fed a high protein meat-free diet, not only is the creatinine excretion fairly constant for any age group, but the *total creatine coefficient*, that is, the creatine plus creatinine (expressed as creatine) per kilogram body-weight excreted per twenty-four hours is extremely constant, averaging 23 milligrams. The creatine coefficient for older persons is very similar in amount. Such a result obviously suggests that,

given a sufficiency of proteins to work upon, the body forms an amount of creatine proportional to its weight.

The Site of Creatine Formation in the Body. It has usually been considered that creatine is formed in muscle, but there is some experimental evidence against this view. Studies by Cameron and Gibson of an individual with extremely deficient musculature, and of amputation cases, have shown persistent creatinuria, with an approximately normal total creatine-creatinine excretion. The creatine excretion in these cases roughly corresponded to the muscular tissue that was absent. Such absent muscle obviously could not be responsible for the formation of this creatine.

Harding's results show that, provided sufficient protein material be fed, there is a constant creatine production at all ages, proportional to the body-weight, and therefore to the total number of body cells. Harding concludes from this constancy that, since there is very different muscular development in children of different ages, the production of creatine is not controlled by the muscular system.

It may therefore be postulated that creatine is formed at a definite rate in some tissue other than muscle, from specific amino-acids, and is then circulated in the blood, 98 per cent. being absorbed by muscle and the other 2 per cent. by other tissue. Muscle becomes almost completely saturated. Man can handle all the supply, and even a little from the diet. In women, with a relatively smaller musculature, the same relative creatine production should lead to an excess above muscle requirement, and such an excess, remaining in the circulation, would be excreted. Actually, as we have seen, creatinuria does occur much more easily in women than in men. In children the same rate of creatine production, with still smaller relative musculature, should give constant excess and therefore constant creatinuria. In children there is constant creatinuria.

Harding says: "Creatine disappears from the urine of the adult man when the creatinine coefficient has attained its average

maximum value, and the muscular system has also reached its average percentage of the total body-weight."

The Site of Creatinine Formation in the Body. High protein feeding causes little increase in creatinine excretion, which (excluding creatinine in the diet) comes chiefly from *endogenous* sources (sources within the body). Species with highest muscle creatine excrete relatively most creatinine in their urine. Muscle contains not only more creatine than any other tissue, but also more creatinine. The creatinine content of other tissues may easily be accounted for by diffusion from blood, since this compound is one of the most diffusible in the body. Incubation of muscle under buffer reaction conditions results in a distinct increase of its creatinine content.

It may be concluded that by far the greatest amount of creatinine produced in the body is formed in muscle by dehydration of creatine.

Gaebler's most recent results (p. 371) are not in complete agreement with this view.

Zwarenstein has recently published evidence tending to show that some creatinine may be formed from uric acid by a transformation of the iminazole nucleus.

The Function of Creatine Metabolism. Since creatinine excretion seems proportional to the muscularity of the individual it might be expected that with increased work there would be increased excretion of creatinine. There is a distinct increase in a working as compared with a resting period, but no evidence that such an increase affects the twenty-four hours' excretion. (It is doubtful, however, if such working periods have been of sufficient duration to warrant a final conclusion.) It has been stated that increased creatinine excretion accompanies increased muscular tone, but here also the evidence is inconclusive.

There is definite evidence relating the metabolisms of creatine and carbohydrate. When an animal is starved creatine is excreted, and to the extent to which it is excreted creatinine excretion

diminishes. The same result follows with carbohydrate starvation (low-protein, non-carbohydrate diet), and the effect produced by complete starvation ceases as soon as carbohydrate (without other food), or anything such as glycerol which will yield glucose in the body, is fed.

Creatinuria cannot be related to acidosis nor to protein deficiency.

The manner in which creatine is held in muscle has long puzzled investigators, since even mincing muscle liberates its creatine while water extraction wholly removes it. It cannot be present in simple solution or the circulating blood would remove it. Fiske and Subbarow, Eggleton and Eggleton, and Meyerhof and his collaborators have recently materially increased our knowledge of the way in which creatine is held in muscle, besides giving us a clue to its function, which we may now consider indisputably is linked with the metabolism of muscle contraction.

All of the 400 to 500 mg. of creatine in each 100 gm. of muscle, except a possible 30 mg. (and even this may not be true creatine but merely some other compound capable of giving coloured picrate), exists as salts of creatine-phosphoric acid, *during muscular rest.* Coincident with muscular contraction this compound hydrolyses to creatine, phosphoric acid, and base, the free base so liberated being sufficient to neutralise most or all of the lactic acid formed from glycogen. During the subsequent resting period, when most of the lactic acid is re-transformed to glycogen, most of the creatine and phosphoric acid recombine.

The hydrolysis and the synthesis of creatine-phosphoric acid seem to vary with the *p*H of the medium containing it. In alkaline solutions synthesis prevails, while with a *p*H of 6 hydrolysis is rapid. The reaction is associated with the glycogen-lactic acid changes, but is not dependent on them, and is actually more essential to muscular contraction than the breakdown of glycogen (*cf.* Chapter XXXII).

It is now possible to put forward a reasonable hypothesis for the mechanism of creatinine formation. It can only be

formed in muscle when creatine itself is free. This only occurs during muscular work. Even under neutral conditions the equilibrium creatine $\rightleftharpoons$ creatinine is in the direction of creatinine formation. Any increase of acidity increases the rate of creatinine production. Every time a muscle contracts, therefore, a little creatine will be transformed to creatinine. This cannot be re-transformed to creatine, but will be excreted from the muscle to form shortly thereafter part of the urinary creatinine. The total creatinine output hence must be a more or less complex function of the total muscularity, and the total work performed by the total muscle-bulk. Shaffer's conception of the significance of Folin's creatinine-coefficient still holds, even though *creatinine* in the light of this new evidence *appears to be an accidental bye-product of the carbohydrate metabolism of muscle.*

The Nature of Creatine-Phosphoric Acid. The constitution of "phosphocreatine" (Fiske and Subbarow), or "phosphagen" (Eggleton and Eggleton), or creatine-phosphoric acid is :

$$HN : C\begin{cases} NH \cdot PO(OH)_2 \\ N(CH_3) \cdot CH_2 \cdot COOH \end{cases}$$

The —NH . PO— linkage may be compared with the ordinary peptide linkage, —NH . CO—. A few compounds with this type of N.P linkage have been prepared in the laboratory, but this is the first compound exhibiting it which has been found in the living organism. Like it, the laboratory compounds are unstable in acid solution.

Creatine-phosphoric acid gives rise to crystalline salts with divalent metals, the calcium salt being stable and crystallising at room temperatures.

As already mentioned, there is evidence that the place of creatine in invertebrates is taken by the amino-acid arginine. Work from Meyerhof's laboratory suggests strongly that in crustacean muscle arginine-phosphoric acid, with a similar linkage, functions like the creatine-phosphoric acid of mammals.

Creatine Metabolism in Certain Pathological Conditions. Febrile conditions result in creatinuria, associated with increased creatinine excretion. Evidently all the factors involved in creatine metabolism are exaggerated.

Creatinuria, accompanied by diminished creatinine excretion, occurs in a number of conditions involving muscle wasting. The actual wasting of muscle will liberate some creatine, while the diminished muscle volume will diminish muscle requirement, without, presumably, diminishing supply.

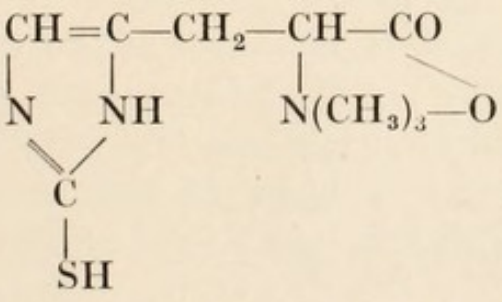
Creatinuria occurs in exophthalmic goitre, and in the comparable condition which follows feeding of thyroid or thyroxine. In these conditions there is an increased general metabolism and also actual tissue destruction.

Creatinuria is an accompaniment of various pathological conditions of the liver, including malignant disease. Cases of increased muscle tonus without wasting show creatinuria. It occurs in the pre-coma stage of the syndrome of abnormal carbohydrate metabolism known as diabetes mellitus.

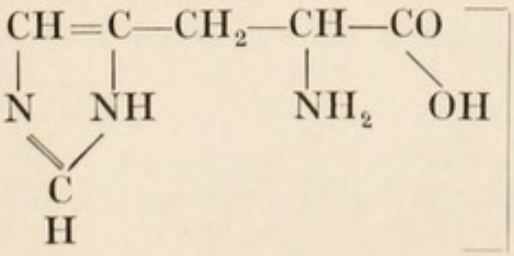
(After nerve section to a muscle the creatine content of this muscle remains constant until its degeneration commences.)

It has been recently reported that creatinuria is an accompaniment of a diet deficient in vitamin C, the creatinine coefficient increasing markedly and also the creatine content of muscle.

Ergothioneine. Our ignorance of many of the important links in the metabolic chain of protein decompositions is exemplified by the recent discovery of the presence in the blood of a betaine of thiol-histidine, thioneine (thiasine, sympectothione)—



Cf. histidine:



which, under the name of *ergothioneine*, was isolated from ergot in 1908. Pig's whole blood contains 0·15 per cent., present entirely in the red cells. Human blood contains about one-tenth this amount. Such a compound would appear to be probably of metabolic importance.

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CHAPTER XXVI

INTERMEDIATE METABOLISM OF THE LIPIDES

THE intermediate metabolism of the fats, phosphatides and cholesterol is closely interrelated; some part of this interrelationship we are beginning to comprehend, but to much we still have no clue of understanding. It must be constantly remembered that, while fats are relatively inert compounds, the phosphatides include some of the most reactive substances that are found in animal and plant tissues.

Fat of the body derived from fats of the diet consists chiefly of the triglycerides of palmitic, stearic and oleic acids, which have been digested in the manner explained in Chapter XI., and absorbed. In addition, tissue fat may be derived from carbohydrates and from proteins. Fat is only stored in the tissues when the food ingested (the potential energy ingested) is in excess of the energy requirements of the body, and not always then, since many individuals show a peculiar idiosyncrasy preventing the laying down of fat.

Absorption of fat to the extent of at least 60 per cent. takes place through the membrane of the villi of the intestine as neutralised fatty acid and glycerol; there is immediate reformation of neutral fat, which is then transferred to the lacteals of the villi, and so by way of the thoracic duct to the general circulation.

The evidence concerning the mechanism by which the other 40 per cent. reaches the general circulation is conflicting. According to Fish the blood in the portal vein contains less fat than that in the jugular vein. According to Cantoni the fatty acid content of the portal blood is always higher than that of the carotid after a meal of fat.

The absorption is probably assisted by the bile salts; these form clear solutions of fatty acids, which are diffusible.

When the diet contains excess energy *and* excess fat the type of fat laid down in the tissues depends on the type of fat fed; if this contains strange fatty acids these will be stored.

Lebedev, in 1882, starved a dog, so that it lost part of its stored fat, and then fed it a diet containing a considerable amount of mutton suet. After some weeks the dog was killed and examined. Its fat was solid at 50° C. A second dog, treated in the same way, but fed on linseed oil instead of mutton suet, gave a fat which was still liquid at 0° C. The resemblance of the properties of the stored fat to those of the fat fed is obvious. Other investigators have fed foreign fatty acids, such as erucaic acid, $C_{21}H_{43}COOH$, and have found the corresponding fats subsequently in the tissues. Maude Powell (1930) has shown that when trilaurin is fed to rats the stored fat may contain as much as 25 per cent. lauric acid.

When fatty acids are fed, instead of fats, only a part is absorbed, and this result suggests that the body can only supply a limited amount of glycerol.

The fat that is actually stored and the fat of the chyle may show some chemical differences from the fat of the food when, under normal conditions, the animal has free choice of food, and the amount of fat ingested is not too large. Two factors govern these differences, the first an apparent selection of the more desirable or useful portions of the (mixed) fat, generally those of lower melting point, and the second, chemical changes in the fatty acids such as saturation and desaturation.

Dogs have been fed on lard, and it has been found that the fat of their fæces had a considerably higher melting point than that of the lard. Hence, since bacterial action on fat in the intestine is *nil* or negligible, the absorbed fat must have had a considerably lower melting point. Dogs have been fed cetyl palmitate, and it was found that the chyle fat consisted of one part of triolein and six parts of tripalmitin, the mixture melting at 36° C. When these dogs were fed ethyl palmitate the chyle fat contained 36 per cent. olein. In both these experiments definite desaturation of the

fatty acid and a further synthetic change must have occurred. When mutton fat, with a melting point of 51·7° C., was fed the chyle fat melted at 38° C.

On the other hand, when dogs were fed olive oil (almost pure triolein), with a melting point of 16° C. and an iodine number of 86, the chyle fat melted at 30° and its iodine number was 72, indicating increased saturation.

The absorbed fat, changed or unchanged during its absorption, reaches the blood to produce an alimentary lipæmia ; under normal conditions the fat content of the blood may then be as much as 2 per cent., present as finely divided particles of one μ diameter, with pronounced Brownian movement.

In diabetes the lipæmia may increase to even 20 per cent.

This fat disappears from normal blood in from eight to fourteen hours ; the mechanism by which it is withdrawn is not definitely known.

Coincident with the rise of blood fat occurs a rise of blood lecithin and a delayed rise of blood cholesterol. Generally, whenever one of the three—fat, lecithin and cholesterol—is increased in the blood the others also increase. (Thus, if cholesterol is fed, and the amount in blood is thereby increased, there is an increase in blood lecithin.) Bloor is of the opinion that in the blood fat changes to lecithin, and that *the lecithin is largely the form of carriage of fat in the blood to the tissues.* Such a transformation would be of practical value, since lecithin is the only compound of the fatty acids, except the toxic soluble soaps, which is miscible with water and can therefore be easily transferred by a watery medium. Both fat and lecithin appear to be carried in the corpuscles in somewhat greater amounts than in the plasma.

We must now distinguish between the true *lipases* which hydrolyse (emulsified) fats readily, and the *esterases*, which only slowly act on fats, but readily split esters of the lower fatty acids and lecithins, and which, as well as the lipases, are of quite general distribution in the tissues. We may

suppose that these esterases cause the transformations to lecithin in the blood (or in the absorbing tissues before the blood is reached), and that lecithin brought to the tissues by blood is broken down by their esterases and the constituents built up to insoluble neutral fat in the fat depôts.

Meigs, working on milk secretion in cows, has shown that during this secretion the difference in the lipoid phosphorus of the blood plasma before and after passing through the mammary glands is sufficient to account for the entire amount of fat secreted into the milk, so that it may be concluded that a large proportion of milk fat originates from blood lecithin.

There is also evidence that the *microphages*, a group of endothelial cells found in the capillaries of the hepatic lobules, capillaries and venules of the spleen, bone-marrow, and hæmal glands, and lymphatic sinuses of the lymphatic glands, and characterised by abundant delicate pseudopodia which can engulf free particles of fat, can also play a part in removing fat from the circulation.

When fat is injected into the circulation a large part is removed rapidly. The liver and lungs seem chiefly responsible.

Fat Derived from Carbohydrate and Protein of the Food

This is obviously formed from glucose. That carbohydrates are a potential source of body-fat was first accurately demonstrated by Lawes and Gilbert in 1852, and their experiments still afford the classical proof.

They took two young pigs of the same litter, killed one and estimated its fat and protein content; they assumed that the figures so obtained could be applied to the other animal. This was fed on a food mixture of known composition, and consisting chiefly of barley and, therefore, of carbohydrate. After several weeks this animal was killed and analysed, and so the amounts of fat and protein formed during the experimental period determined. Assuming that the whole of the carbon in the food-protein that had not been stored or excreted as urea was converted to fat it was found that the actual increase in fat was greater than such quantity plus the food fat, and must therefore have been derived from some other food-constituent, obviously the carbohydrate. Their results are shown by the following figures:—

Total fat increase, 71·2 lb., minus food-fat, 12·4 lb., leaves fat synthesised by body, 58·8 lb.

Food protein, 64·0 lb., minus new-formed body-protein, 6·5 lb., leaves protein available for fat synthesis, 57·5 lb.

Carbon in this available protein, less carbon excreted as urea, 27·4 lb.

Carbon in the fat formed by synthesis 45·3 lb., minus 27·4 lb., yields 17·4 lb. of fat-carbon which must have come from carbohydrate.

This experiment apparently shows also that fat may originate from food-protein. Theoretically, since glucose can be formed from amino-acids, and excess glucose can be stored as fat, an excess protein diet should permit fat storage. Atkinson, Rapport and Lusk have adduced direct evidence.

They showed that starved dogs, in whom there is in consequence of the starvation a low glycogen and fat reserve, when fed large quantities of lean meat (containing practically nothing but water, protein and salts) in excess and continuously, store a mixture of glycogen and fat. When very great excess of meat is fed then fat alone is stored.

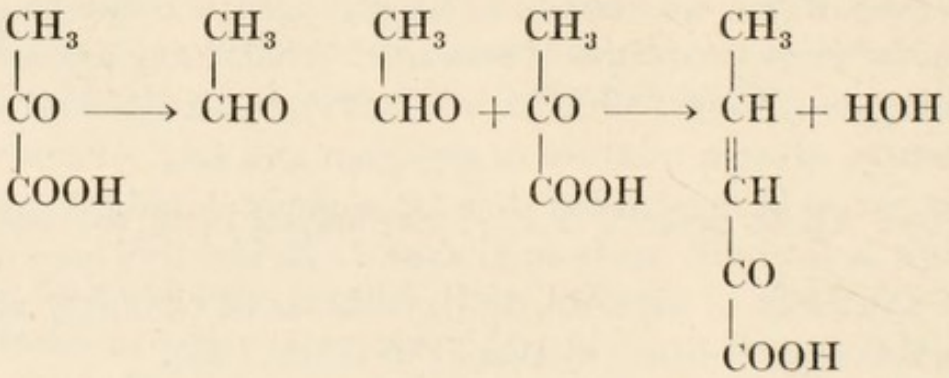
How is fat built up from glucose ? Evidently there must be a synthesis of glycerol, and syntheses of the fatty acids, with subsequent lipase synthesis to neutral fat.

The production of *glycerol* from glucose by the plant organism yeast has been dealt with in Chapter XXI., and the probable stages of the process outlined. We know that if glycerol be fed to the diabetic animal glucose is formed, and it seems certain that the reverse process can take place normally, though the steps cannot yet be outlined. It has been suggested that glucose can give rise to glyceraldehyde, $CH_2(OH) . CH(OH) . CHO$, closely related to pyruvic aldehyde, and which, on reduction, yields glycerol.

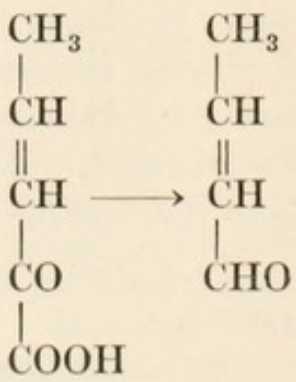
The production of the requisite fatty acids from glucose is also a matter of speculation, but of speculation based on reasonable experimental premises. The oxidation stages of glucose include lactic acid and pyruvic acid, and the body

can apparently easily change either of these two compounds into the other. If excess of sodium pyruvate be administered subcutaneously to an animal, both glucose and lactic acid are found in the urine. If a glycogen-free liver is perfused with pyruvic acid, large amounts of lactic acid are formed.

The following series of changes have been brought about in the laboratory, and it is believed that the liver can also bring them about. Pyruvic acid, through the enzymic (carboxylase) liberation of carbon dioxide, gives rise to acetaldehyde, which by condensation with more pyruvic acid yields an unsaturated keto-acid. The carboxylase, acting on this, produces an aldehyde with two more carbon atoms than has acetaldehyde.



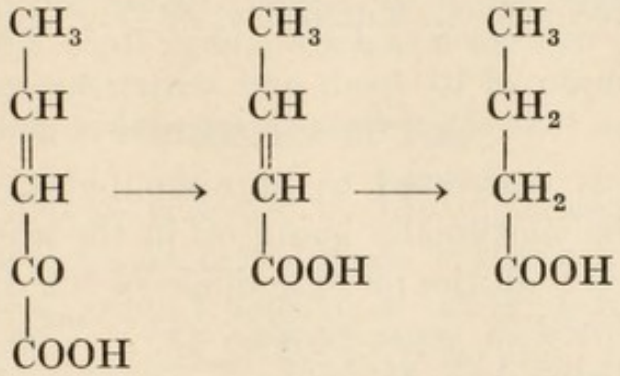
Pyruvic acid *Acetaldehyde* *Unsaturated keto-acid*



The new, unsaturated aldehyde will also condense with pyruvic acid, and so a whole series of unsaturated fatty acids

can be formed, all by successive increases of two carbon atoms.

At any stage the unsaturated keto-acid can be oxidised to an unsaturated fatty acid, and this hydrogenated to a saturated fatty acid.



Such fatty acids will all have an even number of carbon atoms. If the series of changes be extended, the necessary three, palmitic, stearic and oleic acids, will result. It is probable that in any particular species a definite mixture of the three will be formed.

It is, therefore, possible to say that *the constancy of composition of animal fat of a particular species is traceable to two causes, first, the tendency to absorption of the type of fat best fitted to that species*, with alteration or rejection of the remainder, *and second, the building up of a specific fat mixture from glucose from the diet.*

A Fat Deficiency Disease.

Burr and Burr have recently published results which suggest that, at least as far as the rat is concerned, this animal cannot synthetise all the unsaturated fatty acids it needs. When young rats were fed diets completely freed from fat over a period of some months they developed various symptoms, of which failure to grow seems the most certain. Onset of these symptoms was prevented by feeding the animals a little linoleic acid. Hume and Smith think that the abnormal results are due to a deficiency of vitamin B_1; the work of the Burrs must at present be considered as suggestive rather than final.

Fate of Animal Fat

Fat is secreted into the milk. It can be stored as inactive fat. It can be burnt up and so used as a source of energy. It can possibly be changed to carbohydrate.

During the germination of fatty seeds starch and cellulose are formed from the fat of the seeds. Before hibernation such an animal as the marmot lays down a large store of fat formed from the carbohydrate of its food, and during hibernation there is some evidence that the reverse process takes place.

Some fat is converted to phospholipides. This process appears to be continually going on in the absorbing tissues and the blood ; all the phospholipide of the different tissues must be formed in some similar way. This will not only involve synthesis from the component parts of the phospholipide, but also possibly some degree of saturation or unsaturation of the fatty acids, and utilisation of fatty acids more highly unsaturated than oleic acid. We do not know by what process such a probable precursor as glycerophosphoric acid is formed, but it is interesting to note that Greenwald has demonstrated the presence in blood of the related compound di-phospho-*l*-glyceric acid :

$$\begin{array}{l} CH_2.O.PO(OH)_2 \\ | \\ CH.O.PO(OH)_2 \\ | \\ COOH \end{array}$$

The storage of fat takes place in a specific tissue, the *adipose tissue*, which is a modification of connective tissue, and has a copious blood supply. Many other cells may contain stored fat. The liver is a (frequent) temporary storehouse, and fat accumulates there under a variety of conditions, such as during fat absorption, during poisoning of the liver with phosphorus or chloroform, in chronic alcoholism, often in fasting, and in diabetic lipæmia. The fat is laid down in adipose tissue in a way quite similar to its formation in the intestinal cells during absorption. At first fine

droplets are formed, which coalesce to large single globules. The cells adjacent to the blood vessels are first filled, and the filling extends outwards from these. A similar process takes place during fat storage in the liver.

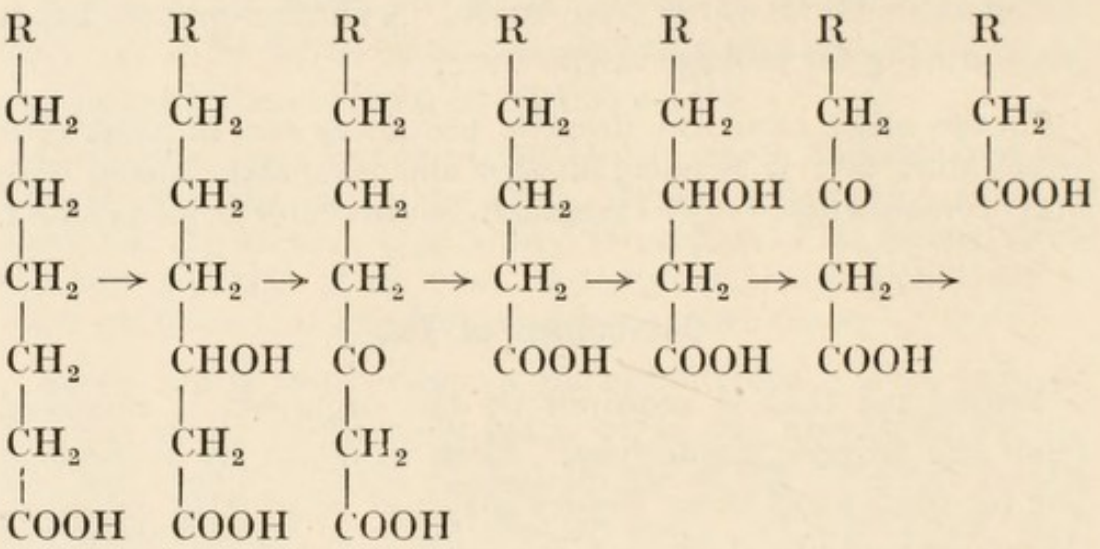
On the other hand, fat droplets are never seen in plant cells which store fat; it is finely divided and intimately mixed with other constituents; only on germination are visible droplets found.

Catabolism of Fat

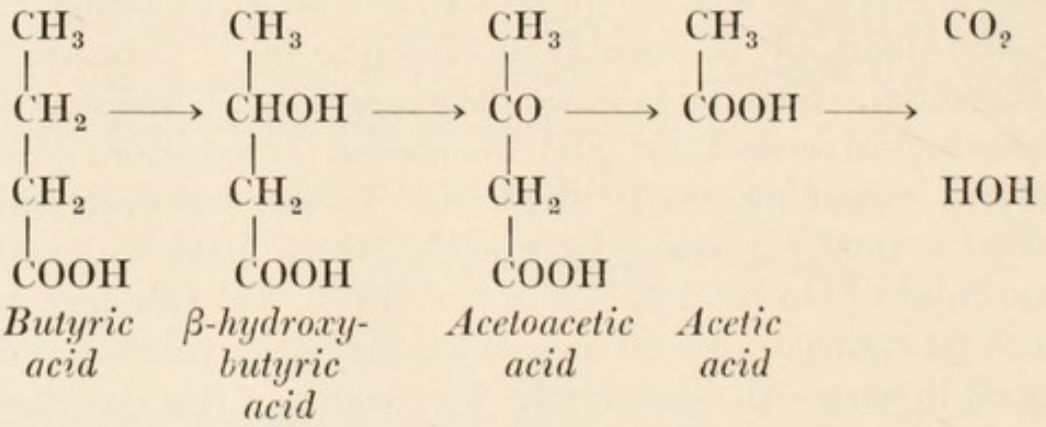
Stored fat that is required by the organism is removed from the adipose tissue first. Even in death by starvation the fat content of other tissues may not be greatly changed. As an immediate stimulant for such removal the depletion of the glycogen store of the liver has been suggested; an antagonism between fat and glycogen metabolism is suspected. We may suppose reasonably that the fat is carried from adipose tissue to liver by the reverse process (in part or whole by lecithin formation) to that which brought it to this tissue. It is probably mobilised to the liver before its catabolism. Such mobilisation is exaggerated in the various pathological conditions just mentioned (starvation, phosphorus poisoning, etc.). Bloor has shown, however, that blood normally contains fatty acids (which make up nearly one-third of the total lipoids of the blood), and this suggests that fat transport occurs in part as fatty acid, perhaps combined in part with cholesterol. Two-thirds of this fatty acid content of blood consists of unsaturated acids.

The series of changes resulting in the oxidation of fat is not yet known with certainty. At first glycerol is split off, in the liver or elsewhere, and is converted to glucose or oxidised directly. The fatty acid may or may not be desaturated before the essential oxidation of it commences. According to Knoop's theory this consists of a series of attacks on the β-carbon atom (the second in order from the acid group), with the resultant etching away of two carbon

atoms by each such series of actions. A hydroxy-acid and a keto-acid are the intermediate products :



In this way, finally, butyric acid is produced, which, through the intermediate stages of β-hydroxy-butyric acid and acetoacetic acid, is converted to acetic acid, and so to carbon dioxide and water :



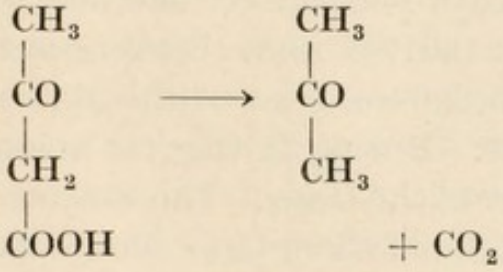
At each stage carbon dioxide and water are formed.

There are evidently two sources of acetoacetic acid, the fats and certain amino-acids (*cf.* pp. 341, 344). Its complete oxidation involves correct glucose oxidation, and apparently about one molecule of glucose must be oxidised to effect oxidation of two molecules of acetoacetic acid.

Shaffer has shown that when glucose is oxidised by hydrogen peroxide in alkaline solutions, acetoacetic acid, if present, is completely oxidised, while in absence of glucose the acid is only

oxidised very slowly. He suggests that this action is analogous with that proceeding in the organism.

Under abnormal conditions, involving the improper oxidation of glucose, acetone is the final product, and we may consider, since normal blood always contains a little acetone, that acetone is always formed to a very small extent from acetoacetic acid, with elimination of carbon dioxide :



Since incorrect oxidation of glucose involves ketone (acetone) production, whilst oxidation of glucose prevents this, and the accompanying accumulation of acid, glucose is said to be *anti-ketogenic*, and the ratio of the compounds giving rise respectively to acetoacetic acid and to glucose in the organism (or in a diet) is termed the " ketogenic-antiketogenic ratio."

There are a number of points that still require elucidation. The order of formation of the hydroxy- and keto-acids is not definitely settled, and the balance of evidence is in favour of the less obviously likely change to the keto-acid being the primary one, and the hydroxy-acid formation being a subsequent change. If the whole series of oxidations were of the nature outlined, then for every molecule of fat oxidised one molecule of acetoacetic acid would be produced. This quantitative relationship has not been demonstrated ; there is a shortage in the amount of acetoacetic acid that results. It would thus appear that fat may be in some small part oxidised through some other series of intermediate channels ; these may, perhaps, involve extensive desaturation of the fatty acids, a type of change for which there is some experimental evidence.

Bloor considers that there is little doubt that desaturation actually does take place in the animal organism, and that it is to be regarded as the first stage in oxidation ; unsaturated fatty acids can be oxidised under the influence of glutathione while saturated acids can not.

Witzemann has recently put forward evidence that in a phosphate-peroxide system (simulating the type of oxidative systems

in the organism) butyric acid is easily oxidised to α-hydroxybutyric acid and thus to acetic acid and carbon dioxide. He thinks that in biological processes *α-oxidation* may accompany β-oxidation to some degree.

Excretion of Fat

The fæces always contain considerable amounts of fatty acids. These are derived from three sources, undigested fat, fat from the cellular material of the alimentary canal, and true excreted fat. During fasting fat amounts to one-third of the dry weight of the fæces. This may be regarded as true excreted fat. Hill and Bloor have shown that when *moderate* amounts of fat are fed the fat of the fæces is to a great extent independent of the diet and approaches in composition to the fæces-fat on a fat-free diet. Such constancy of composition supports the idea of a true fat excretion through the intestines.

If we admit, as therefore seems probable, that there is a normal excretion of a small amount of fat, it follows that fæces-fat cannot ordinarily be regarded as simply unabsorbed food fat. Feeding experiments or tests of the extent of utilisation of particular foods are of doubtful value, as far as fat is concerned, unless the amount and kind of fat that is present in the fæces independent of the diet is taken into due consideration.

Metabolism of Phosphatides

We have seen that apparently lecithins can be easily formed from neutral fat in blood and in at least certain tissues. The requisite phosphate is always available. By what mechanism glycerophosphoric acid is produced, and how it is caused to combine with choline (or cholamine), we have no evidence to indicate. Nor can we yet say anything of the metabolism of choline itself, either as regards its formation or destruction. The same lack of evidence holds for cholamine, sphingosine and other compounds whose radicals are present in the compound lipoids.

Metabolism of Cholesterol

While normal diets undoubtedly contain cholesterol, or sterols easily convertible into cholesterol, evidence is accumulating that the animal organism can synthesise this compound to meet the need for it. It has been shown, for example, that the developing chick in the egg shows increase of total cholesterol which can only have taken place by synthesis. When newly-born dogs are given for four weeks a diet poor in cholesterol, and then killed and analysed, their bodies show an increase in cholesterol amounting to twenty times that administered in their food. Similar evidence is available for the rat; study of cholesterol metabolism in infants supports the view that synthesis occurs.

Cholesterol is present in blood and all the tissues of the body, both free, and combined as esters with the higher fatty acids. The cortex of the adrenal body is relatively rich in the compound and its esters. The intestinal and pancreatic juices and bile, and also blood, contain an enzyme which will hydrolyse cholesterol esters, while pancreatic juice contains an enzyme which will synthetise them from their constituents.

Aside from the obvious purport of the greasy esters in sebum, the function of cholesterol and its esters is not understood, though it seems to be definitely intertwined with certain phases of fat metabolism, and Bloor states that in the plasma most of the unsaturated fatty acids are present as cholesterol esters. It is closely related to cholic acid, though whether the liver forms cholic acid from it for bile salt production still remains to be demonstrated. Fox and Gardner suggest that cholesterol and cholic acid are formed in the body by collateral processes. There is some evidence that cholesterol prevents the onset of polyneuritis. It bears some relationship to vitamin D, since that compound is produced by the action of ultra-violet light on the more unsaturated compound, ergosterol.

Some recent evidence has been published purporting to

show that the cholesterol radical is a prosthetic group in serum globulin.

Cholesterol is excreted in bile, presumably because this medium, in virtue of the bile salts present, is the only one that can hold it in any degree of concentration. There is some evidence that the blood of the portal vein contains more than that of the splenic vein, and still more than that of the subhepatic vein, while blood from the right side of the heart contains more than blood from the left side, whence it has been argued that cholesterol is either fixed or destroyed partly in the liver and partly in the lungs.

Its excretion by the bile is partly controlled by gall-bladder storage, since during such storage a certain degree of re-absorption of cholesterol appears to occur. During passage of the excreted cholesterol through the intestines a considerable proportion is reduced to coprosterol, $C_{27}H_{47}OH$, by bacterial action, the double bond being hydrogenated.

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CHAPTER XXVII

THE METABOLISM OF ALCOHOL, WATER, AND SALTS

The Metabolism of Alcohol

Ethyl alcohol is not affected by digestion. It is absorbed directly from the gastro-intestinal tract, mainly into the portal blood, but partly by the lymphatics. The amount absorbed by the stomach depends on the rate at which it passes the pylorus. Alcohol taken with food remains longer in the stomach, and so a greater proportion will be absorbed there than when taken on an empty stomach. The total rate of absorption is delayed when it is taken with food, fat especially having a delaying action. Since fat lengthens the time of stomach digestion, and so increases opportunity for stomach absorption, this delayed absorption suggests that absorption from the stomach is less rapid than from the intestine. Alcohol is also absorbed and utilised when given by rectum, and when inhaled as vapour.

Alcohol taken with food may have some effect on the digestion, since a dilute solution increases the concentration of hydrochloric acid in the gastric juice. The net results are the same ; the amount of undigested residue in the fæces is unaltered. Stronger solutions of alcohol act as irritants, and may cause increased mucus formation, and even vomiting.

The absorption of alcohol from the gastro-intestinal tract is rapid. Within five minutes of its ingestion a change in the respiratory quotient (see Chapter XXX.) can be observed, indicating that it is already being oxidised. Its concentration in the blood reaches a maximum between one half and two hours following its ingestion. The body oxidises between

90 and 98 per cent. of ingested alcohol to carbon dioxide and water. The remaining 2 to 8 per cent. is excreted as unchanged alcohol in the urine, breath and sweat. A trace is also excreted in the milk of nursing mothers. Alcohol is conveyed by the blood to all the tissues of the body, and very evenly distributed. Apparently all these tissues have the power to oxidise it.

While large amounts of alcohol lead to marked peripheral dilatation of the arterioles, with consequent fall of body temperature, moderate amounts do not produce this effect, but are oxidised and produce heat which otherwise would have to be derived from some other food source. Protein metabolism is not affected, but alcohol when available in the tissues is oxidised in preference to either fat or carbohydrate. The rate of combustion is fairly constant and independent of the amount taken. It seldom reaches 50 per cent. of the total heat production of the body during the period. In a resting individual who has taken in proper dilution about 30 to 45 c.c. of alcohol (or two or three times that amount of undiluted brandy or whisky, depending on the alcoholic strength), about 3·5 c.c. are burned per hour, producing 20 to 40 per cent. of the total heat production of the individual during this period. For nutritive purposes for patients (or normal individuals) the best utilisation for energy purposes will be attained by doses of 10 c.c. or less, which may be repeated on the basis of a body consumption of 3·5 c.c. per hour.

It is generally believed possible that the potential energy of alcohol can be changed into the kinetic energy of muscular work, but this is unlikely, since it would involve a transformation to glycogen and hexose-phosphate. The action is probably indirect, alcohol acting as a carbohydrate sparer, and thus rendering more glucose available for muscle metabolism.

When alcohol is added to the diet of a person doing heavy muscular work the work is not done so efficiently, nor so easily.

On the other hand, power of endurance is definitely increased Actual experiment has demonstrated that an individual who could hold his breath for fifty-three seconds without alcohol could do so for 105 seconds after administration of it. Ordinary feats of endurance, like hanging on to a bar or lifting oneself from the floor, can be carried out much more successfully. The effect of moderate dosage on mentality is well recognised. Inspiration is greater, while accuracy is lessened. But, as a poet has pointed out, one's effusions can always be proof-read next morning.

It has recently been demonstrated that continued administration of alcohol to rats over several generations, while at first producing a greater mortality, finally leads to the production of a stronger and more virile race (the weaklings have perished ; the race has improved). Pearl has shown from United States statistics that at every age from 30 to 100 inclusive, persons in the " all moderate " class of drinkers, whether male or female, have a somewhat higher expectation of life than the occasional moderate drinkers, and that these latter have still a somewhat higher expectation than persons in the " abstainer " class of the same age.

It is, of course, universally recognised that too much alcohol is harmful to the human organism, and that, to be of any practical value for nutritive purposes, it must be taken in small amount.

Alcohol has a definite value in the diet of the diabetic. He can utilise alcohol normally and obtain energy from its oxidation. It does not prevent the formation of acetone bodies by incorrect oxidation of fats in absence of sufficient glucose oxidation, but it can replace a corresponding amount of fat for energy production, and in that way lower acetone formation.

It must be emphasised that in moderate doses alcohol is a true food, and that it has the advantage over other foods that its energy is sooner available to the body after ingestion.

Metabolism of Water

The different tissues of the body contain varying but large amounts of water, the average for the whole body being about 70 per cent. The purpose of this water is obviously to provide a medium in which chemical actions can occur, and physical chemical phenomena such as osmosis and diffusion

can effect the necessary transfers of chemical compounds through tissue membranes. The actual dilution is presumably the optimum dilution for such changes.

Any marked deprivation of water, leading to greater concentration of the chemical compounds present in the tissues, is followed at first by a sensation of thirst, and subsequently by a series of pathological happenings. Of all the varied types of starvation the complete deprivation of water leads most rapidly to a fatal termination.

Water is being continually lost from the body, through all the channels of excretion, but chiefly and reciprocally through kidneys and skin. There is a complete balance between intake and output, provided the intake be sufficient to maintain that equilibrium amount required by the body. Such an intake is, for persons living in temperate climates, of the order 2,000 c.c. per day, and includes not only the water of the various beverages drunk, but also the large amount in " solid " food, most of which contains over 50 per cent. of water.

It is merely a truism that no living process can continue without the presence of water, but it is insufficiently realised to what an extent many processes are favoured by particular dilutions. If we consider the digestive processes alone, it can easily be demonstrated experimentally that dilution of saliva with water accelerates the action of salivary amylase, the optimum dilution being six parts of water to one of saliva. Water acts as a stimulant to gastric secretion, and thus again accelerates digestion at this stage, and more rapid emptying of the stomach. Indirectly, through the increased gastric secretion, and resulting increasing acidity, it acts as a pancreatic stimulant. Thus there is much experimental evidence that water, drunk with meals, benefits digestion.

Hawk concludes from many experiments that " the drinking of a reasonable volume of water with meals will promote the secretion and activity of the digestive juices, and the digestion and absorption of the ingested food, and will retard the growth of

intestinal bacteria and lessen the extent of the putrefactive processes in the intestine " in the average normal individual.

Distilled water, taken with meals, produces the same effect as tap water. Ice water produces only a very transient lowering of stomach temperature, and hot beverages but a very transient rise.

The Metabolism of Salts

While this section will deal essentially with the metabolism of inorganic salts it is convenient to include certain metallic compounds which may or may not be organic.

The number of different elements that are found present in living tissues depends largely on the delicacy of the analytical tests applied in seeking them ; since we are now able to command extremely delicate tests, it is becoming increasingly difficult to determine which elements are essential to life, and which are merely present by the accident of their presence in the ingested food. The crucial test in each case is obviously the feeding of a diet which does not contain the element whose essentiality is being tested, but it is by no means easy to exclude completely all traces of many of these mineral elements. If the test can be applied, then if the element is essential and is withheld, some pathological condition will sooner or later (depending on the animal's store) result.

The difference between the elements found present, and those actually required, is well exemplified by what we know of plants. Plant ash has been found to contain (different plants at different times) sulphur, phosphorus, chlorine, bromine, iodine, fluorine, boron, silicon, potassium, sodium, lithium, rubidium, magnesium, calcium, strontium, barium, zinc, mercury, aluminium, thallium, titanium, tin, lead, arsenic, selenium, manganese, iron, cobalt, nickel, copper and silver. Of these it has till recently been claimed that the plant requires for normal growth only sulphur, phosphorus, potassium, calcium, magnesium and iron, and perhaps chlorine, in addition to the elements, carbon, nitrogen,

hydrogen and oxygen, though here also more rigorous experimental procedures are leading to an extension of this list; for example, it has recently been shown that traces of boron are essential. Apparently plant requirements are met by a water solution of salts in the following proportions: one part of potassium nitrate, one part of potassium dihydrogen phosphate, one part of magnesium sulphate, and four parts of calcium nitrate, with a trace of ferric phosphate. Actually these probably suffice only because they contain as impurities minute traces of a few other elements; the full list of these minor essentials has still to be determined.

The mineral constituents of the adult human body total between 4·3 and 4·4 per cent. of the total weight, and are, in order of decreasing amount, calcium, phosphorus, potassium, sulphur, chlorine, sodium, magnesium, iodine, fluorine, iron, bromine and aluminium, with traces of several others. The new-born child shows relatively lower total ash, calcium and phosphorus, and higher iron. Five-sixths of the total ash is derived from bone, which contains 99 per cent. of the body calcium, 70 per cent. of the magnesium, and 75 per cent. of the phosphorus. The animal has therefore very definite mineral requirements, though the amounts that the diet must provide of these are not yet definitely ascertained. The results of deficiency of such elements are shown in various ways. Thus a salt-poor diet leads to faulty nutrition, unpleasant nervous phenomena such as sweating, lack of appetite, listlessness, disturbed sleep, and, if continued, acetonuria, with a fatal termination. Under such conditions, within a few days calcium and magnesium cease to be excreted in the urine, and the daily chlorine excretion falls to 0·2 gm.

Not only is a sufficiency of the necessary elements required, but in certain cases the diet must contain a definite balance of them. The essential elements will now be considered in turn; some remarks will be added on certain non-essential elements.

Calcium. Although calcium is the mineral element present in largest amount in the body the circulating blood only contains between 10 and 11 milligrams per 100 c.c. of plasma. The red cells contain none, or, at most, a trace of the order of 1 milligram. The plasma of the young animal in the first few months of life contains slightly more, and that of woman in the final stage of pregnancy slightly less. Blood calcium bears a very constant relationship to blood inorganic phosphate ; the latter is also high during the period of most rapid growth and most rapid deposition of bone, and the constancy of bone composition is believed to be traceable to the constant proportions of these blood constituents.

Calcium of the blood plasma exists in part in organic combination (4 to 5 milligrams), and in part as calcium ions and inorganic salts (largely bicarbonate and hydrogen phosphate). Only the non-organic calcium is present in the cerebrospinal fluid. The height of blood calcium is controlled by two factors, the secretion of the parathyroid glands, and vitamin D. It seems possible that the parathyroid secretion controls the organic calcium, and that this holds the other calcium combinations in an interlocked balance, provided that the diet constantly affords a sufficient supply. The chief articles of diet containing calcium are in decreasing order : cheese (0·8 per cent.), almonds, beans, egg-yolk, whole milk (0·12 per cent.), cauliflower, olives, oatmeal, celery and spinach (0·07 per cent.). The chief dietary source for the young animal is milk, which contains its calcium partly in inorganic and partly in organic combination (calcium caseinate).

The lactating woman and cow obviously require a slightly greater amount of calcium provision ; the same is true of the pregnant animal. Pregnant guinea-pigs contain less calcium in the maternal organism than non-pregnant females of corresponding weight ; the depletion increases as gestation proceeds. The drainage of calcium from the body during lactation is large ; it has been shown that a cow in 133 days

lost 20 per cent. of its body calcium. The loss is from bone, which must be regarded not only as a supporting structure, but as a reservoir of calcium and phosphorus. Bone minerals are in a state of flux, easily laid down, and easily removed into solution.

Excluding bone the other tissues contain calcium of the same order of amount as that in blood. It is excreted through the kidneys, to a slight extent through the bile, and in greatest amount through the intestinal mucosa.

The daily requirement of calcium is between 0·4 and 0·8 gm. for the human adult.

Potassium. Most tissues contain more potassium than sodium ; the ratio of the two in blood is about 1·5 to 1, most of the potassium being present in the red cells. There is a similar preponderance of potassium in milk. Vegetables provide most of the potassium of the diet.

Sodium. Sodium is present in a meat diet to an extent comparable with potassium. In plants, while it is as widely distributed as potassium, the relative amount is much smaller, the ratio varying from 1 : 2 to 1 : 700, and averaging less than 1 : 100. Hence sodium is present in relatively small amount in a vegetable diet. In carnivorous animals it largely replaces potassium in blood corpuscles, and evidently plays a more important *rôle.* Such animals obtain a sufficiently balanced sodium-potassium mixture in their meat diet. A vegetarian requires much more sodium to maintain a correct sodium-potassium balance. Man, partly vegetarian, and herbivorous animals require to supplement their food with sodium chloride. Herbivorous animals in a tropical climate will travel long distances to " salt-licks " to obtain their necessary supply of sodium.

The actual amount of sodium chloride required by adult man on a mixed diet is probably about 5 gm. per day, much less than the 10 or 20 gm. that he, through custom, usually consumes.

Magnesium occurs in small amounts in all animal and plant

cells. In vertebrates the body's chief store is in bone. Bone contains but one-eighth as much magnesium as calcium ; muscle and nerve tissues, on the other hand, contain twice as much. The blood content is 4 milligrams per 100 c.c. Magnesium deficiencies do not occur on the average diet. Human milk contains very little, indicating that but little is required, even by the growing organism.

Iron is chiefly required for the building up of hæmoglobin, though other tissue compounds are known which contain it. Since the body conserves its iron so well the daily requirement is very small. Only 8 or 9 milligrams are excreted daily (1 to 5 in the urine) and only this loss has to be made good. The iron content of vegetables ranges from 0·0192 (parsley) down to 0·00015 per cent. (lemon juice). Different samples of the same vegetable show great variations. The general (decreasing) order of content of various classes of foodstuffs is : dried legumes, green leafy vegetables, dried fruits, nuts, cereals, poultry, green legumes, roots and tubers from leafy vegetables, fish, fruits. Vegetables containing little chlorophyll have a low iron content. Salt-water fish contain more than fresh-water fish. Most of the iron in the diet, as, for example, in egg-yolk and spinach, is in organic combination. But inorganic iron can be absorbed and utilised, and it is still uncertain to what extent such organic sources of iron are decomposed before absorption.

The daily requirement is probably not greater than 12 milligrams, with perhaps a slightly greater requirement during pregnancy and lactation.

Copper is an essential constituent of crustaceans, whose oxygen-carrier, hæmocyanin, is a copper, instead of an iron, compound. Oxidised hæmocyanin is blue, the reduced compound is colourless, and we may assume that copper is combined in hæmocyanin somewhat similarly to iron in hæmatin. In animal and plant tissues minute traces are widely distributed. In plants the presence of the element seems more than accidental ; it would appear to function in early growth. It is still uncertain as to whether the presence of copper is of significance in animals other than

crustaceans. A recent suggestion would require a trace of copper as essential for the formation of hæmoglobin.

Manganese is widely and constantly present in animal and plant tissues, and appears to function as a co-enzyme. Human liver has been reported to contain from 0·1 to 0·16 per cent. Most human tissues contain much smaller amounts; blood contains from 10 to 20 milligrams per 100 c.c.

Much more minute traces of *nickel*, *cobalt*, *aluminium*, and *zinc* are also found widely distributed; their significance still remains to be ascertained.

Normal human fæces contain per day about 10 milligrams of zinc, urine one-tenth this amount.

Traces of *lithium* and *arsenic* are normally present in tissues, but their presence is accidental and functionless.

Phosphorus occurs in the phosphate radical in organic and in inorganic combination, as inorganic phosphates, and in nucleoproteins, phosphoproteins, phospholipides, and hexose-phosphates. Lack of phosphate in the diet chiefly affects the inorganic phosphate. Brain- and heart-phosphorus do not decrease even in complete phosphorus starvation. Phosphorus equilibrium has been established on an intake of phosphate equivalent to 2·25 gm. of phosphorus pentoxide per day, and phosphate equivalent to from 3 to 5 gm. is considered sufficient for adult man. Phosphorus is excreted (as phosphate) in urine and fæces; the amount excreted *viâ* the intestine is largely increased on a vegetable diet, and is probably conditioned by calcium excretion. The fæces phosphate is largely that of calcium. An ordinary normal diet contains sufficient phosphate for bodily requirements; inorganic phosphate can be derived by digestion from any of the complex molecules containing phosphorus.

Chlorine is provided by the ingestion of sodium chloride. It is present in all tissues, apparently solely in inorganic combination as chloride. It functions in part as an ionic medium for establishment and maintenance of correct osmotic pressures for the chemical changes of animal metabolism, and in part as a convenient mechanism whereby (through secretion

of hydrogen chloride) a sufficiently high concentration of hydrogen ions can be attained in the gastric secretion.

Some recent work suggests that perhaps chlorine plays some *rôle* in forming organic derivatives during detoxication of complex nitrogenous poisons produced in certain pathological conditions.

There is no permanent storage of chloride in the body above the minimum required for metabolic processes. Increased ingestion of sodium chloride results in slight retention for a few days, and finally equilibrium between intake and output at the higher level. Such slight retention is accompanied by such a retention of water as will maintain the normal osmotic pressure (so that the result is equivalent to the retention of the corresponding amount of " normal saline "). On a salt-free diet and during fasting chloride elimination falls to about 0·2 gm. (as sodium chloride). The body, through fasting, does not normally lose more than 10 to 14 per cent. of its chloride content. Continuous removal of hydrochloric acid from the stomach by tube or fistula leads to symptoms of chloride hunger and malnutrition.

When food is ingested there is an immediate short increase in chloride excretion, due to absorption of sodium chloride in the stomach ; this is followed by a decrease, due to secretion of hydrochloric acid into the stomach, and then a slow increase as chloride is absorbed in the intestine. Blood chloride remains very constant even under most pathological conditions. Slight changes occur during digestion, corresponding to those observed in urine. More marked changes are usually due to variations in corpuscular content, since, for equal volumes, the corpuscles contain only about half the amount of chloride present in plasma.

Sulphur is obtained in inorganic form as sulphate by oxidation of such organic compounds as cystine. It is therefore impossible to estimate the body requirements of inorganic sulphur, since the body can meet them from organic sources.

Inorganic sulphate in diet cannot meet the body need for cystine, which the mammal cannot manufacture for itself.

Iodine is present in blood to the extent of about two parts in ten millions. With the exception of the thyroid gland tissues contain only negligible traces. Dried thyroid tissue contains from 0·01 to 1·0 per cent. of iodine. Although only minute amounts are required in the diet, such minute amounts are absolutely essential, and are especially essential in the growing animal. Deficiency in the diet leads to enlargement of the thyroid and various other pathological and sub-pathological conditions.

Iodine is a constant constituent of marine plants and animals, dried tissues containing amounts up to 1 per cent. Land plants contain much less. Its concentration in a particular tissue seems to occur first in vertebrates. Sea-water contains it, but the purer the water the less its iodine content is the rule for fresh waters. In most countries sufficient iodine is obtained in food and water. But in parts of Switzerland, and Northern India, and in large tracts of the North American Continent, including the wide areas surrounding and to the west of the Great Lakes and practically extending to the Pacific, the water and the purified food of the diet do not supply sufficient iodine for human and animal requirement, and the deficiency must be met by addition of iodide to the diet in some form or other.

Iodide is rapidly excreted through the kidney. Organic iodine compounds are largely broken up during digestion and the iodine for the most part rapidly excreted as iodide.

Bromine is present in minute traces in most tissues, and in slightly greater amount in the thyroid. Blood is stated to contain normally from 1 to 1½ milligrams per 100 c.c. Its presence seems to be without particular significance. Bromide can, however, function about as well as chloride as co-enzyme for amylolytic digestion, and hydrobromic acid is equally efficient for gastric digestion. If bromide is fed in

large amount it seems to replace chloride to some extent in blood and tissues and hydrobromic acid may be found in the gastric juice.

Like iodine, bromine is probably present in tissues in organic combination. Corals contain their iodine largely as di-iodo-tyrosine, and di-brom-tyrosine has also been isolated from the hydrolysed tissues of such animals. In view of the fact that thyroxine is a derivative of di-iodo-tyrosine, it seems possible that the bromine of thyroid may be present in the two corresponding forms of combination.

Fluorine is an essential element, though the body requirements are very small. It is present in some complex inorganic combination in bone and teeth, and especially in the enamel of teeth. The ordinary diet contains a sufficient supply.

The Regulation of Neutrality

One of the functions of the inorganic constituents of the body is to assist in the maintenance of neutrality. The body is constantly producing carbon dioxide, which, in solution, is acid. Other acid oxidation products of carbon, such as lactic acid, and inorganic sulphate formed by oxidation of such compounds as cysteine, in fact, all acid compounds produced in the body, require neutralisation. Such neutralisation has to be brought about by the metallic ions available, chiefly sodium and potassium, and by ammonia.

Elimination of carbon dioxide from the lungs involves no loss of base from the body ; the neutralisation is temporary. Elimination of sulphate and organic acid through the kidneys results in permanent loss of base.

If the sum total of blood base and blood mineral acid be determined there is found to be present in blood a slight excess of base sufficient to neutralise and carry the carbon dioxide from tissues to lungs. In spite of loss of base through the kidneys, however, it is found that the daily requirement of mineral acid is somewhat greater than that of base, there

being an average excretion of base equivalent to 2,350 c.c. $N/10$ alkali, and of acid equivalent to 2,500 c.c. $N/10$ acid. The daily excess of acid requirement is therefore 150 c.c.

There is some evidence that if the diet does not conform to some such equilibrium body processes are not normally maintained. McCollum and Davis have shown that a ration containing considerable excess of acid-forming elements may support growth but is quite inadequate for reproduction.

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CHAPTER XXVIII

THE VITAMINS

UNTIL the early years of this twentieth century it was customary to consider a perfect diet as made up of a mixture, in fairly definite proportions, of proteins, fats, carbohydrates and water, although it was realised that sodium chloride appeared to be necessary and could be included under none of these groups. Experiments to maintain normal life on such a mixture, whose components had been as far as possible purified, failed. Such failures were accounted for by the unappetising quality of the mixtures.

Advances to a completer knowledge are due to the work of several groups of investigators.

In 1887 the Dutch government established a laboratory in Java, to study beri-beri, a disease common throughout the East. Eijkman was appointed its first director. He observed a condition in fowls analogous to beri-beri, but attributed it to food toxins. In 1905, Pekelharing expressed the opinion that there was still an unknown substance in milk, which, even in very small quantities, is of paramount importance in nourishment.

In 1906 Hopkins drew the conclusion that no animal can live upon a mixture of pure protein, fat, and carbohydrate, even when the necessary inorganic material is added. The tissues of plants and of other animals normally forming its food contain many other substances than proteins, fats and carbohydrates, and some of these are dietary essentials. Hopkins then wrote, " It is certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we

have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering.

"Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves and some of them depend upon unsuspected dietetic factors. I can do no more than hint at these matters, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown." This assertion in 1906 has been amply justified.

In 1912 Hopkins published results of many years' work on artificial pure food mixtures, containing casein, starch, sucrose, lard and inorganic salts. On crude food mixtures of these constituents rats can live and show a certain degree of growth, but when the constituents have been carefully purified growth always ceases when they have been fed for a comparatively short period, although the energy value of such foods is ample for growth. Addition of a small amount of milk (not more than 4 per cent. of the total food fed) or of protein-free and salt-free extracts of milk solids, or of yeast, to the purified diet permits normal growth. Hopkins was led immediately to the conception of specific constituents of diets, other than proteins, fats, carbohydrates and salts, which he termed the *accessory factors of the diet.*

Results of a similar kind were published by Osborne and Mendel in 1911 and 1912.

In 1907 Fraser and Stanton reported that if rice polishings were extracted with alcohol a product was obtained which, added to the diet of a beri-beri patient, appeared to produce curative effects. In 1911 Casimir Funk published his results, obtained by fractionating rice polishings, and testing the

fractions on birds in whom polyneuritis had been produced by feeding them polished rice. He obtained amongst the fractions a crystalline substance that appeared to be curative in high degree. This he named " vitamine," " life-amine," since it preserved life, and contained nitrogen, which nitrogen appeared to be present in basic form. In the same year the Japanese workers Susuki, Odake and Shimamura published similar results, and termed their product *oryzanin*.

At this period, therefore, there appeared evidence that one or more food-factors were concerned in growth, absence of rickets, of scurvy, and of beri-beri. A very great amount of accurate work has since that time demonstrated that there are at least six different accessory food-factors, with different properties, and whose absence from the diet produces definite deficiency diseases. They have all been termed *vitamins* (eliminating the final " e " from Funk's term for his preparation, since some are not amines).

These vitamins can evidently be compared with the two other classes of powerful chemical agents that have already been discussed, the enzymes and the internal secretions. Minute amounts of them produce powerful physiological effects ; absence of these minute amounts leads to profound pathological changes.

They are only gradually being isolated in chemically pure condition, and their chemical nature ascertained. Tests for them are still almost entirely biological—the effects they are known to produce in preventing the onset or curing certain deficiency diseases such as rickets, beri-beri, and scurvy, and in facilitating growth in young animals. As their separate existences were gradually established they were named alphabetically **A**, **B**, **C**, etc.

The vitamins can be broadly separated into two groups, one of which is soluble in water and the other is soluble in fats and fat-solvents. They may, therefore, be at present classified for convenience in some such fashion as the following :

I. *Fat-soluble Vitamins.* **A.** Deficiency of this vitamin leads to cessation of growth and the peculiar eye condition known as xerophthalmia.

D. This vitamin is concerned with normal growth and the correct calcification of bones and teeth. Deficiency leads to cessation of growth and rickets and to improper teeth formation.

E. This vitamin is concerned with the functioning of the reproductive organs. Deficiency leads to sterility in the male and death and absorption of the fœtus in the female.

II. *Water-soluble Vitamins.* The **B** vitamins. These include $\mathbf{B_1}$ (or **B**), the anti-beri-beri vitamin, $\mathbf{B_2}$ (or **G**), the anti-pellagric vitamin, and possibly others.

C. Deficiency of this vitamin leads to scurvy.

In addition, it is necessary to consider several substances, together termed *bios*, which promote the growth of the yeast cell. These do not appear to be factors of importance in animal metabolism.

None of these vitamins are affected by digestion. They will now be dealt with individually.

Since their discovery, and in some cases their isolation, have depended upon the results which follow a deficiency of some one or other of them, an *avitaminosis*, these results will in each case be first dealt with. It will however be convenient to prefix a brief statement of the present state of our knowledge concerning each of them.

The general and commonest method of testing for the presence of any vitamin is to feed young animals a diet deficient in that vitamin alone, and otherwise perfectly adequate, until the corresponding deficiency disease has developed to a perfectly recognisable degree. Then the material under test is fed. The minimum amount which it is found will restore such animals to normal condition gives some measure of the relative concentration of the vitamin present. When growth is retarded by vitamin-lack, growth

effects can also be utilised, but greater care is then necessary, since several vitamins are involved in growth changes.

The Fat-soluble Vitamins

Vitamin A. *This is a complex alcohol and a derivative of the plant pigment carotene. Animals transform the pigment to the vitamin. Herbivorous and to a less extent omnivorous animals are dependent on plants for an adequate supply of carotene. The most characteristic deficiency-disease associated with lack of the vitamin is xerophthalmia.* **A** *is growth-promoting.*

Deficiency of vitamin **A** has been shown to lead to an ophthalmia in man, the rat, the guinea-pig, dog, rabbit, swine and chickens. It does not seem to be an essential vitamin for the pigeon. The condition has been termed *xerophthalmia* (Gk. *xeros*, dry) and *keratomalacia* (Gk., *keros*, horn), and is typically a dry and thickened condition of the conjunctiva.

What is the nature of this ophthalmia? Perhaps the most likely series of events is that described by Mori in studies on the rat. He found that the first observable change is a tendency for the lachrymal glands to pass into a resting condition and to cease to produce tears, and believes that the subsequent changes in the eyes can be accounted for by this loss or cessation of function of the tear glands. When the supply of tears fails the conjunctival sac is no longer washed continuously, as under normal conditions, and bacteria begin to grow there in great numbers. This stimulates a migration of leucocytes which accumulate in the eye-chamber, causing an effusion of pus into the anterior chamber of the eye (hypopyon) visible as a yellowness of the pupil. Some of the leucocytes migrate through the outer coating of the eyeball and find their way into the conjunctival sac, and break up, accumulating as a sticky exudate which tends to paste the eyelids together. As this exudate dries the animals have difficulty in opening their eyelids.

Through the dryness of the eye there results hardening of the external coating, resembling the horny layers of the skin. During

the later stages of the disease, following death of the tissues, ulcers form on the cornea. These finally perforate and the lens pops out. There are a variety of changes in other tissues and ultimately the animal dies.

If animals whose eyes are in a damaged condition have fats administered which are rich in vitamin **A** content they may make a most spectacular recovery.

Xerophthalmia may assume epidemic proportions where communities are placed through any cause on a restricted diet. The young are especially liable to suffer. Mori has described 1,500 cases amongst Japanese children between two and five years of age during the three years 1905–07. He found at that time that cod-liver oil relieved the symptoms, and we now know that cod-liver oil is one of the richest sources of **A**.

While the ophthalmia is the most prominent symptom of the avitaminosis it is by no means the most important. The manifold effects which can ultimately lead to death are well displayed by the rat. In order there occur : roughness of coat, emaciation, loss of agility, inco-ordination of movement, lessened protrusion of the eyes. The clinical manifestations of xerophthalmia appear rather suddenly, with copious secretion, which may be serous, purulent, or blood-stained. The eyes almost shut ; the cornea begins to become opaque, the conjunctiva becomes inflamed. At this period the rat is commencing to lose weight. There is profuse discharge from the nose. Respiration is accompanied by râles. The lymph glands of the neck are enlarged, abscess formation often follows, and the abscesses may perforate. Haematuria is common. Occasionally death is preceded by diarrhoea.

Older rats tend to exhibit respiratory disease more frequently, and the ophthalmia less. After long deprivation of **A** calculi and calcareous deposits are found in the renal passages and the bladder. The salivary glands are infected. In females the vaginal smear shows cornified cells persistently (this is merely a transient stage in the oestrus of normal

animals). A partial inadequacy of the vitamin frequently prevents fertilisation or causes failure of implantation of ova.

The diseased conditions associated with this avitaminosis seem to have as an underlying common factor substitution of stratified keratinising epithelium for normal epithelium in numerous sites, such as the respiratory, alimentary, and genito-urinary tracts, the eyes, and the para-ocular glands. These changes may be followed by infection in the various organs and tissues concerned.

Among other conditions in man attributable to *avitaminosis* **A** are night-blindness and respiratory infection. Gastro-intestinal disturbances are less certainly a result. Infants affected with the ophthalmia exhibit a dry, scaly, shrivelled condition of the skin.

Lack of this vitamin thus provokes changes in many parts of the organism, changes which permit invasion by various infective agencies. Some evidence is available that diets rich in **A** tend to lessen susceptibility to infection. It has been termed the anti-infective vitamin, perhaps unwarrantably.

The relationship between carotene and vitamin **A**. Carotene is a widely distributed plant pigment, a hydrocarbon, $C_{40}H_{56}$. Within the past few years proof has been furnished that it is the material from which the animal organism prepares the vitamin. It is doubtful if the vitamin is normally produced in plants at all.

There are four types of experimental investigation which can be applied to check the theory of the relationship between the vitamin and carotene, a specific colour test, the nature of the absorption spectrum, the effect on growth, and the effect on xerophthalmia.

Animal material rich in vitamin **A** gives a brilliant blue colour with a 30 per cent. solution of antimony trichloride in chloroform (a similar colour is developed with arsenic trichloride). There is a marked parallelism between the

intensity of this reaction and the known distribution of the vitamin, as ascertained by biological tests. The test is not quite specific, since von Euler has shown that carotene and many related carotinoid pigments will produce it. It cannot therefore be used in investigations as to the nature of the vitamin, but seems to possess utility in testing plant and animal material for relative richness of vitamin content. For example, as judged by this colour test, Rosenheim and Webster have shown that of liver oils that of the cod is far from being the richest source of **A**.

The effects on growth are studied by placing a group of young animals such as the rat on an absolutely controlled diet, containing everything requisite for growth and normal development except the vitamin that is being studied. Control animals are given this vitamin in addition, and the rates of growth of the two groups compared. It has been shown by von Euler that in such tests addition of purified carotene in amounts of from 0·01 to 0·03 mg. per day per rat will produce an increase in growth exactly parallel to that produced by a sufficiency of vitamin **A**. This result has been amply confirmed by other investigators. Further, it has been found that the purer the carotene, the smaller is the effective dose required (Hume and Smedley-Maclean). Moore (1930) has found that the minimum effective dose for the rat is 0·004 mg. per day. The carotene is effective, whether fed or injected intramuscularly (Rydbom, 1930). These results suggest either that the vitamin is carotene, or that it is formed from carotene, or that carotene can replace it. It is significant that the oxidised derivative xanthophyll is ineffective (Rydbom). Moore has shown that carotene cures xerophthalmia.

Vitamin **A** cannot be identical with carotene, since highly active concentrates of cod-liver oil are barely coloured, and as Moore points out, are readily soluble in all lipide solvents and in natural fats, while carotene of similar physiological potency is markedly coloured and its solubility is much more

limited. Drummond and his co-workers have shown that the vitamin is characterised by an absorption band at about 3,280 Å.U., while carotene shows no absorption in this region, and also that the blue compound developed with antimony chloride gives, for the vitamin, a band at about 6,080 to 6,120 Å.U., the corresponding band for carotene being at 5,900 Å.U.

Moore has shown that the liver oil of rats suffering from deficiency of A invariably gives negative results when tested by the colour test; this excludes both carotene and the vitamin. When these rats have been cured by administration of excessive doses of carotene, the liver oil is only slightly coloured, indicating no great storage of carotene, but shows development of the absorption band at 3,280 Å.U. characteristic of the vitamin, and give a marked reaction with antimony trichloride with a band at 6,100 to 6,300 Å.U., also characteristic of the vitamin.

All these results, along with the chemical evidence of transformation to be set forth below, indicate with reasonable certainty that carotene is the parent substance of the vitamin.

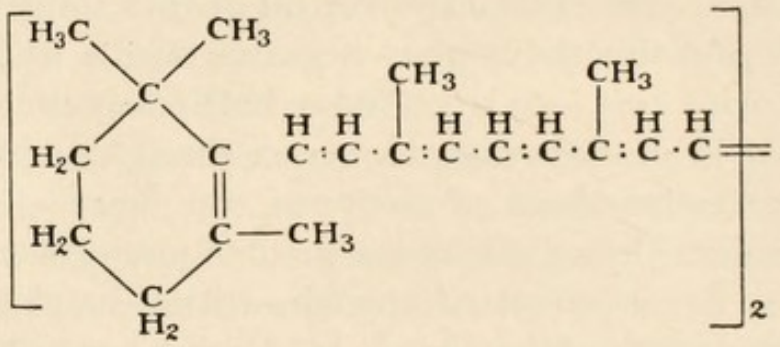
Olcott and McCann state that carotene can be converted into the vitamin by incubation with fresh liver tissue, or with an aqueous extract of liver. Since boiled extracts are ineffective, they consider that the action is brought about by a specific enzyme, which they term carotenase.

Carotene and Related Pigments. The group of plant pigments termed carotinoids, which all possess a "polyene" structure, have been divided into (i.) polyene hydrocarbons, (ii.) polyene alcohols, and (iii.) polyene carboxylic acids.

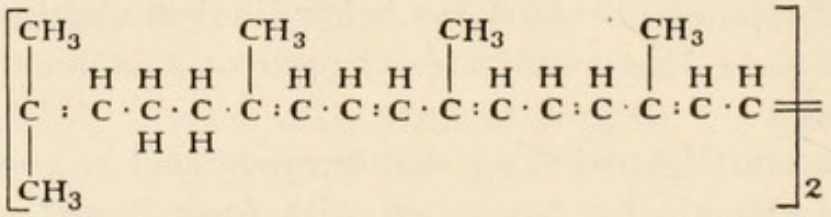
The most important hydrocarbons of this group are the carotenes. They are widely distributed in the green parts and roots of plants, and, from ingestion of plants, in animals. The coloured compound of the corpus luteum is probably carotene. They are easily extractable from plant material by such solvents as carbon disulphide and petroleum ether. In dilute solutions the red colour is reminiscent of that of carrots, and almost completely

matches that of a solution of potassium bichromate. The pure crystals are so intensely coloured as to appear black.

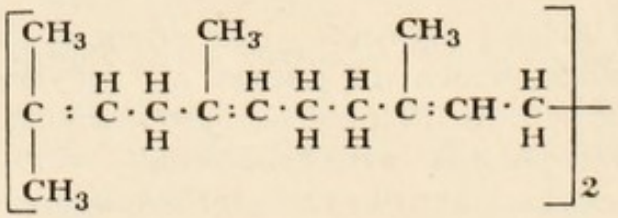
Such crystalline carotene, prepared from carrots, has been shown to be a mixture of two isomers, optically inactive β-carotene with a melting-point of 182° C., and dextro-rotatory α-carotene, with melting-point 172° C. The precise constitution of α-carotene has not yet been decided. Karrer and his associates attribute to β-carotene the formula :—



Lycopin, $C_{40}H_{56}$, is the characteristic pigment of tomatoes, red pepper, and a number of tropical fruits. To this Karrer ascribes the constitution :—



Closely allied to these plant pigments is the squalene, $C_{30}H_{50}$, which constitutes a large part of the unsaponifiable fraction of shark liver oil. According to Karrer, its formula is :—



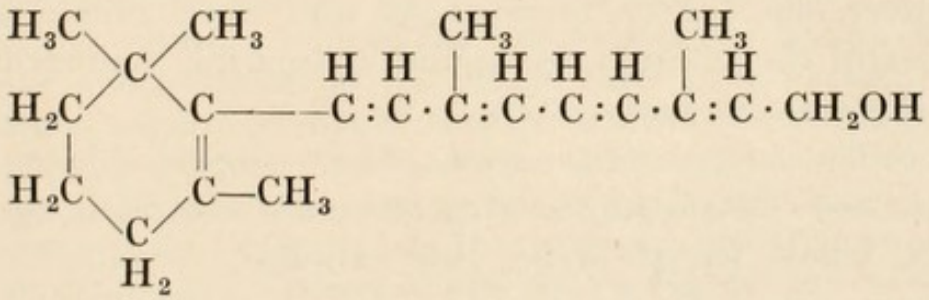
Xanthophyll, $C_{40}H_{56}O_2$, is a typical alcohol of the series, with two hydroxyl groups. It is always associated with carotene in the green part of plants. Various other alcohols of this series, showing greater degrees of oxidation, are violaxanthin, $C_{40}H_{56}O_4$, first isolated from pansies, and present in the rind of oranges and

mandarins, taraxanthin, also $C_{40}H_{56}O_4$, present in dandelion flowers, and fucoxanthin, $C_{40}H_{56}O_6$, the pigment of brown algae.

The carboxylic acid compounds include α-crocetin, $C_{20}H_{22}O_4$, which possesses two (COOH) groups, and is one of the pigments in saffron.

The Chemical Nature and Properties of Vitamin **A.** Two groups of investigators have obtained an almost pure preparation of **A.** Karrer and his colleagues have isolated and purified the vitamin from the unsaponifiable fraction of the liver oil of the mackerel and also from that of the halibut. Their preparation is an alcohol with a molecular weight of 300 to 320, which analyses either to $C_{20}H_{29}OH$ or $C_{22}H_{31}OH$. Drummond, Heilbron and Morton fractionally distilled the unsaponifiable residue of halibut liver oil. The vitamin fraction consisted of a heavy viscid oil, slightly yellow in colour, an alcohol considered to be over 90 per cent. pure, and to have the formula $C_{20}H_{29}OH$. Its potency as a vitamin was relatively of the same order as that of pure calciferol (see next section).

It would appear from the study of this oil that the vitamin probably has the constitution :



and that the reaction whereby it is formed from carotene can be written :

$$(Carotene) + 2H_2O = 2(\mathbf{A})$$

The almost pure oil is easily oxidised by ozonised oxygen, the vitamin activity being destroyed. In its slight natural concentration in animal material it is somewhat more resistant to oxidation, although during such processes as pasteurisation of milk some loss probably occurs.

Its solubility properties resemble those of lipides. It is but little soluble in water. Adipose and certain glandular tissues are especially capable of storing it, variations in such storage being almost certainly due to variations in amounts of other lipide material capable of dissolving it. Hence fish livers, and the oils extracted from them, are amongst the richest natural sources.

The Vitamin **A** *Standard.* The League of Nations Health Organisation have selected carotene as a provisional international standard of reference for determination of the strength of preparations of **A**. The international unit is the potency in **A** of 0·001 mg. of a pure sample of carotene, of which 0·003 to 0·005 mg. should be sufficient to restore growth or cure xerophthalmia in rats in which a condition of **A** avitaminosis has been induced.

Distribution of Vitamin **A**. The old tables of distribution are no longer reliable, as far as the vitamin itself is concerned, except for animal material. For plant material they merely indicate its relative richness in carotene. Some approximate statements can be made, such as the following :—

Excellent sources of the *vitamin* are cod liver oil and other fish liver oils, butter, cream, eggs and cow's milk. Good sources of the vitamin are animal margarine, human milk, beef liver, cheese and oysters.

Excellent sources of *carotene* are raw carrots, spinach and watercress. Good sources of carotene are asparagus, (green) beans, whole wheat bread, raw cabbage, cooked carrots, yellow corn, lettuce, orange juice, peaches, peas and tomatoes.

The Function of Vitamin **A**. Concerning this, at present nothing definite can be stated, in spite of the widespread changes which ensue when there is a deficiency. **A** appears to be either a necessary integral part of certain epithelial cells, or more probably to be essential to their correct function or the correct nature of their proteins.

Some insight has been gained as to the function of **D** from

study of the effects following overdosage. Heavy dosage with **A** has not yet been shown to produce any untoward effects. Under normal conditions the organism probably never produces undue amounts of it, however much carotene is available.

Vitamin D, or Calciferol. *This is a steric isomer of ergosterol, $C_{27}H_{41}OH$, formed from it by the action of ultra-violet light. It can be produced in plants, in food material, and also in animals, whenever ergosterol is present and is subjected to ultra-violet rays. Deficiency of the vitamin is the chief cause of rickets. The vitamin is growth-promoting. Large over-dosage of* **D** *is toxic to most species of animals. Cod liver oil, and many other fish liver oils, are excellent sources of* **D**.

The deficiency disease most commonly associated with **D**, *rickets*, is fairly frequently seen in children and young animals. The entire body is affected, although the most noticeable signs are seen in the bones.

At the beginning of the disease children are usually constipated, restless and irritable, apathetic and disinclined to play. They sleep poorly. Frequently a rachitic child rolls its head about on the pillow until the hair is worn off from the back. The muscles are lax and the tendons and ligaments may become elongated. From this result, and from the softening of the bones, children do not walk or stand at the proper time. The muscles of the intestines are also weakened, and the muscles of the abdomen ; a pot-belly develops. As the disease advances bone deformities appear ; the rachitic rosary or line of knobs on the side of the chest where the rib bones join the cartilages, and later on pigeon-breast deformities. Bosses of new bone are developed on the side and front of the skull, and the head acquires a square shape. The ends of the long bones of the extremities become enlarged, and the legs become knock-kneed or bowed. The bones of the arm bend and there is marked enlargement of the epiphyses at the wrists and ankles. Rickets is seldom fatal, but often the child dies from some complication, especially broncho-pneumonia.

Rickets can be produced in the young growing animal either by deficiency of calcium or of phosphate or of vitamin **D** in the diet. In the presence of ample **D** much smaller

amounts of calcium and of phosphate are sufficient to prevent rickets than when there is only a small amount of the vitamin present. It conserves these mineral constituents to the body.

In rickets there is either low serum calcium, with somewhat low inorganic phosphate, *or* normal calcium with low inorganic phosphate. In the former combination the rickets may be accompanied by tetany. In rickets also there tends to be a loss of calcium and phosphorus from the body, while in the growing child bone requirements especially demand a marked calcium and phosphorus retention.

Rickets can be cured by administration of cod-liver oil and by treatment with ultra-violet light of wave-length between 2,000 and 3,000 Å.U. from any powerful source, such as the quartz mercury lamp or even the open carbon arc lamp. Such treatment brings back the deficient calcium and phosphorus balance to a positive one, while the mineral content of the bones increases. Direct sunlight contains sufficient of these ultra-violet rays to prevent onset of rickets, and plant material, such as clover hay, made by exposure to sunlight, contains vitamin **D**, though when the hay is dried in the dark it shows none of the activity of the vitamin.

Dental caries is, like rickets, due to deficiency of **D**, and since bone demand apparently takes priority over that of teeth, the development of caries may precede bone changes. There is some evidence that pathological results following a deficiency of **D** are accentuated by a large proportion of cereal in a diet.

Chemical Nature and Properties of Vitamin **D**. The earlier work on the fractionation of cod liver oil, and the discovery that the vitamin could be produced in sufficient amount to heal rickets by direct ultra-violet radiation of the patient, or of his food, led finally to the association of the vitamin with ergosterol.

Ergosterol, $C_{27}H_{41}OH$, is a crystalline compound with a similar structural formula to cholesterol (*cf.* p. 93), but with

two additional double bonds. Cholesterol preparations from natural sources nearly all contain a small trace of ergosterol, and the two are only separated with difficulty. Both are precipitated with digitonin.

When ultra-violet light acts on ergosterol a yellowish resin is produced. This " irradiated ergosterol " is a mixture of **D** and other non-active products. Continued irradiation destroys the vitamin. Various very active preparations have been made by such procedures—such as viosterol, radiostol, vigantol, etc. These permit more powerful anti-rachitic treatment than does cod liver oil, since a correspondingly powerful dose of the latter would not be tolerated by children.

A large number of other sterols and sterol derivatives have been treated with ultra-violet light, and in no case has anything with the properties of vitamin **D** been produced ; ergosterol is the sole and specific precursor of the vitamin. It therefore follows that it must be present in practically all fats of animal and vegetable origin, since they all will give rise to **D** on irradiation.

The chief difficulty in preparing pure **D** arose from the fact that the same treatment with ultra-violet light which produces it also destroys it. It was therefore necessary to determine the type and length of treatment which produced the greatest proportion of it in the resinous mass, and then to isolate it from the mixture.

In the last two or three years it has been obtained in pure form by several groups of workers. Webster and his co-workers obtained it by distilling the irradiated ergosterol at about 145° C. in a very high vacuum. They re-crystallised the distillate from aqueous alcohol. Windaus treated an ethereal solution of irradiated ergosterol with maleic anhydride and potassium hydroxide. The portion which remained uncombined yielded crystalline needles of the vitamin on evaporation of the solvent. Reerink and Van Wyk, and Jendrassik have also obtained very pure preparations.

Such work has ultimately led to the preparation of pure **D** on a relatively large scale. On account of its influence on calcium metabolism the name *calciferol* has been given to it.

Calciferol melts at 114·5° C. and is dextro-rotatory. Like its parent substance it has the composition $C_{27}H_{41}OH$, with three unsaturated linkages, so that it must be regarded as a steric isomer of ergosterol. It is not precipitated by digitonin. The minimum daily dose adequate to prevent rickets in rats is of the order 0·00003 mg. The vitamin is lipide-soluble, and is moderately resistant to heat.

"Line Test" for Calciferol. A delicate test is now generally employed to measure the amount of **D**. Rats are rendered rachitic by special calciferol-free diets, and then treated with the material under test for a specified time. They are then killed. A longitudinally-split tibia is treated with solution of silver nitrate and exposed to strong sunlight. This causes deposition of silver at points of calcium-salt deposition. The extent of the "line" so produced indicates the extent of healing and therefore the potency of the preparation.

Standard for Calciferol. The international standard (as set by the League of Nations Health Organisation) is a solution of irradiated ergosterol prepared under defined conditions from a solution containing 1 mg. of ergosterol in 10 c.c. of olive oil. The unit is 1 mg. of this solution. The activity is such that 1 mg. given daily to rachitic rats for eight successive days produces a wide calcium line in the line test.

Some idea of the varying strengths of different materials and preparations, as expressed in such units, is given by the approximate values in the following table:

Fresh cow's milk contains	0 to 0·2	units per gram.
Butter contains.	0 to 1·0	,, ,,
Cod liver oil contains.	60 to 150	,, ,,
Viosterol (250 **D**) contains	15,000	,, ,,
Pure calciferol contains	40,000,000	,, ,,

The Effect of Overdosage of **D**. The production of highly

active preparations of vitamin **D** has led to ample proof of its toxicity in very large doses. This merely illustrates the truism that the difference between the poisonous and healing action of any chemical compound is merely quantitative. If a drug is not pharmacologically inert then a sufficiently large dose should be toxic.

Pfannenstiel, and Kreitmaar and Moll, and subsequently others, have shown conclusively that continued excessive doses of highly active preparations of **D** prove fatal to mice, rats, guinea-pigs, rabbits, cats, and dogs. Death is preceded by loss of appetite, loss of weight, rough fur, and sometimes marked diarrhœa. Rachitic animals are somewhat more resistant. The susceptibility to overdosage varies in different species. Man displays greater resistance, although some fatal cases in children have been reported, birds do not appear to be affected.

Animals killed by this treatment show rich chalk deposits in predisposed sites, especially in the vessel walls, which are often changed to stiff tubes. The effect can be produced in one or two weeks.

Hess and Lewis have reported a hypercalcæmia in one or two rachitic children, treated with moderately large doses. They considered that the effect was produced by stimulation of the parathyroids by the vitamin. It has been proved that markedly excessive doses raise the blood calcium of both normal and parathyroidectomised animals. The suggested connection between the vitamin and the parathyroids cannot yet be considered as proved.

Moderate overdosage of the vitamin is without harmful effect.

Function of Calciferol. Study of the overdosage effects on calcium metabolism suggests that the vitamin normally facilitates the absorption of calcium from the alimentary canal, and, perhaps indirectly, bone mineralisation. Marked overdosage may lead to depletion of the mineral salts from bone. A precise explanation of these changes is not yet forthcoming.

Sources of Calciferol. As with vitamin **A**, sources must be differentiated into those in which the calciferol is actually present, and sources relatively rich in ergosterol, which become rich sources of the vitamin *after* irradiation with ultra-violet light.

Excellent sources of *calciferol* are the liver oils of the halibut, turbot, cod, and pilchard, and the body oils of sardines, herring, and tuna. Egg-yolk is a good source.

Excellent sources of calciferol *after irradiation* are yeast (especially), and egg-yolk. Good sources are dried milk, oils and fats, cereal products, and butter.

Milk from cows which have been fed on irradiated yeast is a good source of the vitamin. Animal margarines are moderately good sources.

Vegetable fats and green vegetables are usually lacking in the vitamin. Vegetable margarines are not good sources.

Note on Cod-liver Oil. According to Drummond, cod-liver oil consists of 99 per cent. of true fats and 1 per cent. of unsaponifiable compounds. This 1 per cent. contains the vitamins, though these make up but a trace of it. One-half of it is cholesterol, and the remainder chiefly spinacene and batyl alcohol.

Vitamin E. *This, the anti-sterility vitamin, has lipide properties. Deficiency of* **E** *leads to sterility in male and female rats and probably in other mammals.*

It is probably of the nature of an oil, and most probably contains no nitrogen. It withstands heat (to 250° C. when dry), oxidation and can be distilled at 180° C. at 50 mm. mercury pressure. Our present knowledge of its properties is largely due to Evans. His experiments have been chiefly with the rat, but the results can probably be extended to other animals, including man.

Deficiency of the vitamin in the diet of the male leads to sterility through destruction of the germ cells, and eventually of the entire seminiferous epithelium. In the female sterility also results, but in an entirely different fashion.

Successful mating can occur, and the preliminary stages of gestation may be normal, but after the eighth day in the rat a series of pathological changes occur in the placenta, as a result of which death of the fœtus occurs on the twelfth or thirteenth day, and it is eventually resorbed. If such a female be thereafter placed on a normal diet containing plenty of this " reproduction " vitamin, fresh mating with normal males will lead to the normal sequelæ, and birth of healthy young.

The investigations of Evans and Bishop, and of Sure, have demonstrated that the vitamin, which is insoluble in water, can be extracted by ether from yellow corn, wheat embryo and hemp seed, cottonseed oil and commercial olive oil. It is present in sufficient amount in lettuce, meat, whole wheat, rolled oats, dry alfalfa, and milk fat (if large quantities of these foodstuffs are fed), to relieve the condition if treatment be commenced in the early stages, but it is not markedly present in whole milk, cod-liver oil, orange juice and yeast, potent sources respectively for **A**, **A** and **D**, **C**, and **B**. The ether extracts of wheat embryo and of desiccated lettuce leaves yield the greatest concentrations yet obtained and have been partly fractionated. The body stores no great amount of it. Such amounts as are stored are chiefly in the body-fat and musculature.

Kudrjaschov (1930) finds that when male rats are kept on a diet freed from vitamin E from the 25th day of age, their testicular degeneration commences in 13 or 14 weeks ; in about 20 weeks there is inhibition of development of the secondary sex characters (suggesting diminishing output of the internal secretion of the testes) and in 30 weeks atrophy of the seminal vesicles and prostate is marked. This atrophy and the effect on the secondary sex characters are identical with the changes following surgical castration in rats of like age.

Evans and Burr (1928) claim to have established another peculiar result of deficiency of this vitamin. Provided the diet of the female rat during lactation contains a plentiful supply of vitamin **B** the young grow normally. But if the mother's diet is

deficient in **E**, then at the end of the third week of life a large proportion of the young exhibit a paralysis affecting the musculature of the body wall and of the posterior extremities. The onset is sudden, and is characterised by a spastic condition of the lower limbs and flex or spasm of the toes. The condition progresses in severity for several days, about one-third of the animals die, while in addition one-half continue to exhibit some degree of paralysis throughout life, since if the disease becomes well established it cannot be cured.

Injection of **E** into infantile female rats is said to induce hypertrophy of the uterus. Spontaneous sterility in cows has been cured, following its injection. Certain compounds such as iron salts appear to antagonise the vitamin. Fats and oils which act as autoxidants accentuate, and anti-oxidants depress its action.

The Water-soluble Vitamins

The Nomenclature of the B Vitamins. The present state of our knowledge of the **B** vitamins is confused, since recent discoveries have upset our earlier ideas. The properties attributed to the original vitamin **B** now have to be distributed between at least two distinct vitamins. The distribution in plant and animal tissues and in foods generally, carefully studied for the original "**B**," when it was supposedly one entity, applies uncertainly to its different components.

The nomenclature is similarly confusing. $\mathbf{B_1}$ and $\mathbf{B_2}$ (and $\mathbf{B_3}$, $\mathbf{B_4}$ and $\mathbf{B_5}$) have been suggested. Numerous writers prefer to use **B** in place of $\mathbf{B_1}$, and **G** in place of $\mathbf{B_2}$.

Vitamin $\mathbf{B_1}$ (or B). *This, the "anti-neuritic vitamin," is a relatively simple compound, which is stated to contain sulphur. Deficiency leads to beri-beri in man, and a polyneuritis in rats and pigeons. It is widely distributed. Yeast and rice-polishings are excellent sources. Green plants are good sources. "Purification" of plant material removes the vitamin. "Polished rice," white wheat flour, degerminated corn meal, and corn grits, are devoid of it. It is moderately resistant to heat and oxidation.*

Deficiency Diseases Associated with $\mathbf{B_1}$ **(B).** The disease beri-beri, especially prevalent in Eastern countries, is attributable to a deficiency of this vitamin, although whether this is the sole cause or some other additional factor is necessary has not been finally settled. Beri-beri is a multiple peripheral neuritis. The initial symptoms are fatigue and depression. There are varying degrees of cardiac disturbance, oedema, and gastro-intestinal derangements.

When pigeons are fed a diet deficient in the vitamin, a corresponding condition, " avian polyneuritis," develops.

The most striking feature of avian polyneuritis is loss of the co-ordinating powers of the muscles. The onset of the disease is generally preceded or initiated by a period during which the bird sits with ruffled feathers and the appearance of illness. The illness is progressive, and at a later stage, when the pigeons are disturbed, there is a tendency in many to be taken with convulsive seizures, during which they turn cart-wheels backwards at intervals. In the acute stage many birds sit with the head greatly retracted.

Complete deprivation of $\mathbf{B_1}$ results, in rats, in early death without preceding convulsions. A *hypovitaminosis*, a chronic prolonged slight deficiency, leads to a characteristic polyneuritis.

Though such symptoms suggest that the pathological lesions are principally situated in the nervous system, McCarrison has presented evidence that other tissues may be even more profoundly affected, and has observed functional and degenerative changes in thymus, testes, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid and brain, the severity of these changes being in the order named. It seems possible that in some of McCarrison's experiments the results are to be attributed to lack of more than one vitamin, but in any case they illustrate the profound changes which may follow deficiency of two or three of these compounds, and lend support to McCarrison's contention that a diet deficient slightly in vitamins, and especially in $\mathbf{B_1}$, may lead to various digestive disorders not generally characterised as due to deficient diet. Such effects must be considered as indirect results, for Drummond and Marrian state that, at least for the rat, the nutritive failure following a deficiency of vitamin $\mathbf{B_1}$ is virtually identical with that resulting from starvation.

One curious result following a deficiency of **B** vitamins is hypertrophy of the adrenal glands; the hypertrophied glands contain more adrenine. No explanation has yet been found for this change.

The vitamin stimulates appetite, so that its deficiency is complicated by the results of inanition. Many of the symptoms of hypovitaminosis of $\mathbf{B_1}$ are probably due to such inanition. Growth is affected.

Such hypovitaminosis affects carbohydrate metabolism, depressing the degree of reconversion of lactic acid to glycogen, so that lactic acid accumulates in tissues (including the brain) and in the blood to reach abnormally high levels. It causes a remarkable drop in the heart-rate of the rat, which may be a consequence of such increased lactic acid content in the heart muscle.

The Chemical Nature and Properties of $\mathbf{B_1}$ (**B**). This is one of the most stable vitamins; it is reasonably resistant to heat and to oxidation. It is destroyed at 120° C., but the decomposition at 100° C. is insignificant. It is soluble in water, alcohol-water mixtures, and glacial acetic acid, but not appreciably soluble in most lipide solvents. Material that is rich in it reduces ferric ferricyanide to produce a Prussian blue colour. The reaction is not specific; it is given by a number of polyphenols.

On account of the solubility of the vitamin in water, when fresh food and especially when vegetables are boiled in water a considerable proportion of the vitamin may be lost to the food consumer. It easily passes through semi-permeable membranes, a property which indicates that it has a relatively small molecular size. It resists treatment with acids, but is easily decomposed by alkalies. Nitrous acid does not affect it.

Various concentrated extracts have been made, especially as the picrate, by Seidell and others. In 1926 Jansen and Donath prepared a crystalline product from rice polishings which they considered to be the pure vitamin and to have

the composition $C_6H_{10}N_2O$. A dose of 0·0003 mg. of the hydrochloride of this preparation was sufficient to prevent the development of polyneuritis in "rice-birds" fed on diets lacking the vitamin. Their work has not been completely confirmed. In 1932 Windaus announced the isolation of the pure vitamin in the form of the crystalline picrolonate. This was prepared from yeast. He found that its composition was $C_{12}H_{17}N_3OS$. It is far more potent in curing polyneuritis in pigeons than any previous preparation. If this work is substantiated the chemical nature of the vitamin should shortly be completely elucidated.

Standard for **B_1 (B).** For the international standard a special product has been selected prepared by adsorbing a sulphuric acid extract of rice polishings on Fuller's earth. One unit is defined as the antineuritic activity of 10 mg. of this product (the earth with the adsorbed vitamin). Ten to 20 mg. are needed to maintain normal growth in young rats, 20 to 30 mg. for a pigeon curative day-dose.

Distribution of **B_1 (B).** Plimmer's work, in which pigeons were used as assay material, suggests the following comparative values, taking dried yeast as arbitrary standard.

Dried yeast	100
Marmite, wheat-germ	60–70
Baker's yeast	33
Bran, buckwheat	20
Oatmeal	11
Other cereals	10

On dry weight basis

Orange juice, tomatoes, watercress	20
Cabbage, leeks, parsnips	10–13
Potatoes	7

Milk is poor in the vitamin. Certain commercial liver and stomach extracts are relatively rich.

Vitamin B_2 (or G). *This, the "anti-pellagric vitamin,"*

has somewhat similar distribution and properties to $\mathbf{B_1}$. *It appears to be identical with the yellow pigment flavine.*

Deficiency Diseases due to Lack of $\mathbf{B_2}$ (**G**). These include human pellagra, " black tongue " of dogs, and a pellagra-like disease in rats.

There is a peculiar disease known as *pellagra*, characterised by erythema involving usually the exposed portions of the body surface, characterised also by gastro-intestinal disturbances, and ultimately by nervous and mental symptoms and marked languor and muscle weakness. Victims of the disease become unable to carry on any occupation calling for any degree of muscular strength. It has long been recognised as a deficiency disease. Some very excellent work of a medical committee of the Egyptian Expeditionary Force working on Turkish prisoners of war led to conclusions which associated the occurrence of the condition with a deficient protein element in the diet. A recent publication by some of the workers in the United States Public Health Service indicated that the deficiency is not a deficiency of protein itself, but of a vitamin associated with certain of the proteins of our diet.

If pellagra is recognised early a plentiful diet containing plenty of meat will cure it within a few months. A daily allowance of 200 gm. of lean beef, or of 15 gm. of commercial dry yeast extract, is sufficient in the diet of adults to prevent pellagra.

Black tongue in dogs has many symptoms corresponding to those seen in pellagra. It can be produced experimentally by diets deficient in the vitamin, and is rapidly fatal. It can be cured by administration of preparations of the vitamin.

" Pellagra " in rats can be induced by a diet deficient in $\mathbf{B_2}$. Such treatment produces stunting of growth, diarrhoea, a soreness of the mouth, and a peculiar dermatitis.

The Chemical Nature and Properties of Vitamin $\mathbf{B_2}$ (**G**). Much of the present evidence concerning the chemistry of the vitamin is conflicting. Earlier attempts to separate it from $\mathbf{B_1}$ were based on supposedly greater heat resistance. Its resistance to autoclaving varies according to the source that is used.

Recent claims have been made that it can be obtained free from $\mathbf{B_1}$ by removing the heat-coagulable proteins

from egg white, and that aqueous extracts of liver concentrate when autoclaved at *p*H 9 for thirty minutes at 125° C. are good sources of $\mathbf{B_2}$ and contain no $\mathbf{B_1}$.

Its identity with flavine has been almost certainly proved. Flavine is a naturally occurring pigment present in milk (lactiflavine) and in egg-white (ovoflavine), and has the formula $C_{17}H_{20}N_4O_6$.

The Distribution of Vitamin $\mathbf{B_2}$ (**G**). Commercial liver and stomach extracts are among the richest sources. Yeasts contain only one-fourth or one-fifth as much. The water soluble fraction of milk is a good source. In descending order it is present in moderate amount in carrots, turnips, egg-yolk, and wheat-germ. Potatoes, cereals, pulses, and leafy vegetables are poor sources.

Vitamins $\mathbf{B_3}$, $\mathbf{B_4}$ and $\mathbf{B_5}$. Studies of yeast fractions have suggested the existence of several additional vitamins. The results of further work must be awaited before these vitamins can be characterised.

Vitamin C, or Ascorbic Acid. *This acid,* $C_6H_8O_6$, *is widely distributed. Fruit juices, especially those of the citrus family, are excellent sources. It has been obtained in crystalline form from both plant and animal material. It is a hexose derivative, and its constitution is approximately known. Deficiency of the vitamin leads to scurvy. It is easily destroyed by heat.*

Scurvy can easily be produced in guinea-pigs on a diet deficient in **C**, is not uncommonly met with in infants on artificial diets, and very occasionally still occurs in adults during expeditions of long duration where fresh food supplies have failed. It was for centuries the scourge of the sailor and the explorer, who discovered empirically that an addition of lime-juice to the diet would prevent its onset.

Scurvy is characterised by a spongy condition of the gums and a tendency to hæmorrhages into the gums, muscles, joints and internal organs. The two most noticeable changes observable at

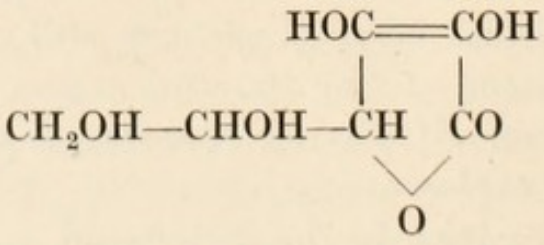
post-mortem are hæmorrhages and fragility of the bones. The latter is not due to defects in calcium metabolism. (The healing of fractured bones seems to require the presence of vitamin **C**.)

Scurvy cannot be induced in certain animals, such as the rat, mice, pigeons and chickens. This is due, it would seem, to the fact that such animals can synthesise their own requirement of vitamin **C**. Rats have been kept from birth on a diet totally deficient in vitamin **C**, and subsequently the presence of the vitamin has been demonstrated in their tissues.

The Chemical Nature and Properties of Ascorbic Acid. Various very active concentrates have been prepared from lemon juice by Zilva, King, and others. Rygh has claimed that the vitamin is a derivative of narcotine, and suggested that during the ripening of various fruits it is formed from narcotine. His work has been repeated in a number of laboratories and his conclusions have not been upheld.

Szent-Györgyi in 1928 isolated a crystalline compound from adrenal cortex, oranges, and cabbage, which proved to have the formula $C_6H_8O_6$ and to be a hexose derivative. He has suggested, and the suggestion has been completely substantiated by his own work and that of Palmer, Tillmans, Harris, King, and others, that this compound is the vitamin. King and Waugh use lemon juice as the source of the crystals.

Ascorbic acid has now been synthesised by Haworth, Karrer and others, and its constitution thus definitely demonstrated to be—



Ascorbic acid is exceedingly soluble in water, is freely soluble in methyl alcohol, and is somewhat less soluble in ethyl alcohol and in acetone.

The crystals are stable *in vacuo*, but decompose slowly in air. The aqueous solution is stable in absence of oxygen, is not decomposed by short boiling, and resists dilute mineral acid. The compound is not acted upon by yeast. It is highly autoxidisable. When oxidised by mild oxidising agents it loses two atoms of hydrogen. The resulting oxidised acid is easily reduced by animal tissues. Hence it probably functions as part of the complex oxidation-catalyst system of tissues.

Szent-Györgyi's yield from adrenal cortex was 0·3 gm. per kilogram. He considered this to be about half the amount present in that tissue. The anterior lobe of the pituitary is also rich in ascorbic acid.

Standard of Vitamin **C.** Fresh lemon juice varies but little at different places and in different seasons in its content of the vitamin. It has hence been chosen as the international standard. The unit is defined as the vitamin **C** activity of 0·1 c.c. of the juice. This represents about one-tenth of the daily dose needed to prevent macroscopic scorbutic lesions in the guinea-pig.

Distribution of Vitamin **C** (*Ascorbic Acid*). Excellent sources are orange juice, lemon juice, grape-fruit juice, raw cabbage, lettuce, spinach, and tomatoes.

Good sources are germinated pulses, fresh peas, bananas, carrots, orange peel, potatoes, and *fresh* (summer) cow's milk.

Pasteurisation decomposes a large proportion of the ascorbic acid in milk (whence an infant's diet frequently needs to be supplemented by orange juice).

It is resistant to the heat used in canning processes, in absence of oxygen.

Bios. Wilders, many years ago, showed that some particular accessory substance was essential to the growth of the yeast plant, and this he termed *bios* (life). As far as is yet known bios is not an essential for animals, and whether bios should be classified as a vitamin depends upon whether that term should be limited to

accessory substances required by animals or should include also plant needs. Bios seems to be a mixture of more than one substance. Its nature is still a matter of dispute.

Recent work is not entirely in agreement with Wilder's original observations. Yeast will grow in absence of the bios compounds ; these accelerate its growth.

General Remarks

The pharmacological actions of vitamins seem to consist in the prevention of onset of certain diseased conditions. Their presence leads to negative results ; their absence leads to positive symptomatic happenings. We have evidence that excess of a vitamin leads to deleterious results for vitamin **D.** Similar deleterious effects following overdosage of some of the other vitamins are to be expected.

Prevention and cure of vitamin-deficient diseases require different treatments. With our present knowledge of vitamins the occurrence of these diseases is unnecessary. They will not occur on a well-balanced diet containing plenty of fresh fruit, such as oranges, grape-fruit, tomatoes, fresh vegetables such as spinach, cabbage and carrots, eggs, fresh milk, whole wheat bread and meat. The dietary of infants must contain orange juice as soon as they are weaned, if not earlier, and the diet of children especially must contain a sufficiency of fresh fruit, vegetables, eggs and milk.

Where one or other of the deficiency diseases has developed it may be justifiable to use as a temporary measure some special material, and even some pharmaceutical preparation, *but only as a temporary measure.* A proper re-adjustment of ordinary diet will maintain the cure. Cod-liver oil is an excellent source of **A** and **D**, yeast of the **B** complex, whole wheat bread and lettuce are excellent sources of **E**, and orange juice of **C**. If the diet is correct, cod-liver oil and yeast will never be required.

It will have been noted that vitamin-deficiency frequently leads to cessation of growth in the young animal. This growth effect is probably secondary to the essential changes induced by the deficiency, though it affords an easy method of detecting the deficiency.

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CHAPTER XXIX

THE CHEMISTRY OF REPRODUCTION

It is of some interest to compare chemically on the one hand the stigma, pollen and fertilised seed of the plant with, on the other hand, the ovum, spermatozoon and fertilised egg of the animal. The gaps in our present knowledge are, however, so great that but a skeletal comparison can be attempted.

The Plant. Practically nothing can be stated about the chemistry of the *stigma*.

Pollen contains protein, fat, carbohydrate and mineral constituents. The protein content varies from 16 to 30 per cent. In addition, small amounts of proteose and peptone are present. The chief protein seems to vary in different plants; fir pollen contains a globulin, ambrosia pollen a glutelin. Traces of plant albumins are usually present. Hydrolysis of the chief protein suggests that relatively considerable amounts of the diamino-acids, arginine and lysine, are present, and since purine bases can always be detected there seems strong likelihood of a protein-nucleic acid combination which may be comparable with the corresponding compound present in the head of the spermatozoon.

Different pollens contain from 3 to 10 per cent. of fats. These consist chiefly of triolein and tripalmitin. The carbohydrate content varies from somewhat over 1 up to 20 per cent., and consists chiefly of starch, with traces of simpler compounds. The ash, amounting to between 3 and 5 per cent. of the original pollen, is especially rich in potassium and phosphate, with much smaller amounts of magnesium,

sodium, iron, calcium (sulphate), and chloride. Traces of manganese have been noted.

The fertilised *seed* consists essentially of plant embryo, and a varying quantity of nutrient material stored either in the cotyledons (seed-leaves) or in the endosperm when this is present. The amount and nature of this store vary greatly in different species. Nothing seems to have been ascertained as to the chemical constituents of the embryo.

The food-store includes a globulin (different in different species), 0·25 to 2 per cent. of phospholipides, of which there are at least two, one of which contains a choline radical (choline is widely distributed in plants), and the other a galactose radical, the carbohydrates starch, sucrose and fructose, fats, chiefly triolein and tristearin, though smaller amounts of many other fats are present, and, of course, water.

Oxygen is required by the ripening seed, and during ripening there is an increase in phospholipide and a marked increase in starch (up to 64 per cent.). The germinating embryo develops its own vitamin supply.

Seeds can be divided into two classes, those in which carbohydrates predominate, such as the wheat grain, the pea, and the chestnut, and those in which fats predominate, such as hemp-seed, flax-seed and the cocoanut. Dried tissue of the first class contains from 30 to 60 per cent. of carbohydrate, from 2 to 5 per cent. of fat, and from 3 to 23 per cent. of protein. Dried tissue of the second class contains from 7 to 24 per cent. of carbohydrate, from 33 to 67 per cent. of fat, and from 9 to 22 per cent. of protein.

The ash of seeds is especially rich in potassium and phosphate, with smaller amounts of sodium, calcium, magnesium (sulphate), silica, iron, chloride, and a trace of manganese.

In contradistinction to animals, the germinating seed of the plant can, in the majority of species, after its own store of food has become exhausted, utilise mineral constituents

only, along with the carbon dioxide of the atmosphere, for further growth.

The Animal. Little or nothing is known so far of the chemistry of the mammalian *ovum*. The *white yolk* of the hen's egg contains protein, nucleic acid, lecithin, etc., and its membrane contains a scleroprotein.

Spermatozoa exhibit a marked difference in the composition of the head and the tail. They are, besides, very resistant to chemical agencies, and are not completely soluble in concentrated sulphuric acid, nitric acid, acetic acid, or boiling concentrated sodium hydroxide. They yield 5 per cent. of ash, of which three-fourths is potassium phosphate.

The heads are rich in nucleic acid. In fishes this is combined with a protamine, and in some species with a histone. In the spermatozoa of higher animals no protamine is found, but sometimes a histone, sometimes some other protein. Besides this nucleoprotein material the heads contain little other organic matter, though traces of phospholipide, cholesterol and fat are present.

The last researches of Albrecht Kossel were devoted to the elucidation of the stages by which the simple proteins of sperm are built up. From studies of carp and herring sperm at different periods of the year Kossel and Schenck showed that in the unripe stages of these sperm there are present different peptone-like complexes containing radicals of arginine, of arginine and lysine or arginine and histidine, and of all three of these hexone bases, and that through the union of two or more of these " peptones " the somewhat more complex protamines and histones are, so to say, assembled, there being an underlying close similarity in the processes in different fish species.

The composition of the " tails " of spermatozoa somewhat resembles in gross that of non-medullated nerve fibres. They contain relatively large amounts of phospholipide, cholesterol and fat.

When semen is allowed to stand in contact with air crystals of *spermine* phosphate separate. These were first noticed by Leeuwenhoek in 1678, were considered by Vauquelin (1791) to be

possibly calcium phosphate, and were converted by Poehl (1891) into the chloroplatinate, and this analysed to $C_5H_{14}N_2 . H_2PtCl_6$. Poehl claimed to have isolated spermine phosphate from the testis and other organs, and considered that spermine was the essential internal secretion of the testis. He endeavoured to exploit it commercially, though his claims have long since been discredited. Spermine has now been studied accurately by Rosenheim and Dudley, and by Wrede.

When a drop of fresh semen is mounted on a microscope slide under a coverslip crystals of spermine phosphate appear in from forty to sixty minutes. These vary from 0·25 to 2 mm. in length, and have a definite crystalline form. They can be obtained from various tissues such as testis, pancreas, and also from yeast. The maximum yield from semen is 0·24 per cent., pancreas contains about one-tenth this amount, and most of the other tissues of the body still smaller amounts. Yeast contains somewhat more than pancreas ; bull's semen contains none. Spermine is identical with " musculamine " from muscle, " neuridine " from brain, and " gerontine " from liver. The so-called " Charcot-Leiden " crystals in fæces are probably spermine phosphate.

Various salts of spermine have been prepared, and determination of the molecular weight has shown that its formula is $C_{10}H_{26}N_4$. It has been synthesised and its constitution shown to be

$$NH_2.(CH_2)_3.NH.(CH_2)_4.NH.(CH_2)_3.NH_2.$$

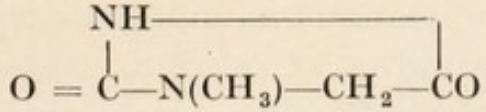
Its function is still unknown. It is accompanied, in much smaller amount, by *spermidine*,

$$NH_2.(CH_2)_3.NH.(CH_2)_4.NH_2$$

which is intermediate between spermine and *putrescine*, a compound found in putrescent organic material,

$$NH_2.(CH_2)_4.NH_2.$$

From 40 kg. of ox testes Collip has isolated 5 gm. of β-methylhydantoin,



Its significance is not known.

In mammals the fertilised *egg* develops into the fœtus within the body of the mother and derives filtered nourish-

ment from the blood of the mother through the chorionic membrane. On the other hand, the fertilised eggs of birds, reptiles, amphibia and fishes contain sufficient nourishment for embryonic development. The hen's egg has been most completely studied, and can perhaps be taken as typical in considering the nature of this nutrient material.

Varying in weight between 40 and 60 gm., it consists of from 6 to 8 gm. of outer skin and shell, from 23 to 34 gm. of " white," and from 12 to 18 gm. of yolk. The shell contains between 3·6 and 6·5 per cent. of organic matter and over 90 per cent. of calcium carbonate, with a little magnesium carbonate and traces of the corresponding phosphates. The presence of phosphate is probably of significance in connection with the laying down of calcium carbonate in the egg-shell (*cf.* Chapter XVIII., p. 263). The skin consists, besides water, chiefly of a keratin.

There is experimental evidence that the shell of the egg is dissolved by the carbonic acid produced by the embryo to yield a soluble calcium salt for the needs of that embryo. Eighty per cent. or more of its calcium requirement for initial skeletal development is provided from the shell.

Egg-white, a faintly yellow, alkaline-liquid, rich in protein, consists of from 85 to 88 per cent. of water, 10 to 13 per cent. of protein, and 0·7 per cent. of mineral salts, with traces of glucose, fats, soaps, lecithin and cholesterol. The proteins, including at least two different albumins and two different globulins, all contain glucosamine radicals, and the albumins contain small amounts of phosphate. In addition, a mucoid—*ovomucoid*—is present. The ash contains potassium, sodium and chloride in approximately equal amounts, with smaller quantities of calcium, magnesium, phosphate (carbonate), (sulphate), silica and iron, and a trace of fluoride.

The corresponding " whites " of fish and frog eggs contain chiefly mucins, with only traces of albumin.

Egg-yolk is a thick opaque liquid, dull or orange yellow in colour, with a flat taste, alkaline in reaction, and of the nature of an emulsion. It analyses approximately to 47 per cent. of water, 15·6 per cent. of protein, 23 per cent. of fat, 10·5 per cent. of phospholipide, 2 per cent. of cholesterol, and 1 per cent. ash.

The chief protein present is *ovo-vitellin*, very complex, apparently a lecitho-nucleo-protein containing over 20 per cent. of lecithin and also glucosamine radicals. It is insoluble in water, but soluble in dilute salt solutions and in very dilute acid or alkali, so that it somewhat resembles the globulins in its solubility properties. *Ichthulin* of carp's eggs is similar to ovo-vitellin. Both contain iron, and peptic digestion of ovo-vitellin splits off an iron-containing compound, which, it has been suggested, is utilised in the embryo in the building up of hæmoglobin, and which has been named *hæmatogen*.

Between one-fourth and one-fifth of the total protein of hen's yolk consists of *livetin*, a simpler protein with the properties of a pseudoglobulin and apparently unrelated to vitellin. Whether still other proteins are present remains to be determined. The fat of yolk consists chiefly of tripalmitin and triolein, with less tristearin, though the proportions of the three present depend on the diet of the hen. Lecithins and kephalins are present.

The colour of the yolk is due to a yellow pigment *lutein*, $C_{40}H_{56}O_2$, which is either identical with, or similar in structure to, the plant pigment *xanthophyll*. Similar or identical pigments are present in blood serum, milk-fat and the corpus luteum (yellow body).

In the ash phosphate predominates, with relatively large amounts of potassium and calcium, and less sodium, magnesium, iron, silica, sulphate, and chloride. The marked predominance of phosphate—two-thirds of the ash—is misleading, since most of it is of organic origin. Some, if

not all, of the sulphate is derived from organic sulphur in all such ashes.

The food store of the egg-white and yolk contains all the necessary material for the embryo chick, and the similar food store in reptile and frog eggs permits similar development, so that at hatching the young animal can use the food of the adult. But the mammal at birth still requires a special nutriment, the milk of the mother, and it is desirable to consider this perfect food. But *while the milk of the mother is the perfect food of the nursling, we must not stress it as giving too great a clue to the perfect food of the adult.*

Milk

The function of the mammary gland is to secrete milk, and in so doing it performs various chemical syntheses which are not carried out by other tissues of the body. Milk is no mere filtered fluid from the blood plasma, but contains the specific compounds casein and lactose, formed in the gland, other probably specific proteins (though milk globulin and serum globulin from the same animal are probably identical), and, to name but one other compound, citric acid, which is probably formed in the mammæ. It is gradually being demonstrated that the mammæ contain a large number of active enzymes.

The constituents of this secretion of the mammæ show considerable variation in amount in different species. For this brief account only human, cow and goat milk will be considered.

Milk consists essentially of an emulsion of fine particles of fat in a watery liquid, whose chief solutes are proteins, lactose and salts. Since it contains most, if not all, of the vitamins, it contains all the essential food factors for the young animal. It is amphoteric in reaction ; its *p*H value is 6·6. Its average composition is given in Table XX.

TABLE XX. COMPOSITION OF MILKS

	Human	Cow	Goat
Water (average) . .	87·5	87	87
Protein . . .	1·5–0·7	4·0–2·5	3·7
Fat	2 –4	2 –4	4·1
Lactose . . .	6 –7·5	3·5–5	4·4
Salts	0·2–0·3	0·6–0·7	0·9

Goat milk contains slightly more inorganic salt and fat than cow's milk. There is a marked difference between cow's and human milk, the latter containing less protein and more lactose, but less inorganic material, indicating that dilution of cow's milk with addition of sugar is necessary when this has to be substituted for human milk.

Polonovski and Lespagnol claim that human milk contains two other sugars, "gynolactose," slightly lævo-rotatory, and much less powerfully reducing than lactose, and a dextro-rotatory sugar. Gynolactose is hygroscopic, and very soluble in water, melts at 205° C., and hydrolyses to glucose and galactose.

Milk proteins consist of casein (see footnote, p. 104), lactalbumin and lactoglobulin, and, in addition, probably a fourth protein soluble in alcohol. The casein amounts to 75 to 80 per cent. of the total. The clotting of milk by rennin is due to the conversion of soluble calcium caseinate into insoluble calcium paracaseinate. (Calcium caseinogenate into calcium caseinate.)

According to Sjögren and Svedberg (1930), lactalbumin, as it exists in cow's milk, only has a molecular weight of the order of 1,000. Purification increases this twelve- or twenty-fold.

The fats consist especially of triolein and tripalmitin (in cow's milk), with smaller amounts of myristin and stearin, and traces of laurin, arachin, dihydroxystearin, butyrin, caprin, etc. Milk fat also contains a little lecithin and choles-

terol, and the yellow compound lactochrome. Traces of phospholipide are present in the aqueous part of the milk, the whey.

In the cow and other purely herbivorous animals the fat of the food has little direct effect on the nature of the milk fat. In carnivorous and omnivorous animals whose diet contains a relatively much larger amount of fat, milk fat is derived more directly from food (and from stored fat). The fat of cow's milk seems to be formed from blood lecithin (*cf.* p. 384).

The mineral constituents include potassium, sodium, chloride, citrate, calcium, magnesium and phosphate, and a trace of iron. Cow's milk contains an average content of 0·18 per cent. of citric acid.

Although milk is a specific secretion, yet many of its constituents must be considered as having dialysed through from the blood plasma. The vitamins must be included in these, along with traces of urea, creatine, creatinine, uric acid and thiocyanate, and a number of the amino-acids.

SECTION V

QUANTITATIVE METABOLISM

CHAPTER XXX

THE ENERGY EXCHANGES OF THE BODY

The realms of Physics and of Chemistry are governed primarily by two great laws, the law of the *conservation of matter*—matter can be neither created nor destroyed—and the law of the *conservation of energy*—energy can be neither created nor destroyed. *Living processes conform rigidly to these laws.*

In order to test the validity of the first law it is necessary to show whether an organism carrying on an active metabolism accounts for all matter taken within itself during a measured period of time so that the difference in weight between the matter ingested and that excreted is exactly balanced by a gain (or loss) of weight by the organism itself. Rigid proof of such a balance is not too easy to demonstrate. We can more readily prove the truth of the statement for each of a series of elements, and by extending the observations to all the elements concerned in living processes, by summation, since we can show that the law holds for each, it follows that the law holds for the totality of matter affected by the organism.

Thus Carl von Voit in 1857 fed a dog over a fifty-eight days' period 29 kilograms of meat containing 986 gm. of

nitrogen, and during this period found that the dog maintained a constant weight, and excreted 943·7 gm. of nitrogen in the urine and 39·1 gm. in the fæces, a total of 982·8 gm. The difference, 3·2 gm., is only just over 0·3 per cent., a figure certainly within the limit of experimental error of such an experiment carried out at that time. But to render such results more rigid it is necessary to know whether the organism affects in any way the atmospheric nitrogen it breathes, and whether nitrogen from food intake is lost in any other way, and, further, whether the nitrogen content of the body is changed at all during the feeding period. We know now that atmospheric nitrogen is not affected, that under certain conditions minute traces of nitrogen may be lost to the organism as urea in sweat, that none is transformed into gaseous excreta, and that a slight amount must be accounted for by growth of hair, nails and epidermis. We know also that the nitrogen content of a dog maintained at constant weight on a diet of lean meat remains remarkably constant. Hence even the slight discrepancy found in such an experiment is partly accounted for, and the law can be regarded as holding rigidly for nitrogen. And so it can be tested and shown to hold for all the other elements concerned, and for the sum of these elements.

In considering the evidence pertaining to the conservation of energy by the living organism we must first remember the different *rôles* displayed by energy, and must employ a common unit to which these can be referred. It is customary to express energy in terms of units of heat. The actual unit employed is the (large) *Calorie*, which is the amount of heat required to raise 1 litre (1,000 c.c.) of water from 15° to 16° C. The relationship between work and heat was determined by the English physicist Joule. The work done in lifting 426 kilograms through 1 metre or 1 kilogram through 426 metres against the force of gravity is exactly equivalent to one (large) calorie. The mammalian organism does not liberate free electrical energy—at any rate in measurable amounts—

and we can neglect energy exchanges other than heat and work.

We must also remember the conception of *potential* as contrasted with *kinetic* (free) energy. All material which, when oxidised, can give rise to heat possesses potential energy. Most of the food we ingest possesses potential energy, though it only provides us with free energy when its temperature exceeds that of ourselves ; obviously cold food and cold water and cold inspired air subtract free energy from us in being warmed after ingestion and inspiration to body temperature. Muscular energy and body heat are derived from the oxidation of food or its derivatives in the tissues.

The potential energy of any oxidisable substance is easily and accurately measured by burning it in an atmosphere of oxygen in a water-jacketed " bomb calorimeter." From the increase in temperature of the water surrounding the calorimeter the heat that has been produced can be calculated. The potential energy of different food materials has been accurately measured and the results are expressed in terms of calories per gram of material. Such values, for the most important foodstuffs (judging importance here by relative bulk of material ingested), and for certain other compounds, are shown in Table XXI.

TABLE XXI. ENERGY VALUES OF DIFFERENT MATERIALS DETERMINED BY THE BOMB CALORIMETER

Carbohydrates :		
Glucose . . .	3·74	Cals. per gm.
Maltose . . .	3·95	,, ,,
Starch . . .	4·18	,, ,,
Sucrose . . .	3·96	,, ,,
Lactose . . .	3·95	,, ,,
Fats :		
(Glycerol) . .	4·32	,, ,,
(Stearic acid) . .	9·50	,, ,,
(Oleic acid) . .	9·42	,, ,,
Butter fat . .	9·23	,, ,,
Olive oil . . .	9·33	,, ,,
Animal fat . .	9·50	,, ,,

Proteins :		
Lean beef . .	5·78	Cals. per gm.
Veal . . .	5·66	,, ,,
Casein . . .	5·85	,, ,,
Egg-albumin . .	5·74	,, ,,
(Alanine) . . .	4·40	,, ,,
(Cystine) . . .	4·14	,, ,,
(Glutamic acid) .	3·66	,, ,,
(Tyrosine) . .	5·91	,, ,,
Ethyl alcohol . . .	7·10	,, ,,
Urea	2·54	,, ,,
Uric acid . . .	2·74	,, ,,
Water	0·00	,, ,,
Carbon dioxide . .	0·00	,, ,,

Since by chemical analysis we can ascertain the amounts of these and similar compounds present in any known weight of mixed foodstuffs, we can, by referring to a complete table of this kind, calculate *the calorific value of a diet.*

The living organism derives from the material that it oxidises exactly the same amount of energy as would the bomb calorimeter, *to the extent to which the oxidation proceeds.* For carbohydrates and fats the final products are normally carbon dioxide and water, oxidation being complete. For these two classes of foodstuffs, as also for alcohol, there is an exact agreement between body and bomb calorimeter values for all material oxidised. (Some carbohydrate and some fat are unabsorbed ; some alcohol is excreted unchanged.) But for proteins a difference exists. They are not completely oxidised in the body. Urea, and to a less extent uric acid, creatinine, and other nitrogenous compounds are formed, and these final products have a potential energy value. The heat value of a gram of protein oxidised in the living organism is therefore the bomb-calorimeter value minus the bomb-calorimeter value of the urea and other incompletely oxidised compounds that the body forms from this protein and excretes. Such values have been accurately determined in various ways. For convenience also, we employ in calculating the energy equivalents of foods the *average values* of the carbohydrate content, the fat content,

and the protein content. Such values are given in Table XXII.

TABLE XXII. AVERAGE ENERGY VALUES OF FOOD-STUFFS

	Bomb Calorimeter.	Man.
	Cals. per gm.	Cals. per gm.
Carbohydrates . . .	4·1	4·1
Fats	9·3	9·3
Proteins	*5·8*	*4·1*
Ethyl alcohol . . .	7·1	7·1

A determination of the total energy exchanges of the body must take into account the following factors :

A. *Energy Intake.*

1. Potential energy of the food ingested.
2. Actual energy acquired from food hotter than the organism.

B. *Energy Output.*

1. Total heat loss from the body
   - (*a*) By radiation, conduction and convection ;
   - (*b*) By actual heat lost with the excreta ;
   - (*c*) By potential heat lost with the excreta.
2. Work done by the organism.

The above are either self-explanatory or have been dealt with, with the exception of B 1 (*c*), which is the heat value of the oxidisable material of the urine, fæces, and exceptionally of the sweat.

Consideration of this complex series of exchanges will be facilitated by prior consideration of what is, for convenience, termed " basal metabolism," the heat production of the *resting* organism.

Basal Metabolism

In measuring basal metabolism we are not concerned with heat exchange, but with the rate of production of heat by the organism, and, involved with this, since the normal "warm-blooded" animal maintains a practically constant temperature, of the rate of heat loss from the organism. In other words, we are measuring the rate of production of heat

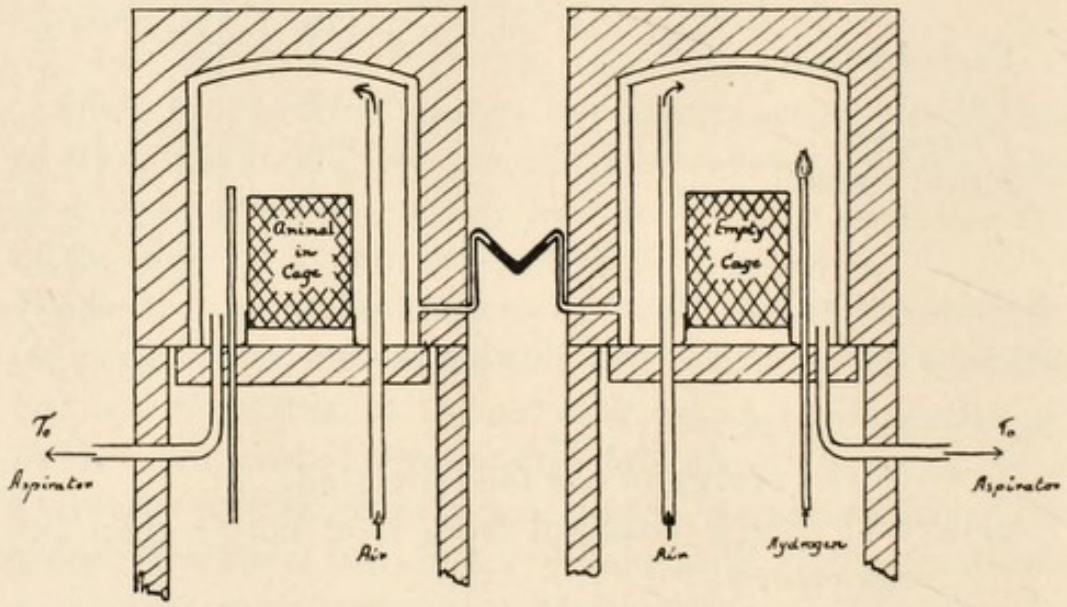


FIG. 10.—Calorimeter for small animals, whose heat production is balanced by that from a jet of burning hydrogen. (After Hale White, *Lancet*, 1897, II., 2.)

which so balances the rate of loss of heat from the organism that a constant temperature results in the organism.

This measurement can be carried out in two ways :

(i.) By direct measurement of the heat produced in a given time ; and

(ii.) By indirect measurement of the oxygen consumption or the carbon dioxide production in a given time.

Direct Measurement of Basal Metabolism. Some essential features of this measurement are illustrated in the apparatus of Haldane and Hale White for *small* animals. This is sketched in Fig. 10.

It consists of two precisely similar chambers with double

walls. The space between the walls is air-tight, and the two air-spaces are connected by a narrow glass tube containing oil of low specific gravity sufficient to fill the bore. Into the one chamber is introduced the small animal that is to be studied, and in the other is burned a jet of hydrogen gas. The rate at which the hydrogen burns is adjusted so that the pellicle of oil remains stationary midway between the chambers. As long as this condition holds it indicates that the gas pressure, and therefore the temperature, is equal in the two air-spaces, and, hence, that the heat production is equal in the two chambers. The amount of hydrogen burned can be measured by passing it through a meter before it enters the chamber, and from the amount of hydrogen burned the heat produced by its burning, and therefore the equal amount of heat produced by the animal, can be determined.

Basal metabolism is defined as the heat production of an individual at rest, physically and mentally. In carrying out determinations the following precautions are necessary.

The measurement must be made in the morning, after the subject has rested prone for half an hour, and has taken no food for twelve, or preferably sixteen, hours and drunk no liquid for four hours. There must be absence of mental anxiety, and as complete absence of mental activity as possible. During the actual test the subject must remain perfectly quiescent in the prone position. The subject must not sleep.

These conditions are essential whether the heat production be measured directly or indirectly. If adhered to they prevent increased oxidation (and therefore increased heat production) from physical or mental activity, and from digestion and absorption of food.

The actual test is carried out in a " metabolism chamber," a chamber which, after the subject has been introduced into it, is closed to the atmosphere, and to which is connected a circuit for introduction of oxygen and removal of carbon dioxide and water. The air is kept moving through the

circuit by a fan. The chamber is double walled. Between the walls are coils of pipe through which circulates cold water. Electrical devices control the temperature throughout this double-walled system, so that the water is circulated just fast enough to compensate for the heat produced by the individual in the chamber. From the heat imparted to the water the total heat produced by the subject is determined.

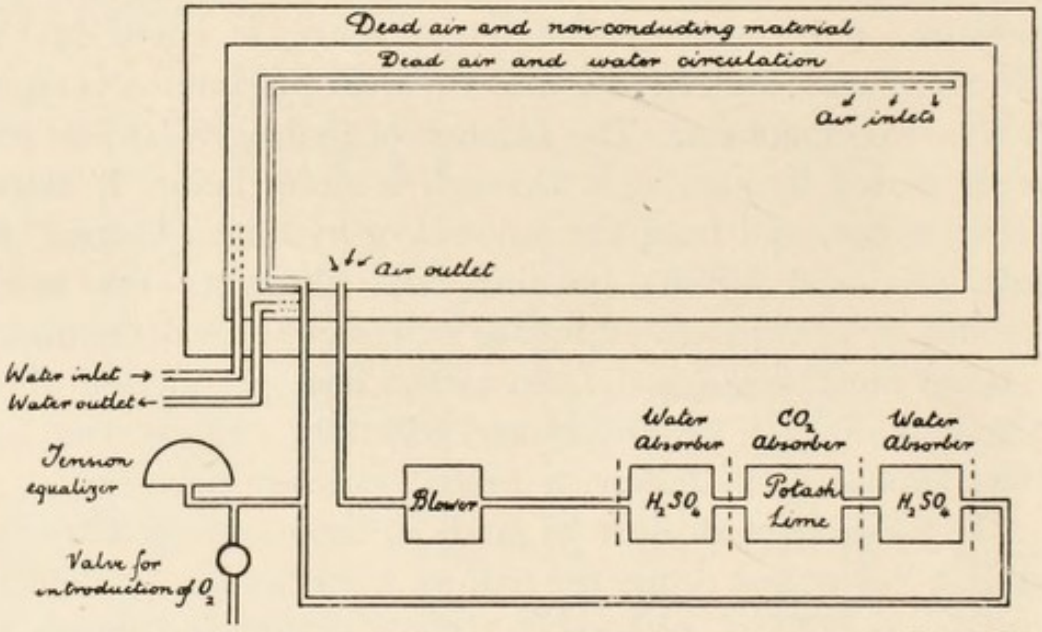


FIG. 11.—Diagram of a bed-calorimeter. After diagrams in Benedict and Carpenter's "Respiration Calorimeters," *Carnegie Institution Publication*, No. 123.

A schematic sketch of such an apparatus is shown in Fig. 11, and photographs of an actual apparatus in Figs. 12 and 13.

Indirect Measurement of Basal Metabolism. The type of apparatus just described can also be used in the indirect measurement. The amount of oxygen added to the closed system in a given time indicates, provided the temperature and pressure be kept constant, the oxygen consumed by the subject. The water vapour formed is absorbed by sulphuric acid (moistening pumice stone with the acid to give a greater absorbent surface) in vessels which can be detached from the circuit and weighed. The dried air then passes through other detachable vessels containing soda-lime, and the increase in weight of these is due to the carbon dioxide absorbed by them.

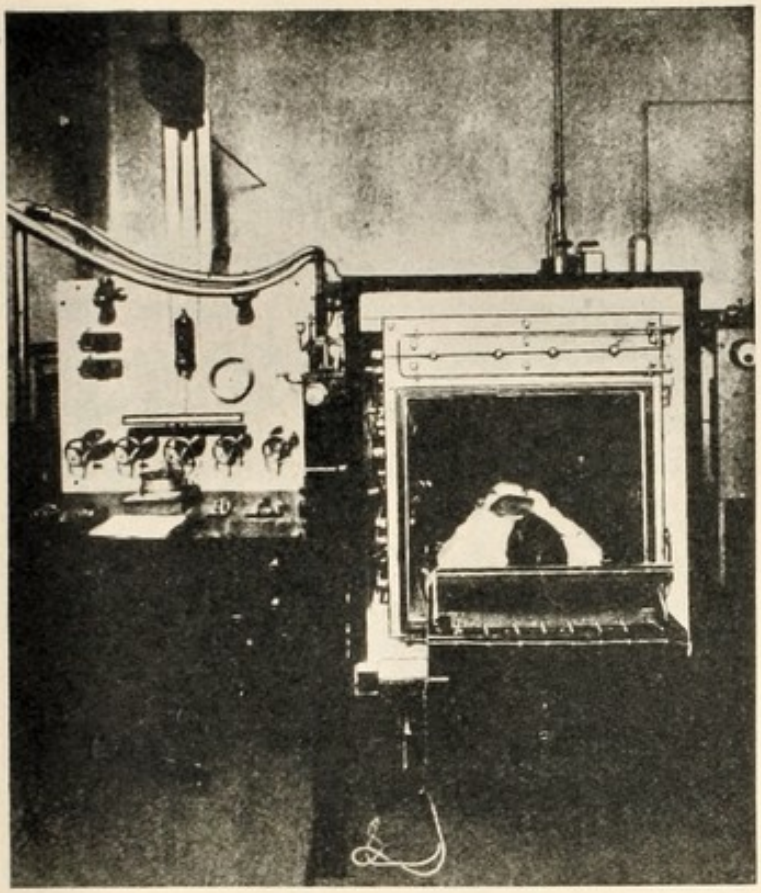


FIG. 12.—Photograph of the respiration calorimeter for metabolism determinations of the Russell Sage Institute of Pathology in Bellevue Hospital, New York. The calorimeter is open, and a patient is on the canvas bed partly in the chamber. The rubber pipes lead to the absorbing apparatus. (From Riche and Siderstrom, *Arch. Int. Med.*, 1915, xv., 816.)

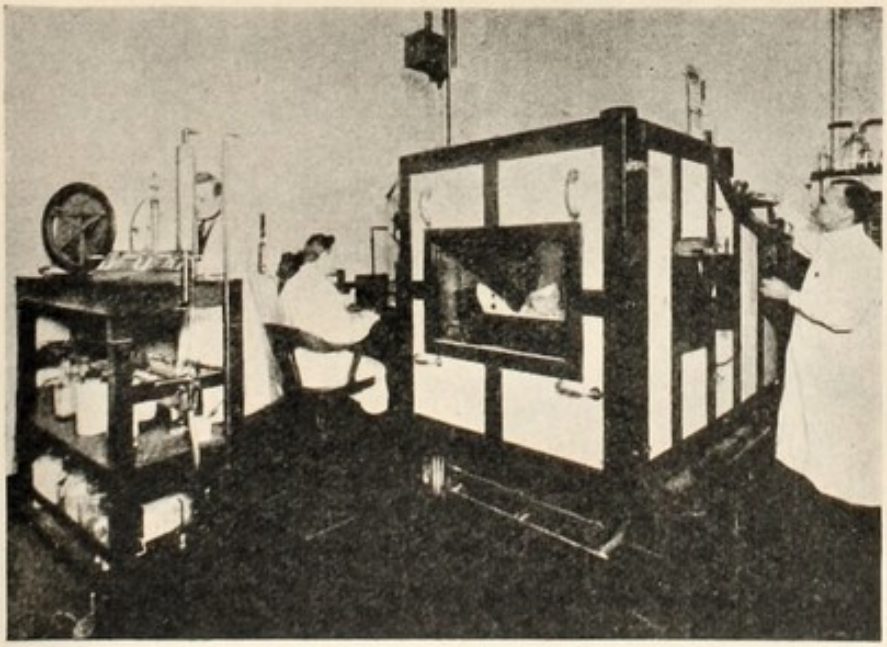


FIG. 13.—Photograph of the Russell Sage Calorimeter during an actual test. The absorbers are shown on the left. (From E. F. DuBois, "Basal Metabolism in Health and Disease," Lea and Febiger, Phila. and New York, 1924.)

We are able to calculate the heat production from the oxygen consumption, or from the amount of carbon dioxide produced, because we know the heat value of the material that the organism is oxidising, and the definite relationship between it, oxygen consumption, and carbon dioxide production. In the resting individual a mixture of carbohydrate, fat and protein is being oxidised. The particular mixture can be determined by measuring *the respiratory quotient.* The respiratory quotient (R.Q.) is defined as the amount of carbon dioxide by volume produced in a given time divided by the amount of oxygen by volume consumed (not inspired, since most of the inspired oxygen is immediately expired). The respiratory quotients for carbohydrate, fat and protein are easily determined from theoretical considerations, remembering that, from Avogadro's hypothesis, under equal conditions of temperature and pressure equal volumes of two gases contain equal numbers of molecules of those gases.

Thus, for carbohydrate, if glycogen is being oxidised—

$$(C_6H_{10}O_5)_n + n\,.\,6\,O_2 = n\,.\,6\,CO_2 + n\,.\,5\,H_2O$$

$$\text{R.Q.} = \frac{\text{carbon dioxide formed}}{\text{oxygen used up}} = \frac{n\,.\,6\,CO_2}{n\,.\,6\,O_2} = 1{\cdot}00$$

For a typical fat, tristearin, $C_3H_5(O\,.\,CO\,.\,C_{17}H_{35})_3$—

$$C_{57}H_{110}O_6 + 81\tfrac{1}{2}\,O_2 = 57\,CO_2 + 55\,H_2O$$

$$\text{R.Q.} = \frac{57\,CO_2}{81\tfrac{1}{2}\,O_2} = 0{\cdot}70$$

For a typical protein we can write an approximate equation such as for lactalbumin :

$$C_{641}H_{1064}N_{166}S_8O_{214} + 690\tfrac{1}{2}\,O_2 = 83\,CON_2H_4 + 561\,CO_2 + 358\,H_2O + 8\,H_2SO_4$$

$$\text{R.Q.} = \frac{561\,CO_2}{690\tfrac{1}{2}\,O_2} = 0{\cdot}81.$$

The respiratory quotient of normal man obviously can only vary between the limits of 0·7 and 1·0.

Calculation of a Respiratory Quotient. If the inspired air analyse to oxygen 20·93, nitrogen 79·03, and carbon dioxide 0·04

volumes per cent., and a sample of (dried) expired air give the corresponding figures, oxygen 16·60, nitrogen 79·40, and carbon dioxide 4·00, then the respiratory quotient can be calculated by the following procedure.

The intake of carbon dioxide is so small that the difference in volume between inspired and expired air will not materially affect it. Hence the carbon dioxide produced by the body and contained in a litre of expired air will be

$$\frac{1000\,(4{\cdot}00-0{\cdot}04)}{100} = 39{\cdot}6 \text{ c.c.}$$

One litre of expired air contains 794·0 c.c. of nitrogen, while a litre of inspired air contains 790·3 c.c. Since no nitrogen has been added or subtracted by the body the 794·0 c.c. of nitrogen must correspond to

$$1000 \times \frac{794{\cdot}0}{790{\cdot}3} = 1004{\cdot}8 \text{ c.c. of inspired air.}$$

This contains

$$\frac{20{\cdot}93 \times 1004{\cdot}8}{100} = 210{\cdot}3 \text{ c.c. of oxygen, the amount taken}$$

into the lungs in the time that 39·6 c.c. of carbon dioxide, formed in the body, is expired from them.

Hence the oxygen retained by the body in this period is

$$210{\cdot}3-166{\cdot}0 = 44{\cdot}3 \text{ c.c.}$$

whence the respiratory quotient is

$$\frac{39{\cdot}6}{44{\cdot}3} = 0{\cdot}89.$$

From such equations as those just cited and the heat values of different food materials that have already been given it can be calculated that 1 litre of oxygen gives rise to 4·69 calories when oxidising fat, 5·05 calories when oxidising carbohydrate, and 3·93 calories when oxidising protein (in the body).

These figures of course refer to such average mixtures of carbohydrates, fats, and proteins as occur in a diet. As an example of the method the value for starch may be calculated. The equation of oxidation is :

$$(C_6H_{10}O_5)_n + n\,.\,6\,O_2 = n\,.\,6\,CO_2 + n\,.\,5\,H_2O$$

Hence six molecules of oxygen are required for every $(C_6H_{10}O_5)$

group, and this has a weight of 162. Hence six gram-molecules of oxygen are required for 162 gm. of starch, and since a gram-molecule of any gas at normal temperature and pressure occupies 22·4 litres, 134·4 litres of oxygen are required for 162 gm. of starch and 1 litre for 1·205 gm. The energy value for a gram of starch is 4·18 cal. (Table XXI.), and therefore for 1·205 gm. is 5·04 cal.

It is found that the normal individual, resting in accordance with the requirements of a basal metabolism test,

TABLE XXIII. RELATIVE HEAT PRODUCTION IN DIFFERENT SPECIES

Species.	Weight, kilograms.	*Relative heat production.*	
		Cals. per kilogram.	Cals. per square metre.
Horse . . .	441·0	0·35	0·91
Pig	128·0	0·60	1·03
Man	64·3	*1·00*	*1·00*
Dog	15·2	1·60	1·00
Rabbit (without ears) .	2·3	2·34	0·88
Goose . . .	3·5	2·08	0·93
Fowl	2·0	2·21	0·90
Mouse . . .	0·018	6·60	1·14

has a very constant respiratory quotient, about 0·82 or 0·83. He evidently is oxidising a very constant mixture of carbohydrate, fat and protein. Since, under these resting conditions, it is known that the amount of protein undergoing oxidation is relatively very small, it is possible to determine with sufficient accuracy the heat production from such a mixture of fat and carbohydrate (neglecting protein) as will give such a respiratory quotient. From this we obtain the necessary relation between the heat production and the oxygen consumption. Under these conditions 1 litre of oxygen is used up in the production of 4·83 calories.

Clinical instruments have been devised which permit direct

measurement of the oxygen consumption per minute by an individual resting under " basal " conditions, and from the relationship just quoted the heat production per minute can be at once calculated. This is found to be related to the surface area of the individual. The relationship between surface area and heat production, and the non-existence of a relationship between surface area and body-weight are shown by the figures in Table XXIII., based on Voit's determinations for the resting animal.

Relative figures are given, those for man being taken as unity.

There thus appears to be a relationship between the heat production in a mass of protoplasm and the heat loss from its surface so adjusted as to maintain the mass at a constant temperature, though this constancy is facilitated by various factors, such as nervous control of the skin circulation, which to some considerable degree govern the heat loss. The relationship is sufficiently rigid to permit the calculation of his normal basal metabolism from the surface area of an individual. An approximation to the surface area can be calculated from the height and weight, the number of calories developed per square metre of surface having been determined with considerable accuracy for individuals of different ages and both sexes.

Numerous experiments have demonstrated that the results of direct and indirect measurement of basal metabolism are in good agreement. The production of heat per square metre of body surface is for young male adults (man) 39·5 calories per hour, for females 37·0 calories. The younger growing individual produces relatively more heat, while, with increasing age, there is a slow fall in heat production.

The determination of basal metabolism is of considerable value clinically, especially in diseases of the thyroid gland. Increase in activity of the thyroid results in increased cell oxidations throughout the body, and hence increased heat production. Decrease in activity of the thyroid gives the opposite effect, there being in total absence of thyroid activity only 60 per cent. of the normal

heat production. Hence a measurement of the basal metabolism frequently gives an accurate idea of the condition of the thyroid. The method in which oxygen consumption is the measured factor of course fails in conditions such as diabetes, where oxidation of carbohydrates is incomplete, and oxidation of fats may also be imperfect, and the respiratory quotient is abnormally low. In such conditions the determination of the respiratory quotient itself becomes of considerable clinical importance ; in diabetes, complicated by extreme acidosis, the derangement of metabolism may become so great as to give a respiratory quotient actually less than 0·7, that for pure fat.

Hibernating animals show interesting variations in the respiratory quotient. Prior to hibernation, when much of the carbohydrate of the food is being laid down as fat as a store of food for use during hibernation itself, the respiratory quotient may exceed unity. This is explained by the following equation, representing the transformation of carbohydrate to fat :

$$19C_6H_{12}O_6 = 2C_3H_5(O \cdot CO \cdot C_{17}H_{35})_3 + 4H_2O + 49O_2.$$

Such a change furnishes the body with so much oxygen that the amount required from the atmosphere may actually fall below the amount of carbon dioxide produced. Correspondingly, during hibernation, when the animal is subsisting largely on its fat, and converting it slowly into carbohydrate, since through this change so much more oxygen is required the quotient may fall to a low figure.

Total Metabolism

The heat production of the body varies with all its different conditions. During sleep the value falls below the

TABLE XXIV. METABOLISM IN VARIOUS CONDITIONS

Condition.	Percentage variation from basal value.
Sleep	down to − 16 per cent.
Digestion . . .	+ 10 to + 20 per cent. and over.
Slight mental effort (memorising) . .	+ 10 to + 20 per cent.
Slight arm movements .	+ 5 to + 10 per cent.
Leg movements . .	Over + 10 per cent.
Vigorous exercise. .	Over + 50 per cent.

" basal " figure. Mental effort, digestion and absorption of food and physical exercise all raise it. Some idea of the effects of these various changes is given by the figures in Table XXIV.

Exact methods of determining the energy exchanges in metabolism have gradually developed from the classic experiments of Lavoisier just before the French Revolution to those of Voit, Rubner, Atwater and Rosa, and, finally, Benedict and the modern American school. Benedict has made the studies of clinical value and is still extending our exact knowledge. Lusk and others have especially extended our knowledge in the direction of energy exchanges under different dietary conditions and variations with disease.

As a means of measuring work accurately Benedict has devised a bicycle ergograph, in which a definite amount of work can be performed and measured against an electrical resistance, the whole being carried out within the metabolism chamber itself. Thus it is possible within the accurate metabolism chambers of Benedict to study every phase of metabolism and to produce an accurate balance-sheet. Such a balance-sheet we can now proceed to study.

The following tables give the summarised results of a four-day experiment on a student carried out by Atwater, and reported by him in the " Ergebnisse der Physiologie " in 1904. Table XXV. gives the results of analyses on the different foods ingested, applied to the amounts actually eaten. Table XXVI. summarises the heat measurements and the analyses of the excreta.

Analysis and Interpretation of Results. The protein figures were derived on the assumption that protein contains on the average 16 per cent. of nitrogen, so that if analytical figures for nitrogen are multiplied by 100/16, *i.e.*, 6·25, the corresponding figures for protein are obtained. The assumption is approximately correct.

Table XXV. shows that there was, during the four days, a total nitrogen intake of 67·1 gm. During the same period

TABLE XXV. CHEMICAL COMPOSITION OF THE FOOD INGESTED AND ITS HEAT VALUE

Food per day.	Weight per day.	Water.	Protein (N × 6·25).	Fat.	Carbo-hydrate.	N	C	H (in dried food).	Heat value.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Cals.
Bread. . . .	450	192·2	35·5	10·4	211·0	5·67	119·34	17·24	1,202
Ginger-snaps . .	75	5·1	4·7	5·4	58·8	0·75	31·67	4·60	318
Graham cakes . .	50	1·7	4·4	5·1	38·6	0·70	22·96	3·40	232
Whole wheat food .	50	4·1	5·3	0·7	39·7	0·84	20·47	2·87	204
Sucrose . . .	140	0·0	0·0	0·0	140·0	0·00	58·94	9·08	554
Crude lactose . .	90	4·6	0·0	0·0	85·4	0·00	36·00	5·54	335
Butter . . .	20	2·4	0·4	16·9	0·0	0·06	12·52	1·99	154
Meat	110	67·9	37·2	3·1	0·0	5·96	21·77	3·17	244
Milk	500	424·0	17·5	27·5	27·5	2·80	42·65	6·15	472
Total per day . .	1,485	702·0	105·0	69·1	601·0	16·78	366·32	54·04	3,715
Total per 4 days . .	5,940	2,808·0	420·0	276·4	2,404·0	67·1	1,465·3	216·2	14,860
Water drunk in 4 days .	8,200	8,200·0	—	—	—	—	—	—	0
Total income . .	14,140	11,008·0	420·0	276·4	2,404·0	67·1	1,465·3	216·2	14,860

TABLE XXVI. CHEMICAL ANALYSES AND ENERGY VALUES OF THE FOUR DAYS' EXCRETA

Material.	Weight.	Protein ($N \times 6{\cdot}25$).	Fat.	Carbo-hydrate.	Water.	CO_2	N	C	H (in dry excreta).	Heat value.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Cals.
Fæces . .	414·5	39·6	15·8	29·8	317·1	0·0	6·33	46·6	6·6	506
Urine . .	3,982·8	0·0	0·0	0·0	3,737·5	—	66·30	50·72	12·9	531
Expired air	17,312·6	0·0	0·0	0·0	10,689·7	6,622·9	0·0	1,806·2	1,197·3	0
Sweat .	—	0·0	0·0	0·0	—	—	1·4	0·3	—	—
Total . .	21,709·9	39·6	15·8	29·8	14,744·3	6,622·9	74·0	1,903·8	1,216·8	1,037

nitrogen was lost to the extent of 74·0 gm., so that there was a nitrogen loss from the body of 6·9 gm., corresponding to a loss of body-protein (from which it must have come) of 43·1 gm. This body-protein would contain, since the average protein contains 53 per cent. of carbon, 43·1 multiplied by 0·53, *i.e.*, 22·8 gm. of carbon.

Table XXV. shows that the total intake of carbon was 1,465·3 gm., and the total loss (Table XXVI.) was 1,903·8 gm., a loss to the body of 438·5 gm., of which 22·8 came from protein, leaving 415·7 to be accounted for. In this experiment, for simplicity, it was assumed that the store of glycogen remained approximately constant, so that this lost carbon was presumed to have come from catabolised body-fat. Body-fat contains on the average 76 per cent. of carbon, so that dividing by the factor 0·76, 415·7 gm. of carbon correspond to 547·0 gm. of fat.

TABLE XXVII. HEAT PRODUCTION

Heat lost by conduction, etc. (measured) .	16,015	Cals.
Heat used up in warming food and drink to temperature of calorimeter (estimated) .	115	,,
Heat used up by body in evaporating water (calculated)	2,927	,,
Total heat production	19,057	Cals.

Hence, during the four days the student, evidently on a diet insufficient to maintain his body-weight, catabolised 43·1 gm. of his body-protein and 547 gm. of his body-fat to maintain his energy exchanges. To estimate the energy available from these amounts we must remember that the energy value of the urine has already been measured, and we must use the factor 5·8 for protein. Then :

43·1 grams of protein correspond to	250·0 Cals.
547·0 grams of fat correspond to .	5,087·1 Cals.

so that from this source 5,337·1 calories were derivable.

The total energy available from the food was 14,860 calories, of which 1,037 calories (the excreta value) were not utilised, so that the net value available from food was 13,823

calories. The total heat production was 19,057 calories (Table XXVII.), and the heat balance provided from the body was therefore 5,234 calories. The difference between this and the amount calculated indirectly is 103 calories, an error of only 103/19057 × 100, or 0·54 per cent. of the total energy exchange.

The water exchange can further be calculated. From Table XXV. the total intake of water was 11,008 gm., and from Table XXVI. the total output was 14,744 gm. Oxidation of the body protein was responsible for the production of 43 × 0·44, equal to 19 gm., and oxidation of body-fat for the production of 547 × 1·11, equal to 608 gm., so that the net loss of pre-formed body-water was 3,736 — 627, equal to 3,109 gm. The total loss of body-weight during the four days should therefore have been (adding fat, protein and water losses) 3·7 kilograms. Unfortunately, the data available do not quote the actual loss of body-weight in the experiment. A certain amount of work was done daily by the student, but the heat value has been included in that measured by conduction in order to lessen the complication of the calculation.

As an additional illustration of the methods employed, an experiment of Atwater and Benedict's may be quoted (Carnegie Institution Publication No. 42, 1905, experiment 70), which was carried out for twenty-four hours on a student.

Material.	*Weight, Composition and Heat of Combustion of Ingested Food.*										
	Total wt.	Water	Water-freed Substances.								
			Protein.	Fat.	Carbohydrate.	Ash.	N	C	H	O	Heat value.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Cals.
Milk .	1652·9	1305·8	49·58	211·86	75·07	10·55	7·94	214·69	33·39	80·49	2545
Plasmon.	5·0	0·5	3·73	0·01	0·34	0·43	0·60	2·21	0·31	0·96	24
Total .	1657·9	1306·3	53·31	211·87	75·41	10·98	8·54	216·90	33·70	81·45	2569

Material.	*Weight, Composition and Heat of Combustion of Excreta.*							
	Total wt.	Water.	Dried Material.					
			Ash.	N	C	H	O	Heat value.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Cals.
Urine. .	1,031·5	991·4	4·54	13·04	8·87	2·37	11·31	103
Fæces .	61·0	40·5	4·25	0·36	12·04	1·91	1·93	149
Total . .	1,092·5	1,031·9	8·79	13·40	20·91	4·28	13·24	252

Total water output in ventilation air current . .	838·30 gm.
Total carbon dioxide output in ventilation air current .	178·05 gm.
Total oxygen consumed	622·40 gm.
Total heat production (corrected for losses to food, dishes, etc.)	2113·45 Cals.

GAIN OR LOSS OF BODY MATERIAL

	Total wt.	N	C	H	O	Ash.
Intake—	gm.	gm.	gm.	gm.	gm.	gm.
Oxygen (air) .	622·40	—	—	—	622·40	—
Water (beverage)	139·00	—	—	15·55	123·45	—
Water in food .	1,306·33	—	—	146·18	1,160·15	—
Solids . .	351·57	8·54	216·90	33·70	81·45	10·98
Output—						
Water (fæces) .	40·48	—	—	4·53	39·95	—
Solids (fæces) .	20·49	0·36	12·04	1·91	1·93	4·25
Water (urine) .	991·37	—	—	110·94	880·43	—
Solids (urine) .	40·13	13·04	8·87	2·37	11·31	4·54
Water (respn.) .	838·30	—	—	93·81	744·49	—
CO_2 (respn.) .	652·86	—	178·05	—	474·81	—
Total . .	2,583·63	13·40	198·96	213·56	2,148·92	8·79
Gain or loss . .	− 164·33	− 4·86	+ 17·94	− 18·13	− 161·47	+ 2·19
Ash of protein .	− 0·45	—	—	—	—	− 0·45
	− 164·78					+ 1·74
Gain or loss of body material—						
Protein . .	− 29·16	− 4·86	− 15·40	− 2·04	− 6·41	− 0·45
Fat . . .	+ 33·54	—	+ 25·52	+ 3·96	+ 4·06	—
Glycogen . .	+ 17·53	—	+ 7·78	+ 1·09	+ 8·66	—
Water . . .	− 188·88	—	—	− 21·14	− 167·74	—
Ash . . .	+ 2·19	—	—	—	—	+ 2·19
Total . .	− 164·78	− 4·86	+ 17·90	− 18·13	− 161·43	+ 1·74

The authors calculated the last set of figures in the following way: The percentage composition of the classes of foodstuffs

concerned was taken as protein, 16·67 N, 52·80 C, 7·00 H, 22·00 O, and 1·53 mineral matter (disregarded); fat, 76·10 C, 11·80 H, and 12·10 O; carbohydrates, 44·40 C, 6·20 H, and 49·40 O; water, 11·19 H and 88·81 O. From these the following equations were derived:

$$0{\cdot}1667\ (\text{protein}) = \text{N}$$
$$0{\cdot}4440\ (\text{carbohydrate}) - 0{\cdot}7610\ (\text{fat}) - 0{\cdot}5280\ (\text{protein}) = \text{C}$$
$$0{\cdot}1119\ (\text{water}) - 0{\cdot}0620\ (\text{carbohydrate}) - 0{\cdot}1180\ (\text{fat}) - 0{\cdot}0700\ (\text{protein}) = \text{H}$$
$$0{\cdot}8881\ (\text{water}) - 0{\cdot}4940\ (\text{carbohydrate}) - 0{\cdot}1210\ (\text{fat}) - 0{\cdot}2200\ (\text{protein}) = \text{O}$$

These equations were then resolved in terms of the elements and the following series obtained:

$$\begin{aligned}
\text{Protein} &= 6{\cdot}0\ \text{N} \\
\text{Fat} &= 0{\cdot}005\ \text{C} + 9{\cdot}693\ \text{H} - 1{\cdot}221\ \text{O} - 2{\cdot}476\ \text{N} \\
\text{Carbohydrate} &= 2{\cdot}243\ \text{C} - 16{\cdot}613\ \text{H} + 2{\cdot}093\ \text{O} - 2{\cdot}892\ \text{N} \\
\text{Water} &= -1{\cdot}248\ \text{C} + 7{\cdot}920\ \text{H} + 0{\cdot}128\ \text{O} + 0{\cdot}460\ \text{N}
\end{aligned}$$

The gains and losses of the elements have already been recorded, and substituting these in the last series of equations the values for protein, etc., are at once found. Thus, for protein, 6N equals $6 \times -4{\cdot}86$, equals $-29{\cdot}16$, indicating a loss of 29·16 grams of protein. The accuracy of the calculation is checked by a calculation of the element content from these figures, and it will be seen to agree very closely with the gains and losses of the elements determined from analysis.

There was thus calculated to be a loss of 29·16 gm. of protein and 188·88 gm. of water, and a gain of 33·54 gm. of fat, 17·53 gm. of glycogen, and 2·19 gm. of ash, in other words, a net loss of 164·78 gm. during the experimental period. The actual loss of body-weight measured directly was 111·00 gm., and the error was traced to a fault in the experimental routine in collection of urine at definite periods, the authors stating that with a subsequently perfected routine a very close agreement is attained.

To analyse next the figures relating to the energy exchanges the heat of combustion of the food used, as actually determined on samples using the bomb calorimeter, was 2,569 calories, but of this 252 calories was not available (the value for the excreta), so that the actual energy available from the food was 2,317 calories. The protein material lost to the body was calculated to have a heat value of 165 calories, while the fat and carbohydrate stored had values respectively of 321 and 73 calories. There was a net storage of energy to the extent of (321 plus 73 minus 165, equal to) 229 calories, so that the energy derived from material actually oxidised in the body should be 2,088 calories. That actually measured was 2,113 calories, the difference being 25 calories, 1·2

per cent. of the total exchange. (In making these calculations the authors took the values for protein 5·65, for fat 9·54, and for glycogen 4·19 calories.)

Atwater, as a result of twelve experiments with resting individuals, each averaging several days, and twenty similar experiments in which some work was performed, obtained an average error between calculation and direct measurement of less than 0·1 per cent., and a maximum error of only 1·7 per cent. in any experiment. Many other observers have obtained similar results, and the principle of the conservation of energy as applied to living processes can be regarded as established.

Muscular Work

In the performance of muscular work additional heat is produced to such a great extent in hard work that the body temperature is actually raised for a period above the normal value. If the additional heat production be measured, and the amount of work done be also accurately determined, it is found that the sum of the two forms of energy is about eight times the actual work done (Atwater).

From what source is this energy of muscular work derived ? Measurements of the respiratory quotient have been utilised to answer this question. It will be remembered that the value of the respiratory quotient gives a clue to the type of oxidation proceeding in the body. Values approximating to unity indicate that the catabolism chiefly affects carbohydrates, while values falling nearly to 0·7 indicate that fats especially are undergoing oxidation.

In 1920 Krogh and Lindhard showed that during moderate work only carbohydrate is directly used for the production of work, and their results also indicated that fat, before it could be used in work production, must be converted into carbohydrate, with an involved loss of 10 per cent. of energy.

Such a conclusion is in full agreement with the chemical changes that are known to occur, and which have been dealt with in Chapter XXII. (*cf.* also Chapter XXXII.).

Specific Dynamic Action

It has long been known that the ingestion of protein stimulates metabolism. As a result more heat is produced than would be derived by oxidation of the actual amount of protein fed. This action is spoken of as *the specific dynamic action of proteins*. Lusk has shown that it is not due to proteins as such, but to specific amino-acids. Alanine, to a greater extent glycine, and still more phenylalanine, are effective in producing it, whilst others, such as glutamic acid, are without effect. The liver cells are essentially concerned in the action. Since heat is produced, oxidation must be stimulated. Various theories have been advanced to explain this increased oxidation and heat production. Such include : (i.) extra heat production arises from intermediate reactions associated with the formation of glucose from the amino-acids concerned ; (ii.) the amino-acids themselves act as metabolic stimuli, elevating the organism to a higher metabolic level ; (iii.) such action is produced by specific products from these amino-acids ; (iv.) they throw extra work on the kidney, for which extra energy must be provided. Of such theories, the first seems most rational, but the matter is far from being settled. The internal secretion of the thyroid plays some intermediate part, since Baumann and Hunt have shown recently that the effect is not produced after thyroidectomy.

When carbohydrates and fats are fed *in excess* a somewhat similar phenomenon occurs. There results an excess heat production, which, however, Lusk attributes to the tissues obtaining such a " plethora," such an increased concentration of combustible material that there is a temporary increase in the rate of its oxidation and therefore of heat production.

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CHAPTER XXXI

DIET

Physiologists of the last century devoted a considerable degree of attention to diet, both from the point of view of its composition and of its heat value.

In the absence of accurate methods which could be utilised to calculate dietary requirements, based on the measured needs of the organism, they proceeded empirically, on the *dictum* " whatever is, is right," to determine what is actually required from statistical determination of what is actually consumed. Figures for daily consumption, of which the following are examples, were obtained.

Farmers average a consumption of energy value of 3,550 calories, there being little difference in value in different parts of the world. Concerning city populations, Rubner calculated that the inhabitants of London consume a value of 2,665 calories, of Paris, 2,903, of Munich, 3,014, and of Königsburg, 2,394.

Atwater, limiting his enquiry to the United States, estimated that farmers' families showed an average consumption of 3,560 calories per individual, mechanics' families, 3,605, professional men's families, 3,630, the U.S. army, 3,851, and the U.S. navy, 4,998. The British army in the Boer War was rationed on a basis of 3,903 calories, and during the Russo-Japanese War the Japanese soldier received 4,313 and the Russian soldier 4,891 calories.

Such figures definitely illustrated differences between individuals carrying on different degrees of activity, and various estimates were made as to the actual average require-

ment in different stated conditions. The following can be taken as typical, for a man weighing 70 kilograms :

When doing sedentary (office) work requirement 2,500 cals.
When doing light muscular work . requirement 2,800 ,,
When doing moderate muscular work requirement 3,000 ,,
When carrying on hard toil . requirement 4,000–5,000 ,,

It is obvious that the requirements vary with weight, with sex, and will be relatively disproportionate for the *growing* child. Estimates were made leading to figures such as the following, for which adult man is taken as standard :

Man	100 per cent.
Woman	83 ,,
Boy over 16 . . .	92 ,,
Boy, 14–16	81 ,,
Girl, 14–16	74 ,,
Child, 10–15. . . .	64 ,,
Child, 6–9	49 ,,
Child 2–3	36 ,,
Child, under 2 . . .	23 ,,

In any case, of course, such estimates can only indicate an approximate average.

With the present knowledge of basal metabolism and the means now available of estimating the added heat requirements for definite amounts of muscular work, it is possible to place such data on a much more secure foundation, though it is interesting to note that the new figures are in approximate agreement with the older empirical estimations.

In Table XXIV. some indication has been given of the increases above basal metabolism resulting for various activities. Lusk has estimated, from actual measurements, that sitting increases the metabolism 5 per cent. above the basal figure, standing in a relaxed attitude 10 per cent., standing at attention 14 per cent., and that normally, with slight activity and while digestion and absorption are proceeding, the metabolism is 30 per cent. above basal.

Starling computed the requirements of an average man

in the following way. He assumed that this average person weighs 155 lbs. (70·3 kilograms) and has a body surface of 1·792 square metres, his basal metabolism being 39·7 calories per square metre per hour, so that his basal hourly production of energy is 71·1 calories. Then—

8 hours asleep at 71·1 cals. per hour	568·8 cals.
8 hours awake, with 30 per cent. added to basal	739·4 ,,
8 hours at work, estimated at 120 cals. per hour above the basal requirement	1,528·8 ,,
Extra energy involved in locomotion, etc.	300·0 ,,
Total	3,137·0 cals.

In the above calculation 960 calories are allowed for the performance of external work. The Food (War) Committee of the Royal Society suggested the following classification for different workers—

Sedentary	Less than 400 cals.
Light work	400 to 700 ,,
Moderate work	700 to 1,100 ,,
Heavy work	1,100 to 2,000 ,,

From the figures of Becker and Hämäläinen they estimated the following values for different occupations—

A tailor requires 2,500 cals..	His food should provide	2,750 cals.
A bookbinder requires 2,800 cals..	,, ,, ,,	3,100 ,,
A shoemaker requires 2,850 cals. .	,, ,, ,,	3,150 ,,
A metalworker or carpenter requires 3,200 cals.	,, ,, ,,	3,500 ,,
A painter requires 3,250 cals.	,, ,, ,,	3,600 ,,
A stonemason requires 4,400 cals.	,, ,, ,,	4,850 ,,
A woodcutter requires 5,000 cals..	,, ,, ,,	5,500 ,,

The figures in the last column allow a 10 per cent. difference for the food as purchased, and as consumed and digested.

Starling concluded that the average man in a mixed population (not mainly agricultural) has an energy requirement of 3,000 calories, and in a similar way calculated that the average woman, if English, requires 2,116 calories, and if American or Canadian, 2,208 calories (this difference being traceable to the different average heights and weights). Lusk

calculated the comparable values for women and children as follows—

Average man (standard)	1·0	3,000 cals.
Boys, 14–20	1·0	3,000 ,,
Average woman	0·83	2,500 ,,
Girls, 14–20	0·83	2,500 ,,
Children, 10–14	0·83	2,500 ,,
Children, 6–10	0·6	1,800 ,,
Children, 0–6	0·5	1,500 ,,

Starling applied these figures to a whole population, and calculated that the average man-value of the population of Great Britain and Ireland in 1911 was 0·835, which, on the daily allowance of 3,300 calories per man, indicates a total yearly requirement of 45·5 billion calories.

Berczeller and Freud (1927) calculated that the daily caloric requirement of the whole world—human beings and domestic animals—was 16,400 millions.

Not only has an interest long been aroused in the total energy requirements of the individual, but enquiries have for long been made into the nature of the food eaten to fill these requirements. For a long period statements had to be based on statistical data, on an analysis of what ordinary groups of people actually ate. Various dietaries were proposed, based on such observations, of which the following are typical—

TABLE XXVIII. OLD STANDARD DIETARIES

	Moleschott.		Ranke.		Voit.		Forster.		Atwater.	
	gm.	Cal.	gm.	Cal.	gm.	Cal.	gm.	Cal.	gm.	Cal.
Protein	130	533	100	410	118	483	131	567	125	512
Fat	40	372	100	930	56	520	68	632	125	1,172
Carbohydrate	550	2,275	240	984	500	2,050	494	1,825	400	1,640
Total Calories		3,180		2,324		3,053		3,024		3,324

It will be observed that in all these diets carbohydrate bulks largest, protein is always over 100 gm., and fat is very variable.

Since we know that the body can easily transform carbohydrate into fat, the relative proportion of fat and carbohydrate can be governed primarily by their distribution in the customary meals we consume, in which carbohydrate is always in considerable excess, the prime factor being that the essential total calorie requirement be met. The optimum amount of protein that should be ingested requires more careful consideration.

Our study of the intermediate metabolism of protein has shown that the body uses it in two ways, to supply the necessary amino-acids to replace tissue " wear," and to furnish energy through transformation to carbohydrates and other oxidisable compounds. The first function is essential, but the second is wasteful, since not only is the body called upon to carry out unnecessary work, but also protein-rich food material is usually more costly than carbohydrate-rich food. Hence it is important to know as accurately as possible the minimal protein requirement.

Since the earlier students of metabolism found that it was possible to maintain an animal such as the dog in an equilibrium at constant body-weight on a " protein " diet only, while in the absence of protein no equilibrium could ever be attained, however much carbohydrate and fat were fed, the animal gradually starving, the unique place of protein in the diet was apparent (though the equilibrium was illusory, since the " protein " fed conveyed the necessary salts and vitamins). Since under such conditions it was found that, after a few days, the nitrogen intake was exactly balanced by the nitrogen excreted, the conception of a *nitrogen equilibrium* resulted. The attainment of such an equilibrium is illustrated by an experiment of Voit on a dog which was fed 500 gm. of meat, containing 17 gm. of nitrogen, daily for some time, and then for a period of seven days three times the amount, and for a subsequent period of five days twice the amount. The nitrogen intake and output are shown in Table XXIX.

TABLE XXIX. THE ATTAINMENT OF A NITROGEN EQUILIBRIUM IN THE DOG

Day.	N-intake.	N-output.	N-balance.
	gm.	gm.	gm.
1	17·0	18·6	− 1·6
2	51·0	41·6	+ 9·4
3	51·0	44·5	+ 6·5
4	51·0	47·3	+ 3·7
5	51·0	47·9	+ 3·1
6	51·0	49·0	+ 2·0
7	51·0	49·3	+ 1·7
8	51·0	51·0	0·0
9	34·0	39·2	− 5·2
10	34·0	36·9	− 2·9
11	34·0	37·0	− 3·0
12	34·0	36·7	− 2·7
13	34·0	34·9	− 0·9

It is customary to state that nitrogen cannot be stored in the body. This is only partially true. During the first period, before equilibrium was attained, 26·4 gm. of nitrogen were retained. During the second period 14·7 gm. were lost. Evidently when protein intake is increased there is a slight preliminary retention of nitrogen, lasting just so long as the greater amount of protein is fed.

The nitrogen equilibrium affords a means of measuring the actual protein requirement. If an individual is given a diet on which he can reach a nitrogen equilibrium, a body-weight equilibrium, and maintain good health, that diet should contain sufficient protein for his needs, and if a series of diets be tested, with diminishing protein content, then the minimum protein requirement should be determinable.

The first advocate of a smaller amount of protein in the diet was Chittenden, of Yale University, who carried out a long-continued series of experiments on three classes of indivi-

duals, University teachers, University students classed as athletes, and men from the Hospital Corps of the American Army. He found that the body can be maintained in equilibrium and in a general state of efficiency on a diet containing from 30 to 50 gm. of protein per day (according to the weight of the individual), a reduction to less than 0·75 gm. per kilogram body-weight.

His results have been confirmed and extended in recent years by Hindhede of Copenhagen.

Hindhede's subject was the laboratory servant, a strong, healthy young man of 70 kilograms weight. While able to perform all his usual duties he lived on a diet consisting only of potatoes, together with margarine and a little onion for flavour, and averaging 4·425 gm. of nitrogen (less than 27 gm. of protein, and only 0·4 gm. per kilogram body-weight) per day. The experiment lasted 178 days, and although in this period 75 gm. of nitrogen were lost from the body it was not possible to discover that the subject otherwise was in any different condition than before the experiment started. He was in nitrogen equilibrium during the greater part of the period, nitrogen loss occurring in one or two short periods in which the larger part of the potato-portion was replaced by fruit. For one part of the experiment, lasting nineteen days, nitrogen equilibrium was maintained on an intake of 3·5 gm. per day (22 gm. of protein). On the potato diet employed it was impossible to reduce the nitrogen intake further without diminishing the heat value below that found essential for the work that he was accomplishing ; this was 4,000 calories per day. The subject was working fourteen to sixteen hours daily, and extremely active.

During a second experimental period he performed hard work as a mason and labourer for ninety-five days. On a diet of 5,000 calories, with an average nitrogen intake of 7·22 gm. per day, he lost 34 gm. of nitrogen during the whole period. During the last ten days nitrogen equilibrium was maintained on 5·72 gm. intake (35·75 gm. of protein), and his condition was perfectly normal.

Hindhede maintained nitrogen equilibrium on himself while doing light work on a protein intake of 16 gm. per day, his diet providing 2,650 calories. On a student doing moderate work equilibrium was maintained on 25 gm. of protein and a diet containing 3,700 calories.

Still more recent work, in complete agreement, is reported by

Kon and Klein (1928). A 65-kilogram man was maintained in nitrogenous equilibrium and in good health for 167 days, with but slight loss of body-weight, on a daily intake of 5·7 grams of nitrogen. A 64-kilogram woman maintained a similar balance for the same period on an intake of 3·8 grams of nitrogen per day.

While it is evident that the average person consumes much more than these "minimum" figures, caution is usually exercised in drawing conclusions from them as to the *desirable* protein minimum, and it has been suggested that such low-protein diets lessen immunity to disease. But it would certainly appear that a diet providing 1 gm. of protein per kilogram body-weight contains an ample amount. On the other hand, there is no evidence that excess of protein is in any way harmful. The mighty Norsemen were great flesh eaters, and the Australians, whose average meat consumption is greater than that of any other large community, can surely be regarded as their inheritors of might, while the Bengali, shown by McCabe's studies to have an average protein intake of 0·7 gm. per kilogram, though apparently a healthy race, are inferior physically to the average European, are particularly deficient in capacity for muscular work in spite of their large carbohydrate diet, and are susceptible to kidney trouble.

The Calculation of a Diet. Knowing the total calorie value to be given, and the relative amounts of protein, fat and carbohydrate that should be used, it is fairly easy to make up correct dietaries by reference to the standard tables that have been constructed for different foodstuffs, both cooked and uncooked. The calorie value can be distributed in various ways.

Supposing three diets contained respectively (i.) 70 gm. of protein and 400 gm. of carbohydrate, (ii.) 90 gm. of protein and 350 gm. of carbohydrate, and (iii.) 125 gm. of protein and 250 gm. of carbohydrate, then the amounts of fat required to total 2,500 calories are calculated as follows—

(i.) Protein . . 70 × 4·1 = 287 cals.
Carbohydrate . 400 × 4·1 = 1,640 cals., a total of 1,927, leaving 573 calories to be provided from 573/9·3 = *61·6 grams of fat.*

(ii.) Protein . . 90 × 4·1 = 369 cals.
Carbohydrate. 350 × 4·1 = 1,435 cals., a total of 1,804, leaving 696 calories to be provided from 696/9·3 = *74·8 grams of fat.*

(iii.) Protein. . 125 × 4·1 = 512·5 cals.
Carbohydrate. 250 × 4·1 = 1,025 cals., a total of 1,537·5, leaving 962·5 calories to be provided from 962·5/9·3 = *103·5 grams of fat.*

Obviously, if any two of the three be fixed the third can be at once determined.

If a diet be called for to meet the following specific requirements—

Protein .	85 grams, equivalent to	348·5 cals.
Carbohydrate	300 grams, equivalent to	1,230 ,,
Fat . .	100 grams, equivalent to	930 ,,
	Total	2,508·5 cals.

then, utilising tables of food values, the meal-values shown on p. 480 can be calculated, though obviously these are only approximate and based on averages.

In considering diet from an energy standpoint no account is taken of salt and vitamin contents, since these provide no energy, however much they may govern its exchanges. In planning a dietary such materials must be selected as will provide a sufficiency of vitamins, while cooking usually provides enough sodium chloride, and the other salts are usually present sufficiently in the ordinary food. The above meals probably do not contain sufficient vitamins.

Alcohol has been sufficiently dealt with in Chapter XXVII. Provided only small doses are ingested, over 90 per cent. is oxidised, and to that extent provides energy. Other food material can be decreased to a corresponding extent.

Content and Calorie Values of Typical Meals

Food.	Weight	Protein.	Fat.	Carbohyd.	Calories.
Breakfast—	gm.	gm.	gm.	gm.	
Bacon, one slice	30	3·15	19·44	0·00	194
Boiled egg, one	50	6·60	6·00	0·00	83
Brown bread, two slices, 4 × 4 × 0·5 in.	160	8·64	2·88	75·36	371
Butter, one ball	15	0·15	12·75	0·00	119
Coffee or tea, one large cup = ¼ cup of milk and two cubes of sugar	—	2·06	2·50	17·12	102
	—	20·60	43·57	92·48	869
Dinner—					
Soup, tomato	125	2·99	9·40	6·36	126
Mutton, boiled, lean, one slice	75	23·18	3·38	0·00	126
Boiled potato, one, av. size	150	3·75	0·15	31·35	145
Carrots, three tablespoonfuls	100	0·53	0·17	3·39	18
Baked custard pudding, containing two cups milk, two eggs, ¼ cup sugar	134	7·31	7·42	20·50	183
Bread, one slice	80	4·32	1·44	37·68	186
Coffee or tea, as above	—	2·06	2·50	17·12	102
	—	44·14	24·46	116·40	886
Supper—					
Bread, two slices	160	8·64	2·88	75·36	371
Butter, one ball	15	0·15	12·75	0·00	119
Egg, one boiled	50	6·60	6·00	0·00	83
Cheddar cheese, one tablespoonful	20	5·54	7·36	0·82	95
Tea or Coffee (as above)	—	2·06	2·50	17·12	102
	—	22·99	31·49	93·30	770
Total food fed	—	87·73	99·52	302·18	2,525
Total called for	—	85	100	300	2,508·5

INANITION

Continued deficiency of any food-constituent essential to life, which the body cannot itself manufacture, constitutes a starvation, and sooner or later the organism will as a result die, whether the item wanting be merely a single amino-acid, such as tryptophane, an insufficiency (total or partial) of calorie-producing material, or a complete absence of food and water.

The survival period and the series of pathological events intervening vary with the nature and degree of the deficiency. Death results most rapidly (within a few days) through lack of water. Total lack of solid food, with a sufficiency of water, only leads to death very slowly. Professional fasters have often carried out fasts lasting several weeks without any subsequent deleterious effects, and non-professional fasters, including many students of metabolism, have frequently fasted for shorter periods without their daily occupation being in any way interfered with. And while during the first twenty-four hours there may be a definite degree of discomfort, subsequently, for at any rate two or three weeks, no discomfort is experienced. Dogs have been caused to fast for much longer periods.

Many studies have been carried out on such subjects, and we know many of the changes such treatment produces in the organism. Naturally one of the results most obviously to be expected is loss of weight. In the longest recorded fast on a dog, Howe, Mattill and Hawk found that in 117 days, during which it was given 700 gm. of water daily, but no solid food, while it remained in apparently normal health, its weight fell from 26·3 to 9·76 kilograms. Subsequently the dog was given a rest on a farm for several months and fully regained its normal weight and original physical condition. It was then subjected to a similar second fast of 104 days, without any permanent harmful results.

During such fasts the organism must draw upon its own tissues for tissue repair essential to life and for material for

heat and work production. Day-to-day examination of urine, measurement of respiratory quotient, etc., indicate the following main changes :

During the first twenty-four hours the liver glycogen reserves are largely depleted and brought towards a minimum figure. As soon as this has happened protein consumption increases, since the organism has to draw upon its protein to provide sufficient glucose to metabolise its fat properly. In absence of much glycogen storage the marked initial rise in protein catabolism takes place in the first and not in the second twenty-four hours.

Thus Benedict found that a subject catabolised 181·6 grams of glycogen and eliminated 5·84 grams of nitrogen in the first 24 hours, in the second similar period the respective figures being 29·7 grams of glycogen and 11·04 grams of nitrogen. A second subject, who only catabolised 64·9 grams of glycogen in the first and 23·1 grams in the second period, excreted respectively 12·24 and 12·45 grams of nitrogen.

After this preliminary withdrawal of glycogen the body settles to a steady state of catabolism of fat and protein. For animals in previous good condition (*i.e.*, with a definite fat reserve) the amount of protein catabolised per day bears a constant relationship to the total catabolism and to the (gradually lessening) body-weight.

Benedict's record of a seven days' fast illustrates the constancy of this protein and fat catabolism (No. 75 on S.A.B.). The essential figures are shown in Table XXX.

The initial body-weight was about 65 kilograms. The methods outlined in the last chapter were used to calculate the various amounts of protein, fat and carbohydrate lost from the body. It will be observed that the differences between the observed and calculated loss of weight and heat production are negligibly small.

During prolonged fasts there is a steady fall in nitrogen excretion. In man Lusk concludes, " About 3 gm. of nitrogen in the urine or a daily destruction of 18·75 gm. of protein

would seem to be the lowest extreme of protein metabolism in the emaciated organism after a prolonged fast." In experiments on animals carried to the extreme there is a sharp *pre-mortal* rise of nitrogen excretion. The actual duration of life in a starvation experiment depends on the initial fat content of the subject. The smaller this amount the quicker death ensues. The actual cause of death seems to be due to a reduction of activity of one or more of the organs essential to the living process, either through too great a reduction of their supply of nutrient material or too great a damage to their tissues.

TABLE XXX. METABOLISM DURING A SEVEN DAY FAST

Day.	Measured loss of body-weight.	Calculated loss of body material.	Protein.	Fat.	Glycogen.	*Heat Production.*	
						Measured.	Calculated.
	gm.	gm.	gm.	gm.	gm.	Cals.	Cals.
1	44·00	29·52	73·4	126·4	64·9	1,796	1,765
2	723·00	722·92	74·7	147·5	23·1	1,790	1,768
3	685·00	690·89	78·1	153·0	5·4	1,785	1,797
4	894·00	907·93	69·8	144·7	25·2	1,734	1,775
5	450·00	452·93	65·2	144·7	8·2	1,636	1,649
6	391·00	392·54	64·4	129·8	21·7	1,547	1,553
7	497·00	489·22	60·8	132·5	18·7	1,546	1,568
Total	3,684·00	3,685·95				11,834	11,875

To what extent do the various tissues suffer during a period of starvation ? Voit answered this question by taking two cats of nearly equal weight, feeding them equally for ten days, so that it may be supposed that the weights and composition of the different organs were approximately equal, then killing and analysing the tissues of one, to serve as standard, and starving the other for thirteen days, and then killing it and analysing its tissues. It lost one-third of its weight during the starvation period. Voit's results are shown in Table XXXI.

The table shows that fat almost completely disappears, and that the tissues of least importance, judged by the extent of their depletion, are in order spleen, liver (but presumably this is due to its huge bulk, and to the initial store of fat and glycogen), testes and muscle. The vital tissues of the heart, and brain and cord, are least affected.

Blood shows but little change in composition during starvation; activity is not much affected till shortly before death, nor is the body temperature until the pre-mortal stage is reached, when it falls.

No evidence has been recorded that during these starvation experiments there are any changes attributable to vitamin-lack. This is presumably for two reasons, the relative shortness of duration of most of such experiments and the initial vitamin-store in the body of the subject.

TABLE XXXI. LOSS OF WEIGHT OF DIFFERENT TISSUES IN A STARVED CAT

Tissue.	Supposed weight of organs before starvation.	*Loss of weight.*	
	gm.	gm.	per cent.
Bone	393·4	54·7	13·9
Muscle . . .	1408·4	429·4	30·5
Liver. . . .	91·9	49·4	53·7
Kidney . . .	25·1	6·5	25·9
Spleen . . .	8·7	5·8	66·7
Pancreas . . .	6·5	1·1	17·0
Testes . . .	2·5	1·0	40·0
Lungs . . .	15·8	2·8	17·7
Heart . . .	11·5	0·3	2·6
Intestines . . .	118·0	20·9	18·0
Brain and Cord . .	40·7	1·3	3·2
Skin and hair . .	432·8	89·3	20·6
Fat	275·4	267·2	97·0
Blood . . .	138·5	37·3	27·0
Remainder. . .	136·0	50·0	36·8

Faddist Diets. Man, like his nearest relatives the higher apes, is omnivorous, and not a vegetarian. Anatomically his alimentary canal differs from those of true vegetarians, cattle, horses, rabbits, etc. From theoretical considerations it is evident that he would obtain the mixture of amino-acids closest to his requirement if he ate the flesh of his own kind, and gets the next best mixture as a flesh eater. The mixture of amino-acids derived from plant food leads to greatest amino-acid wastage and therefore least economy for the organism.

The average vegetarian is merely a pseudo-vegetarian, and consumes such animal food as milk, cheese and eggs. The true vegetarian not only is uneconomical in his protein food-supply, but takes excessive ballast, large amounts of undigestible cellulose, and, as a result, excretes an unduly large amount of fæces, which contain an unduly large amount of non-utilised but utilisable food. The vegetarian is usually less virile, as comparison between the Bengali and the more virile flesh-eating races of India exemplifies.

On the other hand, excessive ingestion of animal protein may lead to excessive bacterial putrefaction in the intestines, and in diet, as in many other of his habits, strict moderation is most commendable and most beneficial for man.

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CHAPTER XXXII

THE CHEMISTRY OF RESPIRATION. PART II

Tissue Respiration and Muscular Activity

In the first chapter of this text-book stress was laid upon the underlying uniformity of biochemical processes, whether applied to the single-celled organism or the most highly differentiated multiple-celled organism such as man. In the intervening chapters many of the chemical changes which occur in living processes have been described, from both the qualitative and quantitative (energy) points of view. But in the final analysis it is necessary again to return to the single cell, as a unit within the organism, in considering closely the formation of heat by biological oxidations and the production of work in that type of cell capable of this function. A recent statement of Meyerhof may be slightly altered and extended to read as follows: "Our present knowledge is not yet sufficient to form a satisfying and coherent description of the chemistry of muscular and other tissue activity, but we may confidently hope that in a few more years a working hypothesis will emerge and bring some degree of order into the mass of new facts which we are accumulating." If and when such a coherent explanation becomes available for even one type of cell, almost automatically we shall be able to extend it to all the others which make up the complete organism, and it is even possible that some light will be thrown upon what is perhaps the most fundamental and is certainly at present the least elucidated problem of all, the cause of growth and the proliferation of the cell, with its important associated problem,

the cause of improper growth, whether that be benign or malignant.

In the meantime it is only possible to outline some of the new facts to which Meyerhof refers, especially those appertaining to oxidation and muscular activity. Before passing to these, since reference to the cell suggests that only a very minute protoplasmic unit is under consideration, it seems desirable to stress the great magnitude, molecularly, of this minute cosmos. The cell is a mass of molecules, and perhaps of molecular aggregations, exhibiting extreme variations in size. Each large colloidal molecule or system carries on surface reactions controlled by osmosis, adsorption and imbibition, by variations in *p*H, and by the selective interchange within and without the cell permitted by the lipide nature of the cell envelope and probably also by various intracellular membranes, at each of which Donnan equilibria play a part.

That such a cell is indeed a little cosmos, a *multum in parvo*, is exemplified by even a partial numerical enumeration of its constituents. Such an enumeration can most easily be made at present for the red blood corpuscle. From the average known weight of the human red cell, which, on the basis of 5 million red cells to a cubic millimetre of blood, a blood cell volume of 45 per cent., and cell specific gravity of 1·055, is $0{\cdot}95 \times 10^{-10}$ gm., and the generally accepted weight of a single atom of hydrogen, $1{\cdot}63 \times 10^{-24}$ gm., it is possible to calculate that a single red blood cell of average size, in venous blood, contains almost a million million molecules of water (as H_4O_2), and the following numbers of atoms and molecules, as the case may be, *in millions :* potassium, 6,300 ; chloride, 2,800 ; bicarbonate, 1,000 ; hæmoglobin, 300 ; phosphatide (calculated as lecithin), 300 ; glucose, 295 ; urea, 295 ; cholesterol, 230 ; glutathione, 52 ; ergothioneine, 44 ; creatine, 26 ; and uric acid, 7 ; the number of hydrogen ions is 220,000, merely one-fifth of a million. Even such a small unicellular organism as the *Bacillus coli*,

with a weight only about one-sixtieth of that of the red corpuscle, has a molecular content of some thousands of millions, a number sufficiently large to permit a multiplicity of metabolic processes.

Oxidations and Biological Oxidations

It has already been pointed out in Chapter XVI. that tissue respiration involves the idea of a combustion, an oxidation. "Oxidation," in its original sense, meant the process of combination of an element or compound with oxygen. "Reduction" connoted the reverse process, the withdrawal of oxygen from a compound containing it. Oxidation and reduction are simultaneous processes; the agent used to bring about "reduction" does this by itself becoming oxidised.

The ideas underlying the conception of oxidation and reduction have been extended to include changes in which the element oxygen is not involved, but which are nevertheless styled oxidations and reductions. Removal of hydrogen from a compound is spoken of as an oxidation; addition of hydrogen as a reduction. When stannous chloride reacts with mercuric chloride it *reduces* the latter to mercurous chloride and then to mercury, and in so doing is oxidised to stannic chloride.

Looking at oxidation and reduction from the broadest point of view, therefore, oxidation is a process which involves the addition of oxygen or some other acidic atom or radical, or the removal of hydrogen or some other basic atom or radical, while reduction is the opposite process. The changes are usually accompanied by alterations in the valency of the element to which the oxygen (or equivalent atom or radical) becomes attached, or from which it is removed, these changes involving transfers of electrons.

Typical oxidising agents, in decreasing order of activity, are nitric acid, solutions of permanganate, and hydrogen peroxide. Oxidation by hydrogen peroxide is considered to

parallel many biological oxidations. Thus Dakin has shown that hydrogen peroxide oxidises indole to indoxyl, glucose to glycuronic acid, and butyric acid to β-hydroxybutyric and acetoacetic acid, all changes which occur in the living organism.

In considering the types of oxidation which occur in the living organism it is convenient to consider first of all the agents effecting these changes, and subsequently to deal with certain of their actions, so far as at present any connected account can be given of them. These agents include surface catalysis, certain specific enzymes, certain compounds of relatively simple and determined composition, such as glutathione, and certain more complex compounds, concerning the nature of which we have some idea, such as the derivatives of hæm which constitute cytochrome.

Whatever the chemical nature of these agents, their action is catalytic, and they illustrate the danger of emphasising too greatly the limitation of the idea of biological catalysts to compounds which are termed enzymes because the act of boiling their solutions results in their decomposition. This decomposition is merely associated with the relatively large and easily altered molecules of these enzymes, and is not inherently connected with their enzymic properties.

Many catalysing agents inducing oxidation are probably systems of two or more substances, each of which alone is inactive. Such systems are termed "autoxidisable systems." An autoxidation is a catalytic reaction, in which a product of the reaction catalyses it to produce oxidation.

Surface Catalysis. To illustrate the possible *rôle* of surface in producing oxidation one need only again refer to the action of platinum black in catalysing the oxidation of hydrogen by gaseous oxygen. That it may well play a *rôle* in oxidations within the cell is shown by Warburg's experiments with solutions of glycine and cystine, and finely divided charcoal. Such solutions, alone, are unaffected by

gaseous oxygen, but in presence of the charcoal they are oxidised to ammonia and carbon dioxide (and sulphate). Presence of traces of iron oxide in the charcoal accelerates the changes ; according to Warburg the metal activates the oxygen.

Enzymic Oxidising Catalysts. There is considerable confusion in the terminology of these enzymes. Various terms such as *oxidase*, *oxygenase*, *peroxidase*, and *catalase* are employed. Catalases do not oxidise. It is doubtful whether much is to be gained by differentiating at present between the enzymes that actually produce oxidation, until the nature of their action is more exactly understood.

Typical of oxidising enzymes are *tyrosinase*, which oxidises tyrosine to a red indole-derivative through the stage dihydroxyphenylalanine, and *dopa-oxidase*, which transforms the latter substance to the same red compound, this subsequently changing to a melanin (see p. 345). *Guanase*, *adenase* and *xanthine oxidase* in mammals cause the oxidation of guanine and adenine to uric acid (see p. 358). The *laccases* bring about the hardening of lacquers by oxidising polyphenols. Laccase preparations usually have a high content of manganese, and in the absence of manganous salts laccase displays but negligible activity.

Brailsford Robertson has pointed out that an excellent example of the promotion of oxidation by the action of metals which are capable of being oxidised in different degrees, is furnished by the familiar reaction between glucose and Fehling's solution when it is improperly carried out. This alkaline copper tartrate when boiled with excess of glucose solution is decolorised, and red cuprous oxide is precipitated. If the solution is then cooled in a shallow vessel, the cuprous oxide takes up oxygen from the atmosphere, and reforms the complex blue cupric tartrate. Re-boiling causes fresh reduction, and re-cooling fresh oxidation, and so on, the cuprous oxide acting as a *carrier* of oxygen to the glucose.

According to the theory of Bach and Chodat the majority of oxidising enzymes are systems made up of an oxygenase, which acts as a carrier of oxygen, and a peroxidase, which causes the

transfer of oxygen from the oxygenase to the compound undergoing oxidation. Frequently the oxygenase can be replaced by hydrogen peroxide, as in the reaction between blood or tissue extracts and tincture of guaiacum, when, in the presence of the peroxide a polyphenol in the tincture is oxidised to a blue compound. It seems quite possible that the so-called oxygenases are not enzymic, but are metallic compounds of varying degrees of complexity, such as the manganous salts in laccase preparations, and the iron-compound hæmoglobin, which can also react with guaiacum. It is, at any rate, certain that such metallic compounds can function in the same way as oxygenases.

Catalase is widely distributed in living tissues. It does not accelerate oxidation, but on the contrary retards it by decomposing peroxides. Its presence in tissues is detectable and its typical action is displayed when hydrogen peroxide is added to tissue extracts ; oxygen is liberated. As will be seen later, it is quite probable that hydrogen peroxide is normally a metabolic product, and that the function of catalase is to depress its concentration.

Bioluminescence, exhibited by many animal organisms, and characterised by maximum transformation of energy into light with minimum (imperceptible) production of heat, is brought about by the oxidation of a protein-like substance, named by Newton Harvey " luciferin," by an enzyme-like, but thermostable substance, " luciferase."

Sulphydryl Compounds. Through the amino-acid cysteine the sulphydryl group — SH is widely distributed in living organisms. This radical probably possesses inherently properties which aid in catalysing oxidation. Anchored in glutathione, it becomes a powerful catalyst. It is not impossible that the powerful action exhibited by insulin and other compounds containing cysteine radicals is traceable to the sulphydryl radical.

Heffter, in 1908, put forward the idea that the sulphydryl group of cysteine took some part in oxidation processes ; this led to the intensive study of reactions associated with this radical. Meyerhof (1918) showed that yeast, killed and precipitated by acetone, and thereafter dried, would, in the presence of thioglycollic acid, $HS . CH_2 . COOH$, take

up oxygen to a far greater extent than is required to oxidise the sulphur of the SH radical.

Numerous investigators have shown that Arnold's reaction, the production of a purple colour with sodium nitroprusside and ammonia, given by sulphydryl derivatives, is also given by aqueous extracts of many tissues. In 1921 Hopkins announced the discovery of glutathione, to which is undoubtedly due this general property of tissues. As is now known (see p. 259) glutathione is a tripeptide, glutamyl-cysteyl-glycine, in which the — SH radical is free to react. Hopkins showed that washed muscle tissue yields a heat-resistant compound, which, when mixed with glutathione, forms a system capable of absorbing atmospheric oxygen. This, he suggested, is brought about by successive oxidations and reductions of glutathione. These may be represented by the equations :

$$2G{-}SH + O = G{-}S{-}S{-}G + HOH$$
$$G{-}S{-}S{-}G + MH_2 = 2G{-}SH + M$$

in which "reduced" glutathione is converted into "oxidised" glutathione through conversion of two cysteine radicals to one of cystine, and *M* acts as a hydrogen carrier.

Meyerhof suggests an alternative scheme, in which a hypothetical peroxide functions :

$$2G{-}SH + O_2 = (G{-}SH){-}O{-}O{-}(HS{-}G)$$
$$(G{-}SH){-}O{-}O{-}(HS{-}G) + M = 2G{-}SH + MO_2$$

and in this scheme *M* acts as an *oxygen-acceptor*.

Traces of iron or copper compounds seem necessary for these reactions.

Meyerhof believes that the oxygen-acceptor in tissue consists of lecithin, and especially the unsaturated fatty acid radicals of lecithin, such as that of linolenic acid (with three double bonds). Thunberg has shown that lecithin is autoxidised by ferrous salts, and Warburg that linolenic acid is oxidised to the same extent, lending support to Meyerhof's

view ; the amount of oxygen taken up suggests that the linolenic acid is converted into a peroxide compound.

Meldrum and Dixon, in Hopkins' laboratory, have shown that pure crystalline glutathione is much less reactive than impure preparations, but that addition of cysteine greatly increases the activity.

The Hæm Compounds and Respiration. Hæm has been shown to catalyse the oxidation of many substances, especially unsaturated fatty acids. Its derivatives, the hæmochromogens of cytochrome, play an important part in tissue respiration. The wide distribution of cytochrome has already been mentioned (see p. 204). Cytochrome readily takes up oxygen from air, and readily loses it in cell metabolism. It is believed to be responsible for the thermostable " peroxidase " reaction of the cell, whereby, in the presence of hydrogen peroxide, compounds such as guaiacum are oxidised. Tissues contain an enzyme responsible for the oxidation of cytochrome, while it is reduced by cell metabolites acting as hydrogen carriers.

Peroxide Formation and Dehydrogenation. Ordinary molecular oxygen, gaseous or in solution, is somewhat inert. Such relative inertness is obvious when the activities of gaseous oxygen and of ozone are contrasted. Various mechanisms have been postulated to explain the transformation of this inert molecule into a form capable of functioning actively.

Traube, and subsequently Bach and Engler, developed the idea of *peroxide formation.* The oxidation of hydrogen on the surface of palladium was represented :

$$\begin{array}{l} PdH + HO{-}{-}H \\ PdH + HO{-}{-}H \end{array} \begin{array}{l} \quad O \\ + \;\| \\ \quad O \end{array} = \begin{array}{l} Pd + H_2O \\ Pd + H_2O \end{array} + \begin{array}{l} H{-}O \\ \quad | \\ H{-}O \end{array}$$

and the hydrogen peroxide so produced could then act upon any oxidisable substance present.

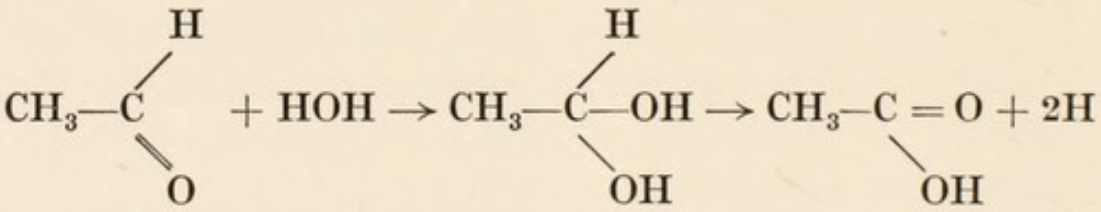
The autoxidation of benzaldehyde into benzoic acid was

represented as due to the intermediate formation of a peroxide which thereby catalysed the reaction :

$$C_6H_5 . CHO + O_2 = C_6H_5 . CO . O . OH$$
$$C_6H_5 . CO . O . OH + C_6H_5 . CHO = 2C_6H_5 . COOH.$$

The application of the peroxide theory by Bach and Chodat to explain enzymic actions causing oxidation has been dealt with.

A contrary view of oxidation has been developed by Wieland, the theory of *dehydrogenation*, in which, instead of oxygen, hydrogen is activated, thereupon uniting with a *hydrogen-acceptor*, the action being brought about by a specific enzyme, a *dehydrase*. Thus the oxidation of acetaldehyde to acetic acid may be represented by an initial combination with water, followed by a dehydrogenation :

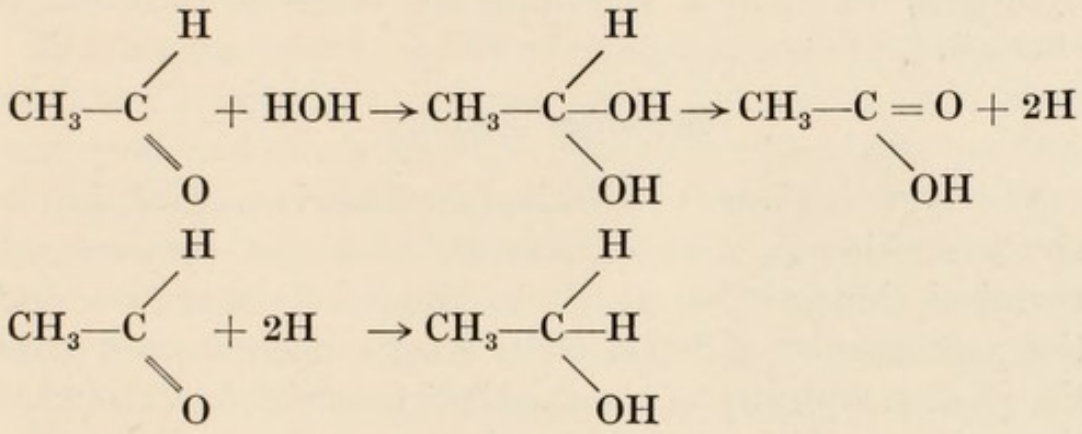


The activated hydrogen, in presence of oxygen (which in this case is the hydrogen-acceptor) gives water :

$$2H + \tfrac{1}{2}O_2 = HOH$$

In absence of oxygen, *i.e.*, under anaerobic conditions, if the hydrogen can be removed from water oxygen becomes available for oxidation. This can be illustrated by the so-called Schardinger reaction of fresh milk. When methylene blue is added to milk, nothing happens ; milk will not oxidise an aldehyde if that be added to it. But when both methylene blue and an aldehyde are added to the milk then some catalysing agency present in milk causes simultaneous reduction of the methylene blue and oxidation of the aldehyde. The method of oxidation of acetaldehyde has just been set forth, and the activated hydrogen immediately reduces the methylene blue to its colourless leuko-base.

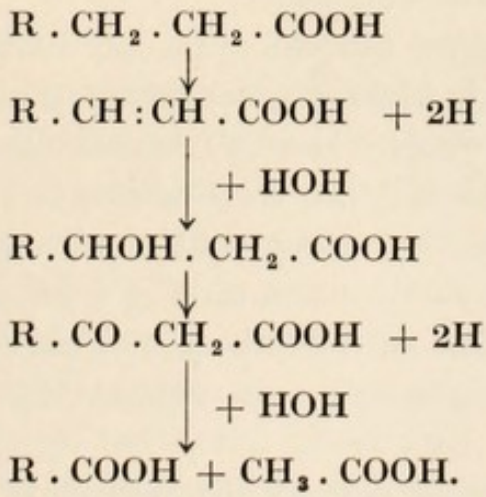
A similar example is afforded by the so-called Cannizaro reaction, in which an aldehyde suffers simultaneous change to alcohol and acid, and which is brought about in the organism by enzymic stimulus :



Thunberg has shown that muscle tissue contains an agent, presumably an enzyme since it is destroyed by boiling, which in the absence of oxygen can cause reduction of methylene blue in the presence of succinic acid or of certain other organic compounds. Succinic acid is oxidised to fumaric acid, the hydrogen thereby made available reducing the dye :

$$COOH . CH_2 . CH_2 . COOH \rightarrow COOH . CH : CH . COOH + 2H.$$

This type of reaction has suggested that the breakdown of fatty acids may take place in the presence of suitable hydrogen-acceptors in the following way :



It seems probable that both peroxide formation and

dehydrogenation are employed by the organism to bring about oxidation.

As examples already quoted indicate, in many anaerobic oxidations water functions by providing both oxygen and hydrogen, so that a simultaneous *oxidation-reduction* is effected.

Muscular Activity

The type of tissue respiration that has been most intensively studied is that of muscle, with the concomitant chemical changes that result in muscular contraction and the performance of work. The results illustrate not only the great complexity of the reactions involved, but also that there is a striking similarity between the carbohydrate metabolism of muscle and the fermentation of carbohydrate by yeast ; they present some glimmering of an idea of the fundamental similarity underlying the whole of such processes.

In considering the bearing of the metabolism of the muscle cell upon its heat production, and upon the contraction of muscle whereby work is performed, it is obviously necessary to consider all the chemical changes involving an oxidation and thereby the production of heat, and all the changes, chemical or physical, which can have a bearing upon the shortening of the muscle fibre.

An isolated striped muscle, stimulated artificially through its nerve, responds after a short latent period with a sharp contraction, followed by slower relaxation. As a result of a rapid series of stimuli the contraction is maintained, as a condition of *tetanus*. Long-continued stimulation brings on fatigue, when further stimulus is ineffective. The continuous contraction of voluntary muscle in the organism is believed to be analogous in nature to the condition of tetanus.

Extremely delicate devices for heat measurement such as sensitive thermopiles and compensation micro-calorimeters

have been employed for measurement of heat production during muscular contraction. Using such instruments it has been shown that a single contraction of a frog's muscle produces an increase of temperature of from 0·001° to 0·005° C.

In the experimental study of muscular activity investigations of the chemical changes and of the energy exchanges have gone hand in hand, such close association between the schools of Hopkins, Embden, Hill, Meyerhof, and others, frequently aiding rapid elucidation of both types of problems. In this account it is perhaps simpler to give first a *résumé* of the present state of our knowledge of the chemical changes, and then to deal shortly with the energetics of muscle.

The two most important reactions associated with muscular contraction are the formation of lactic acid from glycogen, and of creatine and phosphate from phosphocreatine. Of the two the changes associated with phosphocreatine are even more important.

It has been pointed out in Chapter XXII. that the glycogen-lactic acid change is largely reversible. Immediately following normal muscle contraction, lactic acid is formed from glycogen, whilst during the rest period which ensues the lactic acid disappears, a coupled reaction occurring in which about 80 per cent. is reconverted to glycogen, whilst the remaining 20 per cent. is oxidised. In the absence of oxygen, as for example in a muscle under experimental conditions in an atmosphere of nitrogen (or in presence of insufficient oxygen, as in fatigued muscles), lactic acid accumulates, but subsequently, if oxygen becomes available, the lactic acid is used up under precisely the same conditions as those just stated.

The change in phosphocreatine or phosphagen slightly precedes that of glycogen under normal conditions, but is differently affected in abnormal conditions. Meyerhof and his co-workers have shown that while, in absence of oxygen, initially in contracting muscle the rate at which phosphate

is set free from phosphocreatine may be three or four times as great as that of the production of lactic acid, with the onset of fatigue the ratio decreases very rapidly, until in extreme fatigue scarcely any phosphocreatine is hydrolysed, although lactic acid is still being steadily produced. They have found that the rate of hydrolysis of phosphocreatine is parallel to that of the speed of excitation of the muscle. For example, at 8° C. the speed of excitation is half that at 24° C., and so is also the rate of hydrolysis of the creatine derivative.

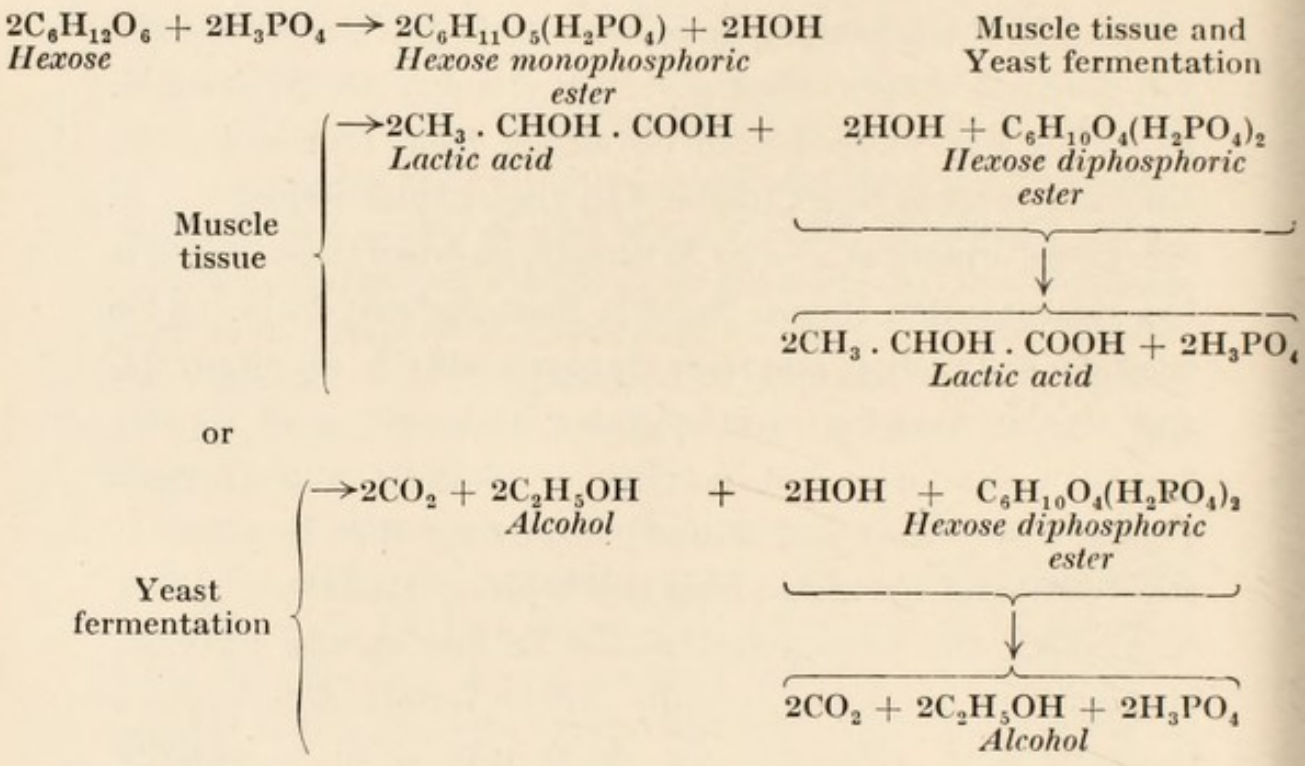
Further illustrating the greater dependence of changes in phosphocreatine on muscle contraction are Lundsgaard's experiments. He has shown that when muscle is poisoned by iodoacetic acid it can contract without producing lactic acid at all, although phosphocreatine is broken down as usual; when it is completely decomposed the muscle passes into rigor.

Lundsgaard experimented with frog muscle. He showed that when frogs are poisoned by the injection of 40 mg. of iodoacetic acid or of bromacetic acid they become completely rigid after one hour. If, however, the sciatic plexus is cut before poisoning, after an hour the limbs still appear normal. When nerve-muscle preparations from such limbs are stimulated, 60 to 100 muscle twitches can be obtained, and these are apparently completely normal in type. The twitches finally cease, contracture develops rapidly, and within a few minutes the muscle is in complete rigor. *Throughout the period no lactic acid is produced. The muscles become distinctly alkaline in reaction.*

This work, which has been fully confirmed, shows conclusively that the hydrolysis of phosphocreatine is the primary change in normal muscular contraction. Meyerhof concludes, and the view has been accepted, that the glycogen-lactic acid changes, including oxidation of part of the lactic acid, merely provide the necessary energy for the resynthesis of phosphocreatine.

Considerable advances have been made in determining the intermediate stages when glycogen changes to lactic acid. Meyerhof has succeeded in obtaining from muscle by the Buchner process (see Chapter II.) the enzyme concerned in this transformation, and by Willstätter's adsorption methods has concentrated it and freed it from carbohydrate. This enzyme, so purified, converts glycogen, starch, amylopectin, and similar complex carbohydrates into lactic acid rapidly and with equal ease, but has little or no effect on the hexose sugars. Meyerhof has found, however, that if yeast is autolysed, and an extract from this is precipitated by addition of alcohol, the precipitate, added to the muscle enzyme, enables it to decompose rapidly all the fermentable hexoses to lactic acid, although the precipitate alone is equally inactive. He terms the active compound in this precipitate *hexokinase*, and regards it as an auxiliary enzyme. Along with the lactic acid hexose mono- and di-phosphates are produced ; after all the sugar has been used up the hexose phosphates are slowly broken down with the production of further lactic acid.

Such results have led Meyerhof to postulate a parallel series of changes for (i.) the formation of lactic acid in muscle, and (ii.) the formation of ethyl alcohol in yeast fermentation. Further stress is laid on such parallelism by the fact that the dialysable thermostable co-enzymes in the two processes are interchangeable and appear to have the same chemical properties. Until recently it was believed that methyl glyoxal was formed as an intermediate product in both processes, but Meyerhof has now come to the conclusion that the true intermediate stage is pyruvic acid, in muscle metabolism as well as in yeast fermentation. Meyerhof's scheme can be shown thus :



Such similarities between apparently widely differing processes suggest a common underlying principle of even wider application.

Considerable confusion has existed in the past concerning the hexose-phosphates postulated to be formed as intermediate products in yeast fermentation and in the production of lactic acid from muscle, nor is that confusion by any means dispelled. Some slight clarifying light has within recent years been thrown upon this aspect of the problem.

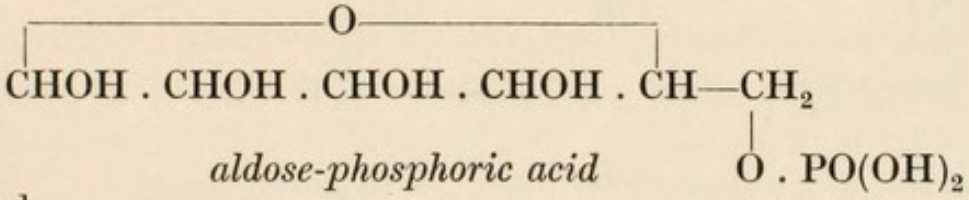
Harden and Young (1905) established the presence of a hexose diphosphate, $C_6H_{10}O_4(PO_4R_2)_2$, amongst the intermediate products of yeast fermentation. This is frequently referred to as Harden and Young's acid. In 1914 Embden and his co-workers showed the presence of a hexose-phosphate in muscle, and, since they considered it to be the immediate precursor of lactic acid, they named it " lactacidogen." It has been shown to be a monophosphate, $C_6H_{11}O_5(PO_4R_2)$. In addition, a hexose monophosphate has been obtained by Neuberg by partial hydrolysis of Harden and Young's acid, a second monophosphate by Robison, amongst the products

of the yeast fermentation of sugar, and a trehalose monophosphate by Robison and Morgan, when glucose is fermented by dried yeast. Further, muscle contains other important phosphates, phosphocreatine, (yeast) adenylic acid (adenine-ribose-phosphoric acid), and, in addition, pyrophosphate, recently (1928) discovered in muscle by Meyerhof's pupil Lohmann, and found to be widely distributed in other tissues.

It might reasonably be supposed that the metabolism of many of these phosphates must be closely interrelated.

Both aldose sugars (of the type of glucose) and ketose sugars (of the type of fructose) can form phosphate esters, and, following Pryde, their formulæ may be written :

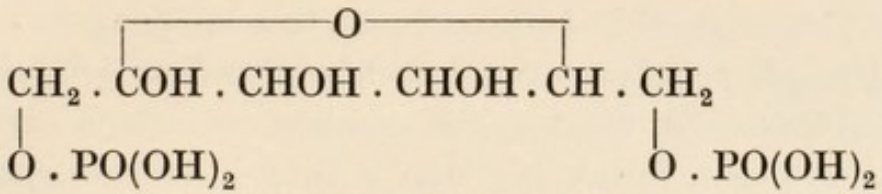
(A)



aldose-phosphoric acid

and

(B)



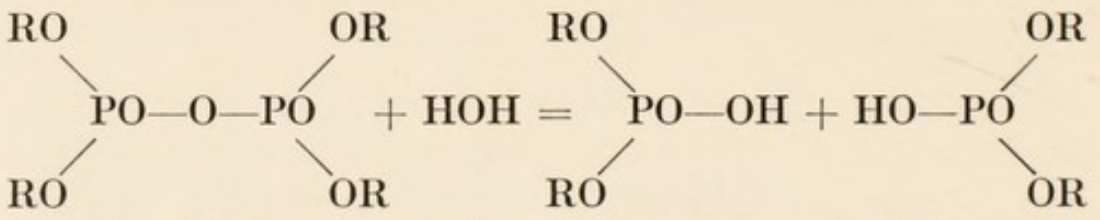
ketose-diphosphoric acid

The work of Lohmann, Robison and Morgan, and Pryde and Waters, has shown that Embden's, Robison's, the Harden-Young, and Neuberg's hexose-phosphates are all mixtures of aldose and ketose phosphates ; in the first two aldose-phosphates predominate to the extent of 80 or 90 per cent., while in the two latter the ratio is reversed.

The predominating constituent of Harden and Young's acid has the structure (B) shown above, while Embden's lactacidogen is mainly represented by (A), and this fraction is probably identical with the corresponding fraction of Robison's phosphate.

According to Meyerhof most of the hexose-phosphates are of only secondary interest from a biological point of view, since they probably take no part in the metabolism of living muscle, if indeed they occur there. It may be hoped that his viewpoint is a sound one.

Lohmann's pyrophosphate plays quite a different *rôle* to the hexose-phosphates. When muscle pulp is autolysed at 42° C. in presence of sodium bicarbonate there is a marked increase in inorganic phosphate. Embden considered that this was due to the hydrolysis of his lactacidogen, but Lohmann has shown that it is due to the hydrolysis of pyrophosphate :



Pyrophosphate *Orthophosphate* *Orthophosphate*

Pyrophosphate is found widely distributed in tissue-cells of all kinds, but is present in greatest concentration in muscle and in yeast, indicating that it may have some part in the catabolism of carbohydrates. It is not free in muscle, but is linked to adenylic acid, and Lohmann has shown further that this complex compound plays a *rôle* as complement of the co-enzyme necessary for the breakdown of glycogen. The purified lactic acid-forming enzyme of Meyerhof decomposes glycogen when phosphate and boiled muscle juice (from fresh muscle extract) containing the co-enzyme are added. A stale extract, boiled, is ineffective, but if then the adenyl-pyrophosphate is added, the co-enzyme in the boiled juice is rendered effective. Adenylic acid itself appears to possess the same property ; the explanation appears to be that muscle juice contains an enzyme which can rapidly synthetise adenyl-pyrophosphoric acid from adenylic acid and pyro-

phosphate. The presence of magnesium ions is also necessary for these changes.

Co-ordination of Agents and Reactions. Any attempt closely to associate the potential oxidative agents outlined earlier in this chapter, with the changes accompanying muscular activity that have just been described, is for the moment doomed to failure, but, we may hope, only for the moment.

The breakdown of complex carbohydrate to lactic acid is associated with a specific enzyme, discovered by Meyerhof, and the subsequent resynthesis of glycogen from most of the lactic acid, under changed energy condition resulting from oxidation of some portion of the lactic acid, may surely be associated with the same enzyme. Necessary to its action are a co-enzyme and pyrophosphate, both common to muscle and yeast. For the oxidation of the lactic acid many possible agents present themselves. Cytochrome, in the presence of hydrogen-acceptors and a dehydrase, is a possibility. Glutathione seems less likely, as a direct agent; its action on proteins and fats is more easily demonstrable than an action on carbohydrates. It may therefore be of significance that the muscle content of glutathione is relatively low.

Glutathione, on the other hand, has been shown to catalyse the oxidation of fats and proteins, and its association with unsaturated fatty acids as oxygen-acceptors along with the formation of unsaturated fatty acids by a specific enzyme (already demonstrated for succinic acid) opens up a promising vista of logical theory to explain the catabolism of fats. Some evidence is also being obtained that the sulphydryl group is an essential stimulus for nuclear and for cell division.

Energetics of Muscle

In the two preceding chapters energy was expressed in large heat units, and dealt with on relatively a large scale.

Energy is now to be dealt with almost microscopically, and the small calorie is more appropriate. This is the amount of heat required to raise 1 gm. of water from 15 to 16° C., and is usually written in *cals.* or *gm.-cals.*

In dealing with energy changes on the large scale, the methods of measurement were by direct measurement of heat produced, and by indirect measurement from knowledge of the chemical changes. Both the direct and indirect procedures have been applied to the study of energy changes in muscle.

Hill, in speaking of the advantages to be gained by utilising experiments involving measurements of heat, writes : " Methods of measuring temperature by means of electrical instruments can be made so refined that there is practically no limit to the sensitivity available ; if necessary, it is possible to read to a millionth of a degree ; it is easy to obtain photographically the deflection of a galvanometer recording the rise of temperature of a muscle stimulated by one shock—a rise of temperature no greater than 0·003° C., and this rise of temperature can be expressed in absolute units, its time-course can be analysed, the heat liberated in the initial phases of contraction can be separated from that liberated in the recovery process."

Hill and Hartree have carried out extraordinarily exact measurements of the heat changes in such processes. To quote further from the former : " A muscle is placed upon a thermopile, in oxygen or in nitrogen, and stimulated. The galvanometer deflects, and its movements are recorded photographically. By suitable methods the deflection can be analysed, to give us a picture of the actual time-course of the production of heat. There are found to be four phases in the heat production. Firstly, at the moment when the muscle is stimulated, there is a large and sudden liberation of heat ; then, as the stimulus is continued, heat continues to be liberated so long as the contraction is maintained ; thirdly, heat is again liberated while the muscle

relaxes. These three phases constitute the *initial process* in muscular contraction, and a striking fact immediately emerges from the observation. Both the magnitude and the time-course of the production of heat in the initial phase are quite independent of whether oxygen be present or not. . . . . Now follows a recovery process. When the muscle relaxes the heat production at first ceases. To outward appearance the muscle has returned absolutely to its former condition. If, however, it be in oxygen, the heat production now flares up again, its rate attains a maximum, and then falls back to zero, which it finally reaches in a period lasting for five minutes to half an hour, depending upon the temperature and on other circumstances."

Hill found that the heats developed in the initial process and in the recovery process were approximately equal. Hill and Hartree have recently found that with fresh muscle the average ratio of heat production in the two processes is 1 : 0·52. These figures give a clue to the ratio of lactic acid oxidised to lactic acid reconverted to glycogen. Accepting Meyerhof's figures (to be referred to shortly) for the heat produced in the initial process when 1 gm. of lactic acid is formed as 385 gm.-cals., and for the heat of combustion of glycogen (as hydrate) to produce 1 gm. of lactic acid as 3,782 gm.-cals., then if one molecule of lactic acid is oxidised for four molecules reconverted the total energy made available from the combustion of 0·2 gm. of glycogen (as hydrate) is 3,782/5, equal to 756 gm.-cals., of which 371 must be developed during the recovery phase, the ratio between the two phases being then 371/385 or 0·96. Similar relationships are shown in Table XXXII. The efficiency of the process, given in the final column, is measured by this ratio.

Such measurements show, therefore, that the functioning of muscle is associated with the combustion of one molecule of lactic acid for every four or five or six molecules reconverted into glycogen, the proportion reconverted probably

TABLE XXXII.

Ratio of mols. lactic acid oxidised to mols. reconverted to glycogen.	Total heat available from glycogen (hydrate) oxidised.	Heat produced in initial stage.	Heat produced during recovery.	Efficiency of process.
	cals.	cals.	cals.	
1 : 3	3,782/4 = 945	385	560	1·45
1 : 4	3,782/5 = 756	385	371	0·96
1 : 5	3,782/6 = 630	385	245	0·63
1 : 5·5	3,782/6·5 = 585	385	200	0·52

depending to some extent upon the condition of the muscle.

Approaching the problem from the indirect method of measurement, it is necessary to consider first the potential energy of the substances concerned, glycogen and lactic acid. Various estimations of the heat of combustion of glycogen have been made. Stohmann obtained the value 4,191 cals., Emery and Benedict 4,227 cals., and Roth and Ginsberg 4,188 cals. If glycogen is completely converted to lactic acid then exactly 0·90 gm. of glycogen $(C_6H_{10}O_5)_n$, with a molecular weight of $162n$, yields 1·00 gm. of lactic acid $(C_3H_6O_3)_{2n}$, with a corresponding weight of $180n$. Hence, accepting the mean of Stohmann's and the Roth-Ginsberg figures, 0·9 gm of glycogen can yield 3,770 cals. According to still more recent measurements made in Meyerhof's laboratory, the heat of combustion of glycogen, expressed for 1 gm. of hydrate in aqueous solution (so that the equivalence is 1·0), is 3,782 cals.

Emery and Benedict (1911) found that the heat of combustion of dilute lactic acid is 3,601 cals., and Meyerhof (1922) found precisely the same figure. Roth and Ginsberg found the value 3,603. With this close agreement the figure 3,602 can be accepted as accurate.

If the figure 3,782 be accepted for glycogen, then complete conversion of 1 gm. of glycogen (hydrate) to 1 gm. of lactic

acid should set free 180 cals. The actual heat set free under anaerobic conditions, which exclude the recovery phase, is according to Meyerhof's determination 385 cals., so that over 200 cals. have to be accounted for.

Heat is liberated when acids are neutralised, and the lactic acid produced in such muscular contraction is rapidly buffered. The third phase of heat production described by Hill and Hartree is probably to be referred to this neutralisation of the lactic acid. The buffering is attributed to protein, phosphate, and bicarbonate (by processes similar to those that have been described for buffer action in blood, *cf.* p. 224).

Meyerhof has shown that 140 cals. are produced when 1 gm. of lactic acid is neutralised by protein, but he considers, since the neutralisation by phosphate or bicarbonate produces only very little heat (only 19 cals. for phosphate), that the combined neutralisation process will not account for more than 100 cals. Hence a balance sheet would read :

	gm.-cal.	gm.-cal.
Actual heat production per gm. lactic acid formed	—	385
Heat set free by conversion of glycogen to 1 gm. of lactic acid	180	—
Heat set free by neutralisation of 1 gm of lactic acid	100	—
Unaccounted for	105	—
Totals	385	385

Hydrolysis of phosphocreatine liberates heat, to the extent of 120 cals. per gm. phosphoric acid liberated. The heat unaccounted for in the balance sheet comes from this source, which is the primary change responsible for the energy of contraction.

In such experiments as those of Hill and Hartree and of

Meyerhof, the heat production is measured under isometric conditions, *i.e.*, under conditions in which muscle is contracting against a weight of sufficient magnitude to prevent appreciable shortening. Under these conditions the work done is practically *nil*. According to experiments carried out by Fenn (1923) when muscle shortens it not only performs work, but produces more heat than under isometric conditions. Thus in a particular experiment, isometrically the work done was zero, and the heat produced 0·00073 erg, while, when shortening was permitted (in so-called isotonic experiments), a weight of 200 gm. was lifted to produce 0·00019 erg, while the heat produced was 0·00115 erg. Fenn's experiments appeared to show that the excess production of heat is roughly proportional to the work done, which would indicate that muscle adapts its energy expenditure to suit the amount of work it has to do.

Such experiments, if correct, suggest that still further experimental inquiry is necessary before a complete balance sheet can be written to include both work and heat. Nor at present can any definite statement be made as to the precise mechanism of the shortening of muscle fibres that sums up to muscular contraction. But the elucidation of these, as of other parts of the problem that are still unsolved, can safely be left in the hands of such savants as Hill and Meyerhof.

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SECTION VI

ADDENDA

CHAPTER XXXIII

AN INTRODUCTION TO THE CHEMISTRY OF IMMUNOLOGY

THE subject of immunology is a small but important branch of protein-chemistry. The methods by which it has so far been studied involve the technique of the bacteriologist. He has allowed himself full freedom in coining a terminology specific to the study. This terminology seems largely unnecessary ; it has led to the hiving off of immunology from biochemistry, and has emphasised too greatly the complexity of the subject.

When toxic compounds such as indole and *p*-cresol are formed within the gut by bacterial action, it has been shown (Chapters XII. and XVII.) that these are absorbed to the circulation, pass to the liver, and, being molecules of small size, they can then and do pass within the liver cells, where they are chemically acted upon so that non-toxic compounds are formed (such as indican, conjugated glycuronates and ethereal sulphates).

The larger protein molecules do not, under normal conditions, reach the circulation. They are almost incapable of passing through the epithelium of the gut, just as they cannot penetrate that of the skin, nor, in all probability, that of the surface of the placenta. When, by any chance, they do reach the circulation they act toxically, producing certain reactions which may reveal themselves by definite symptoms.

The body possesses a second line of resistance to cope with such emergencies. Since these large molecules cannot penetrate and be detoxicated in the cell the cell forms and excretes compounds which can unite with them. These have been inharmoniously named " antibodies," and the proteins which lead to their formation are termed " *antigens*." The proteins native to an animal

are not toxic to it ; they do not stimulate the formation of antibodies, although when altered by chemical treatment with such agents as formaldehyde, nitrous acid or iodine, they may then do so, but they are then foreign to the body. All naturally occurring proteins foreign to the body may lead to the production of antibodies, so that the term " antigen " is obviously superfluous.

The immunologist distinguishes between a toxic compound and a toxin. To him a toxin is a protein, or a substance with some similar complex molecule, with definite poisonous properties, which can cause in the body the formation of a specific immunising compound that will, through some sort of union or interaction, render the toxin non-poisonous, and so render the host *immune* to its action. Obviously his " toxin " is simply an especially toxic compound of large molecular size. The true nature of toxins is still uncertain.

Formation of antibodies can be easily demonstrated by simple experiments such as the following : Three to five intravenous injections of gradually increasing doses (one to five platinum wire " loopfuls ") of killed cultures of typhoid bacilli (containing the toxic protein) are injected into a rabbit at five or six day intervals. About two weeks after the last injection the animal is bled. Its serum then contains large amounts of the specific antibody whose production has been elicited by this treatment. This antibody possesses amongst other properties that of *agglutination*, *i.e.*, it will cause a clumping together of the particular bacteria (the typhoid bacilli) whose toxic protein led to its formation. This may be demonstrated in two ways.

Serum from such an immunised rabbit is diluted (one in fifty), and a drop of the diluted fluid is mixed with a drop of broth culture of typhoid bacilli on a clear cover glass, which is suspended over the cavity of a hollow ground slide ringed with vaseline (to prevent evaporation), and is then examined under the microscope. The bacteria form " clumps," with intervening clear spaces free from bacteria. The reaction may require from one-half to one hour.

A more accurate macroscopic method consists in transferring various dilutions of the serum into small sterile test-tubes, along with broth cultures of the bacteria. A flocculent sediment of bacteria gradually settles, leaving the supernatant liquid clear. (Such types of test are extremely important in diagnosing the presence of pathogenic bacteria in patients, since, as a rule, the antibody will only react with the bacteria whose toxic protein led to its formation.)

If, instead of dead bacterial cultures, germ-free filtrates from

them are employed, then the serum containing the corresponding antibody forms precipitates when added to the filtrate, and this *precipitin reaction* typifies the general reaction of most antibodies with the toxic compounds which elicited them.

Whether or not the antibodies which bring about the two (precipitin, agglutinin) reactions are identical is still undetermined. On general considerations it seems probable that the difference in result is merely due to the different way in which the toxic compound is presented to its antibody.

As might be expected the production of antibody increases to some extent in response to increased injection of the stimulating protein. The response is different, however, in different species, and in different animals of the same species (*i.e.*, it is merely the sum of the total responses of the reacting cells of a particular animal).

While it is believed that all foreign proteins may elicit formation of specific antibodies, it is not yet definitely ascertained to what extent this property is shared by other types of compounds. If, for the moment, we term those compounds possessing the property "active," then most proteoses and peptones are certainly inactive, as is gelatin. Yet study of the degradation products of tuberculin suggests that at any rate the more complex proteoses may be active. While hydrolysed proteins are inactive, the resynthesised "plasteins" (compare Chapter XX.) are active. Large molecular size seems an essential. There is some evidence that the presence of benzene derivatives in the large molecule is a determining factor. It seems particularly of significance that proteins "racemised" by heating with alkalies (so that optical activity is lost) are inactivated, so that reaction between foreign protein and induced antibody is in all probability reaction between two optically active substances, involving their special configurations.

It has been demonstrated that injection of crystalline urease, a protein and an enzyme, leads to formation of a specific antienzyme. Other enzymes produce similar results, specialised examples of antibody formation.

Specific antibodies can be produced by certain complex carbohydrates, and possibly also by certain complex lipides. Examples of the carbohydrates are the capsular polysaccharides of certain pneumococci and streptococci. Active polysaccharides are also present in yeasts. Some of these polysaccharides contain a small percentage of nitrogen, others are nitrogen-free. There is some evidence that the phosphatide fraction of the lipides of the

tubercle bacillus group may give rise to antibodies. (Recent claims which seem revolutionary and still require substantiation are that simple primary amines can induce antibody formation.)

It is claimed that certain toxic glucosides of fungi such as *Amanita phalloides*, and of plants such as the poison ivy, *Rhus toxicodendron*, are active, since rabbits can be immunised to such poisons (*i.e.*, develop sufficient amount of antibody as the result of successive sub-lethal doses that they can subsequently withstand lethal dosage). Man himself is being treated in this way to withstand the effect of poison ivy.

Antibodies are usually considered to be modified serum globulins. It has been suggested that the antigen disturbs the normal mechanism of synthesis of this globulin, perhaps in the polypeptide stages, by modification of the method of linkage or of the spatial arrangement, and so leads to the formation of a new globulin, an antibody.

The most marked feature of immunological reactions is their specificity. With closely related protein-toxins the degree of specificity is, however, not absolute. This may be compared with enzyme action on specific linkages, rather than on specific compounds. Antibodies produced in large amounts in an animal by successive injections of serum of an animal of another species —which stimulate through the proteins present—will react, as shown by formation of precipitates, not only with the specific stimulant—blood proteins from the same species—but also on occasion with closely related stimulants—blood proteins of closely related species of animals. In this way the closeness of relationship of different biological species has been demonstrated, and, incidentally, it has been shown that the biological connection between man and the higher apes is closer than between the higher apes and the monkeys.

In spite of the lack of absolute specificity in such cases, undoubtedly to be attributed to the closely-related chemical structures of the compounds tested, yet the degree of specificity of such reactions affords a test more delicate than any of the usual chemical tests, permitting both determination of the identity or non-identity of certain proteins, and detection of proteins present in concentrations far too small to be revealed by ordinary chemical tests.

Hæmoglobin, of most species, shows sufficient crystallographical differences to indicate chemically distinct compounds. Immunological tests indicate that the hæmoglobins of most species are different. The hæmoglobins of the horse and donkey are crystal-

lographically isomorphous ; immunologically they cannot be differentiated. Based on anaphylactic tests (see below) it has been demonstrated that the legumin from seeds of the pea, vetch, lentil and horsebean is identical. No chemical differentiation has been possible. Gliadin from wheat and rye is identical ; the closely related hordein of barley differs both chemically and immunologically. The four proteins of milk, casein, lactalbumin, lactoglobulin, and an alcohol-soluble protein, are chemically and immunologically different. The milk globulin is chemically and immunologically indistinguishable from serum globulin of the same animal, but the albumin is distinct from the corresponding serum albumin. The caseins of milks of different species of animals are, at any rate, very closely related.

Hektoen can detect by immunological technique thyroglobulin in the lymph coming from the thyroid gland. It cannot be detected in this lymph by any other known procedure. Immunological procedure is the last word in delicate chemical analysis.

The nature of the reaction between foreign protein and its specific antibody has been especially studied for the so-called " toxins " and their " antitoxins." Various theories have been put forward ; none has been universally accepted. The theory in which the reaction is supposed comparable with the neutralisation of a strong acid by a strong base has been discarded. That of Arrhenius and Madsen, that it resembles more closely the reactions between weak acids and weak bases, the equilibrium attained depending on the law of mass action, and the reaction never proceeding to completion, fits the facts more closely, but not completely. So does that of Bordet, in which comparison is made with the reaction between two colloids carrying electrical charges of different sign ; he assumes that the process involves adsorption. Adsorption alone, however, cannot account for the specificity of the reactions which suggests at once the " lock and key " conception of Emil Fischer. In any case the " neutralisation " does not appear to involve the destruction of either component, but only the formation of some non-toxic compound or adsorption complex. In certain *in vitro* experiments it is claimed that dissociation into the active constituents has been brought about by addition of acid even after the lapse of three months.

Precipitation, while typical of these reactions, is not an invariable accompaniment. When the toxic protein is presented in such association that the body has to deal with cellular elements, as,

for example, bacteria, then the antibody, acting in conjunction with some other compound normally present (the *complement*, or *alexin*, from Gk. *alexis*, protection), brings about solution of the material, and hence, in this reaction, is termed a *lysin* (Gk. *lysis*, loosening). It is also variously termed an amboceptor (L. *ambo*, both ; *capere*, to take), an *intermediary body*, and a *sensitisin* (sensitising the toxin to the action of the complement).

The *complement* is present in varying amount in normal blood, and this variation is probably a conditioning factor in susceptibility to infection. It is not specific. Its molecular size is large and its nature colloidal. It is supposed to be of complex protein nature containing both albumin and globulin radicals. The main component may be identical with prothrombin. Complement reacts with many, if not all, antigen-antibody complexes, whether the former be of cellular character or not, though, of course, only in this case does dissolution of the cell material follow. A practical application of this reaction is used in Wassermann's test for the diagnosis of syphilis.

When a *single* injection of a foreign protein is made into the circulation of certain animals, and so, as is believed, induces in them the formation of a certain amount of its specific antibody (immune body), then after a considerable period, as a rule three weeks or more, and seldom if ever less than seven days, such animals become *hypersensitive* to further injections, even in very minute amount, of that particular protein. This state, *anaphylaxis*, is shown by startling symptoms, characterised in guinea-pigs by bronchial spasm leading to death from acute asphyxia, in dogs by severe congestion of the splanchnic area, and in rabbits by acute dilatation of the right chambers of the heart due to spasm of the pulmonary vessels. The general effect appears to be associated with contraction of smooth muscle. Man seems to be less responsive, but, naturally, has not been submitted to exactly graded experiments. Asthmatics seem to be specially susceptible, and many others, though only a small proportion of mankind, respond to certain proteins by skin lesions.

Mention must finally be made of the phenomenon of *phagocytosis*, the chemotactic attraction of leucocytes to any point of bacterial infection in the organism, followed by the ingestion of the bacteria by these cells. Chemotaxis is still only a name describing an action ; we cannot explain it. The actual engulfing of the bacteria by the leucocytes is greatly facilitated by specific substances termed *opsonins* (Gk. *opsonein*, to procure provisions) or *tropins* (Gk. *trope*, a turning), which may be increased in amount

by immunisation, and which appear to be antibodies. We may strongly suspect, though it is not yet proved, that these are the ordinary antibodies specific to the particular foreign proteins involved.

Reverting to the statements in the first paragraph there seems, to the non-immunologist, reasonable ground for stating that the only terms peculiar to this subject which cannot yet be translated into ordinary chemical language are " antibody " and " complement," and these seem to connote, one, a class of proteins with very special properties, and the other, one particular highly complex protein.

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CHAPTER XXXIV

THE UTILISATION OF BIOCHEMICAL PROCESSES IN INDUSTRY

VIEWED as broadly as possible, all industries dealing with the products of living organisms involve biochemical processes, whether these be the production of leather or milk from the cow, of silk from the silk worm, or of cotton from the cotton plant.

In a more limited and more real sense biochemical processes may be said truly to be utilised when the normal, or apparently normal, conditions of animal or plant life are controlled or varied experimentally to produce or increase yields of materials of industrial importance.

Under this definition still comes the whole of agricultural chemistry. As a particular example of specialised application, there is that variation of yeast fermentation of sugar employed by the Germans to obtain large quantities of glycerol for the manufacture of explosives, and so to conserve fats. A different solution of a similar problem was the war-time bacterial decomposition of Pacific giant-kelps by Americans to furnish large supplies of acetone.

Since obviously the organisms which are to be controlled experimentally must be easily obtainable in large enough numbers to furnish products on an industrial scale, saprophytic and parasitic plant organisms, whose activities have been briefly reviewed in Chapter XIV., will be largely utilised. While many of their processes have been employed since the beginnings of man, yet brewing has only recently risen from an art to a science, the production of industrial alcohol has only recently become dissociated from the age-long production of alcoholic beverages by organisms, and the searching for specific organisms which will bring about clear-cut desired reactions is only in its infancy.

It has been pointed out by Sir William Pope that organic compounds prepared by utilisation of the enzymic actions of plants or animals cost much less than when the same compounds are prepared synthetically, the obvious cause being the use of low-grade solar-energy, costing nothing, by the living organism,

which, further, demands neither wages nor a seven hours' day. Hence he considers that the intensive exploitation of such fermentative methods for manufacturing important organic compounds will afford some relief from the ever-increasing cost of high-grade energy from fuel, and the increasing demands of the worker for higher wages and shorter hours, which, however legitimate, do not lower cost of production.

Some of the processes actually in use commercially will be dealt with briefly, and some of the future possibilities mentioned.

Various processes are used for the industrial preparation of ethyl alcohol on the large scale. Thus, moulds are used for the saccharification of starch, and the sugar so produced is either fermented by yeast or converted into alcohol by direct chemical treatment.

During the past decade more than 90 per cent. of the total alcohol produced in the United States has been from molasses. (In 1929 the total amount of denatured alcohol produced in that country was 107 million gallons.) Yeast of a strain suitable for the fermentation is kept in pure culture, and, as required, sufficient is built up by successive transfers for the amount of molasses to be decomposed. The molasses is heated to boiling to destroy other active organisms, then cooled for yeast action. The yeast is successively cultured in increasing quantities of molasses until sufficiently large in bulk for the final action. For this action the molasses is diluted with four volumes of water, sulphuric acid added to a *p*H of about 5, and the yeast molasses mixture added to give a yeast concentration of about 4 per cent. The final fermentation requires from thirty to sixty hours, and is followed by the usual distillation processes.

Vinegar, lactic acid and butyric acid are manufactured by fermentation, and similar biochemical processes have been extended to the manufacture of citric, pyruvic and fumaric acids.

Lactic acid is produced by a large variety of bacteria, many yeasts, moulds, and some other plants. A pure culture from soured yeast of the bacterium *B. Delbrucki*, prepared by Leichmann in 1886, is probably most generally used commercially. It has a high optimum temperature and acidifies rapidly. The raw material consists of any substances containing fermentable sugars, or carbohydrates which may be converted into them. In the United States cane and beet sugar molasses, corn starch, and corn sugar are employed, in Germany potato starch. The starches must be hydrolysed to sugar either by boiling with dilute

acid or treatment with a malt rich in diastase. Gluten from grains or ammonia and phosphate are supplied as nutrient. The bacteria are susceptible to excess of acid, so that the acid must be neutralised as formed, by addition of calcium carbonate initially, or preferably by gradual addition of lime. The process requires five to six days, lime is finally removed by sulphuric acid and the lactic acid solution (about 8 per cent.) concentrated *in vacuo*. Technically lactic acid is used chiefly in the removal of lime from dehaired hides, and to some extent in the dyeing and finishing of textiles, and in the production of ethyl lactate, a high boiling solvent for the nitrocellulose used in the manufacture of pyroxylin lacquers. It may have an important future edible use, in infant foods, near beers, soft drinks, and candies, and also in the preparation of poultry and stock foods.

A commercial process utilises certain moulds to transform glucose or sucrose into more than half its weight of citric acid.

Numerous processes have been patented in recent years in connection with the production of acetone and butyric alcohol from starch by fermentation processes. Necessity for production of butyl alcohol at low cost, in order to obtain butyl acetate required as a solvent in the nitrocellulose lacquer industry, is the guiding cause. *Clostridium acetobutylicum* has been found to be especially efficient in bringing about this conversion, and corn is used to provide the starch. Low-grade corn, not utilisable for feeding purposes, is employed. The bacterium converts 3 lb. of starch into 1 lb. of the mixed solvents dissolved to a 2·5 per cent. solution, and composed of six parts butyl alcohol, three parts acetone, and one part ethyl alcohol. In addition, a mixture of gases consisting of 45 per cent. hydrogen and 55 per cent. carbon dioxide is evolved. All the hydrogen is converted, with part of the carbon dioxide, into synthetic methyl alcohol ; the remainder of the carbon dioxide is finding a use as " dry-ice." The original mixed gases can also be compressed, and under the influence of catalysts will give a commercial yield of methyl alcohol.

In *dairy industries* fermentation processes are necessary, and are becoming properly controlled. In the preparation of butter and cheese a certain requisite degree of acidity is produced by fermentation of lactose. Milk is pasteurised to destroy stray organisms, and then a pure culture of the lactic acid bacillus (known as the " starter ") is added, and fermentation is allowed to proceed until the requisite amount of lactic acid is produced (*cf.* p. 191).

In the allied manufacture of margarine the fats employed are

rendered perfectly tasteless and odourless, and then a correct butter flavour is developed by growing the necessary bacteria in the purest milk and introducing this culture into the melted fats.

In bread-making the diastase, maltase and invertase of yeast all play a *rôle* in converting the raw carbohydrate of the dough into soluble fermentable sugars, which are then decomposed to carbon dioxide and alcohol. The gas expands the dough, giving the porous quality of a well-baked loaf, and further matures the dough by changing its *p*H and so affecting the colloidal and physical properties of the gluten.

Processes are available, by use of cultures of mould organisms obtained from good coffee, which will convert green coffee to a Java-like product within a few days. In the preparation of cocoa the cacao pod, containing from twenty-five to forty seeds, is split, and the seeds, surrounded by a sweet, mucilaginous pulp, are fermented in vats, the bean thereby being improved by hydrolysis of some of its bitter constituents and the loss of mineral matter to the pulp. The seeds are then dried for export.

Pectin is now largely employed in jelly and jam making, both on the commercial scale and by the housewife. It is largely produced from the waste material in cider and vinegar mills, or in the manufacture of citric acid and other citrus products, or in beet sugar manufacture, or directly from carrots, by extraction with organic acids such as citric, tartaric, lactic or malic acid. So produced, it is contaminated with starch and protein, which must be removed in order to produce clear jellies. Oshima and Church claim that this last process can be ideally brought about by the use of the *Aspergillus flavus-oryzæ* group of moulds.

In the tanning of leather by vegetable tans micro-organisms are of great importance. On the other hand, the old-time fermentation of the tanyard to produce a certain degree of acidity is found quite uneconomical on account of the long time that is required, and is being replaced by addition of sulphuric acid or lactic acid. The latter is, of course, produced commercially by micro-organisms.

Fermentation processes are used in the curing of tobacco, the retting of flax, the production of natural indigo, in bread-making, and in the disposal and utilisation of sewage. Agriculture largely utilises micro-organisms in connection with the conversion of nitrogen and its compounds to the desired forms available for the higher plants.

In agriculture, a more direct intervention of man is exemplified

by the conversion of green food into a condition permitting its storage for long periods in the process of ensilage, in which the sugars of such food are allowed to ferment so that a proper proportion of acetic acid is produced. This process, as usually carried out, permits also production of butyric acid and other noxious higher fatty acids, and apparently the fermentation process can be (and therefore will be) so controlled as to minimise the productions of these undesirable compounds.

Straw, fermented by micro-organisms anaerobically, in presence of calcium carbonate to prevent development of acidity, has its cellulose, galactans and lignin so largely converted into methane (with a little hydrogen) that 34 per cent. of the heat value of the straw is obtained in this gaseous combustible form, and this process has already been used on the small scale for lighting purposes (in large country houses). Under aerobic conditions straw ferments to humus, and much use can be made of such processes in production of artificial manures.

Lipman claims that *Thiobacillus thio-oxidans*, which can develop in mineral media and can obtain its carbon from carbon dioxide, is the sulphur-oxidising organism par excellence. Sulphuric acid is formed, and from this oxidation the organism derives a supply of energy for its own life processes, for every thirty-two parts of sulphur oxidised the organism assimilating one part of carbon. It can withstand a concentration of 5 per cent. sulphuric acid. It has been used on a reasonably large experimental scale in solving successfully various agricultural problems. Thus, a culture of the organism mixed with sulphur and added to soils eradicates the potato scab. When mixed with sulphur and ground phosphate rock, and sprayed upon the surface of soil, through the interaction of the acid formed with the phosphate, a surface soil results within sixteen weeks, containing as much as 16 per cent. of soluble phosphate. Soils deficient in sulphate are easily furnished with it if the mixture of sulphur and bacteria is added. Black alkali (impermeable) soils are rendered useful by the treatment, soluble salts being formed which can be washed away. Objectionable vegetation can be destroyed, and, finally, it has been found useful in controlling the intestinal parasites of sheep, swine and poultry.

In certain commercial processes it seems more useful to employ enzyme preparations directly. The " degumming " process in the manufacture of silk, *i.e.*, the removal of the protective coating of sericin without damage to the silk fibre, is easily brought about by treatment with certain enzymes, such as trypsin and pepsin, in

weak alkaline or acid solution, although it is considered advisable to complete the process by short immersion in dilute soap solution.

Invertase preparations are employed in the partial inversion of sucrose in food products containing it, leading to increased solubility and therefore increased possibility of concentration of syrups, which thereby also become more hygroscopic (through the properties of the fructose produced). The sucrose remaining crystallises in smaller crystals, so that both the consistency and flavour of the products is improved by the treatment. It has been suggested that this treatment is therefore of value in the preparation of golden syrup and similar products, and especially in the manufacture of the fondant type of confectionery. Invertase is stated to be used commercially already in considerable quantity in Canada, the United States and Australia.

A curious example of the method of accidental discovery of useful organisms is the investigation of Christoph, leading to the isolation of a mould which, wind-borne from a neighbouring fruit-store to a brewery making a dark Bavarian beer, brought secondary fermentation of the beer to a standstill by withdrawing proteoses and carbohydrates from the culture yeast and inhibiting its production of carbon dioxide. The isolated mould, grown in wort, beer, and similarly fairly acid media, develops only slight amounts of alcohol and gas, but produces some compound with an intensely banana-like odour, and Christoph suggests that it may be usefully employed in making mineral waters, fruit wines, etc.

Foreshadowing vast possibilities is the accidental discovery by the Germans during the war of an organism which can synthetise protein readily from inorganic nitrogen and a simple carbohydrate. This they actually employed to manufacture food on a considerable scale.

Antitoxins and various other important bacterial products exemplify a minor but important use of micro-organisms.

An 18 per cent. yield of mannitol can be obtained from sucrose by fermenting it with a specific organism (*B. invertentemannite lactici*) recently obtained from the fermenting juice of asparagus.

Sufficient illustrations have been given of the considerable extent to which bacteria, yeasts and moulds are already employed commercially. Problems of practical importance which have been suggested as requiring elucidation, and which, when solved, will lead to further employment of such organisms, are, for example,

the utilisation of lactose (for lactic acid production), that still remains in the effluent whey of cheese factories, after as much as possible has been removed in the preparation of " milk sugar " for infant feeding, the production of fermentable sugar from cellulose by a purely biological procedure, and the synthetic production of protein and fat from inorganic nitrogen and cheap carbohydrate (extension of the German work already referred to). Intensive study may reveal some of the mechanism whereby the glow-worm and fire-fly produce " cold light," and permit useful adaptation of the chemical processes involved.

Much further study is required, as Hopkins has pointed out, in determining the best limits of hydrogen-ion concentration for the reactions required in all processes utilising micro-organisms, and the best methods of arresting action at the stage to give the maximum production of the compound required ; many of them are subsequently converted by the same micro-organisms into less desirable products. The remarkable changes in activity that are caused by habituating organisms to new media of composition strange to the original strains also calls for intensive study, and will probably lead to most valuable theoretical and commercial results, as will also accurate selection of micro-organisms for specific, desired reactions. Many, if not most of, such specific activities that are at present employed are haphazard, the results of chance observations.

The higher organisms cannot, from their nature, be utilised to such an extent. Nevertheless a real field of commercial utilisation exists, and is being thoroughly explored and exploited. The preparation of the products of internal secretions from the pancreas, thyroid, pituitary, etc., of slaughtered cattle, hogs and sheep is an important industry, though much work in the nature of accurate purification and standardisation is called for, and though many products that are now marketed are, to write as mildly as possible, of most questionable value.

Many of the active products of animal activity will presumably be subsequently replaced by the synthetic compounds when these can be prepared. In some cases the chemist will still be unable to compete with the animal ; the synthetic product will remain more costly to produce than that obtained from natural sources. This is possibly true for thyroxine. Harington has synthetised it, but, on the other hand, he has found a procedure for materially increasing its yield and cheapening its production from animal thyroids. An opposite example is the essential perfume of musk, which has recently, as the result of long and brilliant research,

been identified as cyclopentadecanone ; the synthetic product (" exaltone ") can be marketed much more cheaply than can natural musk. The chemical identification and preparation of such products, and of the many still unknown alkaloids produced by plants, affords a large future field for the activities of the commercial organic chemist.

In the preceding chapter an indication was given of the potential future use of immunological reactions in chemical detection and identification of proteins. Micro-organisms would appear to be similarly utilisable as biological reagents for the detection and identification of carbohydrates. Castellani and Taylor have shown that fructose is decomposed by the hypomycetes *Monilia krusei*, but not by *M. bateanica*. Maltose is fermented by *M. pinoyi*, but not by *M. krusei*, galactose by *M. metalondinensis* and *M. tropicalis*, but not by *M. pinoyi* and *M. bronchialis*. Lactose is fermented by *M. pseudotropicalis*, but not by *M. macedoniensis*, sucrose by *M. tropicalis*, but the products do not reduce Fehling's solution. Inulin is fermented by *M. macedoniensis*, but not by *M. rhoi*. It is thus possible to devise an analytical procedure with these moulds as reagents, gas production indicating positive reactions, and it is claimed that speedier results are obtained than with chemical procedures.

Enough has been said to indicate the future usefulness of biochemistry and of the biochemist in industry. Evidently the commercial biochemist will require as part of his training a thorough knowledge of the methods of isolation, identification and culture of these micro-organisms.

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CHAPTER XXXV

A BIOCHEMICAL INTRODUCTION TO PHARMACOLOGY

The pharmacologist groups chemical compounds and drugs (which are merely more or less impure chemical compounds or mixtures of compounds) into classes according to their actions on the organism. Such a treatment, bringing together unrelated compounds such as aconite, magnesium salts and bromides as depressants, and of barium salts and digitalis as heart stimulants, while illustrating the viewpoint of the physiologist-pharmacologist, and emphasising the essential application of drugs in therapeutics, tends to obscure what is, from the point of view of the biochemist, the most interesting feature of pharmacology, the relation existing between the constitution of a chemical compound and the pharmacological action that it produces, and thereby tends to delay progress in that science, since future advance must be largely in the direction of utilisation of synthetic compounds whose constitution has been specifically modified to achieve definite desired results ; the brilliant sequelæ of this type of study are exemplified in the researches leading to the production of 606 and similar arsenical compounds used in the treatment of syphilis and trypanosome diseases.

At the same time the usual teaching of pharmacology tends to depreciate the view that the actions produced are traceable to definite chemical compounds, and that these should be used in as pure a condition as possible. The term *drug* is essentially a bad one, acting as a cloak to remediable impurity of preparation, and connected with the Dark Ages, when drugging symbolised medical treatment and the therapeutic use of drugs was empirical and frequently nonsensical, and still leading to such an abuse of definite pharmacological remedies that charlatans can flourish by selling " vegetable drugs " advertised to be safer and more beneficial since uncontaminated with compounds of mineral origin, or, on the other hand, " vegetable compounds," advertised to contain no drugs.

This chapter will include a few examples of the different pharmacological actions induced by closely related series of organic

compounds, preceded by some consideration of the comparative effects of chemically-related ions. We may consider pharmacological actions as producible by two large groups of chemical substances, ions and certain compounds acting essentially in an aqueous medium, and organic compounds, acting frequently through their solubility in lipide solvents, and, therefore, their capability of penetrating lipide membranes.

The periodic classification of the elements groups them into distinct classes whose members are closely related in their physical and chemical properties, and we therefore might well expect that their ionised compounds not only would show that regular gradation of physical and chemical properties that does exist, but also a regular gradation in their pharmacological effects. Series that may well be considered from this point of view are the fluorides, chlorides, bromides and iodides, the salts of lithium, sodium, potassium, rubidium and cæsium, and those of calcium, strontium and barium.

The effects of difference of structure may also be studied in such inorganic compounds by contrasting the results obtained by administration of carbon monoxide and carbon dioxide and of arsenites and arsenates.

The Comparative Effects of Related Series of Ions

In comparing the effects of any ionic series it is obvious that the salts employed must be of the same metal (if anions are compared) or derived from the same acid (if cations are being tested). True comparisons must be based on the actions of equal molecular or ionic concentrations on equal body-weights of the organism used for test. (A similar precaution must be observed in carrying out any quantitative comparative tests.)

The Sodium Halides. In their actions the fluorides stand apart, being markedly toxic, due to the fact that calcium fluoride is very insoluble, and that, therefore, calcium is precipitated from blood and tissue fluids.

When the frog muscle-nerve or heart preparation is immersed in equimolecular concentrations of the sodium halides, isotonic with the body fluids of the frog, fluoride produces rapid death of the tissues, usually preceded by twitching and fibrillary contraction of voluntary muscle. Chloride is practically non-toxic, bromide very slightly, and iodide slightly more toxic, as indicated by the survival periods of the tissues.

Fluoride is rapidly toxic to mammals, through the cause just

mentioned. It induces at first a stimulation of the medullary centres (through removal of the depressant calcium ions, and so an upset of the calcium-sodium-potassium balance normally existing), leading to rapid deep breathing in the rabbit, and vomiting and nausea in the dog. Then co-ordination is affected, respiration slowed, the heart weakens, and death (sometimes in convulsions) is preceded by coma.

The other three halide ions are more closely related pharmacologically, as they are chemically and physically. Small doses of any of the three produce no marked effect, even if continuous. Such small continuous doses of bromide or of iodide lead to some replacement of the chloride ion. Hydrobromic acid (and, when large iodide doses are given, some hydriodic acid) may be secreted in the gastric juice along with hydrochloric acid, and function similarly. The bromide ion is as efficient as the chloride ion as a co-enzyme for pancreatic amylase ; the iodide ion can also so function, but less efficiently.

Strong solutions of chloride by mouth act as irritants in the stomach and may induce vomiting. Strong solutions of bromide or iodide more easily induce nausea and vomiting.

Bromide stands apart from the others in acting as a depressant through direct action on the central nervous system. Nerve irritability is lessened ; various reflexes become weakened, respiration is slowed, and sexual instincts depressed. Drowsiness and sleep may follow. Chlorides and iodides do not affect the central nervous system.

Bromides given man in heavy continuous doses frequently lead to bromism, characterised by skin eruptions of various kinds, and sometimes by a localised blush or erythema, which more rarely is replaced by copper-coloured blotches. The respiratory passages are occasionally affected, there being increased secretion by the bronchial and nasal epithelium. Iodides in large continuous doses may lead to iodism, the commonest symptom being a catarrh of the respiratory passages, especially of the nose. This may be followed by various forms of skin eruption, most commonly purple erythematous patches or papular eruptions. Man exhibits a marked variation of susceptibility to bromides and iodides ; no cause has been ascertained for their capricious behaviour.

Evidently, if the fluorides are excluded, the halides show a definite gradation of effect, bromide being intermediate pharmacologically as it is chemically, though possessing a definite depressant action on the central nervous system not shared by the others.

Chlorides of the Alkaline Metals. The chlorides of lithium, sodium, potassium, rubidium and cæsium are concerned. That of sodium is practically without action, the salts of lithium and cæsium show some resemblance in their actions, and rubidium chloride is very similar in action to potassium chloride. This series therefore shows relationships but no definite gradation in its effects.

Lithium chloride seems to have some depressant action on the motor nerves and to weaken muscular contraction. It slightly weakens the mammalian heart action, but the effect is much less than that of potassium chloride. Subcutaneous or intravenous injection leads to gastro-enteritis and extravasation of blood into the stomach and intestine in animals ; death may ensue. Cæsium salts cause somewhat similar effects in the alimentary tract. Lithium, cæsium and rubidium are in part excreted through the alimentary tract ; the excretion is slow.

Potassium chloride produces a distinctly toxic effect, manifested through the central nervous system and on the heart. The activity of the spinal centres is first increased and then paralysed. There is a direct depressant action on the heart. Rubidium produces similar results.

The Chlorides of the Alkaline Earth Metals. The actions of beryllium salts have been insufficiently studied to enable a definite comparison to be made.

Magnesium chloride given by mouth is rapidly absorbed and excreted ; concentration in the blood is never sufficient to be effective. Intravenous or subcutaneous injection is followed by anæsthesia ; calcium salts absolutely antagonise this action produced directly through the central nervous system, but strontium salts do not. Comparable with this antagonistic effect is the diminution—following injection of magnesium chloride—of the marked intestinal peristalsis produced by barium salts.

Calcium chloride acts as a nerve depressant, its action being especially typified in the quietening of tetany-symptoms. Strontium chloride produces a similar action, though it is less toxic. (Strontium after injection is found replacing calcium to some extent in bone.) Barium chloride is much more toxic. Injected intravenously it produces violent tonic and clonic spasms from stimulation of the spinal cord and medulla oblongata. The calcium salt at first accelerates and strengthens the heart ; in larger doses it brings the heart to a standstill. The strontium salt produces relatively less effect. The barium salt causes the heart to beat more strongly, but more slowly. All three are but slowly

absorbed through the intestinal wall ; all three are chiefly excreted through the intestine. Calcium and barium salts constrict the walls of blood-vessels through which they are perfused, leading to increased blood-pressure. Calcium and barium ions antagonise the effect of the potassium ion. Barium and strontium ions prolong the contraction of muscle.

While no definite gradation of effect can be shown it is obvious that there are marked points of resemblance in the actions of the chemically similar elements, calcium, barium and strontium, though the lightest of the series, magnesium, is definitely apart in its results.

The Effect of Unsaturation

The presence of an unsaturated atom in a compound invariably increases its toxicity. Thus carbon monoxide, with a divalent, unsaturated carbon atom, is vastly more toxic than the corresponding saturated carbon dioxide. Carbon monoxide produces its toxic effect through formation of such a stable compound with hæmoglobin that the function of the latter as an oxygen-carrier may be so depressed that death ensues through oxygen starvation of the tissues. Carbon dioxide can only produce untoward effects through its presence in such amount in the inspired air that its own excretion is diminished and the oxygen content of the gas that is breathed in falls to too low a figure. When its concentration in the blood is definitely increased restlessness and stimulation of the respiratory and vasomotor centres follow ; at still higher concentrations a deep narcosis ensues.

Arsenate, with pentavalent arsenic, is in itself non-toxic, though it is rapidly reduced in the blood of mammals to the toxic arsenite, with unsaturated trivalent arsenic. In agreement, from tests on unicellular organisms it has been found that organic pentavalent arsenical compounds are less toxic than the corresponding trivalent compounds. Experiments on rats and rabbits show that tellurite is much more toxic than tellurate.

The Variation of Pharmacological Effect with Chemical Constitution

Paraffin Derivatives. The gaseous paraffins, methane, CH_4, and ethane, $CH_3 . CH_3$, are inert compounds. Some of their simpler derivatives well exemplify change of pharmacological properties accompanying change of constitution.

Methyl and ethyl chlorides, CH_3Cl and $CH_3 . CH_2Cl$, are local anæsthetics, producing anæsthesia as a result of intense cold developed by their rapid evaporation. Here the pharmacological

action is produced through physical, rather than chemical, means. Ethyl chloride, soluble in lipides, is also used to produce anæsthesia of short duration, short because it is rapidly absorbed and very rapidly excreted. Chloroform, $CHCl_3$, closely allied, is one of the most useful general anæsthetics, penetrating the cell through its solubility in lipide compounds, and thereby especially affecting cells with marked lipide content, such as those of the central nervous system. Its derivative, chloral hydrate, $CCl_3 . CH(OH)_2$, an alcohol, rather than an aldehyde, is a hypnotic, producing deep sleep, as are the similar derivatives " dormiol," $CCl_3 . CH(OH) . O . C(: CH_2) . C_2H_5$, and " isopral," $CCl_3 . CH(OH) . CH_3$. Carbon tetrachloride, when inhaled continuously, produces convulsions.

Methyl alcohol, CH_3OH, while an intoxicant, produces toxic results which are in part specifically different from those produced by ethyl alcohol, $CH_3 . CH_2OH$. Single doses of methyl alcohol are less but longer toxic to animals than corresponding doses of ethyl alcohol. More marked symptoms of gastric irritation are produced, and often convulsive movements. In repeated dosage methyl alcohol is much the more toxic, due to its slower oxidation, and, therefore, to a prolongation of its action. About 40 per cent. is oxidised in forty-eight hours, while in this time 25 per cent. is excreted in breath and urine (*cf.* ethyl alcohol, Chapter XXVII.). Most of the oxidation ceases at the stage of formic acid. In man methyl alcohol produces marked muscular weakness and defective heart action, followed by nausea, vomiting, coma and delirium, of more intense and persistent character than occur in marked intoxication with ethyl alcohol. Death may follow a single large dose, and in many cases, following repeated ingestion of the poison, total and permanent blindness results, an effect peculiar to methyl alcohol amongst such substances, and due immediately to optic neuritis and complete optic atrophy.

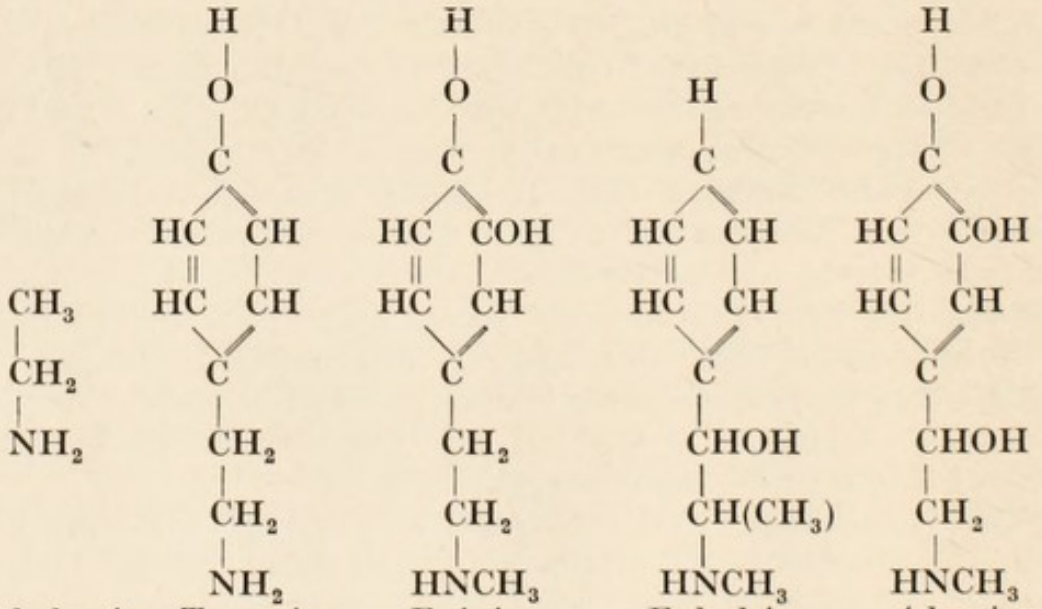
The " anhydride " of ethyl alcohol, ethyl ether, $CH_3 . CH_2 . O . CH_2 . CH_3$, when drunk induces a short intoxication. When inhaled it induces an anæsthesia comparable in most respects with that produced by chloroform.

The oxidation products of these alcohols produce effects of quite another kind. Formaldehyde, $H . CHO$, is one of the most powerful germicides, which, however, while irritant, is not very toxic to higher animals. In large doses it leads to nausea and vomiting, narcosis, coma and death. It is rapidly absorbed, but rapidly oxidised. Its toxic action on living membranes is probably due to its marked power of reaction with free amino-groups, whether in amino-acids or in proteins themselves, the compounds

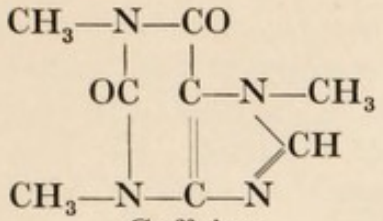
so resulting having markedly altered properties. Acetaldehyde, though equally irritant, is less toxic.

Acetic acid in dilute solution cannot be regarded as poisonous. It is an intermediate product of normal metabolism. Prolonged use of vinegar in a diet may give rise to gastric irritation and various sequelæ. Ingestion of concentrated solutions of acetic acid is followed by irritation of the mouth and stomach, causing vomiting, great pain, collapse and even death. Formic acid, more volatile, is more irritant, and is less easily oxidised in the body.

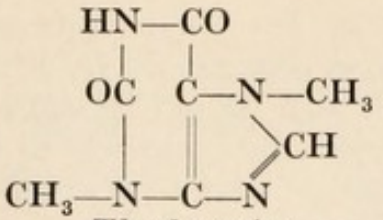
The Sympathomimetic Series of Amines, studied by Barger and Dale, well illustrates a graded and gradually increasing effect in a long series of compounds which are closely related chemically. The effect is exhibited in actions mimicking those following stimulation of the sympathetic nervous system, actions shown feebly by the simpler amines, such as ethylamine, and more and more markedly by the tyrosine derivatives, tyramine, epinine, ephedrine and adrenine.



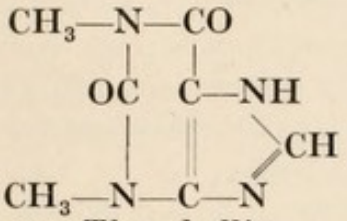
The caffeine series illustrates the possibility of tracing a specific pharmacological effect to a particular part of the molecule. We are concerned with the three methyl derivatives of the partially oxidised purine compound xanthine—caffeine, theobromine and theophylline. Xanthine itself, and uric acid, do not in any way produce similar effects, so that the actions must be definitely connected with the presence of methyl groups.



Caffeine
(1, 3, 7 trimethyl xanthine)



Theobromine
(3, 7 dimethyl xanthine)



Theophylline
(1, 3 dimethyl xanthine)

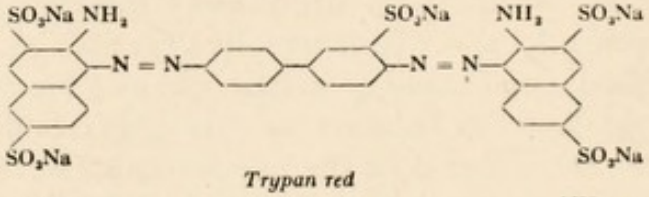
In man caffeine stimulates the central nervous system, especially that part associated with the psychical functions ; thinking is clarified and fatigue and drowsiness disappear. There is a definite diuretic effect, which is usually regarded as brought about by an increased permeability of the glomerular capsules of the kidney. Large doses in animals considerably accelerate the heart through direct stimulation of the cardiac muscle. (In man therapeutic doses do not definitely produce this effect.) Thus there are three separate effects, and corresponding to them are three methyl groups.

Theobromine, with two methyl groups (3, 7), produces the two latter effects, but has practically no action on the central nervous system. Theophylline, also with two methyl groups (1, 3), has a very marked diuretic effect, with apparently no definite action on the heart, but some possible action on the central nervous system, since sometimes epileptiform convulsions follow its use. All three compounds produce a similar effect on voluntary muscle, increasing its response to stimuli, lowering the threshold of effective stimuli, and strengthening the response.

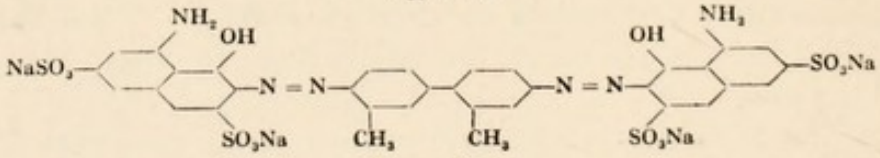
It would appear that in this series of compounds a methyl group in position 1 is mainly responsible for the action on the central nervous system, a methyl group in position 3 is mainly responsible for the diuresis, and a methyl group in position 7 for the heart action. It would be interesting to ascertain whether *paraxanthine* (present in traces in urine from decomposition of caffeine), the 1, 7-dimethyl-xanthine, would in large dosage produce the effect on the central nervous system without diuresis ; this does not appear to have been yet determined.

Search for Compounds with Specific Pharmacological Effects. This was commenced by Ehrlich. His problem was to find compounds that should be markedly toxic to the cells of the parasite, and relatively non-toxic to the cells of the host. The successful results that have been obtained by himself and later investigators seem, however, to be produced by compounds which are not specifically toxic to either host or parasite, but which are capable of combining with compounds manufactured by the host (and possibly of protein nature), whereby they become excessively toxic to the parasite. In other words, this type of research would seem to be to obtain compounds which can form artificial immunological complexes in the body.

Ehrlich and his colleagues tested a long series of organic dyes and their derivatives on a strain of trypanosome transmissible to rats and mice. They found that injection of a benzidine derivative " trypan red " produced some toxic action, while " trypan blue " was distinctly more toxic.



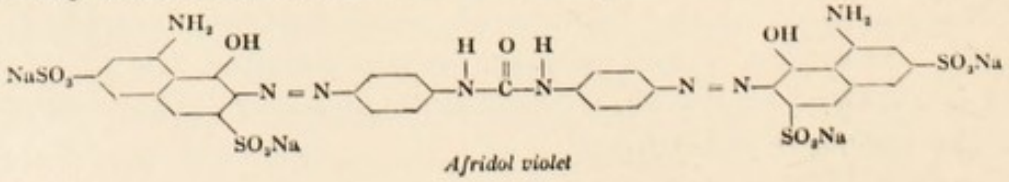
Trypan red



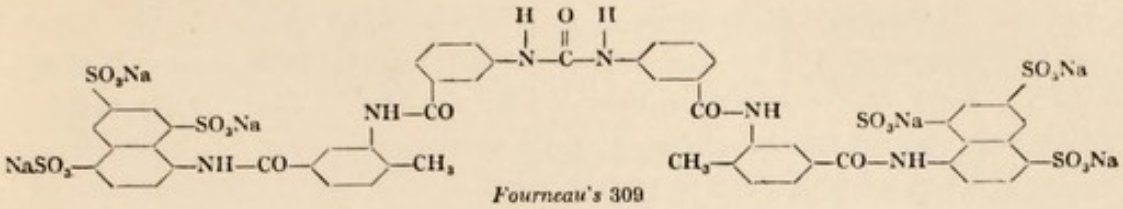
Trypan blue

Trypan blue has recently been shown to have definite specific effect, not on trypanosomes, but on an intracorpuscular parasite infecting dogs and cattle.

A further distinct advance was made by introducing a urea nucleus into compounds of this type. In 1906 Mesnil and Nicolle showed that " Afridol violet " promised to give good results, and recently the firm of Bäyer have placed on the market a compound " 205 " (whose composition is kept secret), which is quite possibly identical with Fourneau's " 309." Both these preparations have remarkable toxicity to trypanosomes. One injection of " 205 " frees a mouse, rat or rabbit from trypanosomes within a few days, and confers marked immunity for weeks or even months.

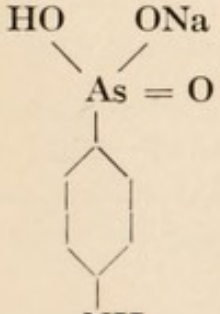


Afridol violet

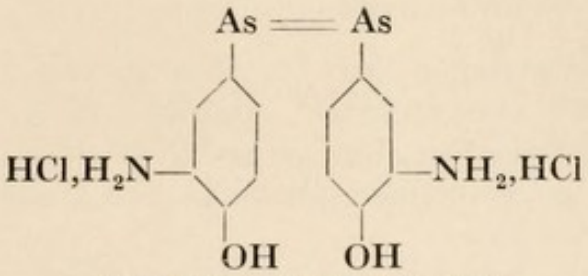


Fourneau's 309

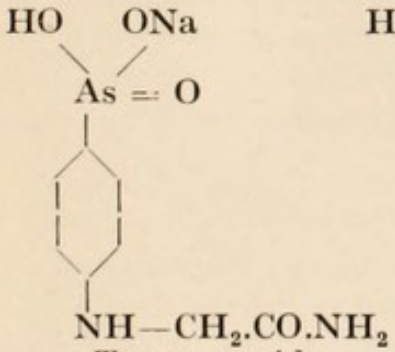
The similar successful results that have been obtained by Ehrlich and others with arsenic derivatives may be exemplified by comparison of atoxyl, toxic to trypanosomes, arsphenamine or salvarsan, still more toxic to spirochætes and trypanosomes, and tryparsamide (of Jacobs and Heidelberger), with which good results are being obtained in the conquest of African sleeping sickness and the treatment of neurosyphilis.



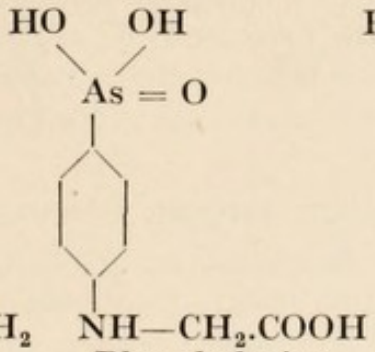
Atoxyl



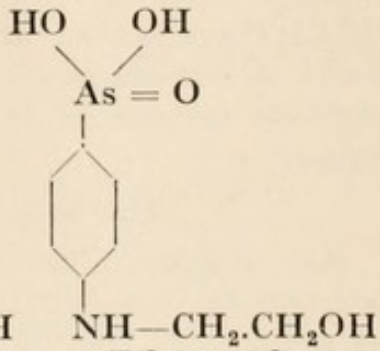
Salvarsan (hydrochloride)



Tryparsamide
(Atoxyl-acetamide)



Phenyl-glycine p-arsonic acid

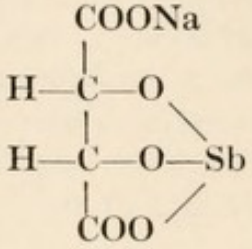


Etharsanol

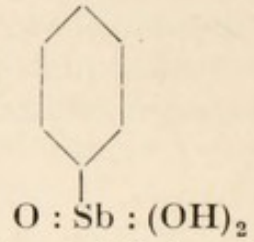
It is interesting to note, as illustrating the extreme sensitiveness of biological reactions to slight change in chemical constitution, that the acid corresponding to this amide, phenyl-glycine-*p*-arsonic acid, has no effect in either syphilis or sleeping sickness, while the corresponding alcohol *etharsanol* is extremely useful in the treatment of trypanosomal infections in animals, but is inert in neurosyphilis.

Since organic arsenic compounds have proved so valuable it is not surprising that derivatives of the allied elements antimony and bismuth have also proved of great service in combating

various diseases. Bilharziasis and kala azar have both been treated successfully by the trivalent antimony compound tartar emetic, and the corresponding sodium salt. The pentavalent antimony compounds show reduced toxicity to the host, and increased toxicity to the parasite, and a number of derivatives of phenylstibinic acid have been used with success in the treatment of kala azar.



Sodium antimonyl tartrate



Phenylstibinic acid

Potassium sodium bismuth tartrate has been tested extensively in the treatment of syphilis. It acts more slowly than the arsphenamines, but seems to be less toxic. Good results have also been claimed for it in the somewhat similar spirochætal disease yaws.

It is, perhaps, not too much to say that when we understand more fully the complete actions produced by a sufficiently large enough series of chemical compounds, and the mechanisms by which these actions are produced, it will be possible to analyse them as summations of the specific actions due to the different radicals present in the molecules tested, as modified by each other.

The Relation between Dose and Effect

Any definite effect produced by a chemical compound on a living organism should obviously bear a relationship to the dosage employed (that is, to the ratio between the weight of the compound used, and the weight of the organism). Little work has so far been carried out to find this relationship. It has recently been shown that in those cases in which the effect sets up a resistance to itself in the organism the relation can be expressed, *as a first approximation*, by the equation

$$b\mathrm{E} = \log (a\mathrm{D} + 1)$$

where E is the effect, D the dose, and a and b are constants. For very small dosages the relation tends to become arithmetical, for larger doses logarithmic. It has been tested satisfactorily for the effects of thyroid, of adrenine, of insulin, of pituitrin, of indaconitine in raising the temperature of the rabbit, etc.

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A FINAL WORD

Here have been presented the known facts of the subject in as well ordered a sequence as their present extent permits. Many doubtful statements still must be confirmed or disproved, many contradictory pieces of evidence harmonised, probabilities replaced by certainties, but the subject no longer presents kaleidoscopic confusion. We can see a definite picture of the chemical processes of life and death.

Plants seize the radiant energy of the sun and with its use build the compounds they use. Animals digest them to build their own. At death the ferments all powerful in life are still all powerful, and the complex organism is disintegrated. The lowest forms of plant life aid the process by which the dead material is restored to the circulation of matter.

The complex activities of the animal have three powerful groups of governors, the ferments, the endocrine secretions and the vitamins, all chemical compounds, and all acting chemically. The more our knowledge of their actions increases the more ordered do we see the panorama of the life of the organism.

All the processes of life are governed by the same quantitative laws that have been proved to hold for non-living matter. Life can create neither energy nor matter, nor cause their disappearance.

We are still far removed from comprehension of what specific atomic arrangement in space confers on some material the attribute of living which other similar material does not possess or has lost, and as far from an understanding of those phenomena—electro-chemical or whatever they be—which confer on the essentially lipide matter of the brain the function of mind, but we have made such progress that we cannot regard even these problems as unsolvable.

In the solving of more material problems progress will con-

tinue to be rapid. More and more does each fresh discovery link together the facts and fill in the picture. And each advance, however small, will have some share in the varied applications of our subject, assistance in the diagnosis and conquest of disease, assistance in the manifold problems of industry, and, not least, enlargement of the realm of pure knowledge.

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