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Chemistry - Physiological

A TEXTBOOK OF BIOCHEMISTRY

Churchill's Empire Series

A TEXTBOOK OF BIOCHEMISTRY

FOR STUDENTS OF MEDICINE AND SCIENCE

BY

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FIFTH EDITION

With 3 Plates and 25 Text Figures

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"Nature, when you cannot take liberties with her,
is always a clog on Art."

GEORGE SAINTSBURY (Introduction to *Tom Jones*)

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Second Edition	*Reprinted*	1930
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Spanish Edition	. . .	1931
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PREFACE TO THE FIFTH EDITION

This edition has been almost completely rewritten. The rapid progress made in many of the fundamental aspects of the subject during the past few years has led to an increasing clarity, which has permitted more logical treatment of the different classes of compounds with which biochemistry is concerned. In consequence, since so much had to be rewritten, it seemed desirable to rearrange large sections of the book, and this has been done.

Ability to acquire a sound knowledge of biochemistry depends on a sound training in organic chemistry, though that training need not be extensive. Such prerequisite training is recognised as essential by most medical schools and is assumed in this volume. Corresponding training in physical chemistry is a most desirable prerequisite, but is not so often given. Hence certain of the facts of physical chemistry which are most needed in the study of biochemistry have been collected together in an early chapter, which those with adequate training in that branch of chemistry can neglect, and others can refer to at need.

The importance of the chemical agencies, the enzymes, hormones and vitamins in performing and controlling the chemical mechanisms of the body has been stressed by discussing them early in the book, and in close connection with each other. The closeness of this connection is exemplified by the fact that at least one vitamin unites with a protein to yield an important enzyme, while another, united to phosphate, functions as an important co-enzyme.

The biochemistry of the classes of compounds labelled carbohydrates, lipides, proteins, etc., is dealt with for each class as completely as possible in a single chapter, which discusses composition, digestion, absorption, utilisation and catabolism. In the same way all the factors bearing on diet have been brought together in one chapter.

In order to indicate to the student that the existence of enzymes, hormones and vitamins is no longer merely a matter of belief, but that many of these compounds have been isolated and crystallised, and their nature fully established, a number of new photomicrographs have been included.

The important and extensive work on oxidation-reduction

systems, which govern the heat production and a great part of the metabolism of the body, has been briefly reviewed, although the present state of knowledge still chiefly indicates the complexity of these systems, without yet yielding a clear picture of how they function in the cell.

Short notes have been added where possible, indicating the way in which abnormalities of metabolism involve the application of biochemistry in diseased conditions.

The important recent work on the chemical nature of many crystalline viruses has been briefly referred to.

As in previous editions, no attempt has been made to give long lists of references. A few references are given at the end of each chapter, for the use of the teacher, rather than of the student. These, as far as possible, are to reviews (which usually provide copious references), or to very recent papers, not yet reviewed. As will be seen, extensive use is made of the "Annual Reviews of Biochemistry."

I am indebted to a number of investigators and publishers for permission to reproduce many of the photographs and diagrams in the book. Acknowledgements have been made in the legends attached to the various plates and figures, and on p. vii.

I desire to thank Dr. F. D. White, Assistant Professor of Biochemistry in the University of Manitoba, for reading the manuscript and proof, and Miss Jean Guthrie, for assistance in reading proof.

I am greatly indebted to Messrs. J. & A. Churchill for the permission they gave me for this complete revision, and hope that it will permit the volume to have continued usefulness.

A. T. CAMERON.

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TEXTBOOK OF BIOCHEMISTRY

CHAPTER I

INTRODUCTION

Biochemistry comes from two Greek words, *bios*, meaning life, and *chymos*, meaning juice. One might use the alternative word *essence* for "juice," and that would suggest that the science deals with the *essence of life*. This, perhaps, would be a little exaggerated, yet, in so far as it infers that biochemistry is of great importance in the study of living processes, it is suggestive of the truth. In the dark ages of Arabian and Mediaeval study there existed men, part charlatan and part philosopher, who endeavoured to transmute base metals to gold—then, as now, the metal of value. They tried to get something for practically nothing, a habit still common, and they frequently succeeded in getting a comfortable living from the credulous, in which also they still have successors. They also pretended to seek, or actually did seek (for some of them were honest) the *elixir, the essence of life*, that something which would prolong life indefinitely, for perpetual life has always seemed to many a goal worth striving for. Perhaps this search had something to do with the name *alchemist* given to them, a term derived from the same Greek *chymos*. They never did learn how to transmute metals; the changing of one element into another was only accomplished by Rutherford within the last two decades, and is a costly process. They never found the elixir of life. All the profound advances in medical science during the last fifty years have only prolonged life a trifle. Raymond Pearl's is still the best dictum: if you want to live to a great age choose long-lived ancestors.

But these alchemists, in striving for the unattainable, learned many chemical facts, and from their pseudo-science gradually developed the science of chemistry, which to-day deals with the composition of substances, and the way in which substances interact with one another. Biochemistry is a part of chemistry, that part which deals particularly with the compounds in plants and animals, and the way in which these compounds interact

with one another in these living organisms. And since death is a part of life, its closing chapter, biochemistry is also concerned with the changes at death and with the decomposition of the organism after death.

The word biochemistry has only been in use for thirty years or so. The science, in its beginnings, was called physiological chemistry. Physiology is a science dealing with the normal organism, and in biochemistry we study more than the normal. We are, especially in a medical course, particularly concerned with disease. The part of biochemistry dealing with disease is frequently termed pathological chemistry, but the difference between physiological and pathological chemistry is often only a quantitative and not a qualitative one, and to stress the division is unwise.

The Importance of Biochemistry in Medicine. Since all living processes are carried on by chemical compounds and ions, both in health and disease, it is obvious that if we can obtain complete knowledge concerning these, and how they interact and behave in normal living processes, and what changes occur in their behaviour in diseased processes, then we shall be much nearer control or prevention of these changes : in other words, we shall be much more successful in curing or in palliating disease. We are still very far from such complete knowledge, but our knowledge of biochemistry is even now great enough to give helpful aid in diagnosis and treatment of many diseases, and is increasing rapidly.

For these reasons it is essential that the student of medicine, whether he aims to become general practitioner or physician or surgeon, should obtain such a good grasp of biochemistry that he can later apply it in his practice whenever its facts can be of the slightest assistance, and so that he can continue to appreciate and apply the newer facts which biochemical research is continually revealing, and which largely apply to medicine. Throughout this text attention will be drawn whenever possible to the practical applications of biochemical facts in the understanding and treatment of disease.

Types of Compounds of Importance in Biochemistry. These can, in large part, be divided into several groups.

(i.) Inorganic compounds : water, acids, alkalies, salts.

(ii.) Carbohydrates or glucides, some very complex, as starch, and others, the sugars, fairly simple compounds, of which glucose, the main ingredient of corn syrup, can be taken as example.

(iii.) The lipides, a large class of substances only loosely related chemically, but unified by possession of some similar physical properties, such as very slight solubility in water, and good or

fair solubility in such solvents as alcohol. The fats form an important division of this group, which also includes a great variety of compounds such as the phosphatides (*e.g.*, lecithin), the cerebrosides (*e.g.*, kerasin), the sterols (*e.g.*, cholesterol), the sex-hormones, certain coloured pigments (such as carotene of carrots), and several vitamins.

(iv.) The proteins (of which the solid part of meat and casein of milk can be taken as examples) and their derivatives, the amino-acids, which, as the term implies, are simultaneously basic and acid.

Physiological Processes. Any living organism, whether it be single celled, like the amoeba, or composed of millions of cells, as we are, manifests in living two important and distinct types of activity. The first is the reproduction of its kind ; the second is the transformation of food material which it ingests, received from outside it, into material for its own growth and repair, and into heat and external work. The higher the species ranks in the evolutionary scale, the less relatively important is reproduction in the life of an individual of that species. We are primarily concerned in this text with the biochemistry of man, and therefore are more concerned with the second type of activity.

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.

We have, then, to consider the chemical changes associated with, and the physical chemical mechanisms concerned in, the physiological processes of digestion, absorption, respiration, carriage of nutrient and other material within the organism (and the changes which take place in that nutrient material, changes

which, in sum, constitute what we call *metabolism*), and the excretion of degradation products, and in addition, such biochemical knowledge as is available concerning reproduction. And we have to discuss in some detail the special biochemical agents which bring about these various changes, the enzymes, hormones and vitamins. But first, before considering digestion, we must know something about the food which is to be digested.

Food. Man lives on a very varied diet—varying greatly according to race, climate and his individual likes and dislikes. While environment, purchasing power of the individual, and not infrequently lack of education concerning nutritional needs, make a perfect diet by no means too common, and even result in too many people consuming an improper diet, yet if the moderately good diet of the majority of people be chemically analysed, it is always found to contain (i.) water, (ii.) carbohydrates, (iii.) fats, with small amounts of some of the other lipides, (iv.) proteins, (v.) inorganic salts, and (vi.) traces of certain essential compounds needed for normal existence, the vitamins or their precursors. Certain vitamins can be listed under other categories, but they need to be stressed by placing them in a separate class.

The bulk of the food we eat is a solid material, insoluble in water. Cooking changes it in part. We say that it is rendered more palatable, but the increase of palatability is bound up with an increased digestibility. Starch and proteins are altered chemically, so that subsequent digestion is easier. In addition cooking kills most of the bacteria and parasites in the food.

Digestion. This takes place in the alimentary canal, which can be regarded as not truly within the organism itself, but a tube extending from mouth to anus, through which food passes, into which digestive juices are secreted from the organism, and from which digested products are absorbed into the organism.

Digestion in the mammalian organism consists essentially of a series of chemical and physical chemical processes by which those compounds of a diet (soluble or insoluble) which cannot pass through an animal membrane are converted into soluble compounds of smaller molecular size and capable of such passage.

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.

Saliva consists essentially of a very dilute solution of an enzyme or ferment called salivary amylase, which converts starch to the sugar called maltose by a process of hydrolysis,

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} + \tfrac{1}{2}nH_2O = \tfrac{1}{2}n\underset{\text{Maltose}}{(C_{12}H_{22}O_{11})}$$

and a glyco-protein (*i.e.*, a complex protein with carbohydrate radicals), a sticky compound called a *mucin*. When food is masticated it is balled together by this mucin, and so is easily swallowed. Mastication intimately mixes it with the amylase, which goes on acting upon the starch in the food for some time after it has reached the stomach.

The smell and sight of appetising food, and even the thought of it, and the presence of food in the mouth and in the stomach, all set up nervous reflexes, one of the results of which is the secretion of gastric juice from cells of the gastric mucosa of the stomach wall.

This *gastric juice* is to all intents and purposes just a dilute solution of hydrochloric acid, averaging a concentration of about 0·4 per cent., and containing in addition slight traces of three enzymes, called *pepsin*, *rennin*, and *gastric lipase*. Rennin clots milk in a very slightly acid medium.

Pepsin splits the big molecules of protein into a number of smaller molecules of *proteoses* and *peptones*, still large, but soluble in water. A fairly acid medium is necessary for this action. Gastric lipase slightly breaks up fats to fatty acids and glycerol.

When the acid gastric juice comes into contact with the neutral food mass in the stomach the acidity is partly neutralised ; after complete mixing the semi-liquid mass has an acidity corresponding to about 0·2 per cent. hydrochloric acid. The permeation by the acid secretion takes from fifteen to thirty minutes for completion. During this time, paradoxically, salivary digestion proceeds within the stomach, until increasing acidity finally halts it.

In infants the gastric juice is only very slightly acid. Peptic activity, the activity of pepsin, is small, but the clotting power of rennin on milk, the chief or sole constituent of the diet, is great. Any deficiency of rennin action can lead to gastro-intestinal disturbances in infants.

During gastric digestion the food mass is slowly moved forwards, partly by pressure from fresh food entering the cardiac end of the stomach, and partly by peristaltic waves set up in the stomach wall by actual presence of the food ; as it is pressed forward salivary and then gastric digestion bring much of its solid contents into solution. At the beginning of gastric digestion the pyloric orifice of the stomach is closed. As a result of increasing fluidity or acidity or both, after a varying time the pyloric sphincter relaxes, the orifice opens and fluid contents gush forth into the duodenum. Their acidity affects the mucous coat of the duodenum, so that a reflex nervous mechanism immediately causes closing of the pyloric orifice again. Alkaline digestive

juices pouring into the duodenum neutralise the acid material from the stomach, thus removing the stimulus to this reflex. The pylorus again opens, more acid contents pass into the duodenum, their acidity again sets up the reflex closing, and again is quickly neutralised. And so, little by little, the digested fluid contents of the stomach reach the duodenum, and finally undigested solid material also passes on.

The fluids which now come into action are three : the pancreatic juice, the intestinal juice and the bile. The first two are always alkaline ; the bile is usually alkaline.

When food reaches the duodenum, one of the reflexes set up brings a discharge of bile from the gall-bladder through the bile duct into the intestine. The presence of acid in the duodenum is believed to set free a compound called *secretin* in the cells of the duodenal mucosa. It is then rapidly absorbed to the circulation. Secretin is termed a *hormone* (Greek *hormao*, I arise), because it acts as a messenger, and can carry a chemical stimulus, producing an effect at a distance from its origin. It has two purposes. Some reaches the liver and causes secretion and outpouring of more bile. Some reaches the pancreas and stimulates the acinar cells of that organ to pour out the pancreatic juice, which rapidly reaches the duodenum by ducts from the pancreas.

Presence of food in the upper reaches of the intestine stimulates certain glands in its wall to secrete intestinal juice on to its surface.

We are now in a position to consider the composition of these juices and their actions.

The pancreatic juice is a dilute alkaline solution of several important enzymes. These include a pancreatic amylase, whose action is very similar to that of salivary amylase, a pancreatic lipase, which may be identical with gastric lipase, and which acts powerfully on fats in a weakly alkaline medium, and trypsin, which has important protein-splitting properties in neutral or weakly alkaline media.

The intestinal juice is a dilute solution of many enzymes. It contains enzymes capable of breaking up nucleic acids, and others which hydrolyse the sugar maltose to the simpler sugar glucose, the sugar sucrose (cane sugar) to glucose and fructose, and the sugar lactose (milk sugar) to glucose and galactose. It contains proteolytic enzymes which split the large fragments of the protein molecules (proteoses and peptones) to smaller fragments and finally to amino-acids. It also contains a compound *enterokinase* which activates trypsin.

Bile is a golden-yellow (to greenish-yellow) solution which contains both functional constituents and material for excretion.

The bile salts aid digestion and absorption. Because they markedly lower the surface tension of aqueous solutions, they facilitate the breaking up of large fat globules into small ones, an important aid in the digestion of fat, since lipase acts at the surface of the fat globule, and does not permeate it. They facilitate absorption of fatty acids through the wall of the intestine. In man the chief bile salts are the sodium and potassium salts of glycocholic and taurocholic acids.

A solution of bile salts is able to dissolve a moderate amount of the compound cholesterol, and bile accordingly is the vehicle by which the body gets rid of its excess of cholesterol. The colour of bile is due to the bile pigments, chiefly red-yellow bilirubin and green biliverdin, degradation products of the haemoglobin of the red blood cells, also excreted through this channel.

Under the mixed action of these three juices the acid contents from the stomach are first neutralised and then digested fairly rapidly. Proteins and partly broken down fragments of proteins are split further, until simple compounds, chiefly amino-acids, result. Fats are split to glycerol and fatty acids (some proportion of which may be neutralised to soaps). Starch is broken down to maltose, and maltose, sucrose and lactose to the simpler sugars glucose, fructose and galactose. The nucleic acids are split to simpler compounds. These enzymic changes are all *hydrolyses*, reactions in which large molecules are caused to combine with one or more molecules of water, this act of combination resolving them immediately into two or more smaller molecules. A good example of hydrolysis is the splitting of cane sugar to glucose and fructose :

$$\underset{\textit{Sucrose}}{C_{12}H_{22}O_{11}} + HOH = \underset{\textit{Glucose}}{C_6H_{12}O_6} + \underset{\textit{Fructose}}{C_6H_{12}O_6}$$

As a result of all these actions the intestinal contents gradually become a solution of amino-acids, simple sugars, fatty acids, glycerol and similar simple compounds, with undigested and undigestible residues suspended in the solution. Bacterial reactions proceed in this medium to varying extent, without materially affecting the supply of nutrient material to the host. (Such bacterial actions will be dealt with in Chapter XIII.).

Intestinal peristalsis moves the contents onwards and downwards, continually bringing them into contact with fresh expanses of intestinal mucosa, and absorption proceeds rapidly. The mucous membrane of the small intestine has a very great absorbing surface, largely due to the immense number of tiny finger-like *villi* which project into its lumen. Each of these

tiny projections has a central duct or *lacteal*, ending blindly near its tip, and opening below into the plexuses of lymphatic vessels in the muscular coat of the mucus. The lacteal is surrounded by capillaries which join to form the venules of the portal circulation.

The unabsorbed and unabsorbable material passes on to the large intestine, where it is concentrated to faeces by absorption of water, and the faeces are finally expelled.

Absorption through the mucous membrane lining the intestine is passage across an animal membrane. The possibility of absorption depends on several factors, of which two of the most important are, that the compound to be absorbed must be of relatively small molecular size, and must be in solution in water. The small-sized sugars, amino-acids and glycerol fulfil these conditions, and absorption of these takes place throughout the length of the small intestine. Bile salts are absorbed and carry with them the fatty acids.

Sugars and amino-acids pass into the blood in the capillaries within the villi of the intestine, and so to the portal vein and the liver. Glycerol and fatty acids recombine to form fats during passage through the cells of the intestinal mucus ; these fats in large part reach the lacteals of the villi, and pass by way of lymph-vessels to the thoracic duct and so to the blood of the general circulation. The liver may store part of the material reaching it, but sooner or later also passes it on to the blood of the general circulation ; galactose and fructose are changed to glucose in the liver, and much of the glucose is temporarily stored there as glycogen, a starch-like compound.

Respiration. The mechanical movements of the lungs draw atmospheric air, with its content of oxygen, into the lungs, through which *venous* blood is passing. Oxygen diffuses across the lining membranes of the alveoli of the lungs into the blood capillaries and is taken up by the haemoglobin of the red blood cells to form oxy-haemoglobin ; this effects the colour change from the purplish-blue of venous blood to the scarlet-red of arterial blood. The oxygenated blood, so altered, passes back to the heart and the general circulation. Oxygen is given up to the tissues throughout the body from the blood in their capillaries, while carbon dioxide passes from them to this blood, and so to the lungs, completing the *respiratory exchange*.

Transport of Material. The arterial blood conveys glucose, amino-acids, fats and other nutrient material, and oxygen, throughout the organism. Cells in different tissues withdraw the compounds they need, replacing them with their degradation products, their waste.

Metabolism. Within the cells throughout the organism a vast number of activities progress. From the material drawn from blood the tissue cells build the enzymes they need, and their own structural material, elaborate compounds specific to their cell-type, and produce more or less energy in the form of heat as a result of their reactions ; muscle cells, in addition, through their specific reactions, enable the accomplishment of work. The sum of all these various activities of the cells of an organism constitutes its metabolism.

Excretion. Cellular activities produce waste products, of which carbon dioxide and urea can be taken as typical examples. These waste products pass outwards from the cells to the nearest capillaries, and so to the venous blood. There are five channels of excretion, the lungs, which excrete carbon dioxide and some water, the kidneys, excreting urine, which removes water and many simple soluble inorganic and organic waste products, the liver, secreting bile, whose excretory contents have been mentioned, the mucosa of the large intestine, through which are excreted such compounds as calcium salts and phosphates, and the sweat glands, whose excretion is very largely water. The faeces contain the material excreted by way of the bile and the large intestine, and in addition, food residues and residues from the digestive juices, unaltered or changed through bacterial action.

Reproduction. The chemical basis of reproduction is largely associated with the actions of certain sex-hormones, which govern the development and functioning of the specific glands and tissues concerned with reproduction.

Biochemical Agents. There are three series of chemical compounds which control a large proportion of the activities of the mammalian organism, the enzymes or ferments, the hormones or endocrine principles, and the vitamins.

Besides the enzymes of the digestive juices, every living cell contains its own enzymes which carry out actions specific to that type of cell. These, in sum, constitute the intra-cellular enzymes. Many of them are concerned, not with hydrolyses, but with oxidation and other types of reaction.

The hormones, to which group secretin belongs, are secreted by special glands, and for the most part are discharged directly into the circulation, wherefore such glands are called *ductless glands*. These hormones vary greatly both in composition and in the nature of their actions. Typical on the one hand are the complex compounds formed by the pituitary gland, protein in nature, which help to control directly or indirectly most of the body's activities, and, on the other hand, the compound adrenaline

of simple constitution which can produce marked increase of blood pressure, and which facilitates breakdown of liver glycogen to glucose.

The vitamins are also very varied in type, though none is highly complex in composition. They are only classed together because of the late discovery of their existence and their essentiality in a diet for normal healthy living. There are about a dozen of these vitamins. They are still named alphabetically, *A*, *B*, *C*, etc., because their chemical natures are only slowly being determined. Their actions are as varied as their chemical natures, but these actions are absolutely essential for normal growth and health.

CHAPTER II

PHYSICAL CHEMICAL CONCEPTS OF IMPORTANCE IN BIOCHEMISTRY

Matter and Energy. The material constituting living organisms, like that in non-living matter, is built up of atoms, existing combined in molecules, and uncombined but carrying positive or negative electrical charges due respectively to loss or gain of electrons, and termed *ions*.

The internal constitution of atoms, and the inherent oneness of matter and energy, though of great general interest, do not appear to be of immediate consequence to the student of biochemistry. The units of matter of which living material is built up are sufficiently static during the life and death of an organism to call as yet for no discussion of events perpending upon transformations of matter into energy or *vice versâ*.

The law of conservation of matter, which states that it can neither be created nor destroyed, holds true for matter in the living organism.

The absolute weight of atoms has now been determined by various processes, and it is agreed that that of the atom of hydrogen (of molecular weight 1·008, by comparison with oxygen as 16·000) is approximately $1{\cdot}66 \times 10^{-24}$ gm. The corresponding weights of atoms of other elements can thus be determined from this and a table of atomic weights.

The proved existence of isotopes of many of the elements (that is, their existence in more than one form, with atomic weights differing by one or more integers) must not be forgotten, however, in estimating such absolute weights. Thus hydrogen exists in two forms, light hydrogen or *protium*, atomic weight 1, and heavy hydrogen or *deuterium*, atomic weight 2. Moreover, the existence of isotopes is already being utilised in biochemical studies. Experiments with compounds containing heavy hydrogen have already yielded important results bearing on the metabolism of fats (*cf.* Chapter V.).

The absolute weight of an electron varies according to its speed; that of relatively slow-moving electrons such as those present in living material has been calculated to be of the order of $9{\cdot}0 \times 10^{-28}$ gm.

The relative *sizes* of atoms and molecules are of some importance in enabling us to obtain a correct conception of the size of the living cell, as contrasted with the number of atoms or molecules contained in that cell.

The human red blood cell has an average diameter of 0·0088 mm. or $8{\cdot}8\mu$ (the unit μ being one-thousandth part of a millimetre). The rim of the circle in Fig. 1 corresponds to that of a human red blood corpuscle magnified 10,000 times. The outlined areas within this circle show the relative sizes of particles of such material as kaolin and gum mastic, and of various bacteria from the large anthrax

bacterium to the small *B. influenzae*. Within the dotted rectangle the magnification has been increased another hundred times, so that the tiny particle of colloidal gold, seen as a dot at magnification 10,000, becomes a square of 1·5 cm. side. This is much larger than the molecule of starch, and that is again much larger than simple molecules of such compounds as chloroform, and still more than hydrogen molecules. In order to express the minuter sizes of these a still smaller unit of length is needed, $\mu\mu$ or $m\mu$, one-millionth of a millimetre, or one-thousandth of μ. Expressed in terms of this unit a molecule of starch

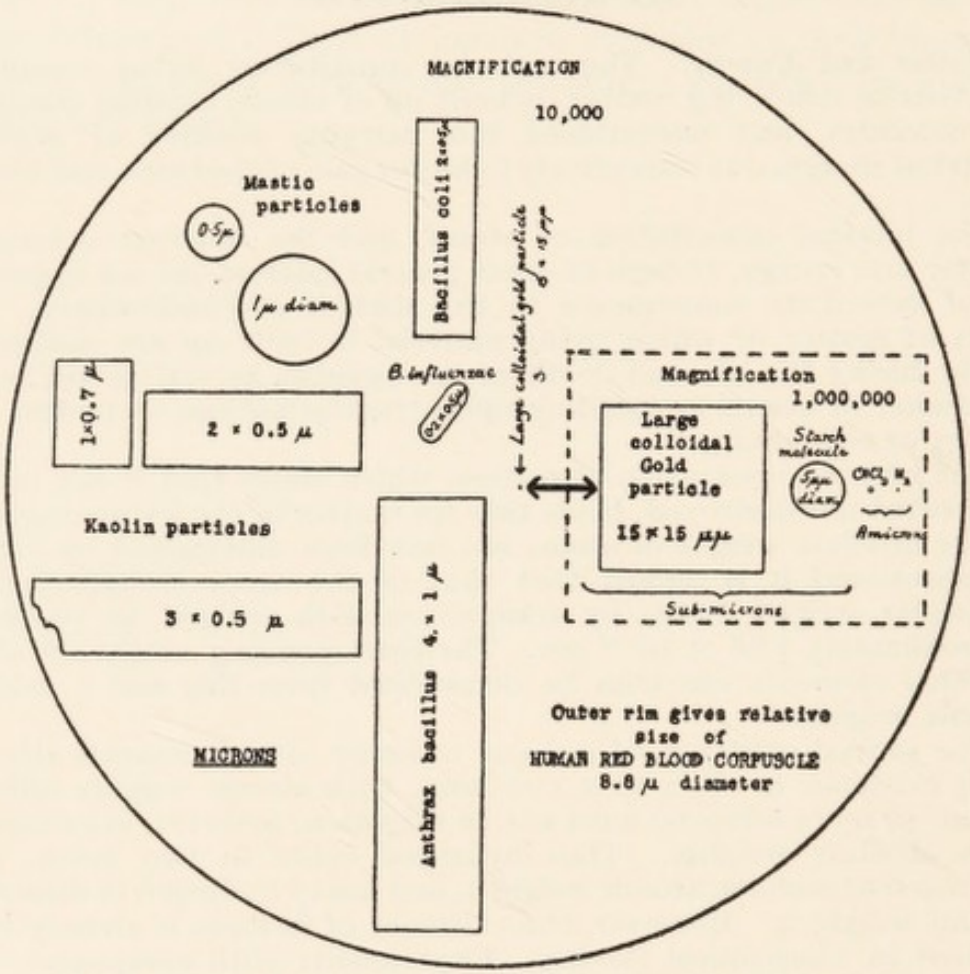


FIG. 1. Scheme illustrating various sizes of particles and molecules. (Modified from W. Ostwald.)

has a diameter of 5 m . It is quite evident that the tiny red blood cell, of which there are, on the average, five millions in each cubic millimetre of blood, is large enough to hold very large numbers of such large molecules as starch.

By simple calculation actual figures can be obtained for the content of some of the constituents of the red blood cell. Accepting the average figures 5,000,000 red cells per cubic millimetre of blood, which contains 45 per cent. of these cells by volume, and an average specific gravity of these cells as 1·055, it follows that 1 c.c. of cells (1·055 gm. of cells) contains $5 \times 10^6 \times 10^3 \times 100/45$ or $1{\cdot}11 \times 10^{10}$ cells, so that the average cell weighs $0{\cdot}95 \times 10^{-10}$ gm. The chief constituent of these cells is the red pigment haemoglobin, present to

the extent of 34 per cent. in average normal adult human blood. Haemoglobin has a molecular weight approximating to 68,000, and thus has a distinctly heavy molecule.

Since 100 gm. of red cells contain 34 gm. of haemoglobin, one cell, weighing $0{\cdot}95 \times 10^{-10}$ gm., contains $34 \times 0{\cdot}95 \times 10^{-12}$, *i.e.*, $3{\cdot}23 \times 10^{-11}$ gm. of haemoglobin.

Remembering that the atom of hydrogen weighs $1{\cdot}66 \times 10^{-24}$ gm., it follows that the molecule of haemoglobin with molecular weight 68,000 weighs $1{\cdot}66 \times 10^{-24} \times 68{,}000/1{\cdot}008$, or $1{\cdot}12 \times 10^{-19}$ gm.

Hence the number of molecules of haemoglobin in one cell is given by the ratio

$$\frac{\text{Weight of haemoglobin in one cell}}{\text{Weight of one molecule of haemoglobin}} = \frac{3{\cdot}23 \times 10^{-11}}{1{\cdot}12 \times 10^{-19}} = 2{\cdot}88 \times 10^{8}$$

or 288 million molecules.

In the same way it can be calculated that the number of molecules of liquid water (H_4O_2) in a single red cell is close to one million million, and that each red cell contains nearly 300 million molecules of the sugar glucose ($C_6H_{12}O_6$) and of the waste product urea (CON_2H_4), and so on.

It is quite evident that the enormous numbers of molecules present in such minute cells (and cells of the various tissues are of comparable size) will permit a vast variety of chemical reactions to proceed, and that this is true even in such smaller unicellular organisms as the bacteria.

Measurement of Energy. It is important to realise from the outset that in biochemistry we are not only concerned with the qualitative chemical reactions proceeding within the organism, but, so far as we can ascertain them, with the quantitative changes also, and since these changes usually involve production of some heat, and sometimes of work as well, it is desirable to define the units in which these forms of energy are measured.

Heat is defined in terms of calories. The (*large*) *calorie* (Cal.) is defined as the amount of heat required to raise 1 litre (1,000 c.c.) of water from 15° to 16° C. The (*small*) *calorie* (cal.), more useful in expressing minute quantities of heat such as that developed in a frog's muscle during a single contraction, is the quantity of heat required to raise 1 c.c. of water from 15° to 16° C. The first experimental comparison of the units of heat and work was made by the physicist Joule, and the ratio of the two, usually, therefore, termed Joule's equivalent, is given by the relation : The work done in lifting 426 kg. through 1 metre (or 1 kg. through 426 metres) against the force of gravity is exactly equal to 1 (large) calorie, or, in the usual units, 1 small calorie of heat equals $4{\cdot}182 \times 10^{7}$ ergs, or 4·182 joules.

Energy can be transformed from one form into another, as from work into heat, but it can neither be created nor destroyed, only so transformed. This principle is the so-called law of *conservation of energy*.

The great majority of chemical reactions either liberate heat or absorb heat. Oxidations occurring within a living organism all liberate heat, so that oxidation and heat production go hand in hand.

States of Matter. In the study of biochemistry we are concerned with all three states of matter, solid, liquid and gas.

Most solid matter in the living organism is present in the *amorphous*

form, non-crystalline. There is some evidence, however, that the solid constituents of bone approximate to a crystalline structure. It is important to remember that we have no sound criteria to determine the degree of purity of amorphous compounds. Crystallisation of such compounds is an important and necessary first step towards achieving their complete purity. Their preparation in a state of purity is essential before proof can be established that they are associated with any particular physiological activity.

The only liquids we have to consider are aqueous solutions, though these, as we shall see, are of varying and great complexity.

Relatively few gases concern us, oxygen, nitrogen, carbon dioxide, water vapour, and, to a much smaller extent, gases such as methane, hydrogen sulphide and so on.

The Kinetic Theory of Gases and Solutions. Molecules of a gas are in constant motion, and through this intrinsic energy they exert pressure on the walls of the vessel containing the gas. According to the kinetic theory of gases, equal numbers of molecules of a gas or of a gaseous mixture exert equal pressures. Different molecular size is not a factor in determining this pressure. Hence the *partial pressure* of a particular gas in a gaseous mixture is determined by the proportion by volume of that gas in the mixture. The standard pressure of the atmosphere is defined as equal to that of a column of mercury 760 mm. in height. The oxygen content of this admixture (neglecting the variable trace of water vapour present) is 20·95 per cent. by volume. The partial pressure of oxygen in (dry) air at standard pressure is therefore equal to $760 \times 20{\cdot}95/100$ or 159 mm. of mercury.

Through the constant movement of gaseous molecules a mixture of gases quickly becomes of constant composition throughout any confined space. If a foreign gas is introduced, it spreads rapidly by a process of *diffusion* throughout the confined space until distribution is again equalised. Collision with molecules of other gases only delays this process to a negligible extent at ordinary pressures, although theoretically with greatly increased pressures and consequently much closer packing of the gas molecules, much increased frequency of collision must slow down the rate of diffusion.

In an aqueous solution the dissolved substances (*solutes*) behave similarly, but the close packed molecules of water markedly slow diffusion ; the larger the size of the dissolved molecules, the more will their diffusion be delayed by frequent collisions. Molecules of solute also, through their motion, impinge on the walls of the containing vessel, and thus each dissolved substance can be considered to set up its own pressure in the solution. This is termed *osmotic pressure*, and there is experimental evidence that this pressure is exactly the same as that which would be exerted by the same number of molecules of solute, could they be converted to a gas occupying the same space as the solution. (There are various theories of the mechanism of causation of osmotic pressure ; that outlined is one of the simplest.)

The average distance (*mean free path*) travelled in a straight line by a gas molecule, before collision alters its direction, diminishes with increase of pressure of the gas. In solutions the average distance in a straight line travelled by a molecule of solute is very small by comparison. Such a molecule, if of small size, progresses by zig-zag motion ; the extent of this motion lessens with increase in molecular size. Extremely large molecules and suspended particles merely seem to

oscillate about a fixed centre. This oscillation is termed *Brownian* movement, so named from the botanist, R. Brown, who in 1827 observed the oscillations of pollen grains suspended in water.

Thermochemistry. The heat produced in chemical reactions is measured in apparatus of varying degrees of complexity, termed *calorimeters* (the name obviously means " measurers of heat "). In biochemistry we are particularly interested in the heat potentially available in the food we eat, its *potential energy*. For example 1 gm. of starch, when oxidised, produces just over four (large) calories, and this is termed its *caloric value*. In estimating heat losses from the body it is also necessary to remember that much heat must be provided by the body to evaporate sweat (almost entirely water) from its surface. The *latent heat of vaporisation of water* is 537·5 (large) calories per gram.

Physical Chemistry of Aqueous Solutions. Solubility is a universal property of all substances, varying from an immeasurably small to an infinitely great amount. The more closely a substance is related chemically to its solvent, the greater will be its solubility in that solvent. Ethyl alcohol, $CH_3.CH_2OH$, can be regarded as water, HOH, in which one hydrogen atom is replaced by an ethyl (C_2H_5) group, and is completely miscible with water in all proportions. Chloroform ($CHCl_3$) and fats are not chemically related to water, and are almost completely insoluble in it.

Substances in aqueous solution may be present either as molecules, or as *ions* formed by *dissociation* of molecules, or as a mixture of molecules and the ions formed from them. A solution of urea, CON_2H_4, or of glucose, $C_6H_{12}O_6$, contains un-ionised molecules. A solution of sodium chloride is largely ionised. It contains a small proportion of NaCl molecules, but most of these have broken up, *dissociated*, into electrically charged sodium and chloride ions :

$$NaCl = Na^+ + Cl^-$$

Dissolved substances affect the properties of the solvent. Its boiling point is raised, and its freezing point is lowered. Each molecule or ion produces an equal effect in such changes, no matter what its size, and the elevation of the boiling point and depression of the freezing point of a solvent are important measurements employed in determining the molecular weight of the solute, or its degree of ionisation. On account of this effect on the freezing point of water, cold-blooded animals can be cooled a little below 0° C. without freezing their body fluids.

Certain definitions can usefully be introduced here. A *molar* solution of a compound is defined as one which contains the molecular weight in grams of the compound dissolved in 1 litre of solution. A *normal* solution contains one gram-equivalent of the dissolved compound in 1 litre of solution, a gram-equivalent being the amount of the compound capable of reacting with or being substituted for one gram-atom (1·008 gm.) of hydrogen, so that equal volumes of solutions of the same degree of normality are equivalent to each other. In a normal solution one cubic centimetre or millilitre (the difference in volume of these is negligible) contains 1 milli-equivalent of the dissolved compound (the solute), made up of 1 milli-equivalent of base and 1 milli-equivalent of acid. For example, 1 c.c. of *N* hydrochloric acid contains 36·5/1,000 gm. of hydrochloric acid, *i.e.*, 36·5 mg., made up of 1 mg. of

hydrogen and 35·5 mg. of chlorine. Hence, since 1 milli-equivalent of (chloride-)chlorine is 35·5 mg., the number of milli-equivalents of (chloride-)chlorine in any volume of solution is given by (total number of milligrams of chloride-chlorine in the solution)/35·5.

Physical Chemistry of Interfaces. If we consider any system of matter that is not homogeneous, but is composed of several different parts each separated off from the other, then each one of the homogeneous parts making up a heterogeneous whole is spoken of, chemically, as a *phase*. These phases are separated by *interfaces*. An interface must exist between two different states of matter, solid/liquid, liquid/gas, and solid/gas. It may exist between two solids in contact, or between two liquids which are not completely miscible. It cannot exist between two gases.

The interface between liquid and liquid, or liquid and gas phases is capable of motion, is *mobile*. It possesses the property of tending to shrink to a minimum area. Typical of this is the assumption of the spherical form by gas bubbles in a liquid, or liquid drops (as fine spray) in a gas, or liquid drops suspended in another liquid. The ratio between surface and volume is lowest in the sphere. The contractile force which seems to produce such a reduced surface is termed *surface tension*, or interfacial tension.

Some idea of the mechanism at work can be obtained if we contrast the condition within a liquid with that at its interface with air. Within the liquid its molecules are subject to equal forces in every direction, but the surface layer of molecules is submitted to a much greater pull inwards into the liquid than that from the negligible effect of molecules in the gas phase. Hence the strain on the liquid surface pulling it into the least possible area.

The force of surface tension can be illustrated by a simple experiment. If a circle of wire is dipped in a soap solution, and then a fine loop of silk is floated in the soap film and the film within the loop is removed by touching it with filter paper, the loop is immediately stretched to a circle.

Most salts and strong alkalies increase the surface tension of a solution, but ammonia and strong acids decrease it. Most organic compounds which dissolve in water lower its tension a little, soaps do so distinctly and the bile salts produce a marked lowering which is of great importance in aiding the intestinal digestion of fats, since it helps to break up fat globules and thus to increase the total interfacial area between fat and the surrounding aqueous fluid in the intestine.

This increase of interface between two phases, produced by dividing one into a series of smaller volumes, can be easily illustrated by such a calculation as the following. 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 chloroform 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 all of them would be just over 483 sq. cm. The area of the interface would therefore have increased nearly five times. Division into 10,000 spheres each of 0·01 c.c. volume would give a total interface of about 2,245 sq. cm., and into one million spheres each of 0·0001 c.c. a total interface of somewhat over 10,400 sq. cm. Yet the diameter of these minute spheres, 0·576 mm. is still more than 100,000 times

that of a large molecule such as starch or haemoglobin, so that the sum of the interfaces between haemoglobin and water in the red blood cell is obviously very great. (In general it can be shown (Fine, *Biochem. J.*, 1931, 25, 647) that when any particle undergoes division into n equal sized smaller particles, the total surface of the smaller particles is $\sqrt[3]{n}$ times the surface of the parent particle.)

Each red cell contains, in round numbers, 300 million molecules of haemoglobin (*cf.* p. 13). The radius of the spherical molecule of haemoglobin has been found experimentally to be 2·44 $m\mu$. From this it can be calculated that the combined surface area of all the haemoglobin in a single red cell is somewhat more than 0·02 sq. mm. The volume of a red cell is $0{\cdot}9 \times 10^{-7}$ cubic mm. Its surface, were it a sphere, would be $0{\cdot}97 \times 10^{-4}$ sq. mm. Hence the combined surface of all the haemoglobin within it is more than 200 times that of its own. And it can similarly be calculated that the total combined surface area of all the haemoglobin in the blood of an adult man is not far from 400,000 times greater than the surface area of his body.

Such large interfacial areas are of great importance in connection with the phenomenon termed *adsorption*.

In a heterogeneous system of two phases it is often found that the concentration of a particular compound is either greater or less at the interface between the phases than it is in either phase itself. The presence of a second phase tends to upset the homogeneous distribution in the first. The difference of concentration at the phase boundary is termed *adsorption* (L. *ad sorbere*, to drink up). If the concentration of the compound in question is increased at the interface, then *positive* adsorption has occurred (with lessened concentration, negative adsorption, we are not especially concerned). The substance adsorbed (drunk up) stays at the surface ; it does not penetrate the second phase. Adsorption may be due to a variety of causes, chemical, cohesional, electrical, etc.

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.

As indicated above, in solutions of biochemical interest the large interfacial areas furnished by compounds with relatively huge molecules afford many opportunities for occurrence of phenomena dependent on adsorption. It is considered to play an important *rôle* in the actions of enzymes, the combination of toxins with antitoxins, the sensitising of leucocytes by opsonins with subsequent ingestion of bacteria by the sensitised leucocytes, etc., while possibly the distribution of a compound in the protoplasm of a cell is largely governed by its concentrations at different interfacial boundaries within the cell.

Reaction Equilibria and the Law of Mass Action. When a chemical reaction takes place it seldom happens that it proceeds to completion and that some one of the initial reacting compounds entirely disappears, unless the products of the reaction are removed from the medium in which it is occurring. In presence of the products sooner or later an *equilibrium* is attained, at which the original reaction is balanced by one between the products, reversing the procedure. (This, however,

may proceed so slowly that the original reaction appears to proceed to completion.)

The equilibrium in a particular reaction depends not only on the specific nature of that reaction, but also on the *masses* of the reacting substances (or their concentrations, when they are in solution). The law of mass action, enunciated by the Scandinavian scientists Guldberg and Waage as long ago as 1864, states, in its simplest form, that the velocity of a chemical reaction is proportional to the product of the concentrations of the reacting substances.

When only one compound is undergoing change, and therefore only one kind of molecule, the reaction is termed *monomolecular*, and from definition the rate of reaction at any time must be proportional to the actual concentration of the compound at that time. This is expressed by the equation

$$dx/dt = k(a - x)$$

which transforms to

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

where a is the initial concentration, x the amount of substance already changed at time t, and k is a constant, and dx/dt is the velocity of the reaction (the fraction of x changing in a fraction of time). The curve representing this equation is shown in Fig. 2.

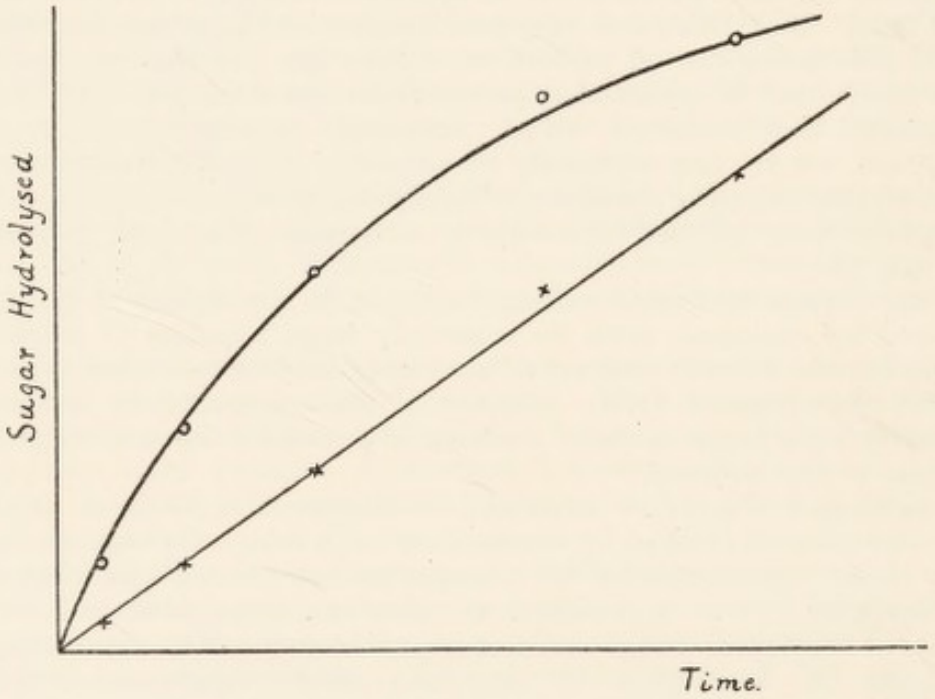


FIG. 2. Curve representing the course of a monomolecular reaction, and the corresponding logarithmic curve (inversion of cane-sugar by acid from Wilhelmy's data). The amount of sugar hydrolysed (x) is plotted against time (t). The straight line represents log $a/(a - x)$ plotted against time.

Many enzymic reactions approximate to this type because, in hydrolyses, such as when for example cane-sugar is hydrolysed to glucose and fructose by enzymic or other action, the concentration of water in the solution is not measurably changed by the minute amount taking part in the reaction.

In reversible reactions of the general type

$$A + B = C + D$$

the velocity of reaction between A and B is expressed by the relation

$$V_1 = (\text{concentration of A}) \times (\text{concentration of B}) \times k_1$$

while the velocity of the reversed reaction between C and D is likewise expressed

$$V_2 = (\text{concentration of C}) \times (\text{concentration of D}) \times k_2$$

(k_1 and k_2 being constants). When an equilibrium is reached the two velocities must be equal, so that

$$\frac{(\text{Concentration of C}) \times (\text{concentration of D})}{(\text{Concentration of A}) \times (\text{concentration of B})} = \frac{k_1}{k_2} = \text{K}.$$

K is termed the equilibrium constant for the particular reaction. It is convenient to indicate " concentration of " by the parentheses, and to write such an equation in the form

$$\frac{(\text{C}) \times (\text{D})}{(\text{A}) \times (\text{B})} = \text{K}.$$

Catalysis. Ordinary reactions may be divided into two classes. The first 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 mixed, or the formation of unionised water which occurs in the neutralisation of a strong acid by a strong base. Such reactions proceed almost to completion.

The second class of reaction requires a measurable time. A good example is the reaction between ethyl acetate and water. It never approaches completion ; 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. Examples of catalysis are the hydrolysis of cane-sugar to a mixture of glucose and fructose in the presence of mineral acids (and therefore 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 or manganous salts.

One of the most important effects of a catalyst is *the shortening of the time of reaction*. The reaction between ethyl acetate and water

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

only reaches an equilibrium after many days. But if a small quantity of mineral acid is added the hydrogen ions catalyse the reaction to an equilibrium within a few hours ; *the point of equilibrium is practically unaltered.*

Catalysts accelerate a reversible reaction in either direction, and give the same equilibrium point.

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.

A catalyst may therefore be defined 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 reduced the speed of oxidation. It has been suggested that a " negative catalyst " produces its results indirectly, by suppressing the action of some true catalyst.

Autocatalysis. The name suggests " self-catalysis," as if the reaction assists itself to proceed ; this suggests that the products of the reaction, or one of them, acts as catalyst for the reaction. Such a state of things can occur when a catalyst A acts on a compound B, to break it up to

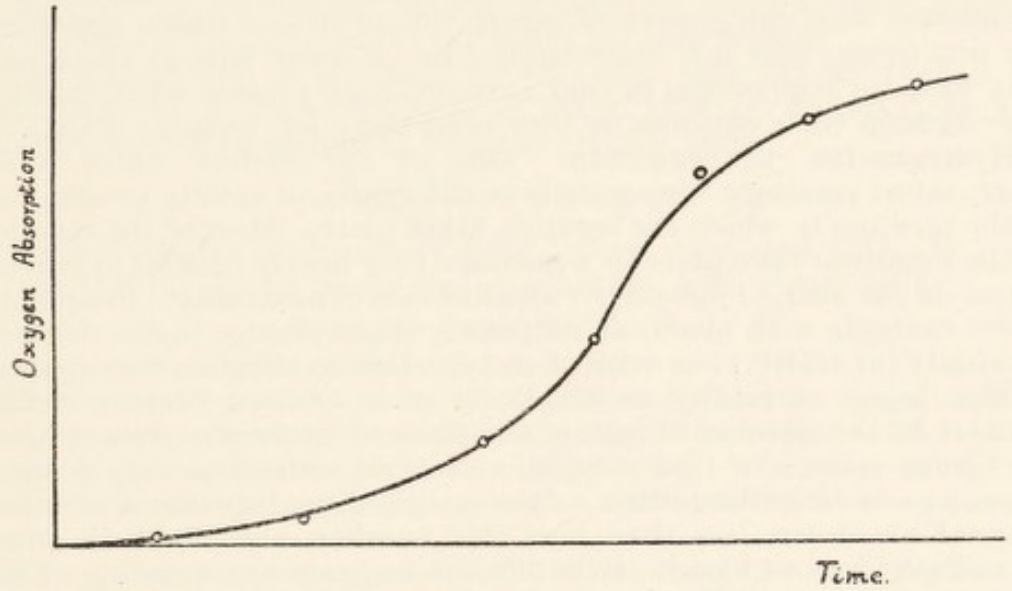


FIG. 3. Curve representing the rate of absorption of oxygen by linseed oil (from data in Glikin's " Chemie der Fette," 1913). The initial part of the curve indicates an autocatalytic effect.

more of A and a third compound C. Then the reaction equation becomes

$$dx/dt = k(a + x)(b - x)$$

where a and b are the initial concentrations of A and B, and x is the amount of B which has been changed in time t, so that $(a + x)$ and $(b - x)$ are the concentrations of A and B after time t.

Many autocatalytic reactions seem to be variants of the ordinary monomolecular reaction

$$dx/dt = k(a - x).$$

Catalysers affect the value of the constant k, both by their specific nature and by the amount of them present. In autocatalysed reactions, when the products are the catalysers, they must be proportional to x, the amount of substance changed. Hence in such a type of reaction the velocity is given by

$$\text{velocity} = kx(a - x)$$

which can be expressed in the form

$$\text{og } \frac{x}{a - x} = ka(t - t_1)$$

where t is the time since the beginning of the reaction during which x

of the original compound has been changed, and t_1 is the time which will have elapsed when half the reaction is completed.

Robertson and others showed long ago that the ordinary growth of individuals, and many other physiological processes give reaction curves which closely conform to this equation. A typical autocatalytic curve is shown in Fig. 3.

In the initial stages of such reactions as that between ethyl acetate and water autocatalysis plays a *rôle*, since acid is formed, and the slight concentration of hydrogen ions resulting facilitates the reaction. Spontaneous oxidations of many fats and oils, and especially of the so-called " drying oils " employed in paints and varnishes, afford other examples.

Autocatalysis seems to be of potentially great importance in connection with the growth of certain plant viruses which apparently are non-living, and not improbably also of some animal viruses ; it may have an application in that extraordinary process whereby tissue cells develop their enzymes as they need them (*cf.* Chapter XIX.).

Hydrogen-Ion Concentration. 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 10 million litres. An average normal specimen of human arterial blood has less than half this number, actually 0·47 gm. in 10 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 section 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 :

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

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 :

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

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 the *mass law*. If we express the concentration of hydrogen ions as (H^+), that of hydroxyl ions as (OH^-), and that of unionised water as (HOH), then

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

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 1 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 1 litre, *i.e.*, 0·0000001 gm. of H^+ and 0·0000017 gm. of OH^- in 1 litre. The relationship is grasped a little easier when expressed as 1 gm. of hydrogen ions and 17 gm. 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 I. 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-ion 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 $9{\cdot}1 \times 10^{-2}$.

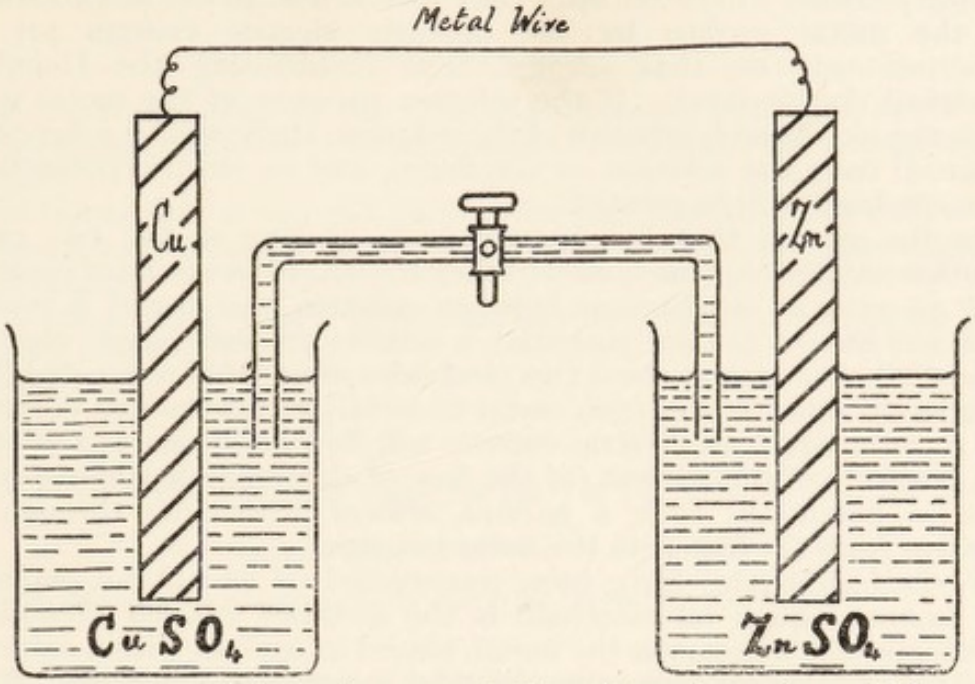


FIG. 4. Schematic representation of two electrodes united to form a cell.

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. 4.

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 potentials 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

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

Such values can also be easily derived from the expression

$$(H^+) = 10^{-pH}$$

if it be remembered that the logarithm of a number to the base 10 is the power to which 10 must be raised to give that number.

Thus, for N/10 hydrochloric acid :

$$(H^+) = 9{\cdot}1 \times 10^{-2} = 10^{0{\cdot}959} \times 10^{-2} = 10^{-1{\cdot}041}$$

whence $pH = 1{\cdot}041.$

Again, for N/10 acetic acid,

$$(H^+) = 1{\cdot}3 \times 10^{-3} = 10^{0{\cdot}114} \times 10^{-3} = 10^{-2{\cdot}886}$$

whence $pH = 2{\cdot}886.$*

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 $4{\cdot}68 \times 10^{-8}$.

Alternately :

$$pH = 7{\cdot}33$$
$$(H^+) = 10^{-7{\cdot}33} = 10^{0{\cdot}67} \times 10^{-8}$$
$$= 4{\cdot}68 \times 10^{-8}.$$

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 gm. 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

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

* I am indebted to Dr. H. R. Noltie for suggesting these alternative methods of calculation.

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.

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.

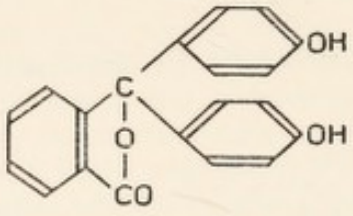
Purest water, *p*H, 7·0 ;
(H^+), $1{\cdot}0 \times 10^{-7}$ or 0·00000010.

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

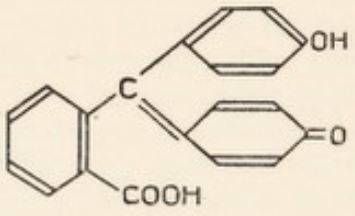
Normal urine (alkaline limit), *p*H, 7·5 ;
(H^+), $3{\cdot}2 \times 10^{-8}$ or 0·000000032.

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 different 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 Phenolphthalein



Red Phenolphthalein

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 about *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.

Typical of 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 :

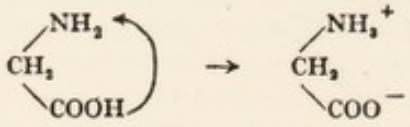
Thymol blue changes from red (*p*H 1·2) to yellow (*p*H 2·8).
Brom phenol blue changes from yellow (*p*H 3·0) to blue (*p*H 4·6).
Brom cresol green changes from olive green (*p*H 3·8) through green to blue (*p*H 5·4).
Methyl red changes from red (*p*H 4·4) to yellow (*p*H 6·0).
Chlor phenol red changes 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.

Amphoteric Electrolytes (*Ampholytes*) (Gk. *amphoteros*, on both sides) are compounds which have the properties of both base and acid. In acid solutions they ionise as bases, splitting off hydroxyl groups, while in alkaline solutions they behave as acids. At some point in the neighbourhood of neutrality, which varies for each ampholyte, their solubility is at a minimum, and they are most easily precipitated or crystallised from their solutions ; this is termed the *iso-electric point*. Certain of these ampholytes near their iso-electric points split off both hydrogen and hydroxyl ions, leaving a neutral ion. In others

an internal rearrangement seems to occur, producing an ion carrying both a positive and a negative charge and thus behaving as a neutral ion. Such have been termed *zwitterions* (Germ., *Zwitter*, hybrid, hermaphrodite). Amino-acids and proteins are important examples of ampholytes. The formation of a zwitterion can be illustrated with the amino-acid glycine :



Glycine *Zwitterion of glycine*

Buffers and the Buffering of Solutions. The term *buffer* comes from the German *Puffer*, a tampon, adopted by Sørensen in 1909 to describe the resistance set up by certain solutes to changes in hydrogen ion concentration. The English term *buffer* (as, for example, of a railway coach) has an even better significance, suggesting something which will damp down the effect of a sudden shock.

If we add to a volume of neutral water, with a *p*H value of 7, an equal volume of N/10 hydrochloric acid, the *p*H is changed to about 1·34 (for the N/20 acid). If we first dissolve some sodium acetate in the water, and then add the same volume of the acid, the *p*H only changes to about 3. The sodium acetate has acted as a buffer.

The essence of buffer action is the damping down of the effect of addition of an acid to a solution by the production of an acid which dissociates to a much smaller extent, or, conversely, the effect of addition of an alkali, through production of another alkali which ionises much less. This depends upon the principle, verified by experiment, that a mixture of solutes in a solution always tends to form the least dissociated compounds possible.

Since a buffer salt, in acting, always produces its corresponding acid (or alkali), buffered systems must always include a salt and its weakly dissociating acid (or alkali).

One of the best buffer mixtures is a solution of bicarbonate in presence of carbonic acid ; this acid dissociates extremely slightly. In a solution so buffered the following equilibria tend to be established :

$$NaHCO_3 \rightleftharpoons Na^+ + HCO_3^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$
$$HCO_3^- + HOH \rightleftharpoons H_2CO_3 + OH^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$
$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$
$$HOH \rightleftharpoons H^+ + OH^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$
$$H_2CO_3 \rightleftharpoons HOH + CO_2 . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Since (3) and (4) only occur to a negligible extent, and (5) produces no ions, (2) determines that the reaction of a solution of sodium bicarbonate shall be alkaline.

If a relatively strong acid (HX) be added to such an equilibrated mixture, the net result can be expressed as :

$$HX + NaHCO_3 = NaX + H_2CO_3 (= NaX + HOH + CO_2),$$
or
$$H^+ + X^- + HCO_3^- = X^- + H_2CO_3.$$

There will be a slight decrease in HCO_3^- ions (in part replaced by further ionisation of $NaHCO_3$), and increased production of CO_2 (5) may result in some loss of it from the solution. These changes have an important bearing on prevention of the condition known as *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 :

$$\text{Na-Protein} + HX \rightleftharpoons \text{H-Protein} + NaX.$$

Buffer action is of the greatest importance in minimising changes of *p*H in the tissues and fluids of the organism.

Dissociation Constant. When the equilibrium equation (p. 19) is applied to the dissociation of a weak acid into its ions

$$\frac{(H^+)\,(X^-)}{(HX)} = K_a$$

then the equilibrium constant K_a is termed the *dissociation constant* of the acid. These constants are usually expressed logarithmically by the dissociation exponents *p*K

$$p\mathrm{K} = \log 1/K_a$$

*p*H and *p*K are related by the equation

$$p\mathrm{H} = p\mathrm{K}_a + \log \frac{\alpha}{1-\alpha}$$

where α is the degree of dissociation of the acid ion. This is given approximately by the Henderson-Hasselbach equation

$$p\mathrm{H} = p\mathrm{K}_a + \frac{\log (\text{salt})}{\log (\text{residual acid})}.$$

A corresponding equation can be developed for bases (α having corresponding significance).

Colloids. Somewhat more than seventy years ago Thomas Hunter, Professor of Chemistry in University College, London, 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 and amino-acids are crystalloids ; starches and proteins 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. Since all sizes of molecules exist, from that of hydrogen with a molecular weight of 2 to, for example, the haemocyanins present in the blood of crustacea with molecular weights of several millions, it is not 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.

We can regard the large colloidal molecules or particles as *dispersed* through the solvent, and they are often referred to as the *dispersoid* or *internal phase*, while the fluid in which they are dispersed is termed the *dispersion medium* or *external phase*.

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.

Particles which can be seen under the microscope, but not by the naked eye, are termed *microns*, and have a diameter of at least $0{\cdot}1\mu$. 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 $0{\cdot}067$ 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.

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.

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.

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 2μ 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 × 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 VI.

Passage of Solutions across Membranes. Passage of molecules across membranes must be inter- or intramolecular. The conception of passage through the intermolecular " pores " seems easier to imagine. The size of such " pores " will vary with different membranes.

In the first chapter it was stated that the purpose of digestion is to break up the molecules of food compounds to others of small size and soluble in water, in order to enable absorption to take place across the mucous membrane of the intestine. Passage across membranes of fluids containing dissolved material is continually taking place in the living organism, and a molecule of glucose or of oxygen must pass across many such membranes in its passage from the mouth or the atmosphere to the cell in which it is to be used.

Two factors are essential for passage across such membranes. The molecule must be in solution in water, and of relatively small size. Gases, for example, in spite of small molecular size, can only pass across animal membranes to the extent to which they can dissolve in the fluids in contact with these membranes (or forming part of them, since all living tissues, including these membranes, are largely composed of water). Gases such as hydrogen and methane, only very slightly soluble in water, are frequently formed by bacterial action in the intestine, and must accumulate therein until they can be expelled.

Other factors also affect membrane diffusion. Carriage of an electric charge may assist or inhibit diffusion. Cations (ions carrying a positive charge) cannot pass through the membrane of the undamaged red blood cell, whose contents of the small potassium ions and of haemoglobin with its huge molecule are alike constant from the time the cell is first set free from bone marrow until it is destroyed. (The hydrogen ion is, possibly, an exception.) On the other hand such anions as HCO_3^- and Cl^- pass across the membrane of the red cell very easily.

If a porous earthenware partition separates two solutions the solutes can diffuse from one to the other practically as if this porous membrane was absent. Its pores are so large that they will only hold back matter in suspension. The same is true of filter paper ; it holds back suspended material, but all matter in solution passes through. It is an observation of general experience, however, that when a fine precipitate collects on a filter paper, especially one of colloidal nature, it chokes it up, and filtration is greatly slowed. The interstices in the precipitate are much smaller than those in the paper itself.

Pfeffer caused deposition of such a precipitate, of copper ferrocyanide, $Cu_2Fe(CN)_6$, within the walls of a porous clay pot, and so produced a *semi-permeable membrane* of the ferrocyanide, supported by the walls of the pot. This membrane is readily permeable to water, but is so

fine-meshed that it holds back even such solutes as cane sugar. When such a "cell," containing a solution of cane sugar or other solute, is immersed in water, the solute cannot pass through the membrane, and its osmotic pressure causes passage of water into the solution, so that the height of solution within the cell may become considerably greater than that in the water outside it, illustrating the actual pressure effect. Differences of osmotic pressure on two sides of an animal membrane are important agencies in causing marked shifts of solution within the organism.

Semi-permeable membranes—sheets or sacs—of all degrees of porosity can be constructed from collodion dissolved in various mixtures of ether and alcohol, the solvent being allowed to evaporate away at different rates. It is thus possible to imitate natural membranes such as those in the living organism, and to construct collodion membranes which will permit the smallest sized protein molecules to pass, but will retain all larger molecules (like those in the glomeruli of the kidney), and others similar to those of the intestinal mucosa, which will hold back all molecules of colloidal size.

Such membranes are frequently used in carrying out a *dialysis*, in which electrolytes of small molecular size are washed away from larger molecules in solution, such as those of proteins, by immersing collodion sacs containing the solutions in running water.

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^+ Cl^- $x\ Na^+$ $x\ Cl^-$ (I)	$y\ Na^+$ $y\ Cl^-$ (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

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

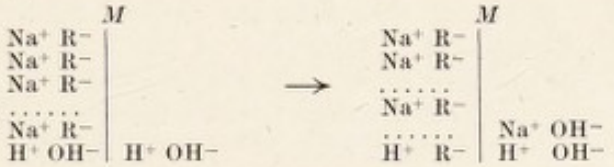
and

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

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.

Hydrolytic Decomposition by Membranes. If there is a solution of a colloid salt NaR on one side of an animal or collodion membrane, and pure water on the other, then if the colloid cannot pass through the membrane it is found that hydrolysis takes place. Since some hydrogen and hydroxyl ions are always present in solution, it would appear that sodium ions with an equal number of hydroxyl ions diffuse away from the colloid :



The nature of the change is indicated in the diagram (*M* indicates membrane). Through diffusion the colloidal solution has become acid,

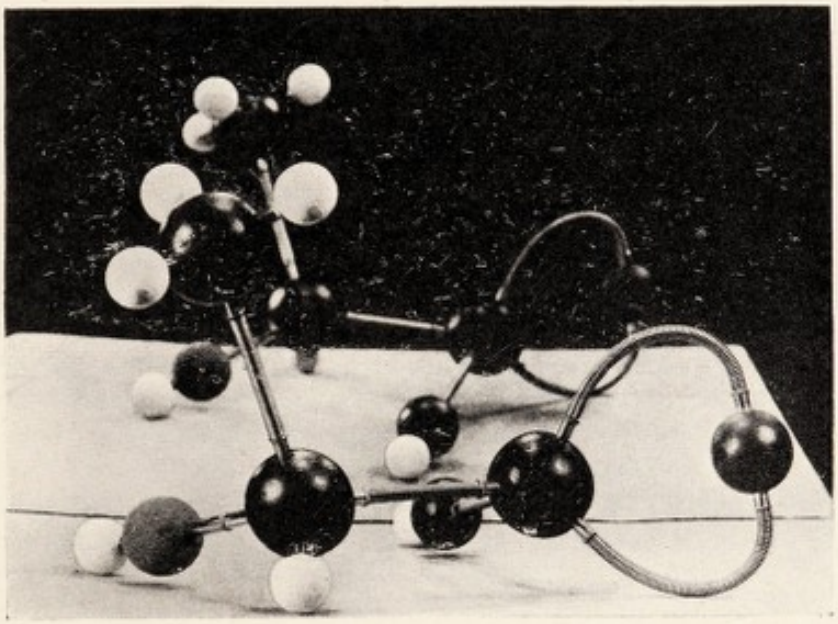
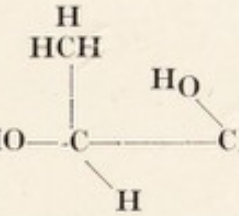


Fig. 5. Model of actic acid,  , and its mirror image. They are not superimposable.

and the water has become alkaline. Such formation of acid and alkali on two sides of a membrane may have a bearing on the production of acid secretions in the living organism.

Stereoisomerism. 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

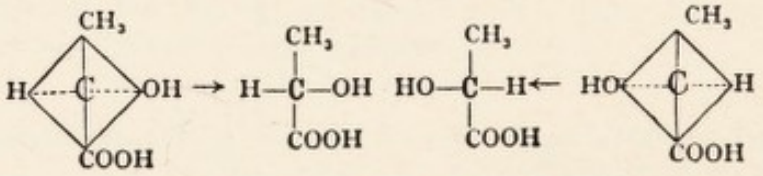
many naturally occurring substances—such as sugars, terpenes and camphors—were capable of rotating the plane in which such polarised light is vibrating, when it was passed through their aqueous solutions.

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 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. 5.

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 laevo-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.

The great majority of compounds which play a part in living processes are optically active, and the biochemical catalysers, the enzymes,

which bring about reactions with these compounds, are themselves optically active ; their chemical activity is affected and controlled by their optical activity.

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.

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

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.

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CHAPTER III

BIOCHEMICAL AGENCIES

INTRODUCTION

THE acts which constitute the life of a unicellular organism, its movements, its methods of obtaining nutrient material, its excretion of waste material, are, for the most part, traceable to chemical agencies. In the multicellular organism, plant or animal, with markedly differentiated tissues adapted to special functions, it is possible to trace particular phenomena to chemical agents, but, until much more is known concerning the mechanisms of functioning of many tissues, it will only be possible to guess at the linked chemical and physico-chemical reactions which guide and control their behaviour. Nevertheless we now know a great deal about certain groups of chemical agents whose combined actions control a large proportion of the processes of living.

The *enzymes* constitute the largest class. Equally important and more general in their actions are the *hormones* and the *vitamins*. In addition, certain substances which are either waste products, or which function as nutrients, also seem to act as chemical agents. Thus, carbonic acid, either through specific action of the HCO_3 ion, or through the increased hydrogen ion concentration which results from saturation of tissues with it, exercises a specific effect on the so-called " respiratory centre " of the brain, stimulating the respiratory reflex so that there results an increase in the rate of respiration—hyperpnoea. When, following forced respiration, the carbonic acid content of tissues is diminished, decreased stimulation of the respiratory centre leads to slowed breathing, and perhaps even a short period of apnoea (cessation of breathing).

It seems very probable that one of the factors causing the secretion of the hormone insulin from the special pancreatic cells which produce it is the amount of glucose in the blood circulating through the pancreas. Insulin is needed for the proper control and use of this glucose. It must be stressed that actions of this kind are not endocrine (hormonal) in nature. To consider such a ubiquitous degradation product as carbon dioxide as a hormone, because it assists in the control of respiration, is quite unjustifiable.

In discussing enzymes, which are all biochemical catalysts, it will also be necessary to consider co-enzymes, compounds or ions frequently essential for action of specific enzymes. Also many other simpler catalysts play important biochemical *rôles* which seem enzymic in character. Typical of these are the tri-peptide glutathione, and the vitamin ascorbic acid.

In this chapter only the general properties of enzymes will be considered. Further consideration will be given to specific enzymes in later chapters in connection with the compounds on which they act.

ENZYMES

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.

The terms ferment and enzyme therefore equally apply to these compounds, though neither is completely satisfactory, for most ferments do not produce an effervescence resembling boiling, while the majority of enzymes do not occur in yeast. Since their actions are catalytic in nature they may properly be styled *biochemical catalysts* or *biocatalysts*, but the term enzyme has been almost universally adopted, and will be used henceforth in this text.

An enzyme is named after the compound or class of compounds on which it acts, 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. A few of the older specific names have been retained, as *pepsin* of the gastric juice, and *trypsin* of the pancreatic juice. The substance which is acted on by an enzyme is termed its *substrate*.

The Catalytic Nature of Enzyme Action. The following criteria are available (*cf.* Chapter II., p. 19)—acceleration of reaction, catalysis of amounts of material much larger than that of the catalyst, non-alteration of equilibrium, and reversibility of reaction. It is easy to find enzyme actions which conform to these criteria.

E. F. Armstrong showed in 1904 that lactase would hydrolyse one-quarter of the lactose contained in a certain volume of 5 per cent. solution in one hour at 35° C., and was therefore much more powerful than the inorganic catalyst hydrochloric acid, since a twice normal solution of this acid required at the same temperature five weeks to produce the same amount of change.

While, until an enzyme has been obtained in pure condition, it is impossible to state precisely the weight ratio of enzyme to substrate, yet 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. Exact figures for urease will be given later (*cf.* p. 47).

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 catalysed by, 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.

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 (*cf.* p. 20).

Does an enzyme initiate 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.

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. 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 in such cases 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 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.

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, but only partial, purification, of the enzymes, since many other compounds will also be thrown down by the ammonium sulphate or the alcohol. Enzymes are colloidal and relatively non-diffusible, properties which suggest 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° C. 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 counter-balanced; 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.)

Zymogen is a term given to the precursors of certain enzymes. These zymogens require treatment of a specific nature to produce the activity associated with their 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, peroxidases* and *dehydrogenases* effect oxidations. *Protein-coagulating* enzymes produce coagulation of proteins (*e.g.*, rennin coagulates the casein 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.

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 formulae, 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 :

$$E+S \rightleftharpoons E—S \rightleftharpoons A + E—B \rightleftharpoons A+B+E.$$

However, Northrop . has shown that neither pepsin nor trypsin combine with their substrates. They initiate a reaction with the ionised part of the substrate, and can combine—reversibly —with the products of the reaction.

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.

Preparation of Pure Enzymes. The preparation of a compound in crystalline form is usually accepted as evidence of a reasonable degree of purity. Recrystallisation increases the purity. Certain enzymes have been obtained in crystalline condition, with retention of their enzymic potency, and recrystallisation has resulted in no loss of potency, but a tendency to increase it. Such enzymic preparations are considered to be pure. Those which have so far been prepared include urease, which decomposes urea with formation of ammonia, and pepsin, trypsin, and chymotrypsin and pancreatic amylase, present in the digestive juices secreted into the alimentary tract. In addition two zymogens, trypsinogen and chymotrypsinogen, have been obtained crystalline. The technique of preparation varies in each case. Two examples will suffice.

In 1926 J. B. Sumner published an account of his successful attempt to prepare crystalline urease ; his work has since been amply corroborated. He extracted Jack-bean meal, which is rich in the enzyme, with a definite mixture of acetone and water (258 to 342 parts by volume), and kept the extract at a temperature just above 0° C. for twelve hours. Microscopic colourless octahedral crystals separated in amounts minute, but sufficient to be centrifuged off and examined. Their appearance is shown in Plate I. Recrystallisation increased their enzymic activity ; they were several thousand times more active than the meal from which they were prepared. Urease crystals will liberate every five minutes from a urea solution at 20° C. 120 times their own weight of ammonia. (As giving some clue to the power resident in an enzyme, in one day 1 gm. of urease crystals, acting on sufficient urea, would produce at this rate 34·5 kg. of ammonia, which would occupy in the gaseous state over 1,600 cubic feet. Most of the urease would still be intact.)

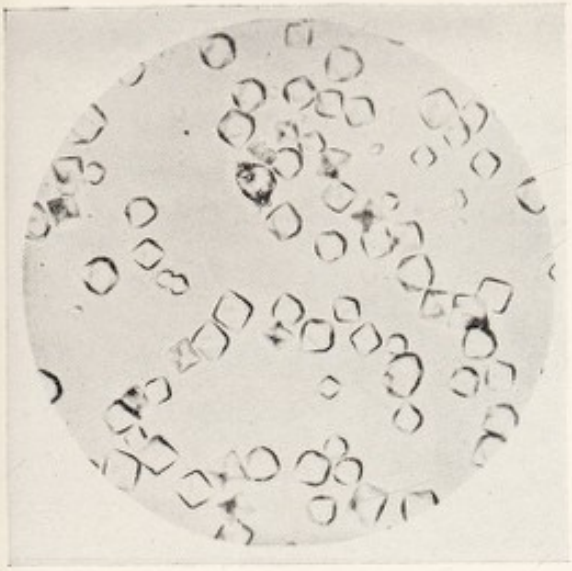
In 1930 Northrop isolated crystalline pepsin from commercial pepsin preparations by dissolving them in dilute sulphuric acid and adding excess of a saturated solution of magnesium sulphate. A precipitate was thrown down and carried the enzyme with it. It was treated with dilute sodium hydroxide until just slightly acid, the enzyme passing into solution. The acidity was again increased, the enzyme precipitated, and it was again treated with alkali at 45° C. The solution, slowly cooled, deposited a heavy crystalline precipitate, with all the properties of pepsin, and these remained unchanged through seven successive recrystallisations. The crystals consisted of microscopic regular hexahedra. Crystalline pepsin is a protein, and its molecular weight determined by the ultracentrifuge is 35,500.

Of those enzymes obtained in crystalline form, or sufficiently pure to be chemically classified, pepsin is an albumin, urease and pancreatic lipase are globulins, pancreatic amylase is a protein as yet unclassified, and rennin is a thio-proteose. Plate I. shows several crystalline enzymes and their zymogens.

The Nature of Enzymes. Willstätter and Waldschmidt-Leitz have advanced views that the intrinsic part of an enzyme is some specifically active radical anchored to a *carrier* which is characterised by large molecular size, but is not necessarily specific. The preparation of pure crystalline enzymes has enabled this theory to be tested. Much experimental work has been carried out with both urease and pepsin, and the results seem now to be fairly conclusive that no *active* compound of small molecular size can be separated from the protein bulk of the crystalline enzyme. Any chemical procedure which alters the enzyme molecule alters its activity. For example, Sumner has shown that when the protease pure crystalline pepsin is allowed to act on solutions of the protein crystalline urease, the rate of decomposition of the urease is paralleled by the rate of loss of its activity. In only three cases as yet, the oxidative enzyme known as " Warburg's respiratory ferment," catalase, and " acetaldehyde reductase," is there definite evidence of a combination between a specific " prosthetic " group and a protein.

The " respiratory ferment " is a compound of a protein and vitamin B_2, and acts as an oxygen carrier. Neither the protein nor the vitamin alone has enzymic activity. Catalase, crystallised by Sumner and Dounce in 1937, is a compound of a protein and either haematin (a constituent of haemoglobin) or a closely related compound, and contains about 0·1 per cent. of iron. Neither the haematin nor the protein alone possess the enzymic properties of catalase. Acetaldehyde reductase, which catalyses

PLATE I

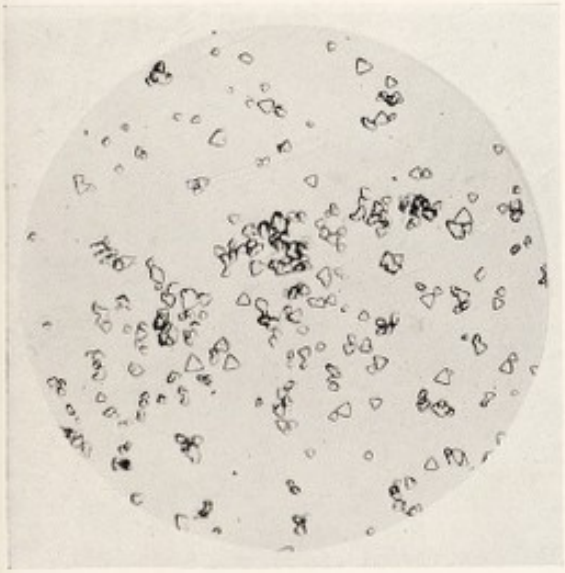


(*a*) Urease.

(*b*) Pepsin.

(*c*) Trypsin.

(*d*) Chymotrypsin.



(*e*) Trypsinogen.

(*f*) Chymotrypsinogen.

Photomicrographs of crystalline enzymes : (*a*) (× 460) from J. B. Sumner, *J. Biol. Chem.*, 1926, lxix., 436 ; (*b* to *f*) from J. H. Northrop, " Harvey Lectures," 1934–35, p. 229 (Williams and Wilkins Co., Baltimore).

[*To face p.* 48.

the reduction of acetaldehyde to alcohol, is a compound of a protein and diphosphopyridine nucleotide (*cf.* p. 286).

Most enzymes are proteins, and since most proteins are not enzymes it is very probable that each enzyme does contain a specific active group with some peculiar arrangement of amino-acid radicals that confers on it its catalytic properties, but which, detached from its " anchor," becomes inactive. It is quite possible that only slight structural alterations are necessary to change many an inactive protein to an active enzyme, as indeed must be the case with zymogens. In this connection it is pertinent to note that Bredig and Gerstner proved in 1932 that the additions of a diethylamine group to cotton-cellulose (a carbohydrate) conferred such catalytic properties on it that it could split off carbon dioxide from keto-carbonic acid, imitating in its action the enzyme carboxylase.

The boundary line between enzymes and other catalysts is somewhat artificial. The presence of a heavy " anchor " perhaps enhances catalytic action, and at the same time it leads to that decomposition of enzymes by heat which is the chief character distinguishing them from other catalysts.

Velocity of Enzyme Action. It was shown in Chapter II. (p. 18) that when a single substance is decomposed into two or more compounds, or is hydrolysed in presence of great excess of water under conditions in which the amount of catalyst is fixed and the temperature is constant, then the reaction is mono-molecular and follows 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 mono-molecular reaction. As example, Hudson's results can be quoted, for the action of sucrase (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 II., p. 38). It will be seen that the figures for k in Table II. are reasonably constant.

The effect of the concentration of enzyme upon the velocity of reaction varies with different enzymes. With sucrase, for example, the rule holds over a somewhat wide range of enyzme 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 III., based upon Hudson's results.

TABLE II. VELOCITY OF TRANSFORMATION OF CANE-SUGAR BY SUCRASE AT 30° C.

Time.	Observed Rotation.	Velocity Constant.
Minutes.	Degrees.	$k \times 10^4$.
0	+ 24·50	—
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	—

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

Relative Concentration of Sucrase.	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.

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 probable that the function of elements such as manganese, present in traces in many tissues, is to act as co-enzymes. Other co-enzymes, such as that needed for the fermentation of sugar by yeast, are organic compounds of varying degrees of complexity (*cf.* p. 286).

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.

From the results of some rather ancient experimental work it was concluded that such tissues contained compounds with definite and specific anti-enzymic properties. It seems rather more probable that the resistance exhibited to digestion by such tissues is due to their physical chemical state. (Anti-enzymes such as antiurease, produced as specific antibodies following the injection of a foreign protein such as urease (*cf.* Chapter XIX.), must not be confused with such presumed physiological anti-enzymes.)

THE HORMONES, INTERNAL SECRETIONS, OR ENDOCRINE COMPOUNDS

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 autoacoids (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 advancing rapidly, but must still

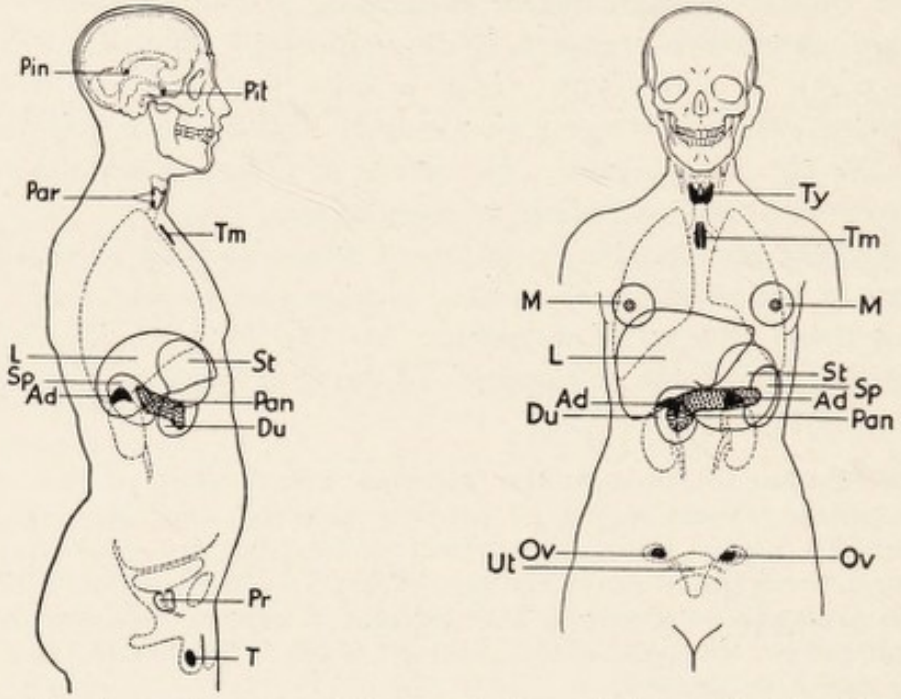


FIG. 6. Approximate positions and sizes of the endocrine glands in man, in relation to other organs. *Ad*, adrenal gland; *Ov*, ovary; *Par*, parathyroid gland; *Pan*, pancreas (with islets of Langerhans); *Pin*, pineal gland; *Pit*, pituitary; *T*, testis; *Tm*, thymus; *Ty*, thyroid; *Du*, duodenum; *L*, liver; *M*, mammary gland; *Pr*, prostate; *Sp*, spleen; *St*, stomach; *Ut*, uterus (placenta absent). After a chart in Barker's "Endocrinology and Metabolism" (Appleton, New York, 1922).

be considered as 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 associated 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

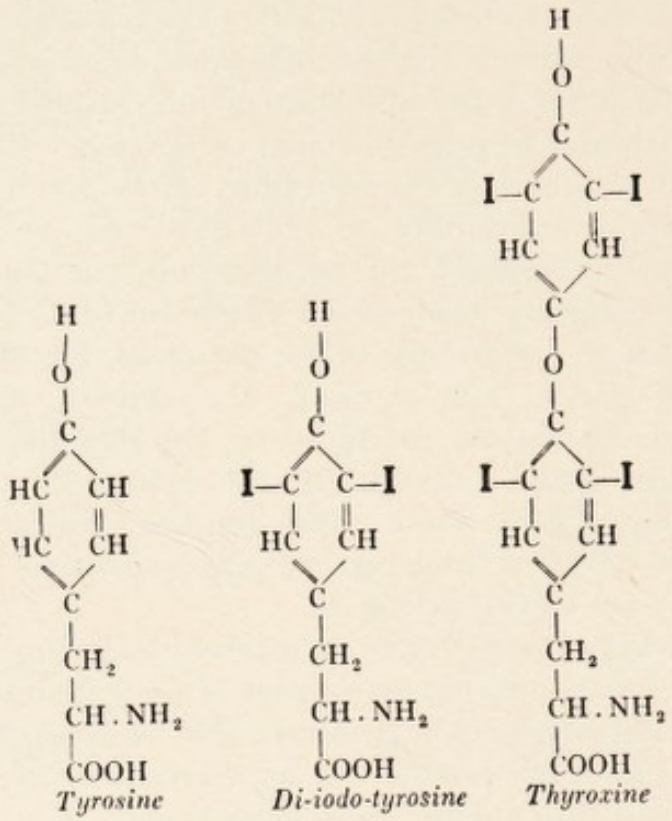
grafts of this tissue to other animals, or the injection of its extracts. These have all gradually led to biochemical studies of the glandular material, and in a number of instances such studies have been successful in yielding the pure chemical compound. Little more than the essential chemistry of the 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 *thymus*, the *ovaries*, and the *testes*. Endocrine secretions are produced by the *placenta* and probably by the *pineal gland*. Claims for the existence of several other secretions have been made, but require substantiation. The approximate position and size of the endocrine glands in the human body are shown in Fig. 6.

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* specifically stores the element iodine, in the form of a complex protein, *iodo-thyroglobulin*. From this the thyroid hormone is set free ; the nature of this hormone has not been completely elucidated, but it is possibly a tri- or tetrapeptide (*cf.* Chapter VI.), containing radicals of di-iodotyrosine, and of *thyroxine*, which is the 3,5.di-iodo-p-hydroxy-phenyl ether of di-iodotyrosine. It contains 65·3 per cent. of iodine, while di-iodotyrosine contains 58·7 per cent. Both give a very specific colour reaction when suspended in water or dissolved in very dilute alkali, upon addition of nitrous acid, which turns their solutions orange or lemon-yellow respectively ; then addition of ammonia or sodium hydroxide changes the colour to rose-red. The relation between these two compounds, and the amino-acid tyrosine from which they are both derived, is shown by the formulae on p. 54. Thyroglobulin on hydrolysis yields both di-iodotyrosine and thyroxine. Thyroxine, qualitatively, exhibits all the physiological properties of the thyroid hormone. There is still disagreement as to whether it possesses the quantitative activity of that hormone. Di-iodotyrosine is practically inert, physiologically.



The thyroid hormone controls to a considerable degree the oxidative processes of the cells throughout the organism. This is indicated by the fact that when the thyroid gland is surgically removed from a mammal its oxygen consumption during rest falls to 60 per cent. of the normal value. Yet certain experimental evidence suggests that the control of oxidation is indirect, and that the real action of the thyroid hormone is to catabolise some intermediate stage of amino-acid degradation in the cells, following which oxidation of the products occurs. *The thyroid hormone appears to be a non-enzymic catalyst.*

It withstands digestion, so that desiccated thyroid gland can be administered therapeutically by mouth. *Most hormonal preparations are ineffective by oral administration.*

The *parathyroid hormone* has been obtained by acid hydrolysis of beef parathyroid glands. It is almost certainly a protein, but it has not yet been obtained in a state of complete purity. It is decomposed by trypsin, so that it produces no effect when given by mouth, unless administered in excessive amount, when a small but uncontrollable proportion may escape enzymic decomposition and be absorbed into the system.

The parathyroid hormone, frequently referred to by a trade name parathormone, is inextricably associated with the metabolism of calcium and inorganic phosphate. Since changes in the organism which affect calcium also affect inorganic phosphate, and *vice versâ*,

it is still uncertain whether the hormone primarily controls inorganic phosphate, or calcium.

When the parathyroid glands are removed in such an animal as the dog, so that there is suddenly produced an acute deficiency of the hormone, the calcium of the blood plasma, normally about 10 to 11 mg. per 100 c.c., falls sharply in from twenty-four to forty-eight hours, to 6 mg. and even less, and this fall is usually accompanied by the onset of a condition termed *tetany*, in which muscular spasms occur through disturbance in an ionic equilibrium in which calcium and sodium ions are primarily concerned. Injection of the hormone abolishes the tetany and restores the blood calcium to a higher figure.

When a preparation of the hormone is subcutaneously injected into a dog at regular and frequently repeated intervals, its blood calcium steadily rises to a figure about twice that of normal, the plasma inorganic phosphate shows a late rise, and the animal finally dies.

Under normal conditions the blood calcium of an animal has a very constant value ; the available evidence indicates that this constancy is largely maintained through the action of the parathyroid hormone on the solid matrix of bone, which largely consists of a calcium phosphate complex. There is also some evidence that, under appropriate conditions, this hormone not only can denude the bone mineral, but can also aid its deposition.

The pancreas contains many nests of special cells, the so-called *islets of Langerhans* (named after their discoverer), which have no secretory duct, but which discharge their secretion into the blood stream. These islets have nothing to do with the external secretion of the pancreas, the pancreatic juice. They secrete the hormone *insulin*, a protein which has been obtained in crystalline form, and which has been shown by the ultracentrifuge method to have a molecular weight of about 35,000. Its molecules have approximately the same size and weight as those of egg albumin. Insulin is unusually rich in radicals of the amino-acid cystine ; their abundance may have something to do with its function. Crystalline insulin contains a small amount of zinc (about 0·5 per cent.), and this also is apparently not fortuitous. A photomicrograph of such crystalline insulin is shown in Plate II.

Insulin helps to control the metabolism of the circulating carbohydrate glucose. When insulin is injected into a normal animal such as a rabbit in sufficient quantity, its blood sugar falls from a figure of the order of 0·1 per cent. to a value even as low as 0·04 per cent., or less, and at such low values the animal exhibits convulsions, actually due to the low blood sugar, the *hypoglycaemia*.

Conversely, when the islets of Langerhans are removed (by removing the whole pancreas), the condition of acute insulin deficiency so caused leads to greatly increased blood sugar—*hyperglycaemia*—and the presence of glucose in the urine (*glycosuria*), and proceeds to a rapidly fatal termination. *Diabetes mellitus* in human beings is the chronic condition corresponding to this acute insulin insufficiency in the experimental animal.

The precise mechanism whereby insulin controls the metabolism of glucose is still undetermined. There is strong evidence that its main function is concerned with 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.

Secretin is a hormone elaborated by cells in the mucous membrane of the upper part of the small intestine, the duodenum and (to a lesser extent) the jejunum. 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 of a dog, rubbed up with sand and 0·4 per cent. hydrochloric acid, 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.

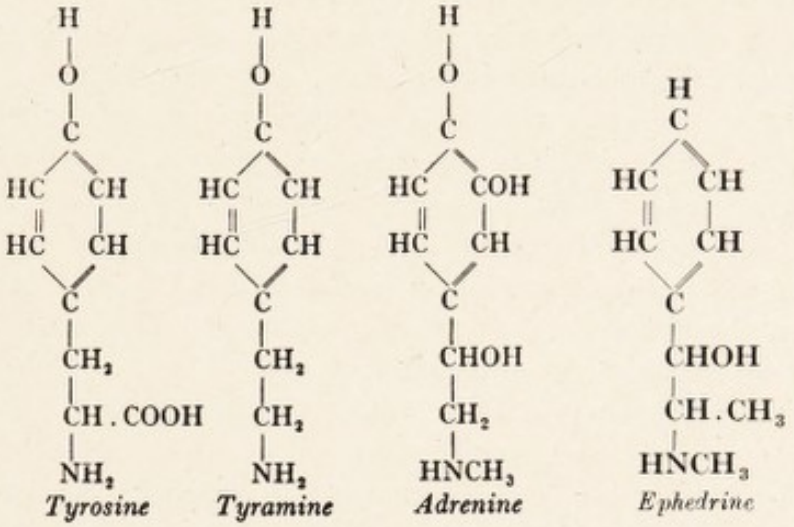
J. Mellanby has suggested that secretin, which is very soluble in bile salts, is carried from the intestinal mucus by these during their absorption from the gut. The view that it is set free following increased acidity in the lumen of the duodenum still, however, appears to be the prevailing one.

Secretin has been crystallised by Ågren and Wilander, and shown to be a polypeptide with a molecular weight of about 5,000, and containing sulphur.

The *adrenal glands* are, as their name suggests, two small glands adjacent to (the upper poles of) the kidneys. Each gland is composed of two entirely unlike tissues ; its interior portion, its *medulla*, is composed of tissue of nervous type ; the exterior and

larger portion of each, its *cortex*, is composed of more typically glandular material.

The *adrenal medulla* secretes *adrenaline* (or *adrenine* or *epinephrine*). This is a simple derivative of the amino-acid tyrosine, and is probably formed through the intermediate stage of tyramine. It is also closely related to the compound ephedrine, derived from the Chinese drug Ma Huang (*Ephedra vulgaris*). The formulae of these compounds are—



The physiological properties of ephedrine are somewhat similar to those of adrenine, but while the former is effective by oral administration the latter is not. Tyramine has similar properties, but in much less degree.

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 laevo-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 presence of potassium persulphate, gives a rose-red colour which permits its detection in dilutions of one part in five millions. Photomicrographs of crystalline adrenine are shown in Plate II.

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 tyranine 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.

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.

It will be observed that both adrenaline and insulin are controllers of glucose metabolism, and that they appear to antagonise each other, insulin favouring glycogen formation, and adrenaline favouring its breakdown (to glucose or lactic acid).

The *adrenal cortex* produces the compound *cortin*. Several crystalline compounds have been prepared from cortical tissue, but it is not yet certain which if any of these is cortin. A suggested formula for cortin is given in Chapter V. (*cf.* p. 119).

Cortin is essential to life. When the adrenals are removed from an animal such as the cat or dog, even with the best surgical technique, death occurs in from seven to ten days, preceded by a characteristic train of symptoms. Addison's disease is the chronic form of adrenal cortical deficiency corresponding to this acute condition. Administration of cortin by injection (and, in larger dosage, by mouth) maintains the experimental animal in health, and benefits the patient with Addison's disease.

(Extirpation of all medullary tissue is not fatal to animals.)

The precise action of cortin is still a matter of dispute. It controls the sodium-potassium balance in the blood plasma, so that when there is a deficiency the sodium content of the blood falls, and that of potassium rises. Perhaps associated with this is control of the volume of fluid in the vascular system; in deficiency this volume falls; the blood becomes concentrated. Cortin also exercises some control over carbohydrate metabolism; this is very probably a secondary effect. In deficiency there is a tendency to hypoglycaemia.

There is some evidence that the adrenal cortex also produces a hormone chemically related to the sex hormones and concerned with control of secondary sex characters (*cf.* p. 122).

Sex Hormones. There are three distinct sex hormones, and a number of degradation and partially detoxicated products (products with lessened activity). All these compounds are derivatives of the sterol cholesterol, and their chemistry will be dealt with in Chapter V. They all possess a characteristic four-ring skeleton.

Testosterone, $C_{19}H_{28}O_2$, the hormone of the testis, controls the development of the secondary sex organs of the male, penis, prostate, and seminal vesicles, and also of the secondary sex characters. In the organism it is changed to androsterone, $C_{19}H_{30}O_2$, which possesses similar properties in lesser degree, and is excreted in the urine in that form.

Oestradiol, $C_{18}H_{24}O_2$, the hormone of the ovary, controls the development of the secondary sex organs of the female, the uterus, vagina, clitoris, and the secondary sex characters. It exercises partial control over the development of the mammary glands, and controls the first half of the uterine cycle in women and the higher apes. In the organism it is changed to *oestrone* or *theelin*, $C_{18}H_{22}O_2$, and *oestriol* or *theelol*, $C_{18}H_{24}O_3$, with similar but feebler physiological properties. The potency of these latter compounds is further reduced by formation of their glycuronates (*cf.* p. 314), a detoxication procedure, in which form they are excreted. Oestriol glycuronate, or *emmenin*, is present in relatively large amount in the placental tissue of pregnant women.

Progesterone, $C_{21}H_{30}O_2$, is formed in the *corpus luteum*, the " yellow body " which results from transformation of an ovarian follicle, after discharge of an ovum. Progesterone controls the second stage of the uterine cycle in women.

The *thymus* produces a special hormone concerned with growth. Its chemical nature has not yet been elucidated. The *pineal* seems to produce a hormone whose action is somewhat opposed to that of the thymus principle.

The *pituitary body* is, in most mammals, composed of three parts, the anterior and intermediate portions, composed essentially of glandular tissue, and the posterior portion, composed of tissue of nervous type. The pituitary can be regarded as the seat of chemical control of the body, but it exerts much of this control through other endocrine glands.

The *anterior pituitary lobe* produces a number of hormones, all either proteins, or closely related to proteins. Of these one controls the general growth of the body, and, especially, bone growth,

another (*thyrotropic*) controls thyroid development and function, a third (*adrenotropic*) similarly controls the adrenal cortex, a fourth (*prolactin*) stimulates the flow of milk from the developed mammary gland, a fifth and sixth (*gonadotropic*) control the development and function of the gonads (ovaries and testes), while, possibly, a seventh (*diabetogenic*) controls carbohydrate metabolism, antagonising insulin, and still another (the *ketogenic*) appears to control and stimulate the oxidation of fats. Nothing is yet known of the precise mechanism of their action.

The *intermediate lobe* produces one or more compounds. Its extracts, when injected into frogs, cause contraction of the melanophores of their skin, and when injected into a certain minnow, *Phoxinus laevis*, produce an intense red coloration between the fins. Injected into mammals, such extracts produce a specific effect on general metabolism, stimulating oxygen-use and oxidation of fatty acids, and apparently depressing oxidation of carbohydrates. A single compound is probably responsible for both the " melanophore " and the general metabolic effect, and may be the agent producing the diabetogenic and ketogenic effects usually attributed to hormones of the anterior pituitary.

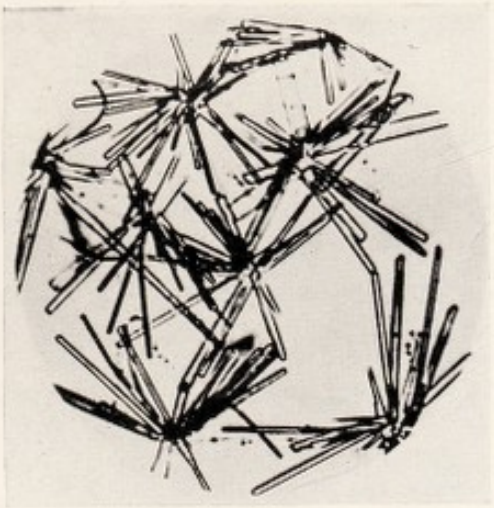
The *posterior lobe* produces two separate compounds, α- and β-hypophamine, of which the trade names *pitocin* and *pitressin* are frequently used. Pitocin has a specific action in causing contraction of the smooth muscle of the uterus, while injection of pitressin into an animal or man leads to increased blood pressure. Pitressin in small doses produces *diuresis* (increased secretion of urine), but in larger doses it acts as an anti-diuretic. These two compounds are complex bases of undetermined nature.

The foetal part of placental tissue elaborates a hormone which closely resembles in its action one of the gonadotropic hormones of the pituitary, and which is hence termed the *anterior-pituitary-like hormone* (APL ; the term " prolan " is also used for it).

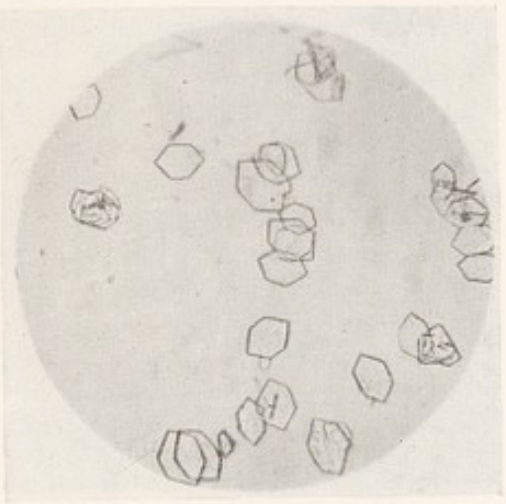
The tabular summary on p. 61 accentuates the variable chemical nature and varying physiological activities of the hormones.

Local Hormones. Impulses transmitted by the sympathetic and parasympathetic nerves are transmitted from the nerve endings to the effector structures by two chemical compounds, which have a very localised action, as contrasted with true hormones which act at a distance from the tissues which set them free.

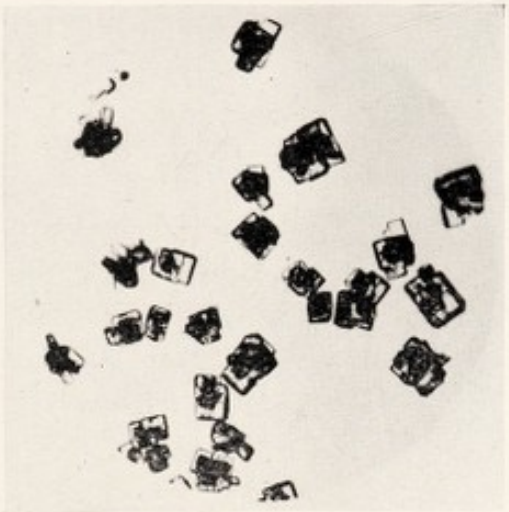
Sympathin, which is either identical with adrenaline, or closely related to it, is responsible for transmission from nearly all the sympathetic fibres, while *acetylcholine*, $CH_3 . CO . O . CH_2 . CH_2 . N(CH_3)_3 . OH$, which can be regarded



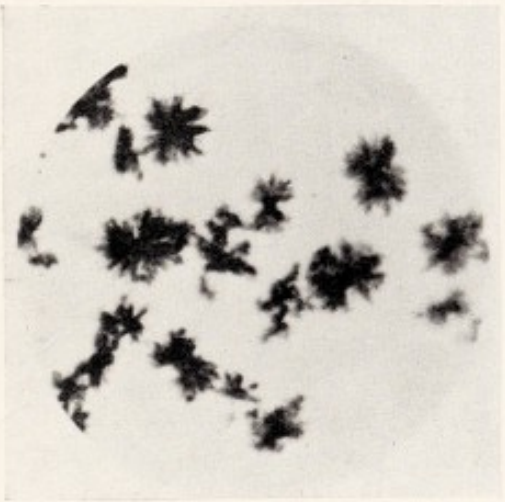
(*a*) Vitamin B_1 (hydrochloride)



(*d*) Adrenaline.

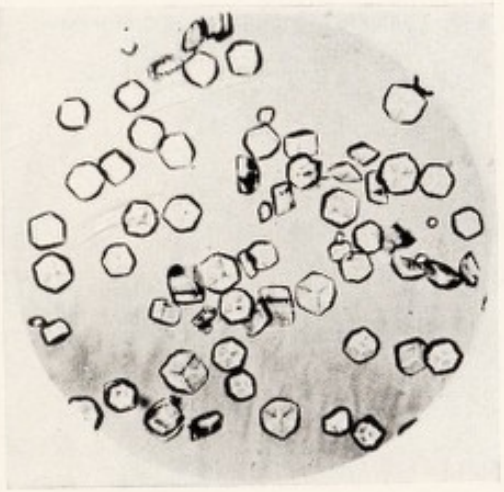


(*b*) Ascorbic acid (vitamin C).



(*e*) Adrenaline.

(*c*) Ascorbic acid (vitamin C).



(*f*) Insulin.

Photomicrographs of crystalline vitamins and hormones: (*a*) vitamin B_1 (thiamin) hydrochloride; (*b*) ascorbic acid recrystallised from acetone and petroleum ether (× 40); (*c*) ascorbic acid recrystallised from butyl alcohol and petroleum ether (× 40); (*d*) adrenaline recrystallised from hot water (× 180); (*e*) adrenaline crystallised from solution in hydrochloric acid by addition of ammonia to slight alkalinity (× 80); (*f*) insulin (× 125). (See p. vii for acknowledgments.)

[*To face p.* 60.

Simple Compounds	Chemical Nature	Type of Activity
Thyroid hormone.	Tri- or tetrapeptide.	Catalysis, in all tissues leading to increased oxidation.
Adrenaline.	Simple base.	Sympathomimetic.
Cortin.	Lipide ; sterol derivative.	Specific, controlling Na-K balance.
Sex hormones.	Lipide ; sterol derivatives.	Specific, on sex tissues, etc.
Complex Compounds		
Secretin.	Polypeptide.	Specific, on pancreas (and liver).
Insulin.	Protein.	Specific, on glucose metabolism.
Parathyroid hormone.	Protein.	Specific, on calcium and phosphate metabolism.
Pituitary hormones.	Proteins.	Specific, on various tissues.

as the acetate of choline (*cf.* p. 114) is responsible for the corresponding transference from parasympathetic nerve fibres. One or other of these compounds is probably concerned with nerve transmissions throughout the body, including passage of impulses across the nerve cells themselves and in the brain.

THE VITAMINS

In the early years of this twentieth century it was discovered, through the work of such investigators as Eijkman, Pekelharing, Hopkins, and Osborne and Mendel, that a perfect diet included more than proteins, fats, carbohydrates, salts and water. Certain other food factors were necessary. Otherwise " deficiency " diseases occurred, such as scurvy (associated with absence of fresh fruits and vegetables), beri-beri (associated with a diet of polished rice), and rickets (associated with the food of poverty and sunless slums).

Funk in 1911 obtained a crystalline fraction from rice polishings, very effective in curing avian polyneuritis, the beri-beri of birds. It appeared to be a basic compound, and he named it " vitamine," the " life-amine." His material was impure, and some vitamins are not basic. But with the elision (in English) of the terminal *e* the name *vitamin* has been applied to all the *accessory food factors.*

Their number is still growing ; there would seem to be at least a dozen of them. They are as unrelated to each other as are the hormones, both chemically and in their functions. Two or three can be produced in the mammalian organism, provided their precursors are available. The only general classification which can be attempted is related to their solubilities. Certain of them are quite soluble in water ; these are the " water-soluble vitamins."

Others behave like lipides and are only slightly soluble in water, but easily soluble in lipide-solvents and in fats. They are termed the " fat-soluble vitamins."

The terminology at present applied to them is very imperfect. When the existence of only two or three was suspected, they were labelled **A, B, C,** etc., in order of discovery. But the original **B** proved to be a mixture of several vitamins. For others, recently discovered, stray letters of the alphabet have been employed. Some proportion of American investigators use different alphabetical letters to those commonly adopted. In addition, as some of the vitamins are being obtained in pure crystalline form, special names are being coined for them, signifying their structure or their function ; different groups of workers coin different names. Hence the terminology is at present confused.

The Fat-Soluble Vitamins

Vitamin A has been obtained as a heavy viscid oil, colourless or almost colourless, by Karrer and his colleagues, from the unsaponifiable fraction of the liver oil of the mackerel and of the halibut, and by Drummond, Heilbron and Morton by fractional distillation of the unsaponifiable residue of halibut liver oil. Still more recently Holmes and Colbert (1937) have obtained it in pure crystalline form from fish liver oils. It is an alcohol, $C_{20}H_{29}OH$; its constitution will be dealt with in Chapter V. (*cf.* p. 125). This alcohol can be formed in the mammalian organism from *carotene*, the red pigment of carrots and other vegetables, and there is some evidence that the change is produced in the liver by a specific enzyme *carotenase*.

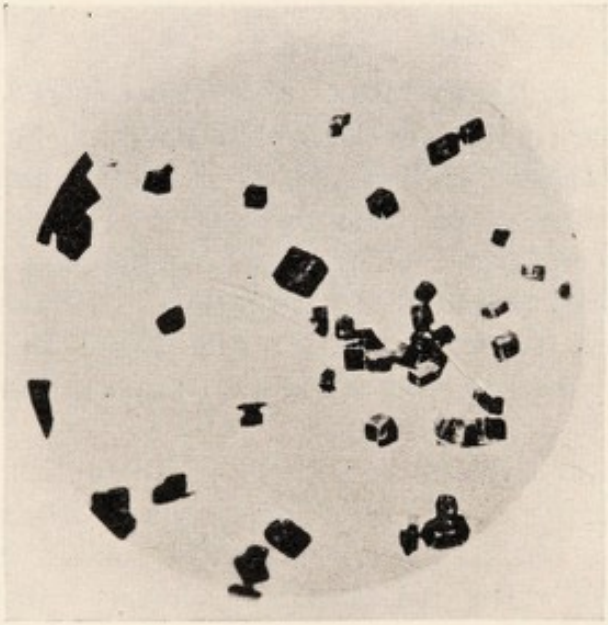


FIG. 7. Photomicrograph of carotene crystals. (From Plate II. of L. S. Palmer's "Carotenoids and Related Pigments," American Chemical Society Monograph Series, 1922.)

$$\underset{\textit{Carotene}}{C_{40}H_{56}} + 2H_2O = 2\underset{\textit{Vitamin A}}{C_{20}H_{29}OH}$$

Man obtains his requirements of this vitamin partly pre-formed,

and partly as carotene, which he transforms. 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. A photomicrograph of pure carotene crystals is shown in Fig. 7.

The vitamin appears to have a specific effect associated with the proteins of epithelial tissue of such glands as the tear-glands and salivary glands, and of such structures as the vagina, preventing these proteins from " keratinisation," *i.e.*, from change into a particularly insoluble and inactive type of protein that is typified by such structures as the nails and hair.

Deficiency of vitamin **A** (*Avitaminosis* **A**) produces, as the most outstanding feature, the condition of *xerophthalmia*, which is one of the chief causes of early blindness in India, is widely prevalent among children elsewhere in the East, and is by no means unknown in occidental countries. In this condition, through keratinising changes in the tear glands, tear secretion ceases. Bacteria then invade the conjunctival sac, stimulating leucocytic infiltration, so that pus effuses into the anterior chamber of the eye, and is visible as a yellowness of the pupil. The decomposition of leucocytes in the conjunctival sac gives a sticky mass which pastes the eye-lids together. Cornification of the exterior coating of the eye follows—whence the term *keratomalacia*, also applied to the condition. If it remains untreated, blindness follows. Administration of any material rich in the vitamin results in an almost miraculously rapid cure.

Various other pathological conditions are said to be associated with avitaminosis **A**, in all of which there would appear to be deficient secretion of specific glands, followed by keratinisation of specific tissues. Partial disturbances of vision, " night-blindness," and hemeralopia or " day-blindness " (inability to see distinctly in bright light), are believed due to a chronic partial deficiency of the vitamin. In this connection it is interesting to note that the *visual purple* of the retina of the eye is related to the carotenoid pigments (*cf.* p. 238).

Edward Mellanby states (1937) that deficiency of **A** in young dogs produces degenerative changes in the ganglia, nerves, and organs of both hearing and balance inside the temporal bone, resulting in various degrees of deafness. Addition of **A** to the diet is only curative if nerve degeneration is slight.

It has been believed that an abundance of the vitamin helps to prevent infection, so that it has been termed the " anti-infective vitamin." This view is almost certainly erroneous in the sense that the vitamin confers any general immunity. It is only effective to the extent that, by maintaining certain secretory mechanisms in normal condition, it prevents bacterial invasion of the tissues washed by secretions from those mechanisms.

There is some evidence that *very excessive* dosage of the vitamin is toxic to animals.

Claims have recently been made that a second vitamin, **A_2**, $C_{22}H_{31}OH$, exists in fish tissues. Its properties are said to be similar to those of **A**.

Vitamin D. Several chemical compounds possess the properties associated with this vitamin. These are all derivatives either of cholesterol, $C_{27}H_{45}OH$, or of ergosterol, $C_{28}H_{43}OH$. Irradiation of ergosterol with ultra-violet light confers on it the properties of the vitamin. From the mixed products Windaus obtained a crystalline preparation which was at first believed to be the vitamin and was termed vitamin **D_1**. British investigators, by other methods, obtained a more powerful crystalline preparation, termed **D_2**, or *calciferol*, $C_{28}H_{43}OH$. It was subsequently found that **D_1** was a mixture of calciferol and an inactive compound " lumisterol." Irradiated ergosterol contains another isomer of calciferol, *tachysterol ;* this does not possess the properties of the vitamin, but is toxic, and when administered to animals produces calcification of the kidneys. It was found that cod liver oil, long a recognised source of vitamin **D**, and calciferol did not produce the same therapeutic effects in different species of animals, and this difference led to further investigation. Finally Brockman has obtained a crystalline vitamin from the liver oil of the tuna fish, and Windaus has prepared the identical compound from cholesterol through the stage of dehydrocholesterol. This is now considered to be the natural vitamin and has the empirical formula $C_{27}H_{43}OH$. It is termed *vitamin* **D_3** (*cf.* Chapter V., p. 122).

The different " **D**-vitamins " have different melting points and specific rotations, and exhibit different spectral absorption bands. They are all physiologically extremely potent. Ultra-violet light seems necessary to produce the true vitamin from its precursor in the mammalian organism. It is possible that more than one natural vitamin **D** exists.

Vitamin **D**, like the parathyroid hormone, is a controller of the metabolism of calcium and inorganic phosphate. We do not know yet precisely how it produces its effects. It has been suggested that the actions of these two controllers are directly

interrelated, but there is still no conclusive evidence to support this theory.

The available evidence supports the view that the vitamin facilitates absorption of calcium from the alimentary tract, and —directly or indirectly—bone mineralisation. In moderate amount it conserves calcium and phosphorus to the body.

Marked overdosage with irradiated ergosterol (which contains both calciferol and tachysterol), and with calciferol alone leads to depletion of the mineral salts of bone, increase of blood calcium, and deposition of calcium phosphate in the walls of blood vessels and other abnormal sites. Marked continued overdosage has

FIG. 8. Rickets following a diet of 175 c.c. whole milk, unlimited white bread, and 10 c.c. linseed oil daily. Duration of experiment five and a half months. Increase of weight during this period 2·67 kg. (From "Vitamins; a Survey of Present Knowledge," Medical Research Council Special Report Series No. 167, H.M. Stationery Office, 1932.)

been shown to be fatal to mice, rats, guinea-pigs, cats and dogs, and some fatal cases in children have been reported.

It is still not known whether overdosage of the natural vitamin can lead to such toxic results.

Deficiency of the vitamin leads to rickets, whence it has been termed the *anti-rachitic vitamin*. Such deficiency may be entirely due to absence of sufficient vitamin or vitamin precursor in the diet, but is accentuated by absence of sunlight—source of ultra-violet light. Hence the frequency of rickets in the slums of large cities in temperate climates.

In rickets there is either normal blood serum calcium, with low inorganic phosphate, or low serum calcium, with somewhat low inorganic phosphate. In the latter case there may be the added complication of tetany (*cf.* p. 55). Rickets is essentially a disease of childhood. In childhood growth, of bone especially,

demands retention of calcium and phosphorus, so that the amount excreted should be less than the intake (a positive balance). In rickets, on the other hand, there tends to be a loss of these two elements from the body (a negative balance). In rickets the bones, due to deficient mineralisation, soften and bend. Children do not walk or stand at the proper time. They become knock-kneed or bow-legged. The epiphyses at wrist and ankle enlarge; other body malformations occur. Muscles are lax; the pot-belly is a typical maldevelopment. Administration of potent sources of vitamin **D** corrects the condition.

Fig. 8 shows a typical case of experimental rickets in a pup.

Osteomalacia in adults is an associated disease, or is actually a form of rickets. *Dental caries* is probably due to low phosphorus intake, with some degree of vitamin deficiency.

There are many excellent sources of one or other of the forms of the vitamin. The liver oils of halibut, turbot, cod and pilchard, and the body oils of sardines, herring and tuna are excellent sources, and egg-yolk, butter and milk are good sources of the natural vitamin. Animal margarines are moderately good sources. Marked exposure of the child to sunlight leads to production of the vitamin within the organism in useful amounts.

Irradiated ergosterol and irradiated yeast and egg-yolk are excellent sources of natural or other forms of the vitamin. Vegetable fats and margarines and green vegetables are deficient.

Vitamin E has not yet been definitely obtained in pure condition. There is evidence that it may be an alcohol, $C_{29}H_{50}O_2$, named by Evans *tocopherol* (Gk. *Tokos*, childbirth, *phero*, to bear; "ol" from "alcohol"), and obtained by him from wheat germ oil and from cotton seed oil concentrates.

Wheat germ and the green leaves of lettuce are excellent sources of the vitamin; ether extracts it from such sources.

This vitamin is specifically concerned with reproductive functions, though apparently its mechanism of action differs in the two sexes. A deficiency of the vitamin in the diet of the male leads to sterility through atrophy of the testes, which are not subsequently restored to normal by a diet rich in the vitamin. Deficiency of the vitamin in the diet of the pregnant female leads to death of the foetus *in utero*, and its resorption, but the effect does not permanently destroy the reproductive capacity of the female. Administration of a diet rich in the vitamin permits a subsequent normal pregnancy.

Various other abnormal effects are being described in the literature, traceable to a chronic but milder deficiency of the vitamin; such include development of a paralysis in old female

rats. There is some evidence that continued deficiency of the vitamin may induce hypofunction of the anterior pituitary.

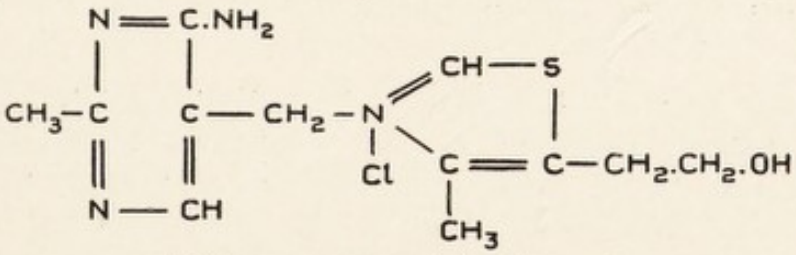
Vitamin K (*Coagulation* or *Anti-haemorrhagic Vitamin*). The Danish investigators Dam and Schønheyder and Almquist and Stoksted claim that chicks require a fat-soluble vitamin which is not identical with **A, D,** or **E,** and which in some way controls the prothrombin-factor in blood needed for blood-clotting (*cf.* p. 224), so that when there is a deficiency of the vitamin the clotting time of blood is markedly prolonged, and subcutaneous, intramuscular and abdominal haemorrhages occur, with anaemia. Administration of material rich in the vitamin restores the blood-clotting time to normal, and cures the anaemia. This vitamin is present in hog-liver fat, in the leafy varieties of vegetables, and, to less extent, in many cereals; its distribution in vegetables differs from that of carotene.

Dam finds that the haemorrhages due to lack of the vitamin can occur not only in chicks, but also in ducklings, young geese, pigeons, and canaries, but do not occur in rats, guinea-pigs, dogs, or man. The condition is probably not related to haemophilia.

The vitamin has been concentrated to the stage of a viscous oil, and Almquist (1937) claims to have prepared it as a colourless crystalline preparation from alfalfa. The crystalline compound contained one or more benzene rings, but no indole nucleus (*cf.* p. 302).

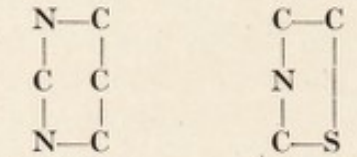
Water-soluble Vitamins

Vitamin B_1 (or *vitamin* **B,** the *anti-beri-beri vitamin*), has recently been named *thiamin*.* From 1911, when Funk's isolation of a crystalline preparation from rice polishings led to the coining of the term "vitamine," constant efforts have been made to obtain the anti-beri-beri vitamin. Jansen and Donath obtained it in pure crystal form from rice polishings in 1926, but did not detect in it the presence of sulphur. Windaus succeeded in crystallising it from a yeast preparation in 1932. He considered it to be $C_{12}H_{17}N_3OS$. Peters also obtained it in crystal form in 1933. Williams finally synthetised it in 1936, and showed that the formula (when obtained from hydrochloric acid solution) is



* The name *ancurin* is also used.

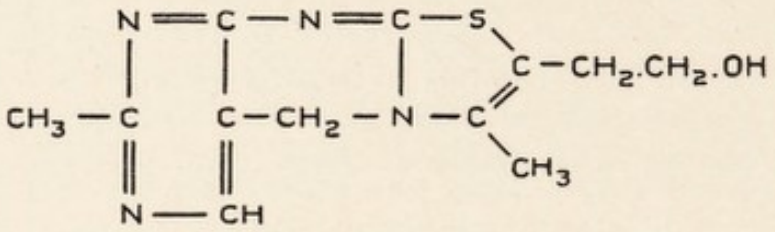
A photomicrograph of crystals of $\mathbf{B_1}$ is shown in Plate II. The free base is possibly $C_{12}H_{16}N_4OS$. This unusual compound is built up from a pyrimidine and a thiazole nucleus. We shall find other pyrimidine compounds among the derivatives of the nucleic acids (*cf.* p. 177).



Pyrimidine ring *Thiazole ring*

In combination with pyrophosphate, thiamin acts as a co-enzyme, *co-carboxylase* (*cf.* p. 286).

Naturally occurring *thiochrome*, a pale yellow, crystalline, blue-fluorescent substance which has been isolated from yeast, can be prepared from vitamin $\mathbf{B_1}$ by the action of alkaline potassium ferricyanide (very mild oxidation)—



Thiochrome

Dried yeast and wheat germ are extremely rich sources of the vitamin, and then in order, baker's yeast, buckwheat bran, oat-meal, and other cereals, orange juice, tomatoes, watercress, cabbage, etc., and potatoes. Milk is a relatively poor source. Otake has obtained a yield of one part of crystalline vitamin from one million parts of rice bran.

The function of the vitamin seems fairly specific. It is concerned with a special stage in the metabolism of carbohydrate, and is apparently an essential factor for the correct catabolism of pyruvic acid, $CH_3 . CO . COOH$ in nerve tissue. Deficiency of the vitamin probably leads to a deficiency of some product of the acid needed for the normal activity of nerve cells (*cf.*, however, p. 287).

Deficiency of the vitamin leads to pathological lesions in the nervous system, resulting in the conditions characterised by *avian polyneuritis* in birds and *beri-beri* in human beings. A slight chronic deficiency seems to be associated with various gastro-intestinal disorders, probably of nervous origin. The

production of polyneuritis in pigeons by a diet of polished rice, and its cure by administration of a preparation rich in the vitamin are illustrated by Figs. 9 and 10.

The Vitamin B Complex includes, besides B_1, some half dozen actual or presumed vitamins, believed to be present in such materials as yeast, and to be necessary for normal growth and function of one or more species of vertebrates. Little is really known of the chemistry or physiological action of most of them. Vitamin B_2 will be dealt with in some detail below. The others

FIG. 9. Pigeon suffering from polyneuritis and showing head retraction (opisthotonos) as a result of a diet of polished rice. (After Schumann.)

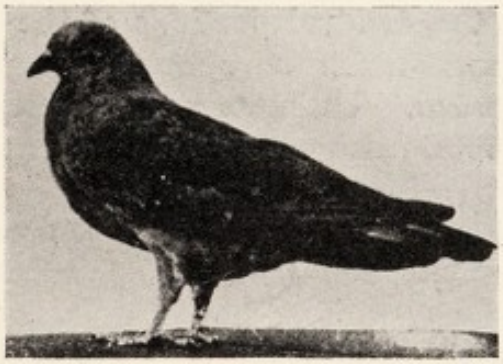


FIG. 10. The same pigeon as in Fig. 9, the day after having received a curative dose of a yeast preparation. (After Schumann.)

at present call for but brief mention. Their separate existences have for the most part been deduced from experiments in which polyneuritis and other conditions have been induced in animals and birds by feeding a diet of polished rice or other deficient diets; administration of crystals of B_1 and preparations rich in B_2 have not resulted in complete restoration, while partial or complete restoration has been effected by administering autoclaved yeast or other specific material.

It cannot be stated at present whether all of the following really are separate entities.

Vitamin $\mathbf{B_3}$, more easily destroyed by heat than $\mathbf{B_2}$, is claimed to be essential for maintenance of body-weight in pigeons and chickens.

Vitamin $\mathbf{B_4}$ is considered to be some factor present in material such as wheat germ, whose absence from the diet of rats results in general muscle weakness and a spastic gait.

Vitamin $\mathbf{B_5}$ is claimed to be essential for weight maintenance of pigeons and to be more stable to heat than is $\mathbf{B_1}$.

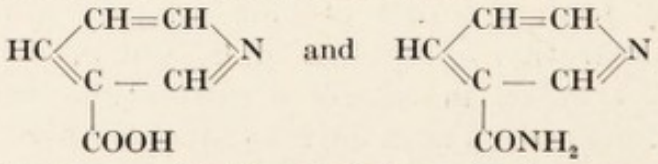
Vitamin $\mathbf{B_6}$ (*vitamin* **H**), present in whey powder and rice polishings, is said to prevent a dermatitis in rats. Leprovsky (1938) claims to have isolated it in crystalline form. The curative dose of his preparation for rats is 5 to 10 micrograms daily.

Vitamin **P-P** (*the pellagra-preventing vitamin*) prevents the occurrence of pellagra in human beings; "black-tongue" in dogs is probably a corresponding condition also associated with deficiency of the vitamin. Some recent evidence indicates that the vitamin may be nicotinic acid *; this has been used beneficially in the treatment of human pellagra and of canine black-tongue (*cf.* p. 286).

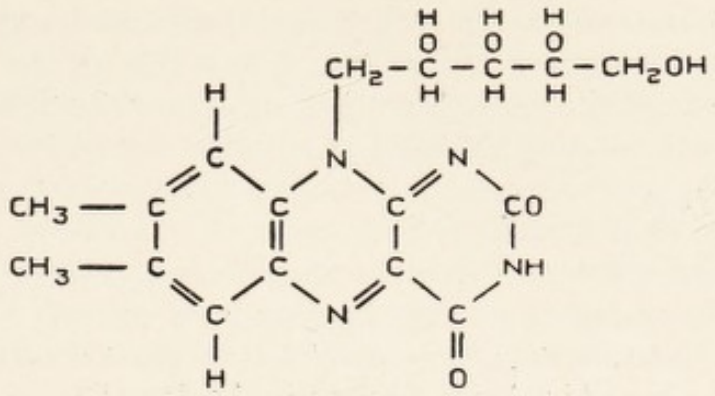
Pellagra is a peculiar disease common in the southern United States and in the sub-tropical East, characterised by erythema, involving usually the exposed portions of the body (face, hands, arms), and by gastro-intestinal symptoms and marked languor and muscle weakness. Ultimately mental symptoms develop. Cures have been obtained by diets rich in meat or administration of yeast extracts.

Vitamin $\mathbf{B_2}$ (**Vitamin G**) is essentially concerned with growth. It is identical with a *flavine*, $C_{17}H_{20}N_4O_6$, which has been obtained from liver, kidney, egg-white, whey of milk, fish-eyes, grass, etc., by Karrer and Kuhn and their co-workers, and has variously been called heptoflavine, ovoflavine, and lactoflavine. This compound, d-riboflavine, containing a radical of the sugar d-ribose (*cf.* Chapter IV., p. 85) has been synthetised by Euler, and has the structure shown on p. 71.

* The formulae for nicotinic acid and its amide are:



The amide is linked as a radical in certain important co-enzymes (*cf.* p. 286).



d-Riboflavine

d-Riboflavine is a naturally occurring pigment, soluble in water, laevorotatory, and melting at 282° C. It is bright yellow in colour, with a characteristic green fluorescence. Various related compounds have been synthetised, several of which have the biological properties of the vitamin. A photomicrograph of riboflavine is shown in Fig. 11.

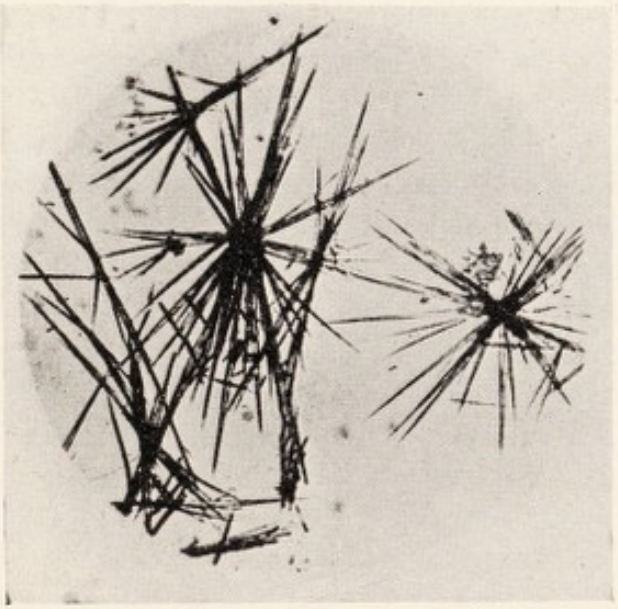


FIG. 11. Photomicrograph of riboflavine (lactoflavine). (After Kuhn.)

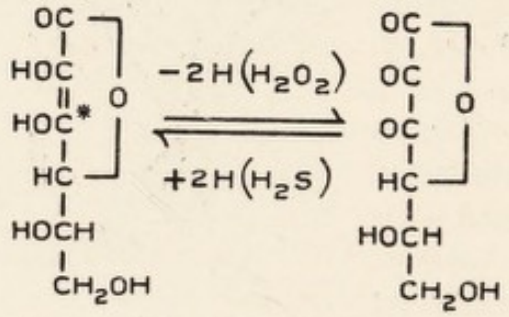
Warburg and Christian have shown that flavine, in combination with a protein, is an enzyme (the "yellow respiratory ferment" of Warburg) with the properties of a dehydrogenase. This seems at present one of the best examples of an enzyme with a prosthetic group. When it is dialysed against dilute hydrochloric acid it is split into flavine and the (water-soluble) protein. *Each of these is completely enzymically inactive alone*, but when they are brought together the activity of the enzyme is largely restored. There is one flavine radical per molecule of this "flavo-protein."

This yellow enzyme has been obtained from bottom yeast and from various mammalian tissues, and has been crystallised in pure condition by Theorell, who estimates its molecular weight at 70,000. Its yellow colour disappears when it is treated with reducing agents, but returns when it is shaken up with atmospheric oxygen. (It is perhaps rather a protein-sized "hydrogen-carrier," than a true enzyme; *cf.* Chapter XII.)

Flavine promotes growth in rats and presumably also in other mammals. It is quite probable that the action of the vitamin is largely produced through the dehydrogenase of which it forms part, an enzyme essential to many reactions in oxidation-reduction systems necessary for correct metabolism in the organism. Flavine is excreted in traces in the urine.

Yeast is an excellent source of the vitamin, as are the " anti-anaemic " preparations of liver and stomach mucosa. Glandular organs, lean meat, and green vegetables are good sources.

Vitamin C (Ascorbic Acid, Cevitamic Acid). The *anti-scorbutic vitamin* is a white crystalline solid, $C_6H_8O_6$, melting at 192° C. It was first obtained in crystalline form from cabbage and from beef adrenals by Szent-Györgyi, and later from lemon juice by Waugh and King. When he first obtained it Szent-Györgyi only considered it as a remarkable catalytic agent. Its identity with the vitamin was only realised a year or two later, coincidently by King and Szent-Györgyi. The naturally occurring vitamin is laevorotatory; the corresponding dextro-compound has no vitamin activity. It is very easily oxidised, and the first oxidation product is easily reduced again to the vitamin, so that both forms are co-existent in the organism, the vitamin acting as a hydrogen-acceptor and carrier. The constitutional formulae of the two compounds are—



$C_6H_8O_6$, *l-ascorbic acid*
Reduced form of the vitamin.

$C_6H_6O_6$, *dehydro-ascorbic acid.*
Oxidised form of the vitamin.

Photomicrographs of crystalline ascorbic acid are shown in Plate II.

Dehydro-ascorbic acid changes rapidly in neutral and alkaline solution, and more slowly in acid solution, to a product which is no longer reconvertible to the vitamin, though it still has strong reducing properties. The acidic properties of the vitamin are due to the hydroxy (enolic) group attached to the third carbon atom (asterisked in the above formula).

The reduced form of the vitamin is widely distributed in plants and animals. It is rapidly formed in the sprouting seed and in rapidly growing stem and root tips. All fresh fruits and tubers

contain significant amounts. The formation of the vitamin precedes the appearance of chlorophyll and carotenoid pigments in plants, though it may well be linked with their activities. Citrus fruits such as oranges and lemons and a Hungarian species of red pepper are especially rich in the vitamin. Its precursor is not yet known. (Reedman and McHenry state that in some plants the vitamin is partly combined with protein.)

It can be formed in certain species of animals such as rats and chicken, but the majority of the higher animals must obtain it in their diets. (Rats cannot therefore be employed to study the effects of deficiency of the vitamin ; the guinea-pig is an excellent experimental animal for that purpose.)

It is present in practically all the tissues of the higher animals, in greatest amount in glandular tissues, and in least amount in muscle and stored fat. The adrenals and pituitary glands, the corpus luteum of the ovary, and the thymus (in *young* animals) are especially rich in it. Human milk is richer in it than is cow's milk, an obviously important fact in considering the diet of infants.

Its specific *rôle* in metabolism is that of transporting hydrogen and thus acting as a reducing agent (and at the same time effecting oxidation). It acts along with definite enzymes, such as an oxidase present in the adrenal cortex and in cabbage leaves. Hubbard squash contains an oxidase so specific for the action of the vitamin as to enable it to react directly with atmospheric oxygen. It is sufficiently powerful *in vitro* to reduce cupric to cuprous, ferric to ferrous, and manganic to manganous ions, —S—S— to —SH, and —CO to —COH. While its various *rôles* in the organism are not yet fully elucidated, it is evidently an essential catalyst for oxidation in tissues generally. It is also believed to regulate, directly or indirectly, the colloidal condition of intercellular substances.

Continued deficiency of intake of the vitamin leads to *scurvy*. In this disease, when produced experimentally in animals, the *oxygen-uptake* of the animal is decreased. Furthermore, tissue-slices from such animals when a solution of the vitamin is added to them *in vitro* show an increased consumption of oxygen. Vitamin **C** is normally excreted in the urine, and when the diet is deficient in it this excretion continues, and the body tissues are markedly depleted before active symptoms of scurvy are seen. It may therefore be concluded that scurvy is primarily associated with some specific deficiency of tissue oxidation, due to deficiency of the vitamin.

Scurvy is characterised by a spongy condition of the gums and

a tendency to haemorrhages into the gums, muscles and internal organs. Such haemorrhages and fragility of the bones (not due to deficiency of bone minerals) are the most noticeable findings at autopsy. It is not yet clear how the normal functioning of the vitamin prevents the scorbutic changes. Administration of any rich source of the vitamin, such as orange juice, rapidly cures scurvy.

Evidence is accumulating that in certain infectious diseases the vitamin **C** content of the organism is diminished, while administration of large doses of the vitamin helps to lessen the severity of the disease.

Vitamin P (Citrin). Szent-Györgyi has adduced some evidence that the " flavone " fraction present in lemon juice (flavones are a group of vegetable dyes) contains an additional vitamin (**P** or *citrin*), deficiency of which favours the haemorrhagic manifestations of scurvy. Silva believes that **P** does not exist, and that the effects noted by Szent-Györgyi are caused by a chronic subminimal dosage of **C**.

General Remarks on Vitamins. At the present time only five vitamins have been obtained in pure condition, **A**, $\mathbf{B_1}$, $\mathbf{B_2}$, **C** and **D**. There is indisputable evidence for the existence of **E**, and evidence varying in strength in each individual case, for $\mathbf{B_3}$, $\mathbf{B_4}$, $\mathbf{B_5}$, $\mathbf{B_6}$, **P–P**, **P**, and **K**. Those whose chemistry is known show no chemical interrelationship, but belong to widely differing groups of compounds. Equally, they exhibit no biological relationships. **C** is indispensable for the action of certain oxidases in all tissues, $\mathbf{B_1}$ is concerned with a specific stage of carbohydrate catabolism, $\mathbf{B_2}$ is a component of an essential enzyme, **D** controls calcium and phosphorus metabolism, and so on.

All these vitamins show definite differences in resistance to heat and to oxidation ; this is of importance in connection with food preparation. Thus **A** is fairly easily oxidised, and **C** still more so. Even such temperatures as those employed in the pasteurisation of milk decompose **C** in presence of oxygen, so that pasteurised milk virtually contains no **C**, and the diet of infants must be adjusted accordingly. **D** is moderately resistant to heat, and $\mathbf{B_1}$ resists boiling and oxidation. In considering diets, it must also be remembered that when food is boiled with water, and the water (with extracted material) is subsequently rejected,the water-soluble vitamins are at least partly lost. The minimum requirements of vitamins in a diet will be dealt with in Chapter XVII.

AUXINS

These are compounds of varied nature, sometimes termed *plant-hormones*, which have been found to be essential for plant growth.

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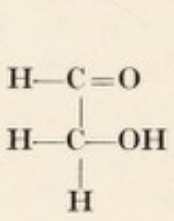
CHAPTER IV

CARBOHYDRATES AND RELATED COMPOUNDS

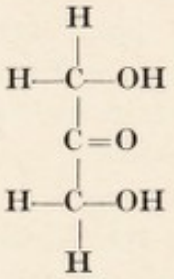
INTRODUCTION

THE name carbohydrate suggests *carbon* and *water*, and most carbohydrates have the empirical formula $C_x(H_2O)_y$. The alternative term *glucide* has been suggested by the International Committee on Biochemical Nomenclature, but has not been widely adopted by English-speaking biochemists.

Carbohydrates may be regarded as aldehyde and ketone alcohols. The simplest of these are $C_2H_4O_2$, glycolyl aldehyde, and $C_3H_6O_3$, dihydroxyacetone :



Glycolyl aldehyde
(An *aldehyde-alcohol* or *aldose*)



Dihydroxyacetone
(A *ketone-alcohol* or *ketose*)

Carbohydrates may be classified as *monoses* or *monosaccharides* (simple sugars), *bioses* or *disaccharides* (more complex sugars), and *polysaccharides* (such as starch). The simple sugars are further classified according to the number of carbon atoms they contain, as *trioses* (three carbon atoms), *tetroses* (four), *pentoses* (five), *hexoses* (six), and *heptoses* (seven). Molecules of disaccharides can be regarded as built up from two monosaccharide molecules by elimination of a molecule of water; polysaccharides are similarly constructed from a number of monosaccharide molecules.

Certain pentoses, hexoses, disaccharides and polysaccharides are of biochemical importance; of them all the hexose *glucose* concerns us most.

Glucose

Glucose, $C_6H_{12}O_6$, frequently termed *dextrose*, occurs in plants and animals. In plants it is found in grapes (whence its name *grape-sugar*), in seeds, in various roots, and in 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.

Alkaline solutions of cupric compounds are reduced by boiling with glucose, cuprous oxide separating as a red or yellow powder, the reaction being essentially $Cu^{++} \longrightarrow Cu^{+} \longrightarrow Cu_2O$. 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 afford an important means of testing for glucose and other *reducing sugars;* the sugar is simultaneously oxidised to an acid.

Glucose reacts with phenylhydrazine, $C_6H_5 . NH . NH_2$, to form a characteristic insoluble crystalline *osazone* (the formula of which is given on p. 81), with a specific melting point. The appearance of the glucosazone crystals under a microscope, and the specific melting point are important in identifying glucose.

When a solution containing glucose is rubbed up with fresh yeast, some of the enzymes in the yeast decompose glucose with production of alcohol and evolution of carbon dioxide (fermentation). The series of reactions is complex, but can be summed up in the equation

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

Only a few sugars are fermented by yeast; the liberation of gaseous carbon dioxide in this reaction can be used as a test for these sugars.

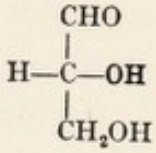
Naturally occurring glucose is optically active, with a specific

rotation of + 52·5° at 20° C. Because it is dextro-rotatory the term *dextrose* is often used for it. It has the formula

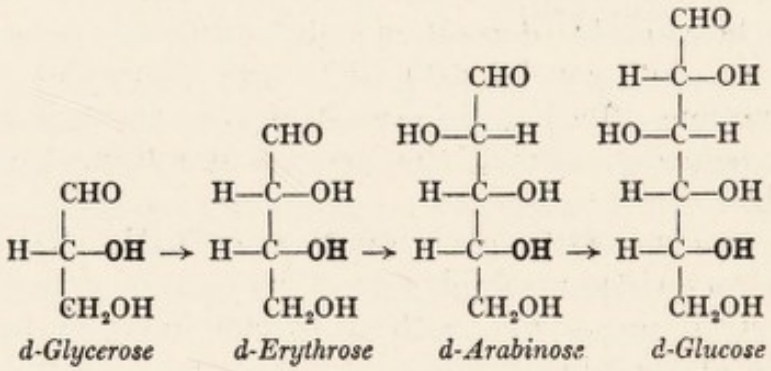
$$CH_2OH . \overset{*}{C}HOH . \overset{*}{C}HOH . \overset{*}{C}HOH . \overset{*}{C}HOH . CHO$$

with four asymmetric carbon atoms (marked by asterisks). Using the projection method illustrated for lactic acid in Chapter II., its space formula can be derived in the following manner.

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 laevo-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

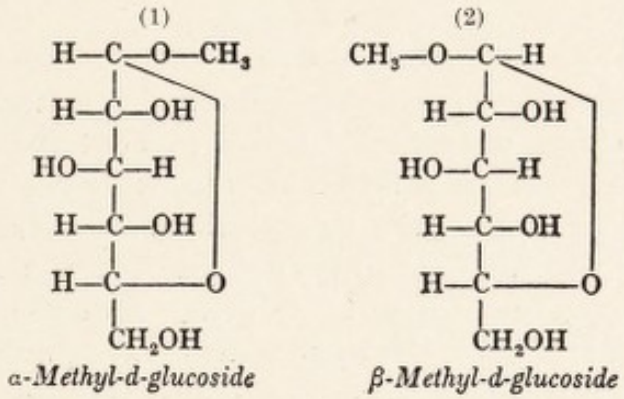
$$[\alpha]_D = + 52{\cdot}5^\circ.$$

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

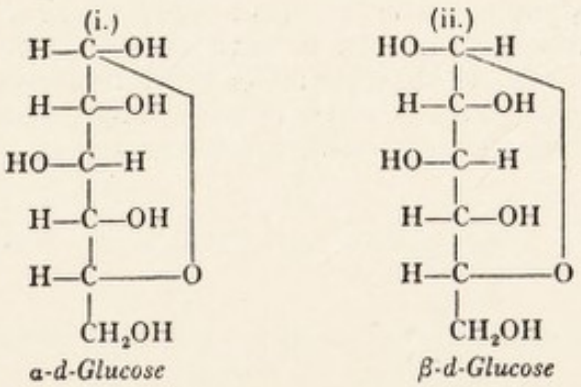
Such an effect at once suggests that there are present in the

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.

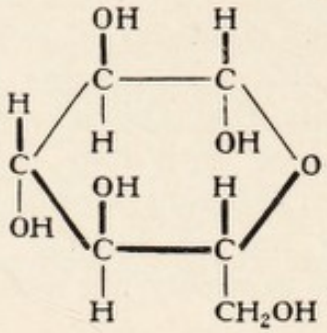
Two methyl-*d*-glucoses (simple *glucosides*) have been prepared, which are believed to be :



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 formulae of these two *d*-glucoses are :



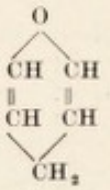
The position of the α- and β-OH groups attached to the first carbon atom is based on physico-chemical considerations, which cannot be dealt with here. The actual spacial arrangement is most aptly illustrated by such formulae as the following for *β-d-glucose* (after Haworth),



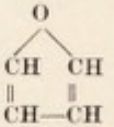
From the equilibrium rotation value it can be calculated that an equilibrium solution contains 37 per cent. of the α-form of glucose, and 63 per cent. of the β-form.

Most Sugars Exhibit Muta-rotation. 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 aldehyde 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.

Note on Nomenclature. It is customary to number the carbon atoms in glucose from the (potential) aldehyde group, and in other sugars from the corresponding carbon atom. Most sugars are δ-lactones, with an ether linkage from first to fifth carbon atoms, and so belong to the *pyranose* series, being, theoretically, derivatives of pyran. Corresponding γ-lactone sugars, with ether linkage from first to fourth carbon atom, belong to the so-called *furanose* series.



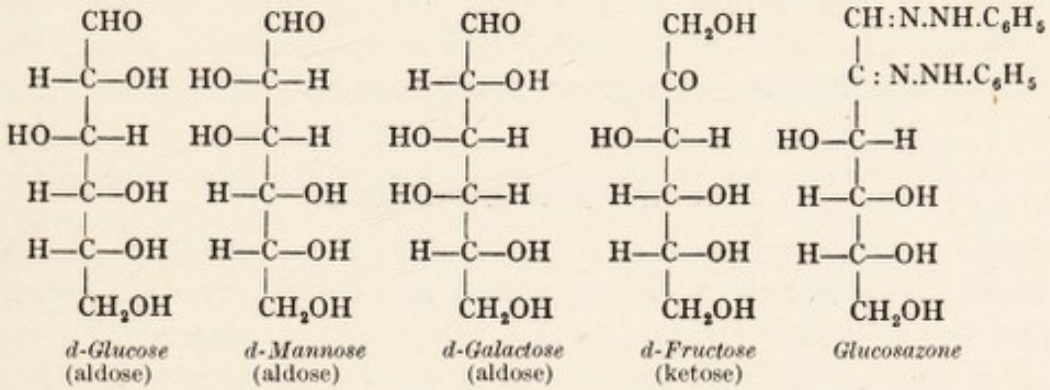
Pyran



Furan

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 laevo-rotatory). Their aldehyde (or ketone) formulae are shown :



The corresponding laevo-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 formulae, *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*, present in some seaweeds, and its radical is present in certain glucosides (such as plant saponins, and the cerebrosides in brain tissue).

Fructose (also termed fruit sugar, and laevulose, 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

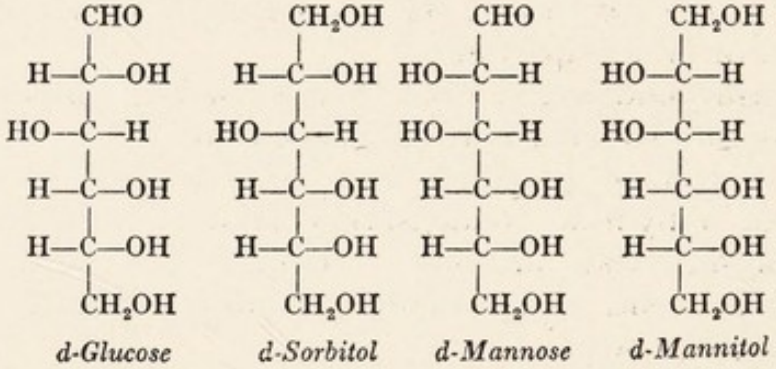
mucilaginous nucleus permeated with a multitude of pointed needles. These crystals consisted of pure fructose.

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 *laevo-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

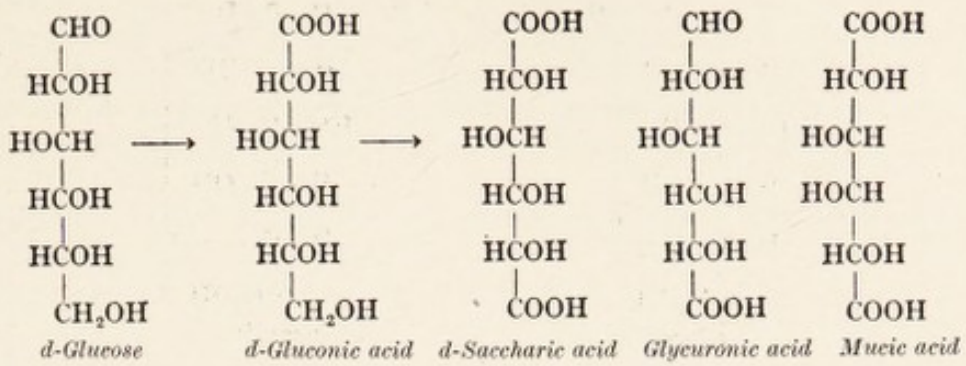
Each sugar corresponds to an alcohol, from which it can be derived by oxidation, and to which it can be reduced by the action of sodium amalgam. The relationships between glucose and sorbitol, and mannose and mannitol, are shown by their formulae :



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.

The aldoses oxidise to acids. Mild oxidation of glucose, as with bromine water, converts it to gluconic acid. Heating with nitric acid converts it to the dibasic saccharic acid. Hydrogen peroxide converts it to glycuronic (or glucuronic) acid. Nitric acid converts galactose to mucic acid, which separates as a hard gritty white powder. Since saccharic acid is very soluble, the formation of insoluble mucic acid can be used as a test for galactose (and lactose).

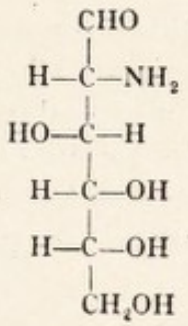


Glycuronic acid is an important detoxicating agent in the animal organism (*cf.* Chapter XIII).

Ketoses oxidise to acids with fewer carbon atoms. Fructose yields a mixture of trihydroxybutyric acid, $CH_2OH \cdot CHOH \cdot CHOH \cdot COOH$, and glycollic acid, $CH_2OH \cdot COOH$.

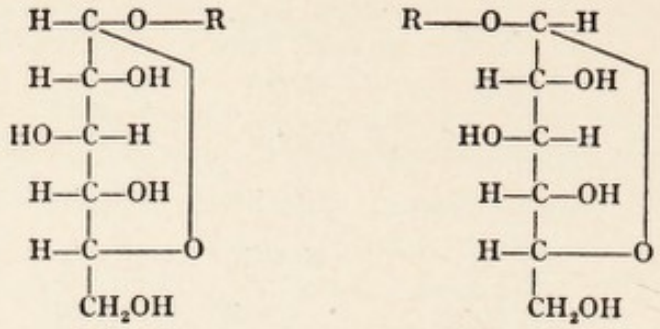
In presence of dilute alkali any one of glucose, mannose, or fructose is converted into a mixture of all three. But more concentrated alkali brings about decomposition with formation of lactic acid (we shall see later that the formation of lactic acid from glucose is biochemically of great importance).

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 formulae 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.

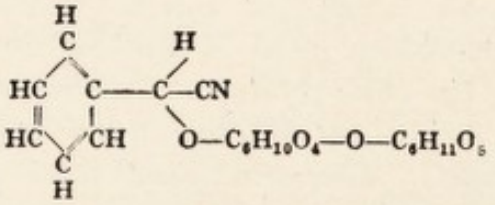
Saponins hydrolyse to glucose, galactose, and sapogenins.

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 formulae 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 lipide radicals.

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 :



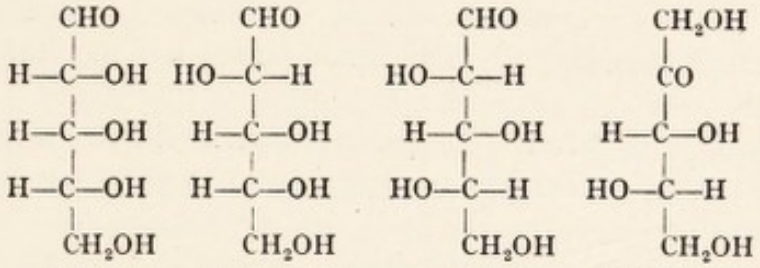
Many glucosides of great pharmacological importance are obtained from leaves and seeds of such plants as *Digitalis purpurea*, *Strophantus*, and *Scilla*. They are generally prepared by extraction with water or alcohol, and most of them are colourless, laevo-rotatory, crystalline,

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 nucleoproteins 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 in rare cases are constant constituents of certain abnormal urines. The principal pentoses of biochemical importance are *d*-ribose, *d*- and *l*-arabinose, *l*-xylose, and *l*-xyloketose.

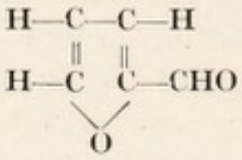


d-Ribose (lævo-rotatory) — *d-Arabinose* (lævo-rotatory) — *l-Xylose* (lævo-rotatory) — *l-Xyloketose* (dextro-rotatory)

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

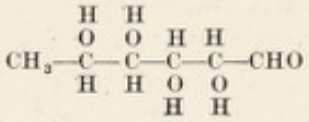
l-Arabinose is obtained from *arabans* by hydrolysis with dilute sulphuric acid. Such arabans are cherry gum, peach gum, and gum arabic. Arabinose crystallises in prisms and has a sweet taste. Wood gums, in the cell-walls of plants, are *xylans*, and yield xylose on hydrolysis. Prepared thus, the xylose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

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 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 algae contain a polysaccharide *fucosan* which on hydrolysis yields the methyl-pentose *fucose*.

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.

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.

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 laevo-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. 89. Numerous attempts have been made to synthetise it, apparently without success.

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. It is not found in plants.

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 reduces alkaline copper solutions, and gives a specific osazone, 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 algae, 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.

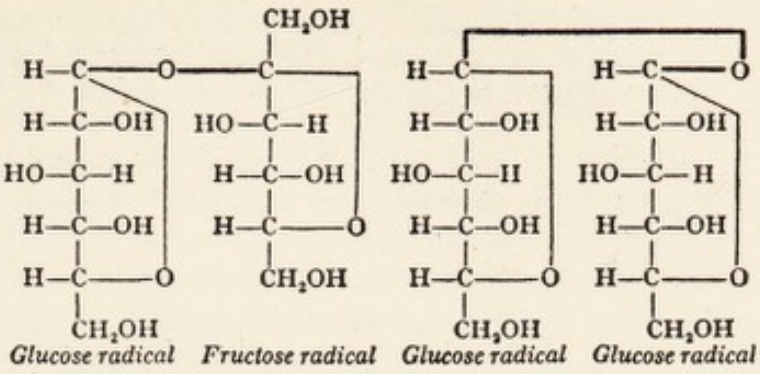
With the exception of sucrose and trehalose all these sugars exhibit muta-rotation. Studies of their constitutions show that they are built up variously from the α- and β-forms of the three hexoses, and that most of them can be regarded as α- or β-glucosides. The actions of enzymes on them depends on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

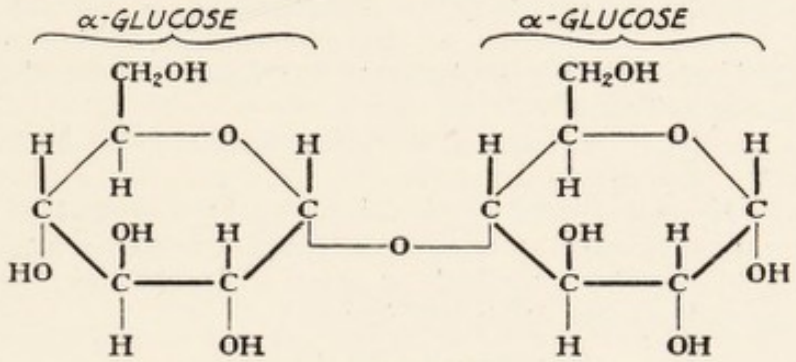
The comparison with a lock and key, frequently made to illustrate the nature of enzyme action, seems quite appropriate, provided we consider the most elaborate form of lock, which can only be turned by one correspondingly elaborate key.

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

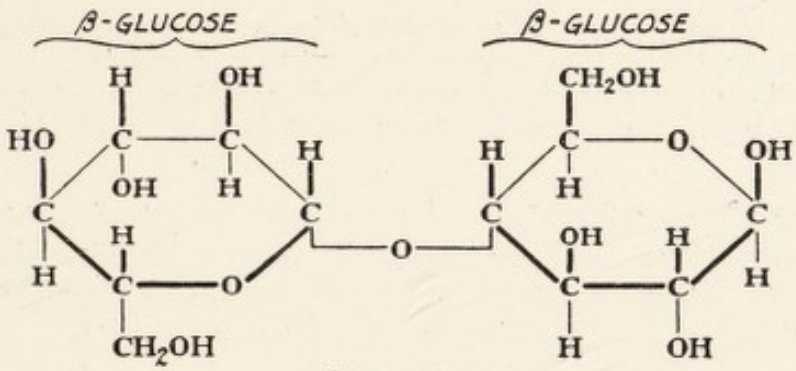


SUCROSE TREHALOSE

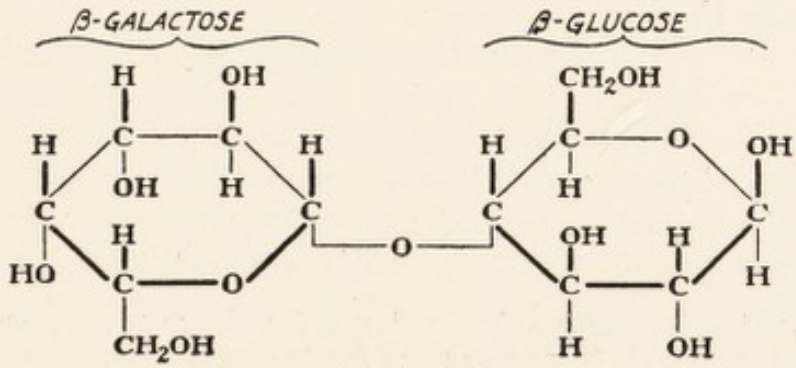
The following formulae for maltose, cellobiose and lactose (after Haworth) are suggestive of the actual molecular arrangement of these compounds in space (*cf.* p. 80).



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 dextro-rotatory, 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.

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.

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, probably formed from galacturonic acid during hydrolysis, and recent work suggests that the pectin molecule consists entirely of a long chain of galacturonic acid units.

The *mucilages* obtained from algae, 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.

Molecules of such colloids as starch and cellulose may " aggregate " to particles (*micellae*) of large " molecular " size, held together by some physical force. Methods for determining molecular weight, such as viscosity and sedimentation procedures, possibly indicate the size of the aggregated particle rather than that of the chemical molecule. Thus starch is built up of amylose and amylopectin (which contains phosphate radicals) each containing some 26 to 30 glucose units. Yet amylose appears to consist largely of particles with a " molecular weight " of about 60,000, while that of amylo-pectin seems to be of the order of 300,000.

Amylodextrin has a chemical molecule containing about 17 glucose units, while other dextrins have been prepared containing from 10 to 7 glucose units. Glycogen is considered to have an average chain length of 12 to 18 glucose units; a number of such chains may be linked together side by side to form a larger molecule. The molecule of cellulose, as determined by the ultra-centrifuge, has a "molecular weight" of about 300,000. It is believed to consist of a uniform chain of β-glucose radicals; some investigators consider that its chemical molecular weight is only about 30,000. Inulin is built up from 30 fructose units, and has a molecular weight of about 5,000.

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. This may apply to starch, but not to cellulose.

It has been suggested that the fibrous form assumed by cellulose (well exemplified in pure filter paper) is due to the β-type of linkage of adjacent glucose molecules, which favours the production of thread-like molecules, while the α-type of linkage in starch favours a closer packed molecule.

The specific rotations of various carbohydrates 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	+ 52·6°	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°	—

Carbohydrases, the Enzymes which act on Carbohydrates

The carbohydrases act on specific linkages, or, more accurately, linkages associated with specific configurations of atoms. The most important of these enzymes are sucrase (a fructosidase), maltases (α-d-glucosidases), emulsin (β-d-glucosidase), lactase (β-d-galactosidase) and amylases.

Sucrase (or *saccharase* or *invertase*) splits sucrose to glucose and fructose, acting on the specific linkage between their radicals in sucrose (*cf.* its formula on p. 89). Sucrase occurs in the intestinal juice of mammals, and in certain plant and animal tissues. Yeast is a good source of it. It is still uncertain whether sucrase is specific for sucrose or will split compounds such as raffinose which contain a sucrose radical. It seems most probable, however, that when enzyme preparations do hydrolyse raffinose, they contain *melibiase*, which splits it to galactose and sucrose, as well as sucrase, which then attacks the sucrose set free. It is also still uncertain whether sucrases from different sources are identical.

Maltases or *α-d-glucosidases* split α-d-glucosides. Since maltose is glucose-α-d-glucoside, these enzymes split maltose to two molecules of glucose. Maltases are widely distributed in plant and animal tissues. Intestinal juice and yeast are good sources. It would appear from recent work by Tauber and Kleiner that there are two types of maltase, true α-glucosidases, which split all α-glucosides, and pseudo-α-glucosidases, which only split easily hydrolysable α-glucosides, including maltose. Yeast maltase, an example of the former, will also synthetise α-methyl glucoside from methyl alcohol and glucose (Bourquelot's synthesis).

Emulsin, from bitter almonds, has long been known to split amygdalin to glucose, benzaldehyde, and hydrocyanic acid. This action is not specific; emulsin will split β-methyl-d-glucoside, and all other β-d-glucosides, though at rates varying with the "aglycone" (non-carbohydrate portion) of the glucoside.

Lactase, *β-d-galactosidase*, hydrolyses lactose to galactose and glucose. Its action is determined by the specific configuration at the attachment of their two radicals (*cf.* formula, p. 89). It is present in intestinal juice, and especially in the cells of the intestinal mucosa. It is also stated to be present in almonds, in lactose yeasts (yeasts capable of hydrolysing lactose), and in certain moulds and bacteria.

Amylases hydrolyse starch and glycogen to maltose. They are present in saliva and pancreatic juice, in the cells of various animal tissues, and in many plants. Two types of amylase exist. The one, termed by Kuhn the α-type (exemplified by pancreatic

and salivary amylases) produces from starch α-maltose (whose specific rotation *decreases* by muta-rotation), while the other, the β-type (represented by the amylases of grains), produces β-maltose (whose specific rotation *increases* by muta-rotation). Kuhn believes that the starch molecule is composed of a chain of alternating α and β linkages ; the two types of enzyme therefore break it up at different points.

Neutral salts are not essential for the action of malt amylase, but are necessary for the activity of salivary and pancreatic amylase. When either of the latter is dialysed against water so that all neutral salts are removed, the amylase remaining behind is enzymatically inactive ; addition of neutral salt restores its activity. The anion of the salt is more important than the cation. Sodium chloride is the natural *co-enzyme* of these amylases. Somewhat less effective, in the order named, are KCl, NaBr, $NaNO_3$ and NaF. Phosphate is not essential for amylase activity.

The optimum *p*H for salivary and pancreatic amylases varies from 6·3 to 7·2, according to salt concentration. That for plant amylases is distinctly lower, 4 to 5·5.

Crystalline pancreatic amylase has been prepared by Sherman, and shown to be a protein.

Certain other *polyases* are known. *Lichenase*, present in malt extracts, in various plants, and in the intestines of snails, hydrolyses lichenin to cellobiose. *Cellulase* from the snail's intestine, fungi, and moulds, hydrolyses cellulose. *Inulase* from moulds splits inulin to fructose.

Natural Synthesis of Carbohydrates

The synthesis of carbohydrates is essentially a function of plant, rather than of animal metabolism. They are formed in green leaves through the action of certain rays in sunlight, chlorophyll acting as a filter for these rays. It is very probable that formaldehyde is first formed, and that this then polymerises to hexoses, from which the plant forms its sucrose, its starch and its cellulose.

$$CO_2 + HOH = HCHO + O_2$$
$$6HCHO = C_6H_{12}O_6$$

Digestion and Absorption of Carbohydrates

Digestion. The carbohydrates present in appreciable quantity in a mixed diet are starch, cellulose, cane-sugar (added as a sweetening agent), glucose (in such material as corn syrup and honey), and fructose (in honey). Traces of other polysaccharides and sugars may be present, including pentoses from fruits.

Starch is digested by salivary amylase (in mouth and stomach)

and pancreatic amylase (in the intestine) with production of maltose. Cellulose is not digested by human agencies; its digestion by bacteria will be considered later (Chapter XIII.). Cane-sugar is converted to glucose and fructose by sucrase of the intestinal juice, lactose is similarly converted to glucose and galactose by lactase, while maltose is hydrolysed to glucose by maltase. (The degree of acidity of the gastric contents is sufficient to hydrolyse cane-sugar slightly but negligibly.)

Absorption. As a result of salivary and intestinal digestion the utilisable carbohydrates of the diet yield a mixture of glucose, fructose, and galactose, glucose being much in excess of the others. These, with traces of pentoses, are absorbed through the wall of the small intestine. There is no appreciable absorption of carbohydrate from the stomach, while little utilisable carbohydrate reaches the ileo-caecal valve. The intestinal mucus of adult man is impermeable to C_{12} sugars. In infants a trace of lactose may be absorbed, but the intestine rapidly becomes impermeable to it.

The absorbed sugars pass to the capillaries of the portal circulation and so to the liver.

Absorption of glucose seems to take place at a fairly definite rate, independent of the concentration of glucose in the gut. In dogs this rate is approximately 0·9 to 1·0 gm. of glucose per kg. body-weight of the animal per hour. The rate at which glucose can be administered intravenously to the dog without loss through the kidneys (and therefore with complete utilisation) has been shown to be 0·9 gm. per kg. per hour, which suggests that there is a close relationship between the rate of absorption from the gut and the rate of utilisation in the normal animal. The maximum rate of intravenous injection in man without occurrence of glycosuria is 0·85 gm. per kg. per hour, so that by inference one may deduce a similar maximum rate of absorption from the intestine. A similar constant rate of absorption has been shown to occur in the rat. Certain investigators, however, do not accept these conclusions, and consider that they have proof that absorption falls off with time.

Conclusions as to a maximum steady rate of absorption seem to apply to total hexose, rather than to individual hexoses, since it has been shown that when solutions containing both glucose and galactose are introduced into loops of dog's jejunum the total amount of carbohydrate absorbed is about the same as when each sugar is administered separately. Glucose is absorbed more rapidly than fructose.

There is evidence that hexoses (glucose, fructose, galactose)

are absorbed more rapidly from the gut than pentoses, and that the cause is the formation of hexose phosphates, this phosphorylation especially facilitating the absorption of glucose. Iodoacetic acid and phloridzin inhibit phosphorylation, and it has been shown that injection of either of these drugs into the rat reduces the rate of absorption of hexoses (but not of pentoses). The evidence for phosphorylation is not yet decisive.

Metabolism of Carbohydrates

Carbohydrates in the Blood. The carbohydrate content of a single meal frequently exceeds the equivalent of 100 gm. of glucose. If this amount of glucose were suddenly thrown into the circulation of a 70 kg. man, who contains some 8·8 per cent. of blood, about 6 litres, then there should be an increase of blood glucose of over 1·6 per cent. Even with the restricted rate of absorption referred to in the previous section (permitting, for a 70 kg. man, absorption of some 60 gm. of glucose per hour from the intestine) there should be a steady marked rise of blood glucose over two or three hours to a high figure well over 1 per cent., unless glucose were rapidly withdrawn from the blood. But it is withdrawn so rapidly that a blood glucose curve over a twenty-four hour period shows only slight variations from fasting level. A typical normal curve is shown in Fig. 12. Various tissues, liver, muscle, skin, withdraw glucose rapidly from blood whenever it rises appreciably above the fasting level.

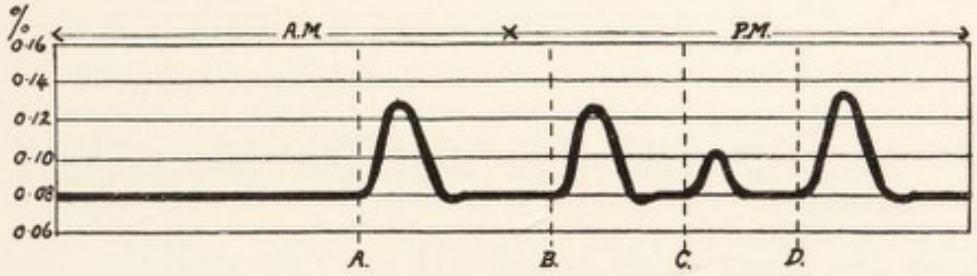


FIG. 12. Typical twenty-four-hours' blood-glucose chart.
A, breakfast ; *B*, lunch ; *C*, tea ; *D*, dinner.

Blood sugar, under normal conditions, consists almost entirely of glucose with traces of pentoses which are not utilisable and are *en route* to excretion. (Following administration of large doses of fructose or of galactose some fructose or some galactose can be detected in the blood of the general circulation, but these are not normal conditions.)

Glucose is present in both cells and plasma ; human plasma contains a relatively greater amount than the red cells. According to recent measurements by Neuwirth the ratio is 1·4 to 1. Actual

figures for blood glucose depend on the method of estimation. When this involves the use of tungstic acid as a precipitant (Folin-Wu procedure) certain compounds such as glutathione and ergothioneine (*cf.* pp. 251, 250) escape to the filtrate which, like glucose, reduce the copper reagent employed for estimation, so that the values obtained are too high. These may therefore be styled "enhanced-glucose values." When zinc hydroxide or similar precipitants are used, the interfering compounds are largely carried down with the protein precipitate, and truer values are obtained. Absolutely "true glucose" values are only measurable by fermenting blood filtrates with yeast; the difference

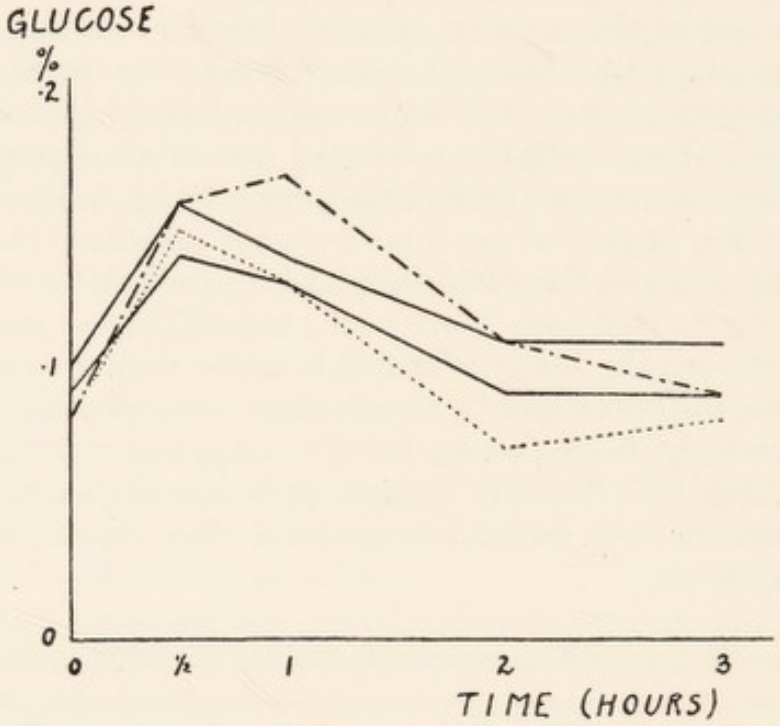


Fig. 13. Typical normal blood sugar tolerance curves, following ingestion of 100 gm. glucose (venous blood; enhanced glucose values).

between the sugar value before and after fermentation gives a true figure for glucose. The difference between "true" and "enhanced" values varies in different individuals, and lies probably between the extremes of 10 and 20 mg. per 100 c.c. of blood (estimated in terms of glucose). Normal "true" values for human blood taken before a meal lie between 0·07 and 0·09 per cent. The effect of a normal meal, or of ingestion of 50 gm. of glucose dissolved in water, is to raise the blood sugar to a figure usually between 0·12 and 0·14 per cent. (true glucose) in normal individuals (*cf.* Fig. 12). This value is usually attained between half and one hour after ingestion of a sugar meal; the blood sugar then falls steadily, and usually, two hours after the meal, it is

back to the original level, and may even be very slightly (and very transiently) below that fasting level.

The effect of a definite quantity of ingested glucose on the blood sugar has considerable clinical application. The curve of the blood sugar so obtained is termed the " sugar tolerance curve." Some typical normal curves are shown in Fig. 13.

When extremely large amounts of sugar are ingested by normal persons, or when, as with patients suffering from diabetes mellitus, the " sugar tolerance " is decreased, the blood sugar rises above 0·16 or 0·17 per cent., and this condition of unusually high blood sugar is termed *hyperglycaemia.* Hyperglycaemia is usually accompanied by a *glycosuria*, the presence of glucose in the urine. The kidney usually dams back glucose, preventing its excretion into the urine. But in hyperglycaemia the kidney cannot dam back all the sugar, the " kidney dam " has been exceeded, and some glucose flows over it.

This " kidney dam," however, is at a variable level. It is usually fairly constant in man and in the non-pregnant woman, but varies considerably in different individuals. In persons with kidney disease it may be slowly elevated. During pregnancy it is frequently lowered. The drug phloridzin lowers it markedly.

Many individuals have, normally, a low kidney dam, and so exhibit glycosuria after meals. They thus exhibit an unusual, but not a pathological condition, termed " renal glycosuria." The *average* height of the kidney dam lies between 0·16 and 0·17 per cent. blood glucose. Probably 80 to 90 per cent. of normal individuals come within these limits.

Glycogen is the storage form of carbohydrates in animals, and in some plants, including yeast. A definite reserve store of glycogen is essential for the maintenance of a constant level of blood glucose during fasting, to replace that withdrawn by tissues from the blood for energy needs. Glycogen is found in most of the tissues of the body, but the chief storehouses are the liver and muscles.

Some rather ancient (1903) but still valid experiments by Schöndorff showed that dogs—after ingesting a rich carbohydrate and meat diet for some time, so that the glycogen stores could be considered amply stocked—exhibited the following distribution of the total body glycogen in percentages : liver, 38 ; muscle, 44 ; bone-marrow, 9 ; skin, 4·5 ; viscera, 3·8 ; heart muscle, 0·17 ; brain, 0·09 ; white cells of the blood, 0·015. The low figures for heart muscle, in constant activity, and for brain are noteworthy. While claims to the contrary have been made, it is doubtful if glycogen exists in blood other than in the leucocytes.

In dog's liver a content of 18·7 per cent. of glycogen has been

recorded after maintenance of the animal on high carbohydrate diet; similar high figures have been obtained for the rabbit. Usually the amount present in the liver of a well-nourished man is of the order of 100 gm., but it is probable that the human liver can store two or three times this amount. During starvation liver-glycogen drops to much lower figures. Measured values for striped muscle of different animals under different conditions vary from less than 0·1 to over 3 per cent. The greater total content found in muscle in such experiments as those of Schöndorff is due to the much greater total mass of muscle as compared with liver.

In the tissues that store glycogen in quantity it is present free in an amorphous granular state in equilibrium with its saturated solution.

The liver can form glycogen from a variety of compounds. When toad's liver is perfused with solutions of glucose, galactose, maltose, glycerol, or formaldehyde, glycogen is formed, but no production is observed following perfusion with sucrose, lactose, or pentoses. Cori and others have shown by perfusion and other methods that rats' livers form glycogen from glucose, fructose and galactose—somewhat more rapidly from fructose than from glucose, but from galactose much more slowly—from dihydroxyacetone, $CH_2OH \cdot CO \cdot CH_2OH$, and from glycerol, $CH_2OH \cdot CHOH \cdot CH_2OH$, rapidly, and somewhat more slowly from lactic acid, and pyruvic acid, $CH_3 \cdot CO \cdot COOH$, from methyl glyoxal, $CH_3 \cdot CO \cdot CHO$, and glyceric aldehyde, $CH_2OH \cdot CHOH \cdot CHO$, and from the sodium salts of succinic, fumaric, and propionic acids. No evidence has been obtained that the liver can form glycogen from fatty acids except from propionic acid and others with an odd number of carbon atoms.

In mammals intravenous injection of sucrose and of maltose leads to glycogen formation. Little or none is produced from lactose, and none from pentoses. It is to be noted that the majority of glycogen precursors are C_6 or C_3 compounds.

During normal metabolism the liver forms glycogen from glucose, fructose, and galactose, brought to it by the portal circulation, and from lactic acid, brought in the blood of the general circulation.

Muscle has a more limited power of glycogen formation. It can utilise glucose and lactic acid.

The conversion of glucose to glycogen in the liver is almost certainly enzymic in character, but the actual enzyme producing the change is not known. Liver contains an amylolytic enzyme, which can be separated from the liver cells and which acts on

both starch and glycogen, but has, in addition, the activity of maltase. The *glycogenase* from rabbit's liver converts glycogen quantitatively to glucose in slightly alkaline medium. Obviously its action is controlled. Otherwise the enzyme would normally rapidly denude liver of its glycogen. It might well be presumed that the same enzyme under changed conditions would synthetise glycogen from glucose. Certain facts render this hypothesis doubtful.

In 1929 von Gierke described a peculiar and rare disease, known now by his name, in which young children develop markedly enlarged livers. At autopsy of such children their livers are found literally stuffed with glycogen. During life, their blood sugar shows marked fluctuations, sinking to very low levels (0·03 per cent.) between meals, though these patients exhibit none of the symptoms usually associated with such extreme hypoglycaemia (*cf.* p. 107). Patients with von Gierke's disease obviously have the power to form liver glycogen, but do not seem to possess the power to break it down to glucose. Either different enzymes are concerned in the two processes, *glycogenesis*, and *glycogenolysis*, or else some co-enzyme or other factor essential for the latter process is absent from the liver of the patient with this rare disease.

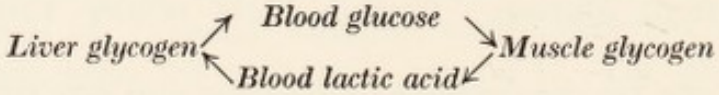
It will be seen later that insulin is essential for the formation of glycogen from glucose in the liver, while adrenaline markedly accelerates the reverse process. Fructose is probably converted directly to glycogen in liver tissues, insulin being unnecessary for the reaction. It is still uncertain whether galactose and lactic acid are directly converted, or indirectly, through initial transformation to glucose. The slower formation of glycogen from these compounds suggests that the latter is more probable.

The store of liver glycogen is mobilised to blood glucose with just sufficient rapidity to maintain the fasting level of the latter. Blood glucose is removed by the tissues that utilise it, and again stored as glycogen. Muscle tissue constantly withdraws it.

Formation of muscle glycogen from glucose is irreversible. This glycogen is broken down, immediately following muscular contraction, through a hexose phosphate to lactic acid. Under normal conditions, during the succeeding period of muscle relaxation, one-fourth to one-fifth of the lactic acid is oxidised to carbon dioxide and water. The energy derived from this oxidation is not only sufficient to account for the work done by muscle in contracting, but suffices also for the reconversion of the rest of the lactic acid to glycogen.

In marked muscular fatigue, when such excess of lactic acid is produced that the available oxygen is inadequate for its

oxidation, or following adrenaline stimulation, when excessive glycogenolysis occurs in muscle as well as in the liver, some proportion of the lactic acid escapes to the general circulation, and is in part excreted, but in part is taken up by the liver and converted to glycogen there, so that a circulation of carbohydrate exists.



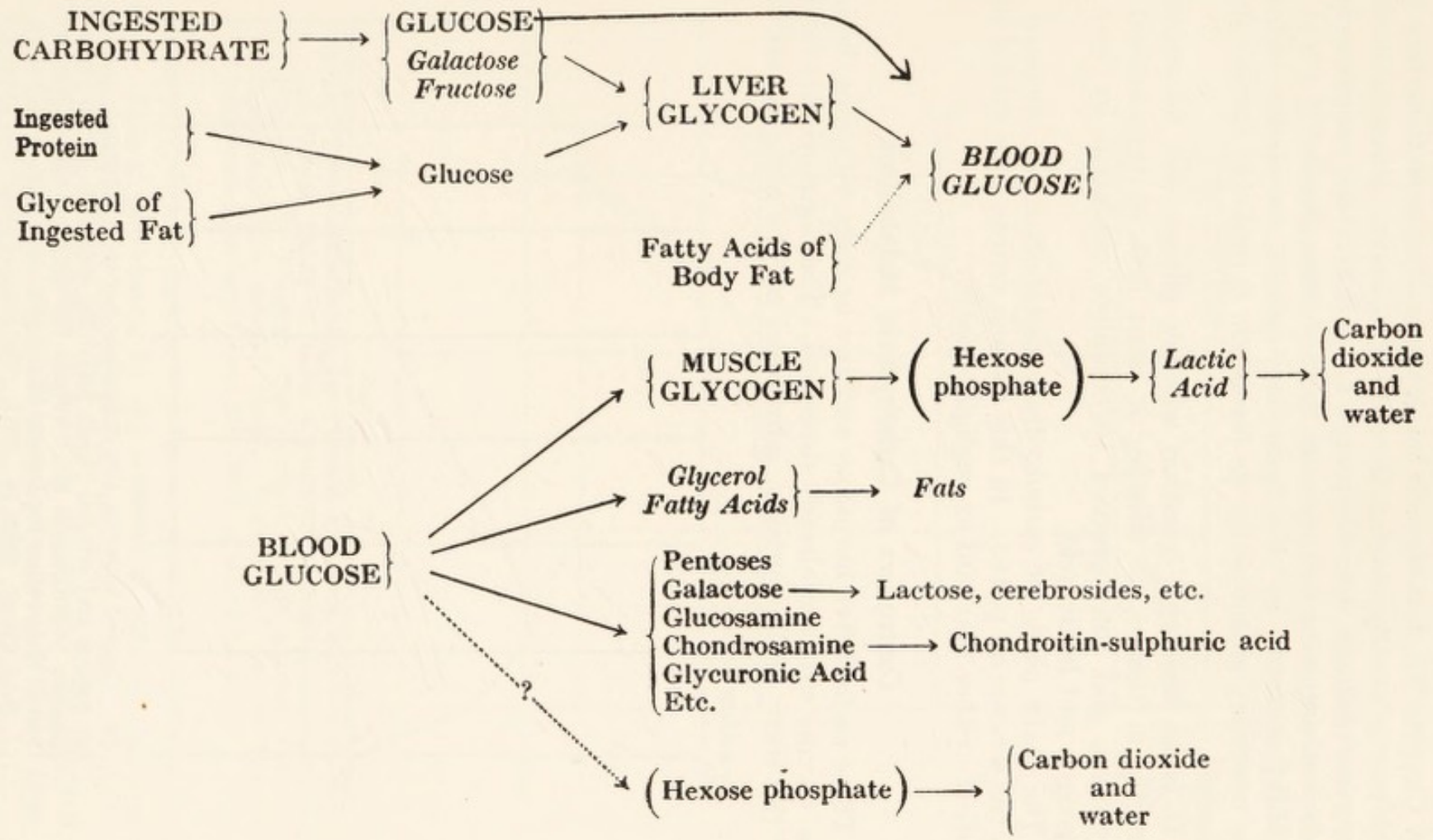
The conversion of muscle glycogen to lactic acid is brought about by a highly complex series of reactions, which will be discussed in Chapter XII.

It is now possible to make a brief summary of the initial stages of carbohydrate metabolism. Glucose predominantly, some fructose and, in adults, a little galactose, pass by the portal circulation to the liver. Some proportion of all three is converted to glycogen there. Much glucose and perhaps a little fructose escape past the liver to the general circulation. Most of this sugar is transiently stored in the skin (Folin, 1927); a little glucose is withdrawn by muscle. The skin slowly releases its transient store, and some hours later this has been very largely transformed to glycogen, either in the liver or in muscles.

Sources of glucose other than carbohydrates. About one-half of the amino-acids from proteins of the diet can yield glucose (*cf.* Chapter VI.). Since the usual diet contains an excess of protein, this production of glucose normally occurs to a considerable extent. Glucose can also be formed from the glycerol portion of fats. Whether or not it can be produced in the body from fatty acids is a highly debatable question. The evidence for such conversion is perhaps a little stronger than the evidence against it, but in any event the amount converted is slight.

Compounds other than glycogen formed from glucose. Whenever the food ingested by an animal is greater than its body needs, and contains an excess of carbohydrate, this excess tends to be converted to fat. The fattening of farm animals has long been based on this principle (*cf.* Chapter V.). Such conversion involves transformation of glucose to glycerol and to the requisite fatty acids.

Such pentoses from the diet as reach the circulation are excreted unchanged. Yet animal nucleic acid contains radicals of the pentose thyminose (*cf.* p. 177), while muscle and other tissues contain nucleotides in which ribose radicals (*cf.* p. 177) are present. These also, it would seem, are formed from glucose. (*Cf.* Chapter VII.)


INGESTED CARBOHYDRATE
GLUCOSE
Galactose
Fructose
LIVER GLYCOGEN
Ingested Protein
Glucose
Glycerol of Ingested Fat
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

The cerebrosides of nervous tissue contain galactose radicals (*cf.* Chapter V.), but there is no evidence that galactose from the diet ever normally reaches the nervous tissue. Mucoids contain mannose radicals (*cf.* Chapter VI.). Both the mannose and galactose are, we must presume, formed from glucose.

Milk, secreted by the mammary glands, contains lactose; the necessary galactose for its formation is probably formed from glucose.

It is an unsettled question whether glucose can be oxidised directly in tissues, or whether it must first be transformed to glycogen, and thus proceed to complete oxidation by way of glycogen and lactic acid.

The main paths of carbohydrate metabolism are summed up by the scheme on p. 103. In this scheme arrows with dotted lines indicate either doubtful or negligible paths.

Controllers of Carbohydrate Metabolism

These include five hormones and two or more vitamins, besides the specific enzymes already described. The hormones are insulin of the islets of Langerhans, adrenaline of the adrenal medulla,

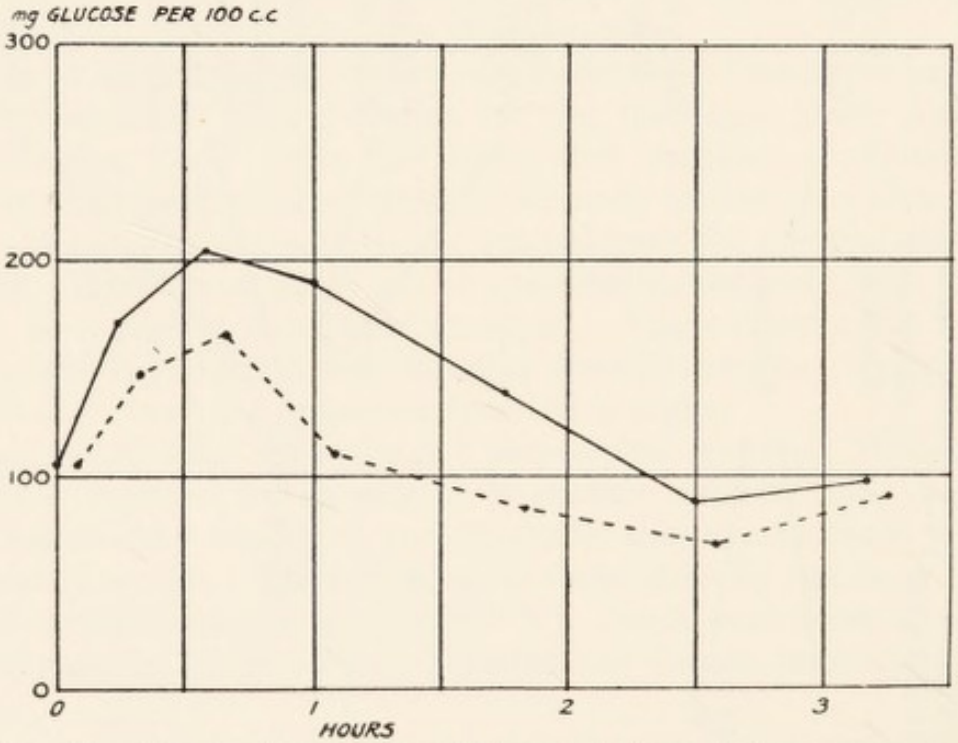


FIG. 14. Curves showing the difference between tolerance curves for venous and arterial blood, following ingestion of 100 gm. of glucose. Continuous line, values for arterial blood glucose. Dotted line, values for venous blood glucose. Data from Foster (*J. Biol. Chem.*, 1923, lv., 291). The venous blood was obtained five minutes after the arterial blood at every period. "Enhanced" glucose values.

the so-called diabetogenic hormone of the anterior pituitary, the thyroid hormone, and cortin of the adrenal cortex.

Insulin seems essential for the correct utilisation of glucose. Following its injection into an animal or man the concentration of blood glucose decreases. Yet *in vitro* experiments show that insulin induces no destruction of glucose in the blood itself.

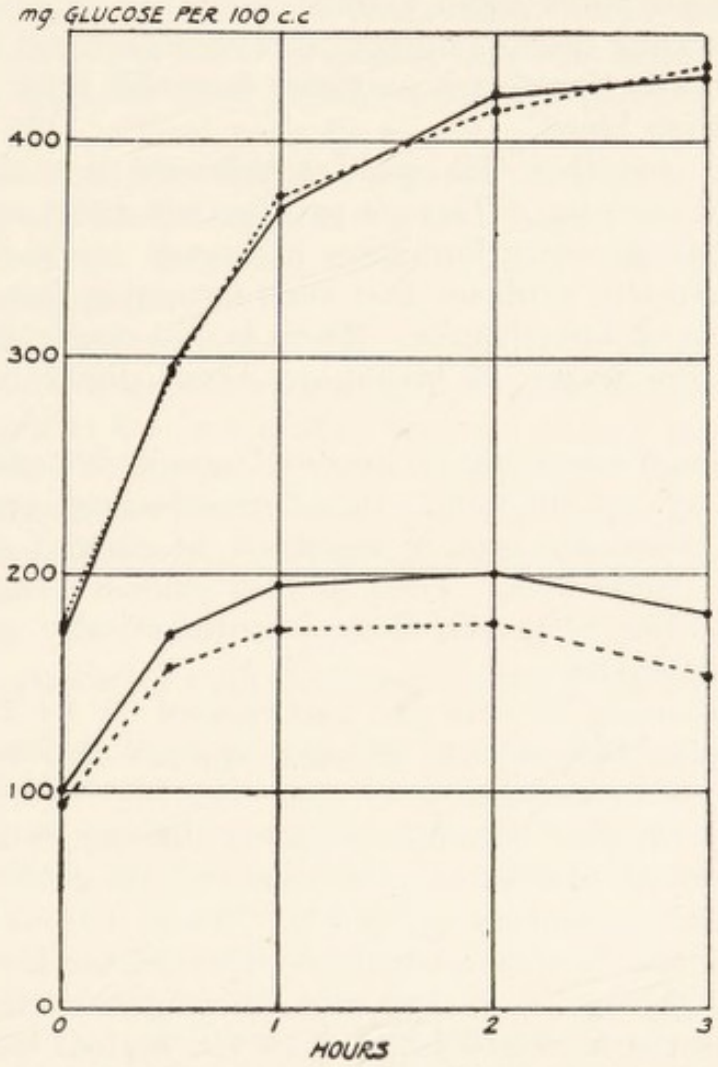


FIG. 15. Curves illustrating the lessened difference between arterial and venous blood in diabetes mellitus, following ingestion of 1·75 gm. per kilogram body-weight. True glucose values. Continuous line, arterial blood. Dotted line, venous blood. Data from Glassberg (*Arch. Int. Med.*, 1930, xlvi., 605).

In Macleod's phrase, insulin creates a vacuum for glucose in the tissues. This is well shown by the contrast of arterial and venous sugar tolerance curves in normal and in diabetic man.

One hour after ingestion of a sugar meal the sugar content of arterial (or capillary) blood is markedly greater than that of venous blood. Glucose is reaching the blood at a greater rate than the tissues can withdraw it (Fig. 14). But in the diabetic

patient, who suffers from an insufficiency of insulin, this difference is considerably decreased, and the greater the severity of the disease (as indicated by the greater height to which the blood sugar rises) the less difference exists between arterial and venous blood glucose (*cf.* Fig. 15). When the diabetic patient is restored to normal condition by insulin administration, not only does his sugar tolerance curve again become normal in type, but it once more exhibits the marked difference between arterial and venous glucose. Insulin therefore is necessary to enable rapid withdrawal of glucose from blood.

We have seen that this rapid withdrawal is mainly due to formation of glycogen. There is satisfactory direct experimental evidence that glycogen formation in muscle needs insulin, and there is reasonable evidence that such formation from glucose in the liver also requires insulin. There is still conflicting evidence concerning the power of insulin to cause direct oxidation of glucose.

The actions of adrenaline on liver and muscle glycogen have been referred to (*cf.* pp. 58, 102). Direct experimental evidence indicates that liver glycogen is mobilised to glucose, and muscle glycogen to lactic acid. There is also evidence that following increased output of insulin there is automatically an increased output of adrenaline.

These balancing actions are participated in by the anterior pituitary. Its diabetogenic hormone appears also to act in an opposite sense to insulin.

While the thyroid hormone probably directly catalyses some phase of protein catabolism, yet indirectly its activity involves carbohydrate metabolism (*cf.* p. 54). When thyroid is administered to animals in continuous heavy dosage, the liver is almost depleted of glycogen. In *hyperthyroidism* glycosuria frequently accompanies the increased secretion of the thyroid hormone.

Cortin of the adrenal cortex is indirectly involved in carbohydrate metabolism. The manner of its action is not yet understood. In adrenal cortical deficiency there is a tendency to hypoglycaemia.

The vitamins are not so much concerned with changes in the carbohydrates themselves, as with essential stages in their oxidations. It will be remembered that $\mathbf{B_1}$ seems to be an essential factor for the correct catabolism of pyruvic acid, at least in nerve tissues (*cf.* p. 68), while **C** acts as a hydrogen carrier for many oxidative procedures (*cf.* p. 73). $\mathbf{B_1}$ seems also to be necessary for the transformation of carbohydrate to fat. Such actions will be discussed at greater length in Chapter XII.

Excretion of Carbohydrates

When normal human urine is heated with such reagents as Benedict's or Fehling's solution, which contain easily reducible organic compounds of copper, a very slight degree of reduction is obtained, which tends to be greater after meals than when the subject is in a fasting state. The reducing substances are chiefly pentoses and non-carbohydrate compounds. After several meals (in late afternoon) a trace of glucose tends to leak through the kidneys, in spite of their highly developed mechanism for its retention. (The clinical tests for reducing sugar in urine are so designed that normal urines give negative results.)

Under normal conditions there is no other excretion of carbohydrates, with the exception of non-utilised and non-utilisable material in the faeces.

When the "kidney dam" is unusually low, glucose leaks through to a much greater extent (*cf.* p. 99), and during pregnancy a glycosuria from this cause often occurs. During lactation, and especially when a breast-fed infant is suddenly weaned, a trace of lactose frequently passes back from the mammary glands to the blood, and then is excreted through the kidneys, the condition of *lactosuria*.

Diseases Associated with Distorted Carbohydrate Metabolism

Diabetes mellitus is essentially due to some damage to the islets of Langerhans of the pancreas, leading to decreased output of insulin. As a result hyperglycaemia and glycosuria develop early, the sugar tolerance lessens (as shown by the elevated curves in Fig. 15), and the marked loss of glucose through the kidneys, involving increased excretion of water to hold it in dilute solution during passage, accounts for the polyuria (frequency of urination), thirst, loss of weight, and hunger characteristic of the disease. As capacity for glucose utilisation lessens, more and more glucose is formed from protein, and increased wastage results therefrom. Finally fat metabolism becomes involved, and the untreated patient dies as a result of distortion of fat catabolism (*cf.* p. 138, Chapter V.).

Hyperinsulinism, the opposite condition, is usually associated with a tumour of islet tissue in the pancreas. This produces too much insulin and hypoglycaemia results, with a characteristic train of symptoms, easy fatigue, lassitude, cold clammy perspiration, hunger, thirst, fear, and in extreme cases behaviour resembling that in alcoholic intoxication, convulsions with complete loss of memory of the events of these later stages, and coma.

Mention has already been made of the rare *von Gierke's disease*. Also very rare are conditions associated with *pentosuria* and *fructosuria*, when, respectively, the patient excretes either arabinose or xyloketose (*cf.* p. 85) through some metabolic abnormalities not yet understood, or fructose, presumably through inability to convert it to glycogen in the liver.

Renal glycosuria has already been mentioned (p. 99), and this, with pentosuria and fructosuria, seem to be harmless anomalies, not truly associated with a diseased condition.

In the condition of *acromegaly*, usually associated with a tumour of the anterior pituitary gland, a diabetes mellitus is often present, which is not due to insufficiency of insulin, but to too great production of its antagonist, the diabetogenic hormone of the pituitary.

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CHAPTER V

THE LIPIDES AND RELATED COMPOUNDS

INTRODUCTION

The lipides consist of a number of classes of chemical compounds, many of which are but slightly related or quite unrelated chemically, but all of which are allied by the physical properties of insolubility or very slight solubility in water, and of fairly marked (though varying) solubility in fats and in fat solvents such as alcohol, acetone, and ether. No satisfactory definition precisely grouping all these compounds together has yet been phrased, while no one term has yet been accepted universally for them. The earlier term " lipoid " has now largely been discarded, but the later coined name " lipin " is still used almost as frequently as the still later " lipide."

It must be remembered, further, that many substances soluble in fat solvents, and but little soluble in water, are not lipides. An example is chloroform.

For convenience, the chemistry of certain derivatives such as glycerol and choline will be dealt with in this chapter.

The very heterogeneous nature of the lipide-compounds prevents a completely logical classification. The following scheme is adopted chiefly because of convenience of treatment.

1. Fats and fatty acids obtained from them.
2. Waxes and the fatty acids and higher alcohols obtained from them.
3. Phospholipides (lipides containing a phosphate radical).
4. Glycolipides (lipides containing a carbohydrate radical).
5. Sterolipides or steroids.
6. Chromolipides.
7. Still unclassified lipides.

Fats and their Derivatives

Fats are widely distributed in plants and animals; their content in different tissues varies 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. They also act as physiological solvents of other lipides.

When fats are hydrolysed they are broken down to the trihydric alcohol glycerol, $CH_2OH \cdot CHOH \cdot CH_2OH$, and a mixture of fatty acids. Of these three abundantly present in fats are the saturated stearic acid, $CH_3 \cdot (CH_2)_{16} \cdot COOH$, and palmitic acid, $CH_3 \cdot (CH_2)_{14} \cdot COOH$, and the unsaturated oleic acid, $CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$. In naturally occurring fats the glycerol radical is combined with three radicals of fatty acids; these are seldom the same. Most commonly, single molecules of fat are represented by one of the following types (where R, R_1, R_2 represent different fatty acid radicals; note the labelling of the carbon atoms of the glycerol radical):

$$\begin{array}{lll} (\alpha)\ CH_2 \cdot OR & CH_2 \cdot OR & CH_2 \cdot OR \\ (\beta)\ \ CH \cdot OR_1 & CH \cdot OR_1 & CH \cdot OR \\ (\alpha)\ CH \cdot OR_2 & CH_2 \cdot OR & CH_2 \cdot OR_1 \end{array} \quad \text{or} \quad \text{or}$$

Although the beta-carbon atom of glycerol may be asymmetric in a fat, as in the following formulae, so far no optically active triglycerides have been found in nature.

$$\begin{array}{l} CH_2 \cdot OR \\ | \\ H{-}C{-}OR \\ | \\ CH_2 \cdot OR_1 \end{array} \quad \text{and} \quad \begin{array}{l} CH_2 \cdot OR \\ | \\ RO{-}C{-}H \\ | \\ CH_2 \cdot OR_1 \end{array}$$

It is evident that natural fats are mixtures of many chemical compounds. Different fats show great variations in the proportions of the various fatty acids derivable from them. This is exemplified by the figures in Table V.

TABLE V

Percentages of Fatty Acids derivable from Different Fats

		Butter	Lard	Olive Oil	Rat Fat	Hen Fat	Linseed Oil
Butyric acid	$C_4H_8O_2$	6	—	—	—	—	—
C_8, C_{10}, C_{12} acids		6	—	—	—	—	—
Myristic acid	$C_{14}H_{28}O_2$	10	—	trace	4	trace	—
Palmitic acid	$C_{16}H_{32}O_2$	41	32	7	30	28	9
Palmitoleic acid	$C_{16}H_{30}O_2$	—	—	—	8	7	—
Stearic acid	$C_{18}H_{36}O_2$	2	8	2	3	7	—
Oleic acid	$C_{18}H_{34}O_2$	34	60	85	53	37	5
Linoleic acid	$C_{18}H_{32}O_2$	—	—	6	2	21	61
Linolenic acid	$C_{18}H_{30}O_2$	—	—	—	—	—	24
Hydroxystearic acid	$C_{18}H_{36}O_3$	1	—	—	—	—	—
Arachidic acid	$C_{20}H_{40}O_2$	—	—	trace	—	—	—

Hilditch has carried out extensive investigations in this field, and finds that in naturally occurring fats the predominating acid radicals are palmitic, oleic and linoleic, with less stearic and linolenic. The " depôt " fats of land animals yield 38 to 40 per cent. oleic, 26 to 31 per cent. palmitic, and 20 to 25 per cent. stearic acid. In vegetable fats there is some approach to a systematic distribution, with at least one unsaturated fatty acid radical per molecule of fat; animal fats show a random distribution.

The properties of the simplest fats, built up from glycerol and a single fatty acid, give a clue to those of fats in general. *Tristearin* (glycerol tristearate) and *tripalmitin* (glycerol tripalmitate) are white waxy solids at ordinary temperatures, melting respectively at 71·6° C. and 65·5° C. *Triolein* (glycerol trioleate) is an oil, solidifying at — 6° C. The physical properties of the corresponding acids are similar. Generally speaking, the physical properties of a natural fat depend on the relative proportions of the fatty acids derivable from it. The more oleic (and linoleic) acid which it yields, the more liquid will it be. Beef fat contains more oleate than mutton fat, and has a lower melting point. Such oils as olive oil are liquid through their large content of radicals of the unsaturated acids.

The specific gravity of fats is less than that of water (upon which they therefore will float, so that an obese person needs relatively less energy to keep afloat). They crystallise from alcohol-ether mixtures in somewhat characteristic forms, such as fine curved needles.

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:

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

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.

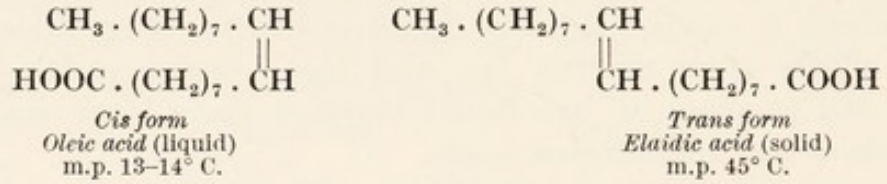
Although the fats are so insoluble in water, yet in the presence

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.

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. A number of similar hydroxy-acids can be obtained from natural fats, while some are important constituents of the cerebrosides.

Elaidic acid, used as a foreign fatty acid in many experiments in metabolic studies of fats, illustrates a common form of isomerism in fats, being an isomer (the *trans* form) of oleic acid (the *cis* form):

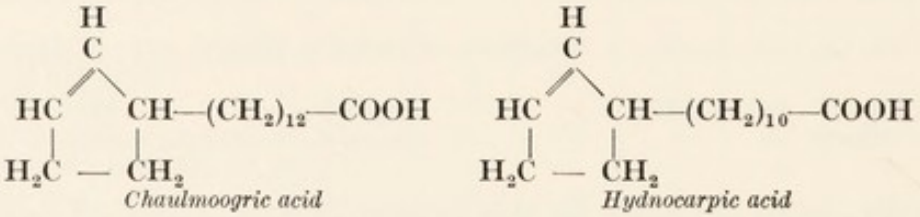


Cis form
Oleic acid (liquid)
m.p. 13–14° C.

Trans form
Elaidic acid (solid)
m.p. 45° C.

Relatively few fatty acids with branched chains occur naturally. Of these isovalerianic acid, $(CH_3)_2 . CH . CH_2 . COOH$, which melts at — 36° C., is present as a glyceride in porpoise-jaw oil, and confers on it the property of remaining liquid at low temperatures.

Two very interesting fatty acids are present as glycerides in the seeds of certain trees growing in the East (genera *Taraktogenos* and *Hydnocarpus*), and are thus present in chaulmoogra oil, which has been used for centuries in India in the treatment of leprosy. These acids, chaulmoogric acid, $C_{18}H_{32}O_2$, and hydnocarpic acid, $C_{16}H_{28}O_2$, are specifically effective in curing leprosy.

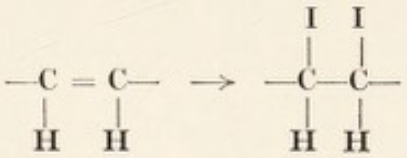


Chaulmoogric acid

Hydnocarpic acid

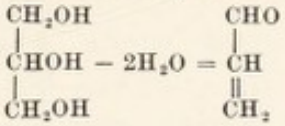
The determination of the *iodine number* is a useful method to ascertain the degree of unsaturation of fatty acids, and similar compounds with unsaturated linkages, since these unite with iodine, decolorising it, and the amount of iodine taken up from solution

by a known quantity of such a compound establishes the number of double bonds, as is indicated by the change—



Glycerol is not a lipide, but 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.



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 gm. of wax diminishes the yield of honey by from 10 to 14 gm. 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 Phospholipides

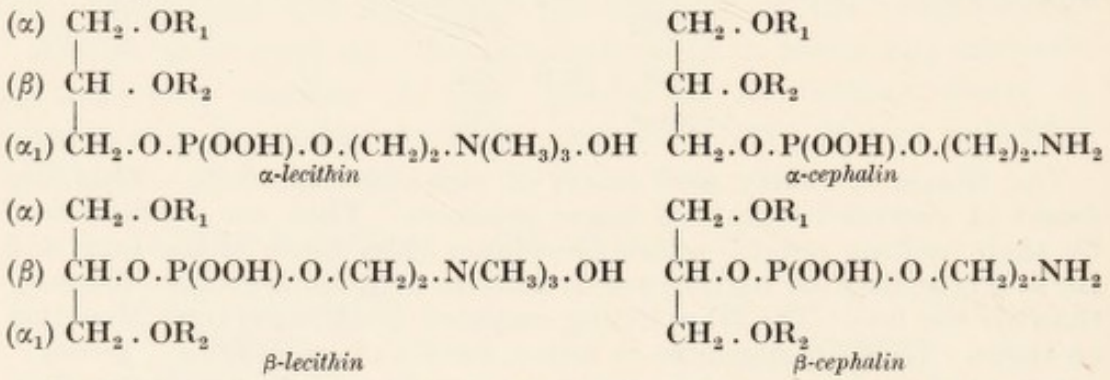
The *phosphatides* or *phospholipides* are those esters of phosphoric acid which resemble fats in their physical properties. There are three groups of compounds, *lecithins* and *cephalins* (closely

related, chemically), and *sphingomyelins*, all soluble in hot alcohol. Their chemical separation depends on their differing solubilities in such solvents as acetone and ether, and on the formation of specific salts with cadmium chloride and similar metallic salts; it is difficult and tedious.

Lecithins are built up from glycerol, fatty acids, phosphoric acid, and choline radicals. In *cephalins* the choline radical is replaced by a cholamine radical.

$HO.CH_2.CH_2.N(CH_3)_3.OH$ — *Choline* $HO.CH_2.CH_2.HN_2$ — *Cholamine*

Two types of lecithins and cephalins are known. (In the following type formulae R_1 and R_2 represent different fatty acid radicals.)



Lecithin preparations are always mixtures of several lecithins. When hydrolysed they yield the saturated palmitic and stearic acids, and the unsaturated oleic, linoleic, and linolenic acids, and also the still more unsaturated arachidonic acid, $C_{20}H_{32}O_2$. Lecithins from different sources yield varying proportions of these acids. About 80 per cent. of the lecithins in brain and egg-yolk are β-lecithins, and 45 per cent. of the cephalins are β-cephalins.

Till recently it was considered that each molecule of lecithin and of cephalin contained one saturated and one unsaturated fatty acid radical. This does not appear to be an absolute rule.

Pure lecithin is a white waxy solid, but it oxidises so easily that ordinary preparations vary in colour from yellow to dark brown. Cephalin crystallises from pyridine in pure white needles, but also easily oxidises to coloured products. Both are hygroscopic, becoming greasy and tenacious. Both imbibe water and swell. When immersed in water they "bud," developing "myelin figures."

Lecithins and cephalins are amphoteric (*cf.* p. 29), in virtue of an acid phosphate group and a basic nitrogen group. They are characterised by extreme lability, in other words, by extreme reactivity; this is of the greatest importance for the functioning of the tissues which contain them.

Lecithins are present in all the tissue cells of the animal body, and in plant tissues generally. They are especially abundant in brain and other nervous tissue, in liver, and in egg-yolk. The cephalins have a similar but somewhat less wide distribution. They have an important *rôle* in the clotting of blood.

Derivatives of lecithins and cephalins. Cobra and rattlesnake venoms contain a specific enzyme, a *lecithinase*, which splits off one molecule of fatty acid from lecithin, producing *lysolecithin*.

$CH_2 . OH$
|
$CH_2 . OR$ (saturated fatty acid radical)
|
$CH_2 . O . P(OOH) . O . (CH_2)_2 . N(CH_3)_3 . OH$

Lysolecithin

Pure lysolecithin is a white waxy hygroscopic compound, crystallising in glistening needles. It is soluble in chloroform, alcohol, acetic acid, and alkali, and gives an emulsion with water. It has powerful haemolytic action on the red blood cells. The corresponding lysocephalin is less active.

Most of the tissues of the animal body possess several lecithinases (differing from that in snake venom), which, between them, can split off both fatty acid radicals and hydrolyse the residue to liberate such compounds as glycerophosphoric acid and choline.

$CH_2 . OH$	$CH_2 . OH$
|	|
$CH . OH$	$CH . O . PO(OH)_2$
|	|
$CH_2 . O . PO(OH)_2$	$CH_2 . OH$
α-glycerophosphoric acid	*β-glycerophosphoric acid*

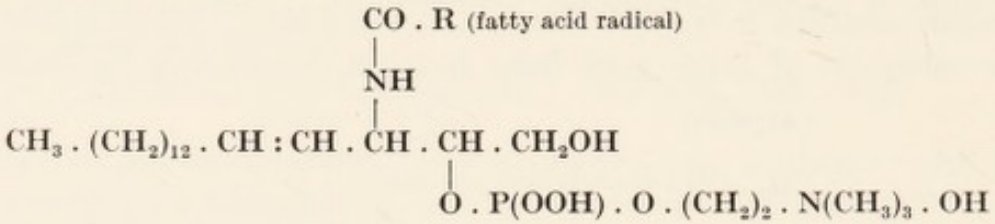
Glycerophosphoric acid is a syrupy liquid, strongly resembling glycerol in physical, and phosphoric acid in chemical properties. *It is not a lipide.*

Choline is a syrupy, strongly alkaline liquid, non-lipide, and easily miscible with water and alcohol. It yields crystallisable salts with hydrochloric acid and with platinum chloride. Boiling its aqueous solution decomposes it into trimethylamine, ethylene oxide, and ethylene glycol. (Trimethylamine is partly responsible for the characteristic odour of stale fish, but in the decomposing fish it is formed from trimethylamine oxide, a constituent of fish muscle.)

Choline has been found in traces in tissues, probably in large part through decomposition of their lecithins during chemical examination. It appears to play a specific *rôle* in fat metabolism in the liver. A derivative, acetylcholine, is an important chemical transmitter of nerve impulses (*cf.* p. 60).

Cholamine, $HO . CH_2 . CH_2 . NH_2$, (amino-ethanol, amino-ethyl alcohol), also non-lipide, is a colourless, viscous oil, miscible with water and with alcohol in all proportions, and strongly alkaline.

The *sphingomyelins* are not glycerides. They yield two molecules of base and only one of acid on hydrolysis, and may be described as diamino-monophosphatides. Their general formula is



They are thus built up from choline, sphingosine (see below) and a fatty acid. The three fatty acids derived from them are stearic acid, lignoceric acid, $C_{23}H_{47}COOH$, and the unsaturated nervonic acid, $C_{23}H_{45}COOH$.

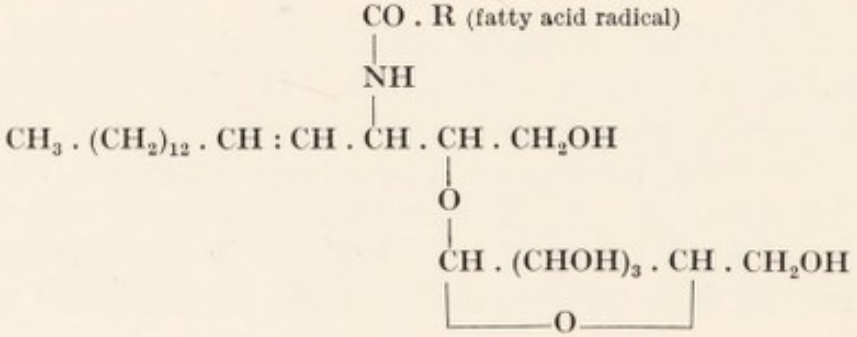
The sphingomyelins are common constituents of the cellular material of the animal kingdom—for example, brain, kidney, liver and egg-yolk—but have not yet been found in the plant kingdom. They crystallise in very thin plates, which often congregate in rosette forms, and dry as white powders of waxy character. The principal impurities are galactosides. Pure sphingomyelins give a negative test for galactose. They are amphoteric.

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

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

The Glycolipides

The *cerebrosides* (*galactolipides*) have the general formula



so that they can be regarded as sphingomyelins in which a radical of choline-phosphate is replaced by one of galactose. Four cerebrosides are known, kerasin, phrenosin, nervone, and oxy-nervone. The corresponding fatty acids are lignoceric, α-hydroxylignoceric, $CH_3 . (CH_2)_{21} . CHOH . COOH$, nervonic,

$CH_3 . (CH_2)_7 . CH : CH . (CH_2)_{13} . COOH$, and α-hydroxynervonic acid. These are all C_{24} acids.

The cerebrosides are white, non-hygroscopic, stable compounds. So far, only phrenosin has been obtained in the crystalline state. They are all optically active, and all exhibit imbibition in water, yielding myelin figures which change to transparent globules. They are much less soluble than other lipides; boiling alcohol extracts them from tissues which contain them. Heating with baryta splits off the fatty acid radical, leaving *psychosine* (sphingosine-galactose). They are important constituents of the white matter of nervous tissue (whence the name), and are also present in the spleen, egg-yolk, and pus.

Lignoceryl-sphingosine (kerasin minus its galactose radical) has been isolated from the liver, spleen, and lungs of cattle. The yield from lung tissue was 0·06 per cent. of its dry weight. The amount present in spleen is about twice that of kerasin (0·03 per cent.). Thannhauser terms such compounds *ceramides*, and regards them as the parent substances of both the cerebrosides and the sphingomyelins.

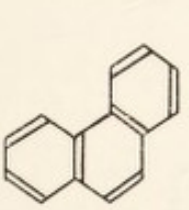
Numerous claims have been made for the existence of other glycolipides containing such carbohydrate radicals as glucosamine and trehalose; definite statements about such compounds cannot yet be made.

Numerous claims have also been made for the existence of lipides containing sulphur, *sulpho-lipides;* their existence is also, as yet, non-proven.

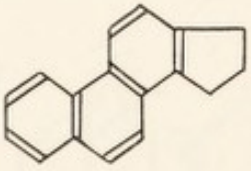
The Sterolipides or Steroids *

This extremely important class includes (*a*) the sterols, (*b*) the bile-acids, (*c*) the sex-hormones and adrenal cortical hormones, (*d*) the **D** vitamins, and (*e*) certain cardiac poisons and sapogenins. Certain carcinogenic compounds are closely related to them, and will also be briefly mentioned in this section.

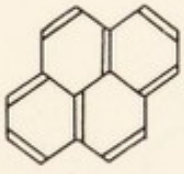
The sterolipides can all be considered as derived from



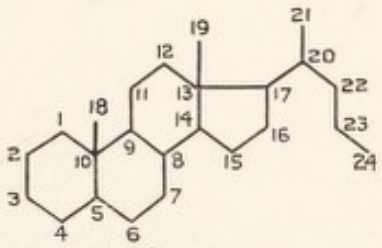
Phenanthrene



Cyclopentenophenanthrene

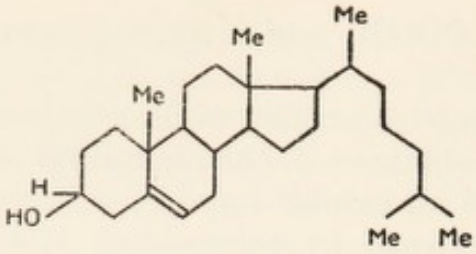


Pyrene

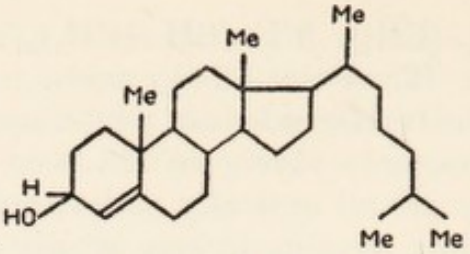


Cholane

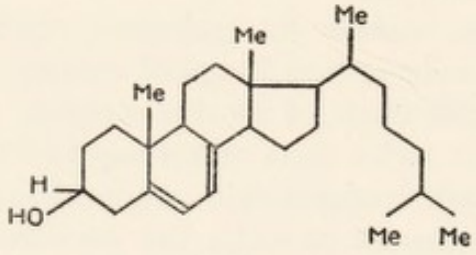
* The term "steroid" has recently been suggested for these compounds by Callow and Young. For the more self-explanatory term "sterolipide" I am indebted to Dr. O. F. Denstedt.



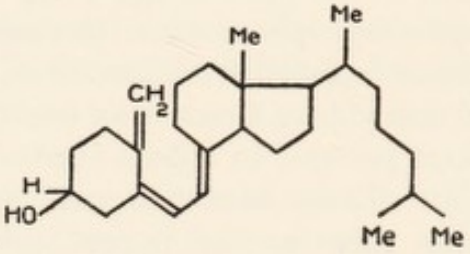
Cholesterol, $C_{27}H_{45}OH$



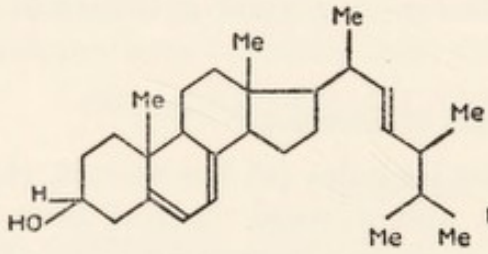
Allocholesterol, $C_{27}H_{45}OH$



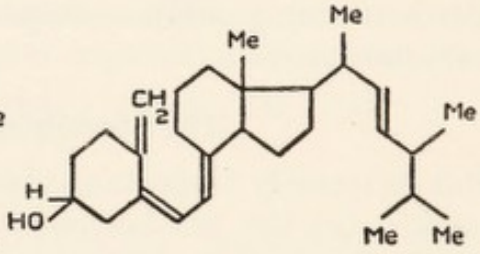
7-Dehydrocholesterol, $C_{27}H_{43}OH$



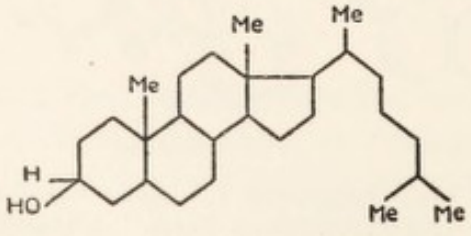
Vitamin $\mathbf{D}_3$, $C_{27}H_{43}OH$ (*from cholesterol*)



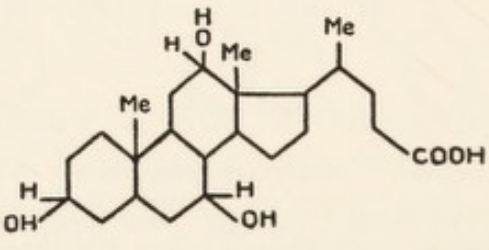
Ergosterol, $C_{28}H_{43}OH$



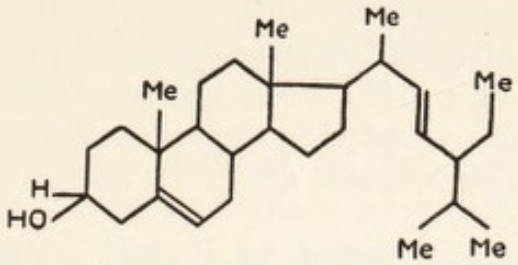
Vitamin, $\mathbf{D}_2$, $C_{28}H_{43}OH$ (*from ergosterol*)



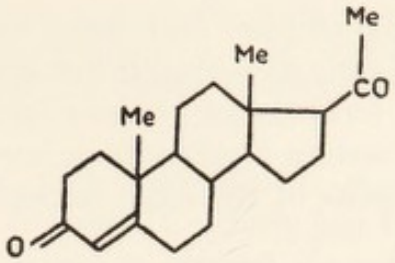
Coprosterol, $C_{27}H_{47}OH$



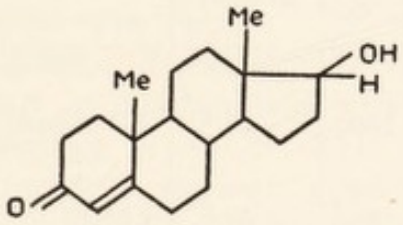
Cholic acid, $C_{24}H_{40}O_5$



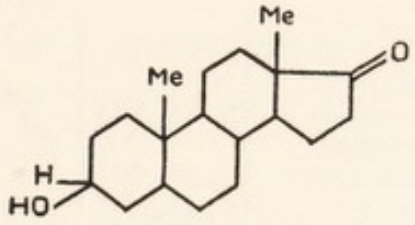
Stigmasterol, $C_{29}H_{47}OH$



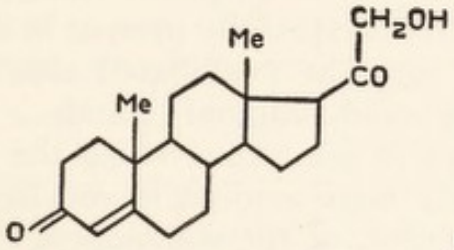
Progesterone, $C_{21}H_{30}O_2$
(*from corpus luteum*)



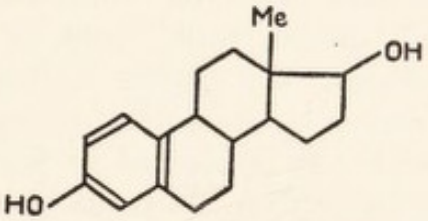
Testosterone, $C_{19}H_{28}O_2$
(*from testis*)



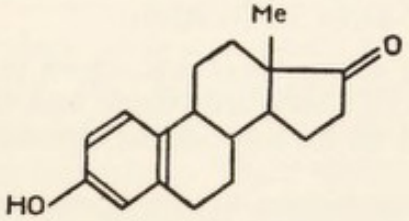
Androsterone, $C_{19}H_{30}O_2$
(*from male urine*)



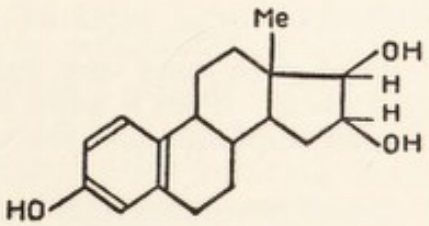
Cortin (?), $C_{21}H_{30}O_3$
(*from adrenal cortex*)



Oestradiol, $C_{18}H_{24}O_2$
(*from ovary*)



Oestrone (*Theelin*), $C_{18}H_{22}O_2$
(*from female urine*)



Oestriol (*Theelol*), $C_{18}H_{24}O_3$
(*from female urine*)

phenanthrene, and from cyclopentenophenanthrene. Some carcinogenic compounds are derivatives of the closely related pyrene. The formula for cholane, given on p. 117, shows the numbering of the carbon atoms which is usually adopted in sterols.* Formulae for some of the most important sterolipides are given on pp. 118–119.

Sterols. At least a dozen different sterols have been obtained from plant and animal sources. Some of the most important are listed in Table VI.

TABLE VI. SOME IMPORTANT STEROLS

Name	Formula	Number of Double Bonds	M.P. °C.	Specific Rotation	Sources
Cholesterol (Cholestenol)	$C_{27}H_{45}OH$	1	150°	−38·8°	All animal cells
Allocholesterol (Coprostenol)	$C_{27}H_{45}OH$	1	—	—	
Coprosterol	$C_{27}H_{47}OH$	0	102°	+23·5°	Faeces
Ergosterol	$C_{28}H_{43}OH$	3	163°	−133°	Ergot, yeast
γ-Sitosterol	$C_{28}H_{49}OH$	1	146°	−42·4°	Fats of higher plants
Stigmasterol	$C_{29}H_{47}OH$	2	170°	−45°	Soy bean, calabar bean

Cholesterol, typical of the sterols, is a constituent of all animal cells. It or some other sterol is found in all plants cells, except those of most bacteria. Cholesterol is especially present in animal fats, bile, blood, milk, yolk of egg, the medullated sheaths of nerve fibres, the liver, kidney and adrenal glands. It is accompanied by its esters, except in bile, from which the esters are absent. It is present in fairly large amount in cod liver oil. It constitutes from 64 to 98 per cent. of the commonest type of human gall-stones, and is found in aetheromata of the arteries, in tuberculous 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. Cholesterol 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

* The current conventions used by organic chemists in writing constitutional formulae of cyclic compounds have been employed in this and succeeding chapters.

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.

Dehydrocholesterol is found along with cholesterol, and is probably the immediate precursor of vitamin **D**, which has been prepared from it by irradiation. Sitosterol is present in wheat, rye, and linseed oil. Ergosterol is not widely distributed, and only occurs in appreciable amounts in yeast and ergot.

Cholesterol Esters. Those commonly met with are esters of palmitic, stearic, and oleic acids. These greasy compounds, in addition to being present with cholesterol in most tissues and fluids except bile, form a large proportion of sebum, the secretion of the sebaceous glands.

Bile Acids. These derivatives of cholesterol occur in bile in great part in combination with taurine and glycine (*cf.* Chapter VI.), through a —CO . NH— (peptide) linkage, and as the potassium and sodium salts. The bile of most carnivorous animals contains only cholic acid, as taurocholate, but human and herbivorous biles contain the conjugated salts of cholic, desoxycholic, hyodesoxycholic, chenodesoxycholic, and lithocholic acids. (In human bile the first two of these predominate, and 80 per cent. of the acids are conjugated with taurine and glycine.) The distribution of some of these acids is shown in Table VII.

TABLE VII. BILE ACIDS

Name	Formula	No. of OH Groups	M.P. °C.	Specific Rotation	Source of Bile
Cholic acid .	$C_{24}H_{40}O_5$	3	195°	+37°	Man, ox, goat, sheep, antelope
Desoxycholic acid	$C_{24}H_{40}O_4$	2	176°	+55°	Man, ox, goat, sheep, antelope
Chenodesoxycholic acid . .	$C_{24}H_{40}O_4$	2	140°	+11°	Man, ox, goose, hen
Hyodesoxycholic acid . .	$C_{24}H_{40}O_4$	2	197°	—	Hog, hippopotamus
Lithocholic acid .	$C_{24}H_{40}O_3$	1	186°	+32°	Man, ox

The bile acids are white crystalline compounds, slightly soluble in water, but readily soluble in alcohol and chloroform. Their alkaline salts are water-soluble. The conjugated salts are very soluble in water. Their unusual capacity for lowering the surface tension of aqueous solutions has already been referred to (*cf.* p. 16).

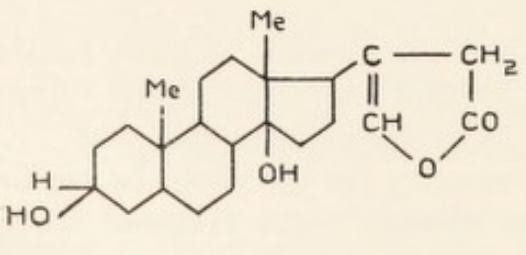
Desoxycholic acid has the curious property of uniting with fatty acids in the ratio of 8 molecules to 1 of fatty acid, to give *choleic acids*.

The Sex Hormones and Adrenal Cortical Hormones. These have already been dealt with in Chapter III. They also are to be regarded as derivatives of cholesterol. The structural formulae of the sex hormones are given on p. 119. They illustrate the close relationship between stigmasterol and progesterone which has permitted the laboratory preparation of the latter on the large scale for therapeutic use.

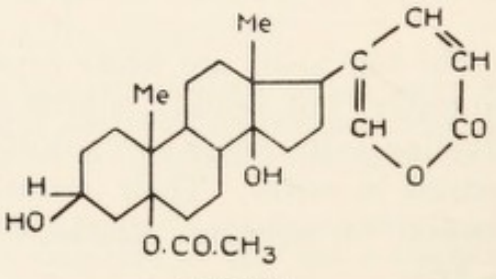
While the constitutional formula of cortin has not yet been finally determined, there is suggestive evidence that it is a cholesterol derivative, and a possible structural formula is shown on p. 119. The alternative name "corticosterone" suggests the relationship to the sterols. In addition, the adrenal cortex forms *adrenosterone*, $C_{19}H_{24-26}O_3$, an unsaturated di-ketone, with fairly marked "male" activity, so that when it is produced in undue amounts by tumours of the adrenal cortex, virilism and hirsutism develop.

The D Vitamins. These have been dealt with in Chapter III. The probable constitutional formulae of the natural vitamin ($\mathbf{D_3}$) and of calciferol ($\mathbf{D_2}$) are given on p. 118, along with that of the precursor of $\mathbf{D_3}$, dehydrocholesterol. Still another active vitamin **D**, has been produced by Windaus by irradiation of 22-dihydroergosterol; its formula corresponds to those of the others and Windaus has named it $\mathbf{D_4}$.

The Cardiac Glycosides and Sapogenins. The so-called plant "cardiac glycosides," with marked pharmacological and toxic actions on the heart, hydrolyse to one or more sugars, and sugar-free residues, *aglycones*, or *genins*. The pharmacological properties are associated with the aglycones themselves. These aglycones, and also certain poisons from the skin glands of toads, possess chemical structures which relate them to the sterols. Typical of them are digitoxigenin, from digitoxin, one of the digitalis glucosides from the red fox-glove, and bufotalin from the skin glands of the toad *Bufo vulgaris*.

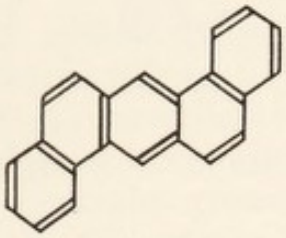


Digitoxigenin

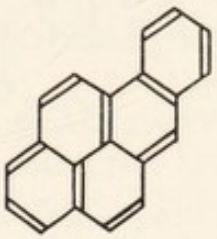


Bufotalin

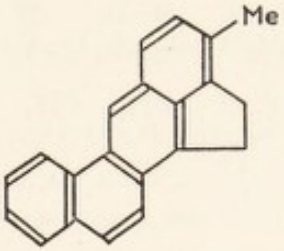
Carcinogenic Hydrocarbons. A number of phenanthrene derivatives are potent agents in inducing cancerous growths in mice and other animals, either following injection, or through long continued " tarring " of the skin. Of these two of the most potent are the synthetically prepared 1.2.5.6.dibenzanthracene, and 1.2.benzpyrene, obtained from tar. With them may be compared methylcholanthrene, a derivative of desoxycholic acid, which, as we have seen, is present in small amounts in human bile. Methylcholanthrene is also carcinogenic, though in less degree.



1.2.5.6.*Dibenzanthracene*



1.2.*Benzpyrene*



Methylcholanthrene

Chromolipides and Derivatives

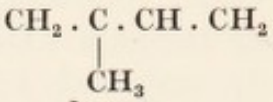
The chief chromolipides are the carotenoids and related pigments ; they are all intensely coloured. Carrots, green leaves of plants, and algae are especially rich in them, and they are essentially plant pigments. They are also found, derived from plant material in the diet, in many tissues and fluids of the animal organism. The most important of these compounds are listed in Table VIII.

TABLE VIII. IMPORTANT CAROTENOIDS

Name	Formula	Colour	M.P. °C.	Plant Source	Animal Source
α-Carotene .	$C_{40}H_{56}$	Red	172°	Palm oil ; carrots.	Human depôt fat
β-Carotene .	$C_{40}H_{56}$	Red	182°	Carrots, etc.	Corpus luteum, adrenal cortex, pituitary, depôt fat
γ-Carotene .	$C_{40}H_{56}$	Red	—	Marsh dodder	
Lycopene .	$C_{40}H_{56}$	Red	—	Tomatoes, red pepper.	Human depôt fat
Xanthophyll (lutein).	$C_{40}H_{56}O_2$	Yellow	—	—	Human depôt fat, placenta, egg-yolk, bird's beak
Zeaxanthin	$C_{40}H_{56}O_2$	—	—	—	Hen's egg-yolk
Fucoxanthin	$C_{40}H_{56}O_6$	Brown	—	Brown algae	—

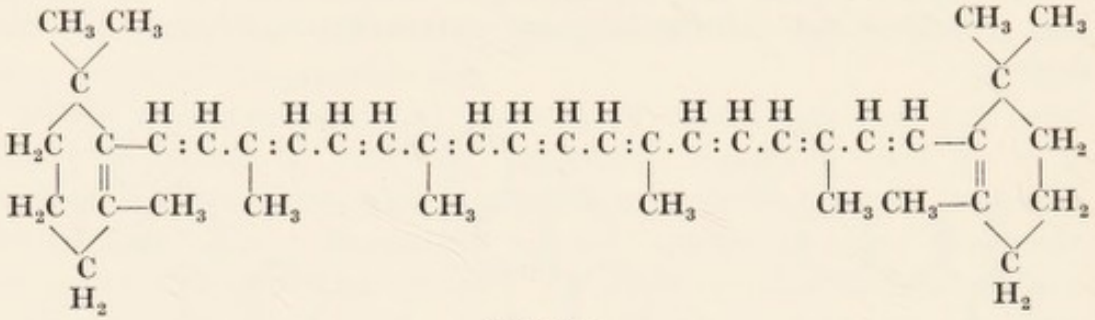
These compounds all have a complex " polyene " structure,

and can be regarded as reduced polymerised isoprene units (indiarubber is a still more highly polymerised isoprene derivative).



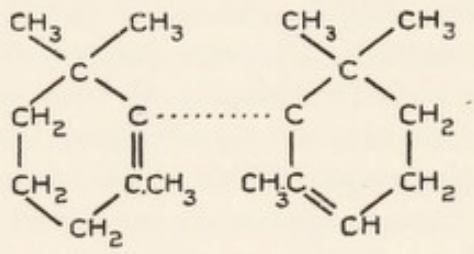
Isoprene

One of the most abundant of the carotenoids is β-carotene :

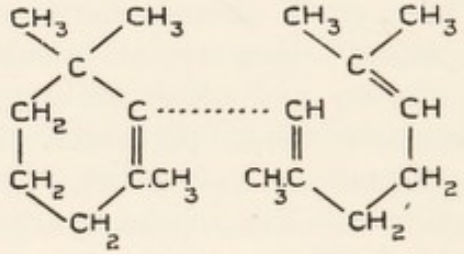


β-Carotene

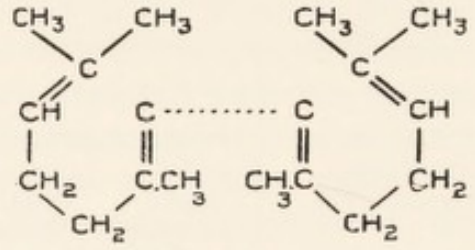
All have the same long central chain, so that if this be represented by a dotted line their formulae can be written—



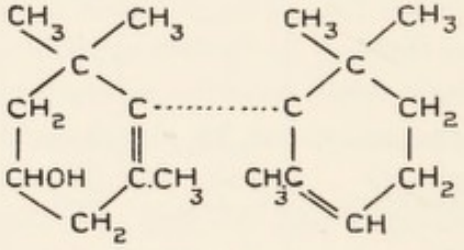
α-Carotene



γ-Carotene



Lycopene

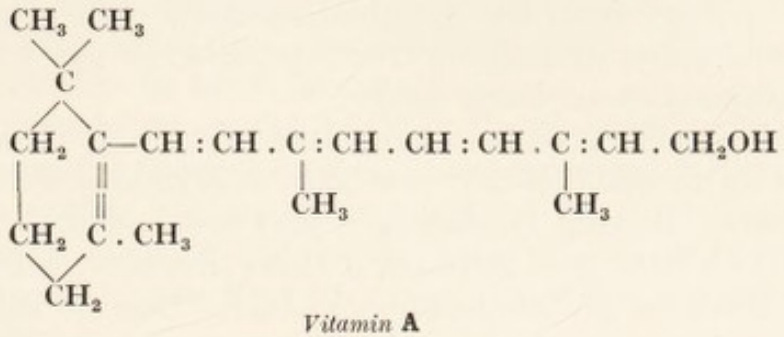


Xanthophyll

Zeaxanthin bears the same relation to β-carotene as xanthophyll does to α-carotene.

The carotenes and lycopene are difficultly soluble in alcohol, and not very soluble in ether, acetone, or acetic acid, but are readily soluble in carbon disulphide, chloroform, and benzene. Xanthophyll and others with hydroxy groups are more soluble. β-carotene is much more abundant in plants than the α or γ forms. One molecule of β-carotene will yield two molecules of

vitamin **A**, whereas α and γ carotenes will yield only half this amount per molecule.



Vitamin **A**

The general properties of vitamin **A** have been dealt with in Chapter III.

It is interesting to note that the shells of lobsters, crabs, crayfish, and some other crustacea contain a blue pigment, which consists of a complex protein compound. This is decomposed by boiling water, the protein portion being coagulated, and the prosthetic chromo-lipide group being set free as *astacene*, a red pigment, with constitution probably somewhat analogous to that of the carotenoids.

Still Unclassified Lipides

Vitamin **E** (see Chapter III.) is almost certainly a higher alcohol with lipide properties ; if vitamin **K** actually exists it also comes within the lipide group.

The Enzymes Concerned in Lipide Digestion and Metabolism

These include the *lipases* and *esterases*. Lipases hydrolyse glycerides of the higher fatty acids, while esterases hydrolyse other fatty acid esters. There is, however, no rigid dividing line between the two groups.

Gastric lipase does not hydrolyse fats readily ; apparently the optimum *p*H for its action is about 5. There is some evidence suggesting that this enzyme is really identical with that of the pancreatic juice, and that apparent differences in action are due to differences in the media.

Pancreatic lipase can be extracted from the defatted pancreas by water and glycerol, or by 10 per cent. sodium chloride solution. From such extracts it is quantitatively precipitated by saturation with magnesium sulphate. It rapidly hydrolyses ordinary fats and other glycerides, and also other esters. Its optimum activity is at about *p*H 8, but varies with the substrate. Its zymogen, *prolipase*, has been separated from pancreatic extracts by dilution

with water, when it is precipitated. Prolipase is activated by serum and tissue extracts, and is decomposed by heat.

Ricinus lipase, from the germinating seeds of the castor bean, *Ricinus communis*, hydrolyses true fats between *p*H 4·7 and 5·0, but has little action on lower esters.

The lecithinases have been mentioned (p. 115). One peculiar to snake venom splits off only one fatty acid radical from lecithins and cephalins, leaving lysolecithins and lysocephalins. Another splits off both fatty acid radicals, a third liberates choline, and a fourth hydrolyses glycerophosphoric acid. One or other or all of the last three are present in many tissues, kidneys, small intestine, spleen, liver, testes, pancreas, etc., in descending order of amount.

Cerebrosidase, which hydrolyses cerebrosides with liberation of ceramides (p. 117) requires to be activated by such compounds as cysteine, or glutathione, or ascorbic acid.

Digestion, Absorption, and Metabolism of the Fats

Food contains fats, with traces of phospholipides, sterols, and chromolipides, and still smaller traces of other lipides.

Gastric lipase is unable to produce much effect on non-emulsified fats, but the slight action which it does produce results in the stomach contents containing a slight amount of free fatty acid by the time they pass on to the duodenum.

Pancreatic lipase rapidly hydrolyses neutral fats to glycerol and fatty acids ; the latter, in sufficiently alkaline medium, will be converted to soaps, but recent work suggests that such a degree of alkalinity is not attained in the intestines. Since the lipase can only attack a fat globule at its surface, its action is greatly accelerated by substances such as bile salts and traces of fatty acids, which promote emulsification through their effect of lowering surface tension. In the absence of bile in the intestine through pathological or experimental causes, fats are split very slowly, and most of the split fatty acid remains unabsorbed.

Absorption of glycerol is probably rapid and easy. The fatty acids, normally insoluble in an aqueous medium, are rendered soluble and diffusible by presence of the bile salts, through some mechanism which is still unexplained.

There is experimental evidence that during the passage of fatty acids through the intestinal mucosa their degree of saturation may change, and that the mixture of acids may become (in different species) less saturated, or more saturated. This evidence is not final, for many of the known facts can be equally well

explained by an assumption of differential absorption of saturated and unsaturated acids.

During passage through the mucosa the acids are reconverted to neutral fats, through a phospholipide stage. This is indicated by such evidence as the following :—

When iodised fats are fed a rabbit, the phospholipides extracted from its intestine three days later contain iodised fatty acids.

When a rabbit is killed during absorption of olive oil from its gut, the amount of phospholipide in the lymph of the intestinal region is greater than that in controls in which no absorption is taking place.

Poisoning by iodoacetic acid is known to tend to inhibit the synthesis of phospholipide ; it also stops absorption of fat.

Staining reactions show that while fatty acids are present in the epithelial cells at the tips of the villi of the small intestine within twenty minutes after feeding fat to rats, yet at the sixth hour the epithelial cells are completely filled with droplets entirely composed of neutral fat. But after poisoning with iodoacetic acid (and thus preventing or greatly lessening phospholipide formation) at the end of the sixth hour less fatty material is present in these epithelial cells than normally, and it is largely composed of free fatty acid.

The intestinal mucus can provide or synthetise a certain amount of glycerol, for when free fatty acids are fed neutral fat appears in the chyle.

At least 60 per cent. of the absorbed fat appears as neutral fat (accompanied perhaps by some phospholipide) in the chyle of the lacteals of the villi, and passes through lymph channels to the thoracic duct and the general circulation. There is evidence (as in experiments with cats with the lacteals tied off) that some of the absorbed fat reaches the blood of the portal circulation, but whether the whole 40 per cent. can be so accounted for is still uncertain.

Transport of Fat. Absorbed fat, whether passing by the thoracic duct or the portal circulation, reaches the general circulation to produce, under normal circumstances, an alimentary *lipaemia* which may amount to as much as 2 per cent. It is present as finely divided particles of about 1μ diameter, which show pronounced Brownian movement. Between meals the lipaemia slowly lessens.

Coincident with the rise in blood fat the phospholipide content of the blood plasma increases, and there is a delayed rise in cholesterol, and also, probably, an increase in the proportion of esterified cholesterol. It seems almost certain that Bloor's

theory that such changes are inherently associated with and facilitate transport of fat is correct, and that the plasma phospholipides, or some portion of them, act as transporting agents. The precise mechanism is not yet ascertained.

This view is supported by such evidence as the following. Sinclair has shown that elaidic acid (*cf.* p. 112), a few hours after it has been fed to an animal, may account for as much as 30 per cent. of the fatty acid radicals of the phospholipides of the blood plasma, though the total phospholipide content of the plasma may not be increased. In such experiments none of the elaidic acid radicals are present in the red blood corpuscles, indicating that they do not function in the transportation of fat.

Probably only a proportion of the plasma phospholipides functions in this way, since recent evidence indicates that at least half of these are sphingomyelins, which possess none of the fatty acid radicals present in true fats.

By whatever mechanism fat is transported, it appears to be withdrawn from blood to the fat depôts at a fairly rapid rate.

Distribution of Fat in the Organism. The "adipose tissues" are found in the subcutaneous tissues, the intermuscular tissue, the omentum and mesentery, and surrounding such organs as the heart, lungs, liver, kidneys, adrenals, ovaries, and testes. Adipose tissue contains about 90 per cent. of neutral fat, with traces of cholesterol, chromolipides, and fat-soluble vitamins. Its amount is very variable.

Tissues in general contain only small amounts of neutral fat.

Formation of Fats from Non-Lipide Compounds. The fattening of live-stock by excess feeding of diets predominantly carbohydrate has long been a recognised farm procedure. The classical experiments of Lawes and Gilbert in 1852 with young pigs gave scientific proof of the conversion of carbohydrate to fat in this process.

Since glucose is the medium of exchange of the carbohydrates, the actual synthesis of fat in the animal must be from glucose. It is elsewhere shown that glucose can be formed from about half of the amino-acids derivable from diet proteins (*cf.* pp. 102, 107), so that, theoretically, an excess of protein in diet should be capable of inducing fat deposition. This can happen. Atkinson, Rapport, and Lusk have shown that dogs, starved to ensure a low glycogen and fat reserve, and then fed continuously large quantities of lean meat (which is practically water, protein, and salts only), stored both glycogen and fat.

The formation of fat from glucose involves formation of both

glycerol and fatty acids. The precise steps involved in both changes are not yet known.

In the fermentation of glucose by yeast small amounts of glycerol are normally produced. The animal organism undoubtedly possesses a similar power. Glyceraldehyde, $CH_2OH . CHOH . CHO$, may be an intermediate stage, its reduction yielding glycerol :

$$\underset{\textit{Glucose}}{C_6H_{12}O_6} \rightleftharpoons \underset{\textit{Glyceraldehyde}}{2C_3H_6O_3} \rightleftharpoons \underset{\textit{Glycerol}}{2C_3H_8O_3}$$

During the normal catabolism of carbohydrate in the body pyruvic acid, $CH_3 . CO . COOH$, is an important intermediate product (*cf.* p. 296). This keto-acid is decomposed by the enzyme carboxylase, present in many tissues, with the production of acetaldehyde, $CH_3 . CHO$. Condensations such as the following can be brought about in the laboratory, and suggest a mechanism for fatty acid formation.

$$CH_3 . CO . COOH \xrightarrow{\text{(carboxylase)}} CH_3 . CHO \xrightarrow{\text{(condensation with pyruvic acid)}} CH_3 . CH : CH . CO . COOH \xrightarrow{\text{(carboxylase)}} CH_3 . CH : CH . CHO \xrightarrow{\text{(condensation with pyruvic acid)}} CH_3 . CH : CH . CH : CH . CO . COOH \xrightarrow{\text{(carboxylase)}} CH_3 . CH : CH . CH : CH . CHO.$$

At any stage such an aldehyde can be oxidised to an unsaturated fatty acid and then reduced to a saturated acid ; all such acids will have an even number of carbon atoms, an essential for the production of the natural fatty acids.

It is not improbable that the constancy of fat composition in different species of herbivorous animals is largely due to formation of their fatty acids by such syntheses, which could yield a mixture of fatty acids specific for each species.

Fat deposition is a highly selective process. The nature of the deposited fat depends primarily on the species of animal, and only secondarily on the character of the fats in the diet. The adipose tissue cells play an active *rôle* in the deposition. They contain a lipase, and an enzyme capable of dehydrogenating stearic acid. They can split fat and change the character of fatty acid. Saturation and desaturation play a definite *rôle* in determining the nature of stored fat, though the sites of such actions are not completely determined. Desaturation would appear to be limited in extent, since, for example, the mammal cannot produce certain essential unsaturated acids such as linoleic acid (*cf.* p. 137). The most definite evidence that saturation and desaturation of ingested fatty acids can occur in the body is afforded by the recent experiments with heavy hydrogen.

The Use of Heavy Hydrogen in Studying Lipide Metabolism. Schoenheimer and Rittenberg and their collaborators have taken

advantage of the existence of isotopes (*cf.* p. 11) to carry out a series of important studies concerning chemical changes and syntheses of lipides in the animal organism. They employ *deuterium* (heavy-hydrogen, *D*, with a molecular weight of 2, as contrasted with ordinary hydrogen, protium, *H*, with a molecular weight of 1). The ratio of the two forms of hydrogen in nature is 1 : 5,000. Water from numerous sources tested contains them in the same ratio, and the water from animal sources with the same density as tap water also therefore contains the same proportion of deuterium. The living organism does not therefore differentiate between the two forms of hydrogen.

The chemical properties of the two isotopes are almost identical. Fatty acids and sterols prepared with 1 or more atoms per cent. of deuterium show no differences detectable by physical methods from the ordinary compounds. (Atom per cent. of deuterium is the percentage ratio of the number of deuterium atoms to the total number of all hydrogen atoms in the molecule.) On combustion of such compounds containing an excess of deuterium they can easily be distinguished, since the resulting water contains an amount of " heavy water " equivalent to the deuterium content and this will increase the specific gravity of the water. Thus natural stearic acid gives water with a heavy water content of 0·02 per cent., while stearic acid with 4 deuterium atoms in its molecule (one-ninth of its total hydrogen atoms) gives water with 11·11 per cent. of heavy water. The density methods available permit a precision of 0·001 per cent. in the determination of deuterium.

Schoenheimer and Rittenberg point out that in order to " label " a compound for metabolic experiments the deuterium must be introduced into the molecule in such a position that it is not interchangeable with the light hydrogen of ordinary water. Thus the H of R . COOH, ROH, RNH_2, and RCHO cannot be used, since when it is replaced by D this will quickly become replaced again by reaction with ordinary water. In most other positions in the molecule deuterium atoms are not subject to such interchange.

Schoenheimer and Rittenberg have obtained the following results by employing compounds whose molecules are labelled with deuterium.

When mice are fed for short periods fats containing deuterium, a large proportion of the deuterium is found deposited in the fat depôts, much less in the water of the body fluids, and only a small amount in the lipides of the internal organs ; it would therefore seem that much of the fat of the diet, even when

this is not in excess, is deposited in adipose tissue before utilisation.

Saturated fatty acids containing 11·2 atoms per cent. of deuterium were fed to mice. Subsequently the unsaturated fatty acids prepared from these mice had a deuterium content of 1·16 atoms per cent., an indication that *unsaturation can be produced in the organism.* Conversely, when unsaturated fatty acids rich in deuterium were fed mice, saturated acids prepared from the mice subsequently also contained deuterium in amounts greater than normal, indicating that *saturation can also be produced in the organism.* When mice were fed *deuterostearic acid*, the saturated acids obtained from them contained *deuteropalmitic acid*, indicating that *the mouse can transform stearic into palmitic acid.* On the other hand, when *deuterocaproic* or *deuterobutyric* acid was fed mice, their higher fatty acids subsequently contained no deuterium, which was found to be entirely present in the heavy water from the body fluids, an indication that the C_6 and C_4 acids are rapidly destroyed.

Mice were maintained at constant weight on a high carbohydrate diet of whole wheat bread, and heavy water was injected into their body fluids. It was found that the fatty acids of the animals took up deuterium in stable positions fairly rapidly, and to a maximum amount in six to eight days. Since the weights of the animals remained constant, indicating that there was no appreciable amount of extra fat deposited, Schoenheimer and Rittenberg conclude that there is a rapid and continuous turnover of fatty acid in the organism. " Instead of comparing the fat tissues to a cellar in which food is stored for times of emergency, it seems more correct to compare them to an ice box in which a part of the food is kept during the short intervals between meals."

Fate of Body Fat. This must be either excreted or catabolised. Catabolism of fats may be defined as the summation of all the processes by which they are either oxidised to simpler compounds, or permanently changed to non-fatty compounds.

Excretion of Fat. The faeces always contain considerable amounts of fatty acids, along with some neutral fat. These are derived from undigested fat, excreted fat (and, negligibly, from cellular *débris* from the alimentary canal). During fasting the fatty material amounts to about one-third of the dry weight of the faeces. This is true excreted fat. Hill and Bloor have shown that when moderate amounts of fat are fed an animal the fat of the faeces is largely independent of that in the diet and approaches in composition that on a fat-free diet. Such results indicate that under normal conditions faecal fatty material should be

considered as in large part excreted fat, and not undigested fat. This conclusion no longer holds when, through abnormal conditions, bile ceases to reach the intestine, or there is a deficiency of pancreatic lipase.

Formation of other Substances of Metabolic Importance from Fats. The glycerol from fats can be converted to carbohydrate such as glucose. It is still a matter of controversy whether fatty acids can be converted to carbohydrate. The arguments for and against this view are complex, and cannot be dealt with satisfactorily in brief compass. Suffice it to state that the evidence is no longer so definitely against non-conversion as it was a decade ago, but that it is improbable that large amounts of fatty acids are changed to carbohydrate.

Fatty acids are also utilised in the formation of phospholipides, not only transiently during fat absorption and transport, but permanently, in tissues not concerned with these functions.

Oxidation of Fats. Fats are mobilised from the fat depôts as needed by the organism. Evidence has already been adduced that this is a constant process. They are presumably hydrolysed by the same agents as function in their formation, and they are transported to the tissues concerned with their oxidation by the same mechanisms as those following their absorption from the intestine.

Within recent years studies by Quastel and others of the effects produced by tissue slices on particular fatty acids have led to valuable conclusions. The liver seems to be the most important organ in the body for oxidation of fatty acids ; such organs as the kidneys, spleen and testes seem to have some power of oxidation ; brain tissue has none.

The classical theory of Knoop held that fatty acids were oxidised in successive stages, in each of which the beta-carbon atom was attacked and oxidised, so that as a result two carbon atoms were " etched away " at a time.

$$R . CH_2 . CH_2 . COOH \rightarrow R . CO . CH_2 . COOH \rightarrow R . COOH$$

Since body fats only contain fatty acids with even numbers of carbon atoms, according to Knoop's theory ultimately from each molecule of fatty acid one molecule of butyric acid must result, which is oxidised to acetoacetic acid, and finally to carbon dioxide and water.

$$CH_3 . CH_2 . CH_2 . COOH \rightarrow CH_3 . CO . CH_2 . COOH \rightarrow 4CO_2 + 3H_2O$$

While this theory of Knoop seems correct in essentials, recent work suggests need for some modifications.

The study of tissue slices has shown that the liver is much more powerful than the kidneys in forming acetoacetic acid from fatty acids, but is much less powerful in carrying the oxidation further. Kidney tissue oxidises acetoacetic acid more powerfully than splenic tissue, while the liver and testes are relatively inactive. (Presence of calcium and potassium ions facilitates the kidney action.)

It was long considered that beta-hydroxybutyric acid was an intermediate stage in oxidation between butyric and acetoacetic acids. This view is no longer held, but it has been shown that the hydroxy acid can be formed from acetoacetic acid by reduction, both in the liver, and, to less extent, in the kidney.

Jowett and Quastel, finding that, molecule for molecule, higher fatty acids yield more acetoacetic acid through action of liver tissue than does butyric acid, have proposed a theory of " multiple alternate oxidation," in which they consider that some specific enzyme produces simultaneous oxidation at alternate carbon atoms in the fatty acid chain, with probable production of more than one molecule of acetoacetic acid per molecule of (higher) fatty acid. Thus :

$$\ldots\ldots CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$$
$$\xrightarrow{\text{(by oxidation)}}$$
$$\ldots\ldots CH_2.CO.CH_2.CO.CH_2.CO.CH_2.CO.CH_2.CO.CH_2.COOH$$
$$\xrightarrow{\text{(by hydrolysis)}}$$
$$. + CH_3.CO.CH_2.COOH + CH_3.CO.CH_2.COOH + CH_3.CO.CH_2.COOH$$

Acetoacetic acid appears to be the only beta-keto acid produced in significant amount in the body.

In addition to the theories in which acetoacetic acid is considered to be the essential product of oxidation of fatty acids, another suggests that oxidation at the " omega " carbon atom plays some part ; some evidence is accumulating in favour of this view. Thus it has been demonstrated that this type of action can occur with acids with from 8 to 11 carbon atoms, dicarboxylic acids resulting, which are then subjected to beta-oxidation, with ultimate production of such acids as succinic acid, $COOH . CH_2 . CH_2 . COOH$.

Summary of Fat Catabolism. Present knowledge suggests transport from the fat depôts, partly as phospholipides, to the liver, oxidation of fatty acids in the liver, chiefly to acetoacetic acid, and subsequent further oxidation of that acid, chiefly in the kidneys. In addition there may be some degree of formation of other products in the liver, including beta-hydroxybutyric and succinic acids. Glycerol is either oxidised or converted to carbohydrate.

Hormonic Control of Fat Catabolism. There is evidence that one of the hormones of the anterior pituitary gland stimulates oxidation of fatty acids to acetoacetic acid. This has been termed the " ketogenic hormone."

Choline and Lipide Metabolism in the Liver. When rats are fed diets rich in fat, low in protein, and very low in choline compounds such as lecithin, in two or three weeks they will develop fatty livers containing up to 24 per cent. of total fat, instead of the normal 3 to 4 per cent. Best and his co-workers have shown that this abnormal change is largely prevented by the daily addition of 2 or 3 mg. of choline to the diet, and it has further been shown that certain proteins rich in methionine, such as casein, also have some preventive action.

When cholesterol is fed to rats to the extent that it forms 2 per cent. of their diet, along with sufficient fat to ensure its absorption, there results an accumulation of cholesterol esters and neutral fats in the liver. Similar administration of choline diminishes, but may not completely abolish this effect.

When depancreatised dogs are kept alive with insulin, it has been customary to maintain them in good health by feeding them raw pancreas. The good effect of the pancreas was erroneously believed to be due to the supply of pancreatic enzymes thus administered, ensuring adequate digestion in the intestine of the experimental animal. Best and his co-workers have ascertained that its true effect is to provide a supply of choline.

Depancreatised dogs kept alive with insulin, but without pancreas in the diet, store fat and cholesterol esters in the liver even to the extent of 60 per cent. of its wet weight, while their blood shows marked diminution of lipide and almost complete disappearance of cholesterol esters. (A somewhat similar picture is seen in acute yellow atrophy of the liver.) When choline or its derivative lecithin is administered, the content of liver lipides is lessened and that of blood lipides is raised.

Thus it would appear that the organism, minus its pancreas, cannot synthetise choline in sufficient amounts for its needs, if at all.

Milk Fat. The milk of female mammals is rich in lipides, chiefly neutral fats. These fats contain fatty acid radicals comprising the whole range of acids with even numbers of carbon atoms from butyric, $C_4H_8O_2$, to cerotic, $C_{26}H_{52}O_2$. The lower acids are probably formed in the mammary glands. Little is known about the higher acids. During milk formation there is no detectable withdrawal of phospholipides from the blood, so that the bulk of milk fat must derive its acids (chiefly C_{16} and C_{18} acids) from those of the blood fat or cholesterol esters.

Digestion, Absorption, and Metabolism of Lipides other than Neutral Fats

Although, as has been seen, the metabolism of neutral fats is inextricably associated with that of certain lipides, yet separate consideration assists clarity.

Digestion and Absorption. The mucous membrane of the small intestine is relatively rich in lecithinases. Lecithins present in the diet are probably partly hydrolysed during absorption.

Trypsin can hydrolyse the " peptide " linkage in galactolipides, producing psychosine ; during ordinary digestion the extent of this action is probably negligible.

Pure cholesterol is not easily absorbed from the intestine ; presence of bile salts renders its absorption easy ; it is probably partly esterified during absorption. Cholesterol esters are hydrolysed in the intestine and re-synthetised during absorption, passing to lymph channels. Other sterols, such as phytosterol, ergosterol, and coprosterol, are only absorbed very slightly.

The carotenoids and fat-soluble vitamins are absorbed unchanged.

Distribution of Non-fatty Lipides. Terroine has drawn a distinction between the reserve fat (his *élément variable*) and the lipides which form essential components of the living cells (his *élément constant*). The latter, virtually the sum of the non-fatty lipides, are present in fairly constant amount, qualitatively and quantitatively, in each of the different cell-types of any species. This distinction of Terroine, of course, merely accentuates the fact that neutral fats are reserve stores of energy, without other special function, whereas non-fatty lipides are present in tissues for functional purposes, and their amounts are just as much under control as the solid matter of normal bone, or the creatine content of muscle.

Nervous tissues are rich in phospholipides, galactolipides, and cholesterol and its esters ; their lipide content is very constant and there is little fat or chromolipide present. In other tissues (excepting adipose tissue and liver) non-fatty lipides predominate, chiefly phospholipides, cholesterol, and its esters. The lipide content is fairly constant in them ; only the neutral fat and chromolipides show much variation.

In spite of the varying content of fat and cholesterol esters in the liver, it has a fairly constant content of functional lipides. There is a marked tendency for liver phosphatides to contain radicals of acids of higher molecular weight than those present in the liver glycerides (Hilditch).

Metabolism and Function. We are still very ignorant of the

functions of these compounds. The specific manner of their distribution, and the essential presence of certain of them in every living cell indicate their great importance.

It has been shown that the phospholipides (lecithins and perhaps cephalins, but not sphingomyelins) play an essential *rôle* in fat absorption and transport, and probably in fat deposition. Their functions are not limited to this. The presence of relatively large amounts of lecithins in nervous tissues undoubtedly indicates important functions, probably associated with their innate lability. It will be shown later that the cephalins have a *rôle* in blood-clotting. The sphingomyelins perhaps play some *rôle* in association with the cerebrosides. Their close chemical relationship has already been pointed out (*cf.* p. 116). The distribution of the latter suggests important function in connection with nerve tissue, while the relatively large amount of sphingomyelins in plasma suggests rather a subsidiary *rôle* as supply for ceramides (*cf.* p. 117).

Bloor and Sinclair have shown that phospholipides in liver and muscle can act as oxygen-carriers. The phospholipide and cholesterol content of certain organs such as the corpus luteum of the ovary, the uterus, etc., seem to parallel their physiological activity (Bloor, Boyd).

Cholesterol has many functions. It appears to assist in carriage of fats through ease of formation and decomposition of its esters. It is almost certainly the source of such vitamin **D** as is formed in the animal organism (through the intermediate stage of dehydrocholesterol). It serves as precursor to the bile acids, and the hormones of the ovary, testis, and adrenal cortex. Its esters are important constituents of the greasy " sebum " secreted from the sebaceous glands on to the skin and they confer on sebum its characteristic protective properties.

Cholesterol and the lecithins probably play a joint but antagonistic *rôle* in regulating the permeability of cell membranes and in stabilising cell colloids.

The function of the hormones of lipide nature is dealt with elsewhere (*cf.* pp. 58, 59).

While the carotenes serve as precursors of vitamin **A,** it is doubtful whether these or any other of the carotenoids have other functions in the animal. Their presence in tissues is accidental, due to their presence in ingested food. Thus the yellow colour of that " yellow body " the corpus luteum, formed in the ovary after ovulation, is absent from the corpora lutea of such species as the mouse.

We are ignorant of the site and method of formation of almost

all the non-fatty lipides. It would appear that lecithins and cephalins are formed with ease from fatty acids in many tissues, although whether a glycero-phospho-choline framework is pre-formed, or formed from its constituents at need, we do not know. Choline may be formed in the pancreas.

The galactosides, associated chiefly with nerve tissue, require galactose for their formation; this is apparently produced at need from glucose, but how and where we do not know. We know nothing concerning the formation of sphingosine.

Cholesterol is in part obtained from the diet, but animals can synthetise it at need. When it is fed in excess to cats, one-half of the part absorbed is destroyed, but the rest is found deposited in all organs except the brain, and especially in the liver.

Young mice fed bread alone have been found to synthetise as much cholesterol in a month as they initially contained. The developing chick in the egg shows a gradual increase in total cholesterol which can only have taken place through synthesis. When newly born dogs were given for four weeks a diet poor in cholesterol, and were then killed and analysed, their bodies showed an increase in cholesterol twenty times that given in their food. Similar evidence is available for the rat, while studies of cholesterol metabolism in infants support the view that synthesis occurs in the organism.

Excretion. Cholesterol is excreted in the bile into the intestine, this channel of excretion being possible through its marked solubility in bile salts. During passage through the intestine it is partly reduced to coprosterol (*cf.* p. 118) by bacterial action, so that the faeces contain both compounds.

Some Diseased Conditions Associated with Abnormal Lipide Metabolism

A Fat Deficiency Disease. Burr and Burr have shown that when young rats are fed diets completely freed from fat over a period of some months they develop various abnormal conditions, including failure to grow, and ultimately die. Onset of symptoms can be prevented by administration of a little linoleic or linolenic acid. Schuringa has also observed faulty growth and early death when rats are fed diets containing only 0·01 per cent. of fat. Thus, at least as far as the rat is concerned, there is a limit to power of formation of essential fatty acids, and a limit to power of desaturation.

Perhaps related to this disease in rats is the observation of Brown and Hansen (1937) that 5 per cent. of the total fatty acids (free and in combination) in the blood plasma of normal children

is linoleic acid, and 3 per cent. is archidonic acid, whereas in eczematous children these amounts are definitely decreased; administration of oils rich in unsaturated acids will cure the eczematous condition.

Obesity. Whenever the intake of food is greater than the need, whether through dietary excess or through deficient exercise, animals tend to store the excess as fat, and a condition of obesity results. In man, for some unknown reason, this result cannot be predicted with certainty. Some proportion of men and women remain thin in spite of dietary excesses. In man a large proportion of cases of obesity is probably traceable to some endocrine cause, and particularly to some dysfunction of the pituitary gland.

Diabetes Mellitus. In this disease disturbances of fat metabolism are secondary to those of carbohydrate metabolism (*cf.* p. 107), but the ultimate cause of death in untreated diabetes is associated with distorted fat catabolism. When the amount of glucose which is being catabolised in the body falls below a certain minimum, the acetoacetic acid from fat catabolism commences to be incompletely oxidised. Instead of being converted to carbon dioxide and water, it is more and more changed to acetone, with carbon dioxide split off in the process.

$$CH_3 . CO . CH_2 . COOH \rightarrow CH_3 . CO . CH_3 + CO_2$$

This is a slow reaction; acetoacetic acid accumulates. Probably more of it than usual is reduced to beta-hydroxybutyric acid. These two acids pass into the circulating blood, and are neutralised and excreted (*cf.* Chapter II., p. 30).

$$HX + NaHCO_3 = NaX + HOH + CO_2$$

This excretion removes blood base, and so lessens the capacity of the blood to remove carbon dioxide from the tissues. There result increased respiration (air hunger), coma, and finally death.

The cause of this upset in fat catabolism is not known. Two theories have been propounded to explain it. One, expressed poetically, says that fat burns in the fire of carbohydrate, and that, in absence of that fire, fat smokes. In other words, the correct oxidation of acetoacetic acid can only proceed when some particular phase of carbohydrate oxidation is proceeding at the same time, an example of interlocked reactions. The other theory simply suggests that normal fat catabolism can only proceed at a certain maximum rate, and that when through deficient carbohydrate oxidation, the fat has to be burned up at a greater rate than this maximum to meet body needs, the combustion becomes faulty.

The minimum amount of carbohydrate needed to prevent undue acetone formation corresponds to about one part by weight of glucose to three parts by weight of fat catabolised. The ratio of carbohydrate to fat catabolised is spoken of as the " ketogenic-antiketogenic " ratio. Fats, ketogenic, tend to produce a *ketosis* (to produce the acetone bodies, acetone itself, and acetoacetic and beta-hydroxybutyric acids), while carbohydrates tend to prevent a ketosis, and thus to be anti-ketogenic.

Other Diseases. Certain rare diseases are interesting in that their study will probably throw more light on the intermediate metabolism of some of the non-fatty lipides. In *Gaucher's disease* the patient, usually a child, has, characteristically, an enlarged liver and spleen ; at post-mortem the spleen is found to contain large amounts (up to 10 per cent.) of the cerebroside kerasin, which is normally present in amounts only of the order of 0·03 per cent. Experiments with animals have shown that when cerebrosides are injected they accumulate especially in the spleen. In *Niemann-Pick's disease* the phospholipide and cholesterol contents of the viscera, bone-marrow, and brain are increased, while neutral fat almost entirely disappears. Increased deposits of the sphingomyelins is perhaps the most characteristic change. In *Schüler-Christian's disease* bone tissue is replaced by cholesterol deposits, and cholesterol esters replace other lipides in many tissues.

Sobotka has suggested that, through lack of some essential enzymes, these diseases represent conditions where " transport lipides " can no longer be changed chemically, and so accumulate in certain definite sites.

Gall-stones. Bile gradually becomes concentrated during its stay in the gall-bladder ; when through various causes, usually pathological, a focus is present which may initiate deposition of solid, " stones " commence to form. The majority of human gall-stones contain crystalline cholesterol, with varying amounts of calcium salts of the bile pigments. The cholesterol content may amount to over 90 per cent. of the stone.

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CHAPTER VI

THE PROTEINS AND THEIR DERIVATIVES

INTRODUCTION

THE term *protein*, and the older and now discarded term "proteid," are derived from the Greek *protos*, first, and signify the relative importance of this class of foodstuffs. Proteins are indispensable in a diet. Carnivorous animals, for example, can dispense with fats and carbohydrates for a lengthy period, for if sufficient protein is fed them, they can make their own fats and carbohydrates. (In the days when the term "proteid" was coined such other essential requirements as the vitamins were not dreamt of.) Within recent years the term "protide" has been suggested to replace protein, but the suggestion has not been widely adopted.

Proteins contain carbon, hydrogen, nitrogen, and oxygen; most proteins contain sulphur, and a few contain other elements, such as phosphorus, iron, and iodine. Analysis shows that the elements present usually vary between the following extremes, C, 50·6 to 54·5 per cent., H, 6·5 to 7·3 per cent., N, 15·0 to 17·6 per cent., O, 21·5 to 23·5 per cent., S (when present), 0·3 to 2·2 per cent., and P (when present), 0·4 to 1·0 per cent.

The proteins of egg-white are so typical of proteins in general that, since they constitute a large part of the solids in egg-white, they were chosen by German investigators to name the class (Eiweiss-stoffe). When the white of egg is carefully separated from its yolk, and mixed with an equal volume of saturated ammonium sulphate (thus giving a half saturated solution of ammonium sulphate), a precipitate of *egg-globulin* (L. *globulus*, globule) is formed. If the filtrate is slowly concentrated at room temperature, after a while another protein separates as a white solid; if this is dissolved in water, and then ammonium sulphate is added until a faint cloudiness appears, on standing crystals of *egg-albumin* (L. *album*, white) separate.

Other typical proteins include *casein* of milk (L. *caseus*, cheese), a phospho-protein, precipitated from diluted milk by very dilute acetic acid, and *haemoglobin*, of the red cells of the blood, a complex protein containing iron.

Proteins, with few exceptions, are colourless, amorphous compounds, as usually prepared. Many have been obtained in crystalline form. They are of very large molecular size, and, in consequence, are colloids. They give a series of typical colour reactions, most of which are due to the presence of specific amino-acid radicals, and not to proteins as proteins.

Classification of Proteins

Present classifications of proteins depend chiefly on their physical, and partly on their chemical properties. These classifications are not completely satisfactory. Those in chief use by English-speaking biochemists are based on systems suggested by British and by American Committees many years ago, and the subject might well be reviewed in light of newer knowledge. The three main divisions are (i.) simple proteins, (ii.) conjugated proteins, and (iii.) derived proteins or products of hydrolysis of proteins, some of which are not protein in character at all.

Simple Proteins. These hydrolyse almost entirely to amino-acids, although some of them appear to contain phosphate and carbohydrate radicals.

1. *Albumins.* These are soluble in pure water and the solutions are coagulated by heat. Their solutions are almost neutral in reaction. Typical of the class are egg-albumin, plasma albumin from blood, and lactalbumin from milk. They are all precipitated from solution by complete saturation with ammonium sulphate. Plants contain relatively small amounts of " albumins " which do not markedly differ in solubility properties from the next class, the globulins, and perhaps should be grouped with them.

2. *Globulins.* These are insoluble in pure water, but easily soluble in dilute solutions of such salts as sodium chloride. They are typified by the globulin of blood plasma, ovo-globulin from egg-yolk, edestin from hemp seed, excelsin from brazil nut, amandin from almonds, peach, plum, and apricot nuts, and legumin from the pea, horse-bean, vetch, and lentil. Many of the plant globulins are readily crystallisable. Globulins are precipitated from solution by half-saturation with ammonium sulphate, and their solutions are easily coagulated by heat.

3. *Glutelins.* These are insoluble in neutral salts, but easily soluble in dilute acids and alkalies. Glutenin from wheat is the only well-characterised member of this class so far obtained.

4. *Prolamins* (or " alcohol-soluble proteins," a less accurate description). These are insoluble in water, absolute alcohol, and other neutral solvents, but are soluble in 70 per cent. alcohol.

They are termed prolamins because on hydrolysis they yield relatively large amounts of the amino-acid proline (10 to 14 per cent.). To its presence they owe their solubility in aqueous alcohol. They occur solely in plants. Typical are zein from maize, hordein from rye, and gliadin from wheat.

5. *Scleroproteins* (Gk. *skleros*, hard; also termed "albuminoids"). These are characterised by marked insolubility. They occur solely in animals. Typical are silk-fibroin, elastin from tendinous material, spongin from sponges, and keratin from horn and hoof.

6. *Histones*. These are soluble in water, but insoluble in dilute ammonia. Their solutions are alkaline. Their properties are intermediate between those of albumins, and of the next group, the protamines. Typical are globin, from haemoglobin, and thymus histone.

7. *Protamines*. These are the simplest known proteins. They contain no sulphur, are soluble in water, and their solutions are strongly alkaline. They are all derived from the sperm of fishes. Typical are salmine (from Rhine salmon sperm), sturine (from the sturgeon), clupeine (from the herring), and scombrine (from mackerel sperm).

Conjugated proteins all contain a *prosthetic group* (Gk. *prosthetos*, added to) in the molecule, some radical which is not amino-acid in character.

8. *Phosphoproteins*. These are proteins containing phosphate radicals which are neither in phospholipide nor nucleic acid combination. They are insoluble in water, in dilute acid, and in half saturated solutions of ammonium sulphate, but are soluble in dilute alkali. They are typified by casein of milk (0·7 to 1·0 per cent. phosphorus in caseins from different species), and vitellin, present in the blood plasma of laying hens, and containing 0·95 per cent. of phosphorus.

Since certain albumins also contain phosphate radicals, there seems no good reason for not also listing such phosphoproteins as casein among the simple proteins, and the British classification does so list them.

9. *Nucleoproteins*. These are present in all cell nuclei, and are compounds of one or more molecules of protein with a nucleic acid. They are acid in character, readily soluble in dilute alkali and in dilute mineral acids, but are only soluble with difficulty in excess of acetic acid. (The nucleic acids will be dealt with in Chapter VII.)

10. *Glucoproteins*. These contain prosthetic groups which include one or more carbohydrate radicals. *Mucins* are present

in various secretions, such as saliva. *Mucoids* are present in bone, tendon, etc. Mannose and galactose radicals are present in the prosthetic group of mucoids and mucins, but are also present to a smaller extent in many other proteins, so that it is very doubtful if " glucoproteins " should be put into a distinct class.

11. *Chromoproteins* (*i.e.*, " coloured proteins " ; *haemoglobins* in the American classification) contain a coloured prosthetic group. The typical representative is haemoglobin of the red blood cell, a compound of the histone globin with haem, an organic iron compound.

12. *Lecithoproteins.* The existence of this class is not definitely proved. Certain mixtures of proteins and phospholipides, present in blood plasma and in fish eggs, are separated with such extreme difficulty that some investigators believe they are held together in chemical combination rather than by some physical means.

Derived Proteins and Products of Protein Hydrolysis

13. *Proteans* are defined as insoluble products resulting from the incipient action of very dilute acids or even of proteases on proteins. Thus edestin yields edestan. Further action of dilute acids, or of alkalies, produces *infraproteins*, or *metaproteins*, soluble in dilute acids and alkalies, but insoluble in neutral solutions. Still further action produces *proteoses*, *peptones*, *polypeptides*, and ultimately *amino-acids*.

14. Heat or alcohol, acting on proteins in solution, converts them into *insoluble proteins*.

Molecular Weights of Proteins

The earlier methods for determining such molecular weights were based on the content of some particular chemical element or radical, present in small amount. The weights calculated by such methods were usually far too small. Within recent years a number of different physical methods have been employed with greater success ; in particular, Svedberg's ultracentrifugal method has given information of great value (*cf.* Chapter II., p. 34).

Measurements by this method of proteins in uniform solution (*i.e.*, with only protein molecules of one size present) have given the results shown in Table IX. When one remembers that the accuracy of such a method as that involving the ultracentrifuge is at most only within 3 or 4 per cent., so that, for example, the molecular weight of egg albumin should be written 40,500 $\pm$ 1,000, it will be seen that many of the figures in Table IX. approximate

to multiples of the unit 17,500, suggesting some degree of regularity in the weight relationships of the proteins.

TABLE IX. MOLECULAR WEIGHTS OF PROTEINS IN SOLUTION

Protein	Source	Class	Molecular Weight	Multiple of 17,500
Lactalbumin	Milk	Albumin	17,500	×1 = 17,500
Cytochrome C.	Muscle	Chromoprotein	17,000	
Gliadin	Wheat	Prolamin	26,000	
Zein	Maize	Prolamin	35,000	×2 = 35,000
Bence Jones Protein (see p. 174)	—	Globulin	35,000	
Insulin	Pancreas	—	35,000	
Pepsin (Enzyme)	Gastric Juice	Albumin	37,000	
Lactoglobulin	Milk	Globulin	38,000	
Egg Albumin	Hen's Egg	Albumin	40,500	
Haemoglobin	Horse Blood	Chromoprotein	68,000	×4 = 70,000
Plasma Albumin	Horse Blood	Albumin	69,000	
Plasma Globulin	Horse Blood	Globulin	150,000	×8 = 140,000
Catalase (Enzyme)	Beef Liver	Chromoprotein	248,000	×14 = 245,000
Excelsin	Brazil Nut	Globulin	294,000	×16 = 280,000
Edestin	Hemp Seed	Globulin	309,000	
Amandin	Almond Nut	Globulin	329,000	
Urease (Enzyme)	Jack Bean	Globulin	483,000	×28 = 490,000
Thyroglobulin	Thyroid Gland	Globulin	650,000	×36 = 630,000
Haemocyanin	Octopus	Chromoprotein	2,785,000	×160 = 2,800,000
Haemocyanin	Snail	Chromoprotein	6,700,000	×384 = 6,720,000

Comparisons of results from different procedures are now possible for a few proteins, and they suggest that reasonable reliance can be placed on figures derived from ultracentrifuge data (Table X.).

TABLE X. MOLECULAR WEIGHTS OF PROTEINS DETERMINED BY VARIOUS METHODS

Method	Egg-Albumin	Haemoglobin	Plasma Albumin	Plasma Globulin
Osmotic pressure	34,000	68,000	68,000	150,000
Ultracentrifuge	40,500	68,000	69,000	150,000
Surface tension	35,000	68,700	66,000	—
Velocity of diffusion	34,000	69,500	—	—
(Chemical methods)	(33,800)	(50,000)	(45,000)	(81,000)

Hydrolysis of Proteins

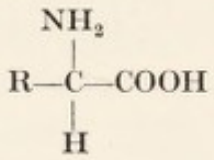
When proteins are heated with a reflux condenser in presence of dilute mineral acids, they are gradually split to smaller and

smaller fragments by a series of hydrolyses. At first *proteoses* are formed, all soluble in water, but still precipitated by complete saturation with ammonium sulphate. As hydrolysis proceeds *peptones* are produced, no longer precipitated by ammonium sulphate, though phosphotungstic acid and tannic acid will precipitate them. Then, through the stage of *polypeptides*, finally a number of *amino-acids* are set free. Hydrolysis with baryta solution, and with proteolytic enzymes, leads to similar results.

The stage of hydrolysis can be followed by the biuret test. Biuret, a compound formed when urea is sublimed, has the formula $NH_2 . CO . NH . CO . NH_2$. When to a solution of biuret is added an equal volume of 40 per cent. sodium hydroxide, and then a trace of copper sulphate solution, a red-purple colour results. It will be seen later that proteins have a comparable series of linked nitrogen and carbon atoms. When the biuret test is applied to proteins, a purple colour results. Proteoses give a less intense purple, peptones a pink colour, and amino-acids do not give this colour reaction.

The Amino-Acids

Twenty-two amino-acids can be obtained from the dietary proteins. Still others may exist, though all those essential to the diet have been isolated (*cf.* p. 172). Twenty of the acids are substituted ammonias of the type RNH_2. The other two are really imino-acids, of the type RNHR′. The majority are mono-carboxylic acids, three are di-carboxylic acids, and several are di-amino compounds. The type formula for most of them is



and, since the amino-group is attached to the α-carbon atom (the carbon atom adjacent to the carboxyl group), they are α-amino (or α-imino) acids. The list of these acids follows.

Mono-amino-mono-carboxylic Acids

1. *Glycine* (glycocoll, amino-acetic acid, $C_2H_5NO_2$ or $NH_2 . CH_2 . COOH$. It gives a well-crystallised hydrochloride, $HOOC . CH_2 . NH_2, HCl$, a reaction typical of many of these amino-acids.

2. *d-Alanine* (α-amino-propionic acid), $C_3H_7NO_2$, or $CH_3 . CH(NH_2). COOH$. Specific rotation of + 2·7°.

3. *l-Serine* (β-hydroxy-α-amino-propionic acid, or β-hydroxy-alanine), $C_3H_7NO_3$ or $HO . CH_2 . CH(NH_2) . COOH$. Specific rotation $-6{\cdot}8°$.

4. *d-Threonine* (γ-hydroxy-α-amino-butyric acid), $C_4H_9NO_3$ or $HO . CH_2 . CH_2 . CH(NH_2) . COOH$.

5. *l-Methionine* (γ-methyl-thiol-α-amino-butyric acid), $C_5H_{11}SNO_2$, or $CH_3 . S . CH_2 . CH_2 . CH(NH_2) . COOH$. Specific rotation $-7{\cdot}2°$.

6. *d-Valine* (α-amino-isovaleric acid), $C_5H_{11}NO_2$ or $(CH_3)_2 : CH . CH(NH_2) . COOH$. Specific rotation $+6{\cdot}4°$.

7. *d-Caprine* (norleucine, α-amino-normal caproic acid), $C_6H_{13}NO_2$ or $CH_3 . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH$. Specific rotation $+4{\cdot}5°$.

8. *l-Leucine* (α-amino-isocaproic acid), $C_6H_{13}NO_2$ or $(CH_3)_2 : CH . CH_2 . CH(NH_2) . COOH$. Specific rotation $-10{\cdot}4°$.

9. *d-Isoleucine* (α-amino-β-methyl-β-ethyl-propionic acid), $C_6H_{13}NO_2$ or $CH_3 . CH_2 . CH(CH_3) . CH(NH_2) . COOH$. Specific rotation $+9{\cdot}6°$.

10. *l-Phenylalanine* (β-phenyl-α-amino-propionic acid),

$C_9H_{11}NO_2$ or  $—CH_2 . CH(NH_2) . COOH$. Specific

rotation $-35{\cdot}1°$.

11. *l-Tyrosine* (β-parahydroxyphenyl-α-amino-propionic acid),

$C_9H_{11}NO_3$ or $HO—$  $—CH_2 . CH(NH_2) . COOH$. Specific

rotation $-13°$ (in dilute hydrochloric acid).

Mono-amino-di-carboxylic Acids

12. *l-Aspartic acid* (amino-succinic acid), $C_4H_7NO_4$ or $HOOC . CH_2 . CH(NH_2) . COOH$. (This compound is laevo-rotatory in alkaline and dextro-rotatory in acid solution.)

13. *d-Glutamic acid* (α-amino-glutaric acid), $C_5H_9NO_4$ or $HOOC . CH_2 . CH_2 . CH(NH_2) . COOH$. Specific rotation $+12{\cdot}0°$.

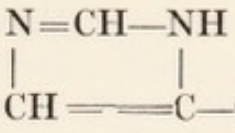
14. *d-Hydroxyglutamic acid* (α-amino-β-hydroxyglutaric acid), $C_5H_9NO_5$ or $HOOC . CH_2 . CH(OH) . CH(NH_2) . COOH$. Specific rotation $0{\cdot}8°$.

15. *d-Lysine* (α-ω-diaminocaproic acid), $C_6H_{14}N_2O_2$ or $NH_2 . (CH_2)_4 . CH(NH_2) . COOH$. Specific rotation $+14{\cdot}6°$.

16. *d - Arginine* (α - amino - δ - guanidino - valeric acid), $C_6H_{14}N_4O_2$ or $NH_2 . C(: NH) . NH . (CH_2)_3 . CH(NH_2) . COOH$. Specific rotation $+21{\cdot}3°$. Arginine, on hydrolysis with baryta, yields urea, CON_2H_4 and another important amino-acid, *ornithine*, $NH_2 . (CH_2)_3 . CH(NH_2) . COOH$.

17. *Citrulline* (α-amino-δ-carbamino-valeric acid), $C_6H_{13}N_3O_3$ or $NH_2 . CO . NH . (CH_2)_3 . CH(NH_2) . COOH$.

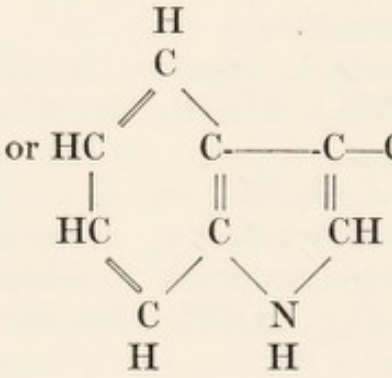
18. *l-Histidine* (β-iminazolyl-α-aminopropionic acid), $C_6H_9N_3O_2$



. $CH(NH_2) . COOH$. Sp. rotation − 39·7°.

Amino-acid with Indole Nucleus

19. *l-Tryptophane* (β-indole-α-aminopropionic acid), $C_{11}H_{12}N_2O_2$



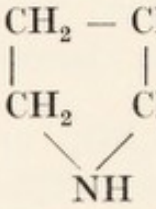
. $CH(NH_2) . COOH$. Sp. rotation −33°.

Di-amino-di-carboxylic Acid

20. l-*Cystine* (di-cysteine), $C_6H_{12}S_2N_2O_4$ or $HOOC . CH(NH_2) . CH_2 . S . S . CH_2 . CH(NH_2) . COOH$. Specific rotation − 242·6° (a 1 per cent. solution in 0·1 *N* hydrochloric acid). With cystine should be associated its reduced product *l-cysteine* (β-thio-α-aminopropionic acid), $C_3H_7SNO_2$, or $HS . CH_2 . CH(NH_2) . COOH$, whose specific rotation is − 1·0°.

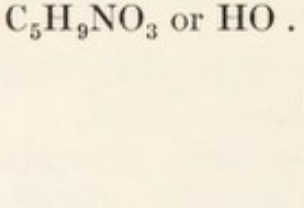
Imino-acids

21. *l-Proline* (pyrrolidine carboxylic acid), $C_5H_9NO_2$ or



. Specific rotation − 77·4°.

22. *l-Hydroxyproline* (γ-hydroxy-pyrrolidine carboxylic acid), $C_5H_9NO_3$ or

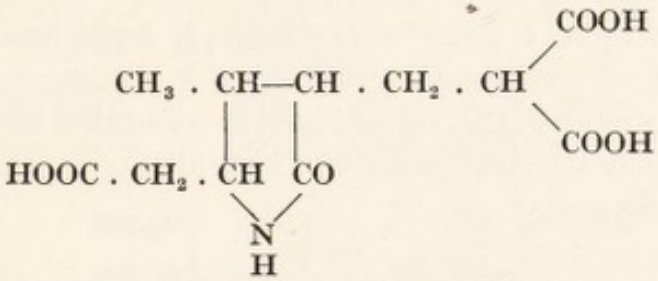


. Specific rotation − 81°.

A number of other amino-acids of natural origin exist, some of which are of considerable physiological importance, while others have probably no significance as far as animal metabolism is

concerned. In the first group come the α-amino-acids di-iodotyrosine and thyroxine (*cf.* Chapter III., p. 54), dihydroxyphenylalanine (*cf.* p. 171) and the amino-acid creatine (*cf.* Chapter X., p. 244). In the second group are such compounds as aminobutyric acid (present in the hydrolysed products of the sclera of the whale), hydroxylysine, in the hydrolysed products of isinglass from the swim-bladder of the sturgeon, and of gelatin, and the unusual acid canavanin,
$NH_2 . C(: NH) . NH . O . CH_2 . CH_2 . CH(NH_2) . COOH$, present in the free state in jack bean, and, according to Kitagawa, who isolated it, hydrolysed by a liver enzyme to canalin, $NH_2 . O . CH_2 . CH_2 . CH(NH_2) . COOH$. From hydrolysis of djenkol nuts, the cysteine derivative djenkolic acid has been isolated ; its constitution is $CH_2 . [S . CH_2 . CH(NH_2) . COOH]_2$.

Dakin and West have isolated from liver extract a tricarboxylic amino-acid, whose constitution is stated to be of the type



and whose function is still unknown.

General Properties of the Amino-Acids. With the exception of glycine, which does not possess an asymmetric carbon atom, all the twenty-two acids obtained from proteins of the diet are optically active ; their specific rotations have been already stated.

Their solubilities in water vary greatly. Glycine, alanine, hydroxy-glutamic acid, lysine, arginine, histidine, proline and hydroxyproline are very soluble, phenylalanine and tryptophane very soluble in hot water, cysteine, serine, methionine and valine are fairly soluble, aspartic and glutamic acids, leucine and isoleucine only slightly soluble, while norleucine, tyrosine and cystine are almost insoluble.

Most of these acids are insoluble in alcohol. Hydroxyproline, histidine, leucine, and tryptophane are slightly soluble, methionine more so, and proline very soluble in alcohol.

The sweetness of the alcohols derived from hexoses has illustrated the fact that sweetness is not peculiar to sugars (*cf.* p. 82). Several of the amino-acids are sweet in aqueous solution, notably glycine, alanine, proline and hydroxyproline. Valine is bitter sweet, while isoleucine, phenylalanine and tryptophane are slightly bitter.

Almost all the amino-acids crystallise in characteristic forms. Photomicrographs of several are shown in Plate III.

All the α-amino-acids react with nitrous acid to yield hydroxy-acids and nitrogen.

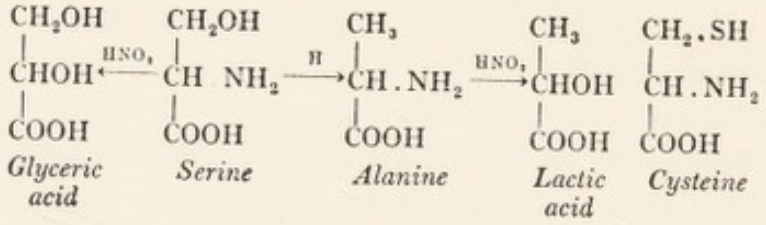
$$R . CH(NH_2) . COOH + HONO = R . CH(OH) . COOH + N_2 + HOH$$

Since gaseous nitrogen is liberated in amount corresponding to twice the nitrogen content of the amino-acids, this permitted Van Slyke to devise an important and accurate method of measuring the *amino-acid nitrogen* of blood and other fluids.

Properties of Some Specific Amino-acids. *Glycine.* Its radical is present in relatively large amounts in gelatin, silk-fibroin, elastin and spongin. Glycine reacts with benzoyl chloride, $C_6H_5 . COCl$, in alkaline medium to form hippuric acid, $C_6H_5 . CO . NH . CH_2 . COOH$; a similar reaction takes place in the body when benzoic acid, $C_6H_5 . COOH$, is detoxicated to form hippuric acid.

Alanine. Its radical also is present in relatively large amounts in certain scleroproteins, such as silk-fibroin and keratins.

Serine. The relationship of this amino-acid to a number of other important compounds is illustrated by the formulae :

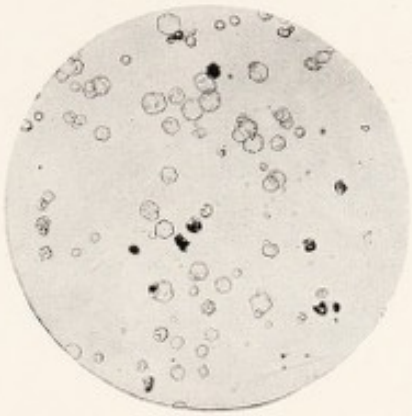


The phosphorus of casein, and probably also of other phospho-proteins, is present as a phosphate ester combined through the hydroxy-group of hydroxy-amino acids, such as hydroxy-glutamic acid, serine, and hydroxy-amino-butyric acid. Most of the phosphate radicals in casein are localised in a relatively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

Although *cysteine* is not a primary hydrolytic product of proteins, 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, which by loss of carbon dioxide, yields *taurine.*

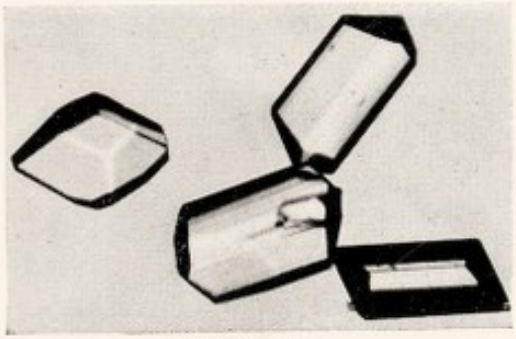
$$HS . CH_2 . CH(NH_2) . COOH \rightarrow HO . SO_2 . CH_2 . CH(NH_2) . COOH$$
$$\rightarrow HO . SO_2 . CH_2 . CH_2 . NH_2.$$

PLATE III

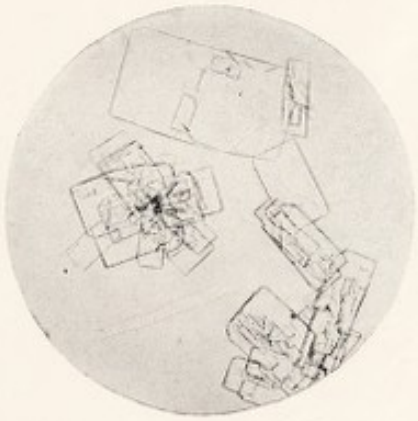


(*a*) Cystine.

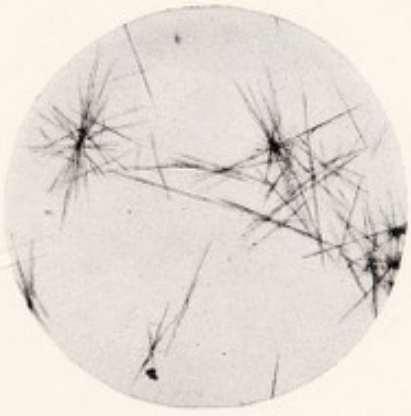
(*b*) Leucine.



(*c*) Glycine.



(*d*) Aspartic acid.



(*e*) Tyrosine.

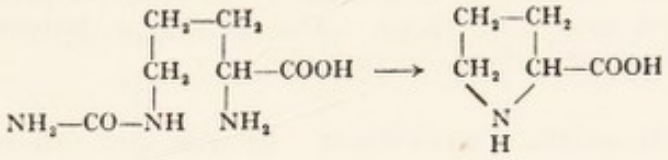
Photomicrographs of recrystallised amino-acids, to illustrate their differing crystal habits. *Magnification* × 45.

[*To face p.* 150.

The *sulphur* of proteins is due to radicals of cystine, long known, and methionine, very recently discovered. Methionine is, however, the more important of the two, since it is an "essential amino-acid," while cystine is not (*cf.* p. 173).

Aspartic acid has an amide derivative, *asparagine*, widely distributed in plants. It gives a blue violet biuret reaction, and has the formula $HOOC . CH_2 . CH(NH_2) . CO . NH_2$.

Citrulline, when heated with concentrated hydrochloric acid, is converted to proline.



Citrulline *Proline*

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

Specific Colour Reactions. The biuret test has already been mentioned (*cf.* p. 146).

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 such proteins as 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 such 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. This reaction is given by the amino-acids tyrosine, phenylalanine and tryptophane, and is characteristic of the phenyl radical C_6H_5 present in these acids.

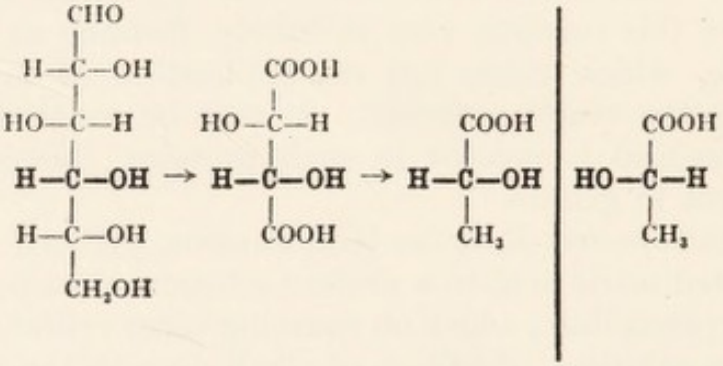
The Glyoxylic Acid Reaction (or Hopkins-Cole reaction) is carried out by mixing with a protein solution in a test-tube an equal volume of solution of glyoxylic acid, $CH(OH)_2 . COOH$, and adding carefully, so that mixture is avoided, a little concentrated sulphuric acid. At the junction between the 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.

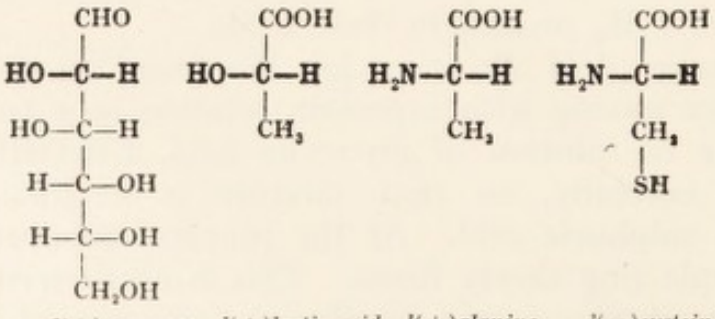
Histidine (and *tyrosine*) give Pauli's *diazo-reaction*, the development of a red colour with diazo-benzene sulphonic acid in presence of mild alkali. On acidification with hydrochloric acid, reduction with zinc dust, and, finally, addition of strong ammonia, a characteristic golden colour is produced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

Sakaguchi has recently found a reaction 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.

Configuration of the Amino-acids. 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 laevo-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 and the actual rotation is indicated by the plus or minus sign prefixed to the name.



d(+)glucose *d(−)tartaric acid* *d(−)lactic acid* *l(+)lactic acid*



d(+)mannose *l(+)lactic acid* *l(+)alanine* *l(−)cysteine*

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 four typical proteins, our present knowledge shows that 100 gm. of these proteins yield the weights in grams of the respective amino-acids shown in Table XI. Many of these figures are derived from colorimetric estimations on the hydrolysates; the amounts of the amino-acids actually *isolated* from the hydrolysates are usually smaller.

TABLE XI. DISTRIBUTION OF AMINO-ACIDS IN PROTEINS

	Egg-albumin	Edestin	Cow Casein	Gelatin
(Ammonia) . .	1·34	—	1·61	0·4
Glycine . . .	0·0	3·8	3·4	25·9
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	1·4	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	8·1	8·8
Histidine . . .	1·4	2·1	2·6	0·9
Tryptophane . .	1·3	2·5	2·2	0·0
Proline . . .	3·56	4·1	7·63	9·5
Hydroxy-proline .	—	2·0	0·23	14·1
Total . .	63·4	97·8	105·1	94·6

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 considerably higher than 100 should be obtained. More perfect figures are available for the protamines, salmine and clupeine, and these, with less complete data for some other protamines, are given in Table XII. (The figures for clupeine are calculated from recent data of Felix and Mager.)

TABLE XII. PERCENTAGES OF AMINO-ACIDS DERIVED FROM PROTAMINES

	Salmine	Sturine	Clupeine	Scombrine
Alanine . . .	trace	trace	3·5	trace
Valine . . .	4·3	—	9·2	—
Leucine . . .	trace	trace	—	—
Serine . . .	7·8	—	3·9	—
Proline . . .	11·0	—	9·2*	trace
Arginine . . .	87·4	58·2	83·1	87
Lysine . . .	0·0	12·0	0·0	0
Histidine . . .	0·0	12·0	0·0	0
Total . .	110·5	82·2	108·9	87

* Including hydroxyproline.

Some part of the discrepancy usually existing between analytical results and theory is possibly to be explained by the exclusion of still undiscovered amino-acids, and of radicals of other compounds that are not amino-acids.

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 :

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

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

and generally :

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

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.*

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

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

Alanyl-glycylalanine

Obviously this procedure should be theoretically capable of indefinite extension.

The actual methods needed in the laboratory to build up simple and more complex *polypeptides* are naturally more complicated. These belong to the realm of pure chemistry. Numerous methods have been devised, and many polypeptides have been synthetised. Some, with 18 or 19 amino-acid units, and molecular weights of over 1,000, have properties at least resembling those of proteoses, and may even give opalescent solutions like those of proteins themselves. They only give the typical colour reactions when they contain radicals of the amino-acids to which these are due, *e.g.*, tyrosine, phenylalanine, and tryptophane.

Within recent years many important polypeptides, and derivatives with non-amino-acid units, have been synthetised, and used extensively in unravelling the enzyme complexes concerned with protein digestion.

When synthetic polypeptides are hydrolysed they break down to amino-acids; as just inferred "peptidases" act on them just as they do on the fragments from normal protein digestion.

A number of di-, tri-, and tetrapeptides have also been isolated from mixtures resulting from *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.

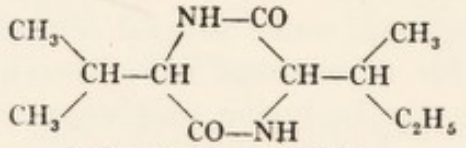
Such compounds as the asparagine-tripeptide yield on hydrolysis free ammonia, and since many proteins also yield a certain amount of free ammonia when hydrolysed, this would seem to suggest the presence of a certain number of acid-amide linkages in the protein molecule.

There is strong evidence that, while the chief linkage in protein molecules is the peptide linkage —CO . NH—, yet actually, this is frequently changed to its *enol* form :

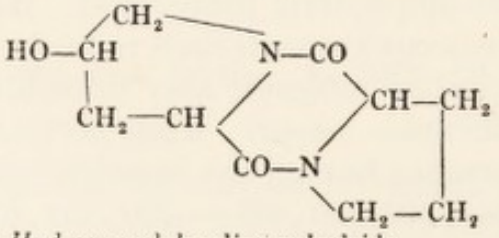
Changes of this kind are known to occur readily in many simpler compounds, and such a change explains the possession by proteins of a considerably greater power to neutralise acids than would be supplied by a few free amino-groups (since the hydroxyl group can react with acids).

Another variation of the peptide linkage can exist, involving condensation to ring structures, the formation of " anhydrides."

Abderhalden emphasises the probable formation of anhydride groups 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 hydroxy-prolyl-proline anhydride, readily soluble in water and alcohol, sparingly soluble in pure ether, and reacting faintly acid to litmus.

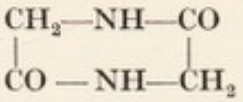


Isoleucylvaline anhydride



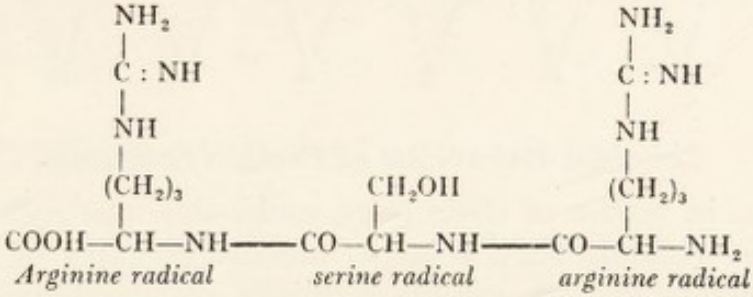
Hydroxyprolylproline anhydride

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. Preformed anhydride linkages probably do occur in the scleroproteins which are especially resistant to the action of digestive enzymes, and of which silk fibroin is a good example.

Table XII. indicates that the simplest proteins, the protamines, are built up from relatively few amino-acids, the basic acids predominating. Kossel believed that these protamines are built up from " protone " units, in each of which were 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 Mager (1937) have shown that clupeine has a molecular weight of 4,470, and is composed of 22 arginine radicals and 11 radicals of mono-amino-acids (2 of alanine, 2 of serine, 3 of valine, and 4 of proline and hydroxyproline, of which 3 are probably proline). Thus one protamine has been almost completely accounted for.

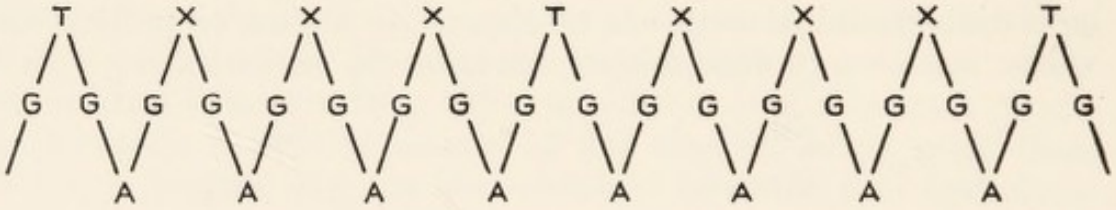
These authors have observed that clupeine unites with insulin and certain other compounds in stoichiometric proportions. Its

compound with haemin (*cf.* p. 218) has catalase properties. Clupeine flavine phosphate also possesses enzymic properties (*cf.* p. 288).

Felix and Mager have made almost as great an advance in the study of *iridine*, the protamine of the rainbow trout (*Trutta iridis*), which is built up from the same amino-acids combined in somewhat similar proportions.

Evidence is accumulating that the amino-acid units in different classes of proteins conform to certain patterns. Thus, according to Block and Vickery, keratins yield histidine, lysine, and arginine in the molecular ratios of 1 : 4 : 12, while the haemoglobins give a corresponding characteristic ratio of iron : arginine : histidine : lysine, equal to 1 : 3 : 8 : 9.

Furthermore, Bergmann is obtaining evidence that there is not an infinitely large series of combinations of amino-acid radicals in the proteins, but that in a long peptide chain each amino-acid radical recurs at constant intervals. He finds, for example, that in silk-fibroin glycine radicals form alternate units, each alanine radical is separated from the next by 3 other units, and each tyrosine radical by 15 other units, so that if *G* is a glycine radical, *A* an alanine radical, *T* a tyrosine radical and *X* an arginine or some other radical, a segment of the molecule of silk-fibroin will have the structure :



Colloidal Behaviour of Protein Solutions

Proteins, in virtue of their large molecules, are colloids. Like many other colloids they tend to form aggregates of molecules, micellae, which do not exhibit ordinary stoichiometric relations in their reactions with acids and salts. But, as Jacques Loeb showed, appropriate conditions of *p*H can be found for each protein, in which it behaves as if composed of single molecules, and reacts with acids and bases like ordinary chemical compounds. According to the actual *p*H it will form dissociable metal proteinates or protein-acid salts, in virtue of its amphoteric properties. Each protein has a definite iso-electric point (*cf.* p. 29), at which it is least soluble, and will precipitate (or crystallise) from solution carrying with it the minimum amount of impurity.

The iso-electric point of a protein can be determined by electro-

phoresis. At that point not only is ionisation minimal, but the ionisation of metal proteinate and of protein-acid salt is equal in degree, so that 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
Oxyhaemoglobin	$1{\cdot}8 \times 10^{-7}$	6·7
Edestin	$1{\cdot}3 \times 10^{-7}$	6·9
Gliadin	1×10^{-9}	9·0

Synthesis of Proteins in Plants

The animal depends on the plant not only for its carbohydrate, but also for the formation of a number 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) :

$$\mathrm{H.C.OH + O:N.OK \longrightarrow H{-}C(=O){-}N(OK){-}H \longrightarrow H{-}C(=N{-}OK){-}OH + O}$$

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 :

$$\mathrm{H.C.OH + H.C.OH \longrightarrow HO{-}C(H){-}C(H){-}OH \longrightarrow CH_2(NH_2){-}COOH}$$

Baly has actually obtained α-amino-acids by such procedures.

Enzymes Concerned in the Hydrolysis of Proteins

The old classification of the proteolytic enzymes found in the digestive juices into pepsin, trypsin, and erepsin, has proved

quite inadequate and inaccurate. Exhaustive studies by Bergmann and others have led to division of the proteolytic enzymes into two groups, *proteinases*, which attack the protein molecule but not simple polypeptides, and *peptidases*, which attack polypeptides of various types and degrees of complexity.

Proteinases. The chief proteinases are pepsin, trypsin, chymotrypsin, kathepsins, papains, and bromelin. Along with these rennin will be included for convenience.

Pepsin has been obtained in pure crystalline form and is a protein (*cf.* p. 48). The "chief" cells of the gastric mucosa form its precursor *pepsinogen* or *pro-pepsin*, which is activated by dilute hydrochloric acid. This activation is due essentially to an adequate concentration of hydrogen ions.

Pepsin splits native proteins of ingested food to the large fragments termed proteoses and peptones, but does not carry hydrolysis further to any appreciable extent. (In certain prolonged experiments pepsin has been shown to set free small amounts of free amino-acid.) It liberates about 20 per cent. of the amino-nitrogen of proteins, *i.e.*, it splits about one-fifth of the peptide linkages. It does not act on the simpler polypeptides. The optimum *p*H for its action is about 2, but varies with the substrate.

The limited degree of pepsin action suggested that it did not act on the ordinary peptide linkage, since otherwise there seemed no reason for such limit in its action. But no evidence of other type of linkage could be found, and it has been postulated that pepsin needs the presence of a fairly long chain of peptide linkages in order to split one of them.

Both *trypsin* and its precursor *trypsinogen* have been obtained in pure crystalline condition, and shown to be proteins (*cf.* p. 48). Trypsinogen is secreted into the pancreatic juice, and is activated by *enterokinase* to trypsin. The nature of enterokinase, and the manner of its action on trypsinogen, are not known. It is usually stated to be a constituent of intestinal juice, though more recently the theory has been propounded that a precursor, pro-kinase, is present in the pancreatic juice, and is activated by the cells of the intestinal mucosa, or becomes active on long standing. This would explain the fact that pancreatic juice, on standing, also develops active trypsin.

The pancreas also elaborates a second proteolytic zymogen, chymotrypsinogen, which is converted to the active enzyme *chymotrypsin* by the action of trypsin.

Trypsin and chymotrypsin have similar activities. Trypsin will digest casein, edestin, gelatin, and peptone, but is without

action on the smaller peptides. Its optimum *p*H for digestion of casein is between 8 and 9. In the course of its action free amino-acids are split off, so that it can act on terminal peptide linkages.

Kathepsins are the proteinases in the cells of animal tissues; *papains* are plant proteinases, and are typified by papain of the melon tree (*Carica papaya*). *Bromelin* of the pine-apple has very similar activity. The optimum *p*H values for the activities of these enzymes are between 4 and 7.

Rennin is a specific enzyme of the gastric juice of the young animal, and is especially present in the fourth stomach of the calf. (Tauber claims it is only found in the calf's gastric secretion.) The long continued controversy as to the possible identity of pepsin and rennin was settled with the production of crystalline pepsin, and the demonstration that this pure pepsin digested rennin and destroyed its activity at the same time.

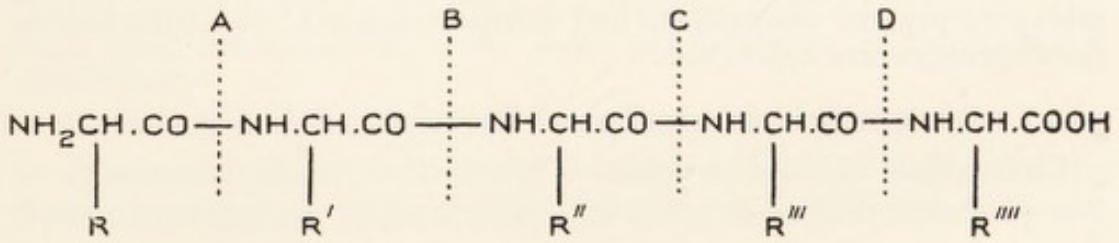
Rennin has the specific property of clotting milk. This process is usually considered to be due to the hydrolysis of casein to paracasein; in the presence of calcium ions paracasein is precipitated as the calcium salt and holds within its meshes the other constituents of milk as a clot.

Proteinases such as pepsin and trypsin will also clot milk. In fact trypsin hydrolyses casein so rapidly that unless very dilute solutions of the enzyme are used the paracasein is digested before it can be precipitated and no clot forms. The essential difference between the action of rennin and of proteinases is the much more powerful effect of rennin in clotting milk, and its inability to produce other enzymic effects.

Rennin is a thioproteose.

Peptidases. A number of these enzymes are known. Others will probably be discovered. Pancreatic juice contains *carboxypeptidase*. Intestinal juice contains a number of peptidases, the sum of whose actions makes up the effects which used to be attributed to "erepsin." They include an *aminopeptidase*, a *dipeptidase*, a *prolinase*, and a *prolidase*.

The actions of these enzymes can best be considered in relation to such a polypeptide as is exemplified by the general formula—



Aminopeptidase acts at a peptide linkage where there is a unit with a free α-amino group, as at *A* in the above formula. Having split off this unit, the adjacent one is open to attack by this enzyme. Carboxypeptidase acts at a peptide linkage where there is a free carboxyl group, as at *D* in the above formula. It hydrolyses the polypeptide at this point, freeing another —COOH group, and can then attack the adjacent linkage (at *C*). Dipeptidase hydrolyses ordinary dipeptides such as leucylglycine, but cannot attack those containing proline (and presumably hydroxyproline) radicals. Prolinase attacks such compounds as prolylglycine, while prolidase hydrolyses such as glycylproline.

Digestion and Absorption of Proteins

As the food mixture in the stomach is gradually permeated by the acid gastric juice, the proteins, in virtue of their amphoteric properties, unite with hydrochloric acid to give protein hydrochlorides, and so delay somewhat the acidification of the mixture. (Any regurgitation from the intestine accentuates this delay.) When, however, the acidity is sufficiently great, pepsin, already activated in the secreted juice, attacks the proteins, splitting them to soluble proteoses and peptones.

Fluid material passing to the duodenum therefore contains proteoses and peptones, and undigested proteins. Under the combined attack of trypsin and chymotrypsin these are reduced to polypeptides, free amino-acids being liberated in the process. The various peptidases come into action and split the polypeptides, so that ultimately there is almost complete conversion to amino-acids.

The amino-acids are absorbed through the mucosa of the villi and pass by the portal circulation to the liver, and on by the general circulation to all the tissues of the body.

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.

Intermediate Metabolism of Proteins and Amino-acids

Circulation of Amino-acids. Our first accurate knowledge of the presence of amino-acids in blood, and of variations in their

content in relation to food absorption, dates back to 1912, when Van Slyke adapted the reaction of these acids with nitrous acid (*cf.* p. 150) to the measurement of small amounts of amino-acid nitrogen. (In such measurements only the α-amino-acid nitrogen is determined, and the determinations apply only to the sum of, and not to the individual amino-acids.)

Van Slyke showed that during fasting the amino-acid nitrogen of the blood of the dog was about 4 mg. per 100 c.c. After a meat meal it rose to 10 mg. per 100 c.c., and then fell rapidly to the fasting figure, during which time there was *a rise of blood urea.*

During their passage through the liver amino-acids, unlike glucose, are relatively little abstracted. Thus in one experiment Van Slyke found that the fasting blood of the femoral artery of a dog contained 3·7 mg. of amino-acid nitrogen per 100 c.c. ; after a meal this figure increased to 8·6 mg., while the corresponding figure for blood from the portal vein was 9·5 mg., so that the liver only abstracted 0·9 mg. per 100 c.c., or 15·5 per cent. of the quantity absorbed.

Injection experiments with amino-acids show that they are abstracted by many tissues. Thus in one experiment, half an hour after injection of amino-acids corresponding to 4 gm. of amino-acid nitrogen, blood amino-acid nitrogen had increased by 41 mg. per 100 gm. blood, that of liver 62 mg. per 100 gm. liver, that of muscle 27 mg., and that of kidney tissue 61 mg. per 100 gm. of these tissues.

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 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, 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 ?

The amino-acids taken up by tissues are in part anabolised to compounds essential for the animal, while the excess is in part transformed to glucose, and in part oxidised to waste products.

Anabolic Processes. Tissue cells build up amino-acids to specific proteins, new material in the growing animal, and material for repair of tissue waste in both the growing animal and the adult. The specific tissue proteins so formed frequently exhibit marked chemical differences. Thus those of fibrous tissue are 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.

It seems most probable therefore that each of these tissues possesses its own specific enzymes. These may well be identical with the tissue kathepsins responsible for tissue autolysis (*cf.* p. 161 and Chapter XV, p. 330) ; the normal and primary action of these kathepsins during life may well be synthesis.

In addition to formation of proteins many simpler compounds needed by the organism are formed from amino-acids. Thus the thyroid forms di-iodotyrosine and thyroxine from tyrosine. The adrenal medulla forms adrenaline from tyramine, which is probably produced from tyrosine in the kidneys. Many tissues form melanins from tyrosine (*cf.* p. 171). The tripeptide glutathione, glutamyl-cysteyl-glycine, is present in many tissues, formed from its constituent amino-acids. Carnosine and creatine are formed, the first from histidine, the second probably from arginine (*cf.* pp. 249, 247). The liver forms taurine from cysteine (*cf.* p. 150) and glutamine (for detoxication purposes) from glutamic acid (*cf.* p. 313).

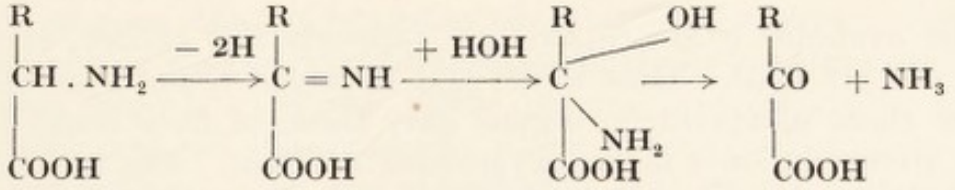
Catabolic Processes. The catabolism of amino-acids essentially

involves their deaminisation, with production of ammonia, and utilisation of the partially oxidised residue for formation of glucose or other useful products, or its further oxidation to carbon dioxide and water.

Formation of Ammonia. If the pulp of liver or various other organs is mixed with solutions of many of the amino-acids, the ammonia content of the mixture increases. Although definite evidence exists that such organs as the kidney possess the power of deaminisation, the studies of Mann and his associates on the effect of the removal of the liver in the dog indicate clearly that in the mammal the liver is almost exclusively the site of deaminisation. After removal of the liver no further deaminisation occurs, and the production of urea (formed from ammonia) ceases. If amino-acids are injected into the blood, they can be recovered unchanged in amount from blood tissues and urine several hours later.

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.

In the normal animal the amount of ammonia in the circulating blood is 0·1 mg. per 100 c.c. or less, and this is in itself an argument against any extensive deaminisation elsewhere than in the liver, since, as will be shown immediately, ammonia is converted to urea in the liver alone; were appreciable amounts of ammonia to be formed in other tissues its transference to the liver would involve a much higher blood content. The chief process by which ammonia is formed in the liver is probably through a series of changes suggested by Knoop and Neubauer.



Other series of changes are possible. Thus many moulds and bacteria possess a specific enzyme aspartase, which converts aspartic acid to fumaric acid and ammonia:

$$HOOC \cdot CH_2 \cdot CH(NH_2) \cdot COOH = HOOC \cdot CH : CH \cdot COOH + NH_3$$

Reactions of this type may take place to a minor extent in the mammalian liver.

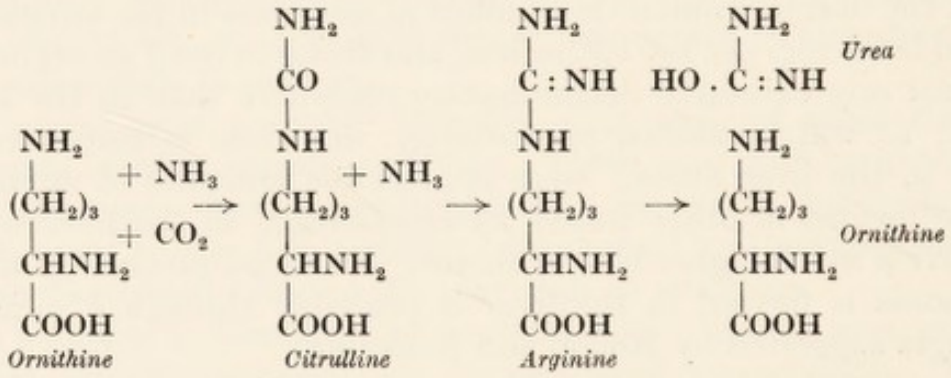
Since the tissues all rapidly abstract amino-acids from blood following a meal, it must be supposed that the unwanted excess is slowly given back to the blood and removed by the liver.

As a result of deaminisation ammonia and deaminised acids remain to be disposed of.

Formation of Urea. The experiments of Mann, and such observations as those of Rabinowitch, already quoted (p. 165), and of Krebs and Henseleit (see below), suggest that the liver is practically the exclusive site of urea production.

Various mechanisms have been suggested in the past to explain conversion of ammonia to urea. The oldest suggested that the stages were ammonium carbonate, ammonium carbamate, urea, a molecule of water being lost at each step. Werner suggested that cyanic acid was the intermediate stage. Neither of these theories proved completely acceptable.

It has long been known that the liver contains an enzyme arginase which decomposes arginine to urea and ornithine (*cf.* p. 147). The recent discovery of citrulline by Wada led Krebs and Henseleit to suggest that a cycle of changes from ornithine through citrulline to arginine, and then once more to ornithine, resulted in the conversion of two molecules of ammonia to one of urea, and that this cycle is the actual mechanism for the production of urea in the liver.



The available evidence strongly supports this theory, though it is not yet final. Thus Krebs and Henseleit found that of the tissue slices of seventeen organs only those of liver could form urea from ammonia in an oxygenated medium. *The action was markedly catalysed by both ornithine and citrulline.*

Supporting evidence is the fact that birds, whose liver contain no arginase, form very little urea.

The enzymes which induce the changes from ornithine to citrulline and from citrulline to arginine are not yet known.

Origin of Urinary Ammonia. The work of S. R. Benedict and of others has proved that urinary ammonia is not derived directly

from blood ammonia, but is formed in the kidneys from urea, as a mechanism for conserving blood base.

$$CON_2H_4 + HOH = 2NH_3 + CO_2$$
$$NaX + NH_3 + HOH = NH_4X + NaOH$$

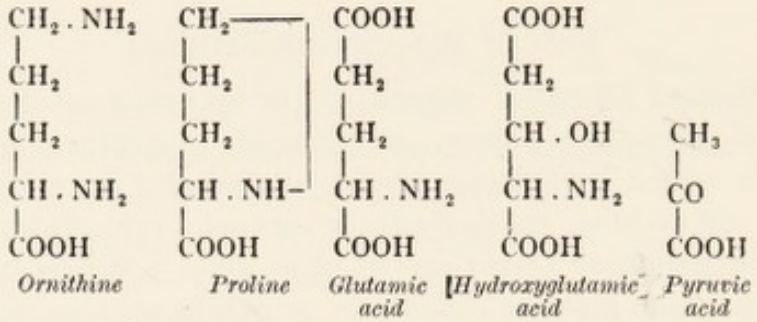
Fate of Deaminised Residues

Production of Glucose. Much of the experimental evidence in support of *glucogenesis* from amino-acids is derived from studies of animals which have been rendered diabetic by removal of the pancreas, or whose carbohydrate stores have been markedly depleted by repeated injections of phloridzin. When such animals are starved the glucose which is continuously excreted must, within a few days, have its origin in other than carbohydrate sources. When proteins are fed them, increased amounts of glucose and of urea are excreted, and the ratio of the increases suggests that 100 gm. of mixed proteins can yield a maximum of 58 gm. of glucose.

By feeding individual amino-acids, especially to the diabetic animal, it can be ascertained in the same way which of these can yield glucose, and it has been shown that glycine, alanine, serine, cysteine (and cystine), aspartic acid, glutamic and hydroxyglutamic acids, proline and hydroxyproline, and arginine (and ornithine) can do so. Hence at least ten of the amino-acids from hydrolysed proteins are glucogenic.

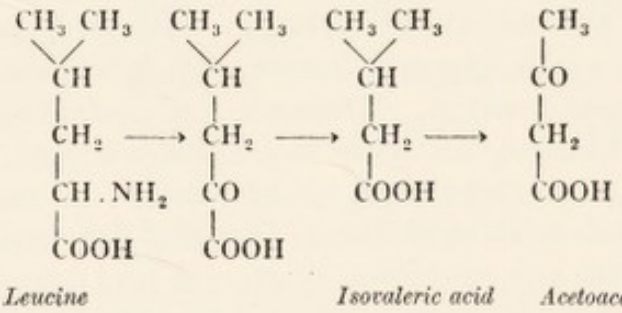
The yield of glucose from the amino-acids that can produce it always approximates to that obtainable if three carbon atoms of the amino-acid were involved. Thus alanine, with three carbon atoms, yields glucose quantitatively. Aspartic acid, with four carbon atoms, yields glucose corresponding in amount 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.

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.



Production of Acetoacetic Acid. It has been shown by similar methods to those indicated in the previous section that leucine, phenylalanine, and tyrosine can yield acetoacetic acid. (There is doubtful evidence for isoleucine and histidine.) Such acids are therefore ketogenic, while the ten yielding glucose are anti-ketogenic (*cf.* p. 139). There is some evidence to suggest by what paths acetoacetic acid is formed from these amino-acids.

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 :



Phenylalanine and tyrosine produce acetoacetic acid by a very different route.

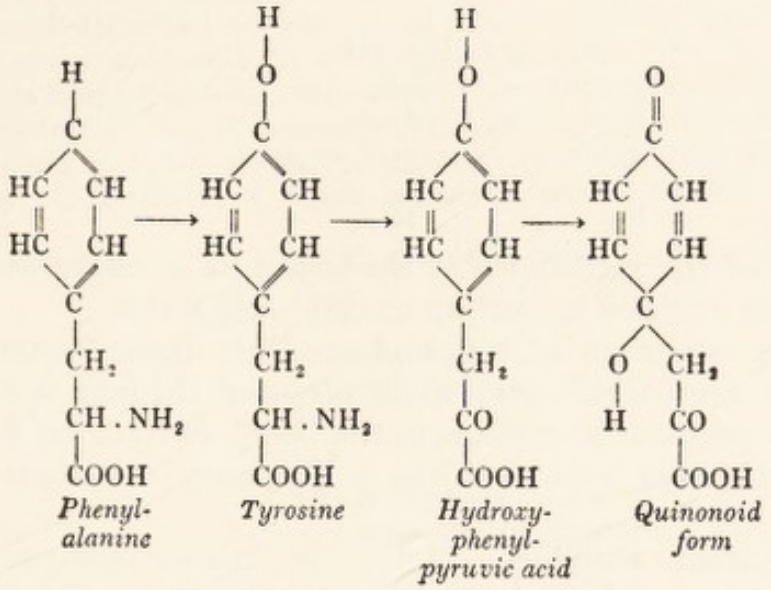
Most compounds with a *benzene nucleus* are not completely oxidised in the body. This benzene nucleus is not easily broken, and usually only the side-chain is oxidised, and then only completely if the *first* carbon atom adjacent to the ring can be oxidised. When benzoic acid is fed, hippuric acid is excreted (*cf.* p. 313). When phenylpropionic acid is fed, hippuric acid is excreted, and the probable series of changes is :

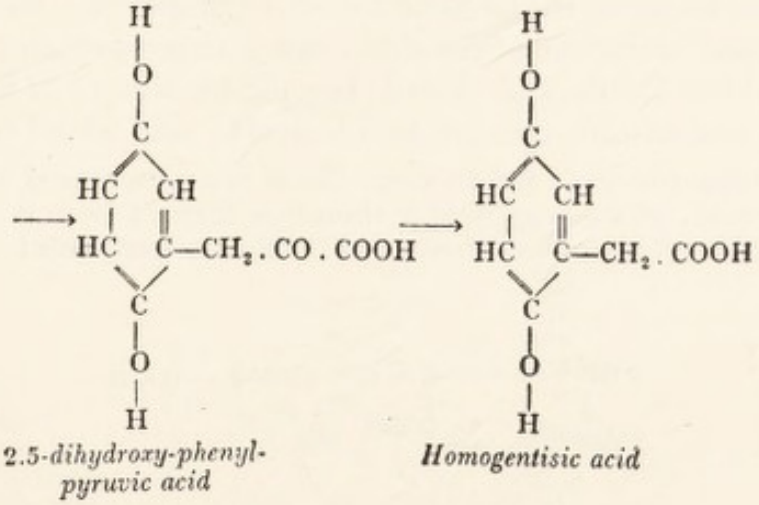
$$C_6H_5 . CH_2 . CH_2 . COOH \longrightarrow C_6H_5 . CO . CH_2 . COOH \longrightarrow C_6H_5 . COOH \longrightarrow C_6H_5 . CO . NH . CH_2 . COOH$$

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 phenylacetyl-glutamine. 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.

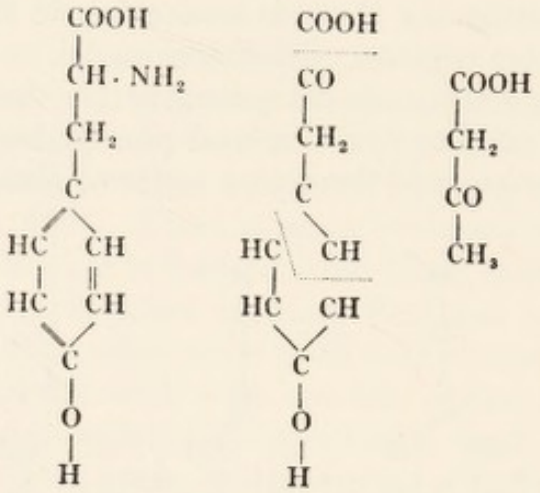
In the condition known as *alkaptonuria* the human organism cannot completely oxidise tyrosine and phenylalanine, but forms from them *homogentisic acid* through a series of changes which are believed to be :





It was originally considered that the formation of homogentisic acid in the alkaptonuric 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 alkaptonuric* 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 aceto-acetic acid :

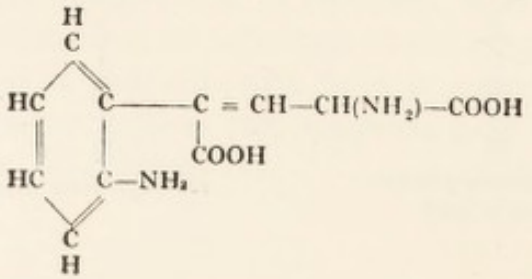


The fate of the remainder of the molecule is unknown. Some or all of it is oxidised to carbon dioxide and water.

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.

Fate of other Residues. 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. 326).

When tryptophane is fed to dogs there is an increased excretion of kynurenic acid, which is evidently therefore formed from it (*cf.* Chapter XIV., p. 325). Kotake has shown (1931) that a compound



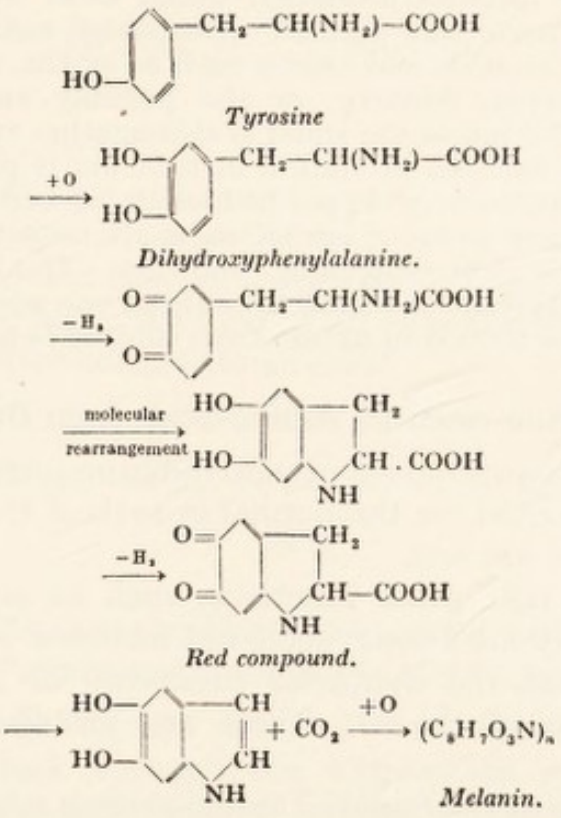
which he terms *kynurenin*, is an intermediate step in this formation. Most, if not all, of the change takes place in the liver. The bile of dogs contains kynurenic acid often to a greater extent than their urine. When injected, it is recovered quantitatively, suggesting that it is one end product of tryptophane catabolism (in the dog). There is also some evidence that kynurenin is utilised in the formation of the urinary pigment urochrome (*cf.* 326).

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. Abderhalden has confirmed this work (1937).

Melanin Formation, The term *melanin* is applied to those black insoluble pigments occurring widely distributed in nature and exemplified by the retinae 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 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 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. 171 indicates.

Very important also is the work of Bloch, who has studied the distribution of an oxidase, which, since it oxidises *di*-(hydr)*oxyphenyl-a*line, 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.

Since the mammalian organism does not contain a tyrosinase it must be assumed that in mammals "dopa" is first formed from tyrosine, and is then converted by dopa-oxidase to melanin by the steps just indicated. It has been shown that ultra-violet light can bring about the change from tyrosine to dopa in such sites as the skin.

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.

Essential and Non-essential Amino-acids from Dietary Proteins

Of the twenty-two amino-acids derived from proteins of a normal diet, ten are essential for the normal growth of the rat, while the remaining twelve are not.

It is possible that other functions, such as reproduction and detoxication, may need some acids not included in the ten. It is also possible that the synthetic capacities of other mammals differ from those of the rat. These are problems still needing solution.

Our knowledge of the essential amino-acids is very largely due to

W. C. Rose. (His studies of this problem led him to the isolation of threonine.) In his studies, rats are fed carbohydrate, fats, inorganic salts, vitamins, and selected lists of pure amino-acids.

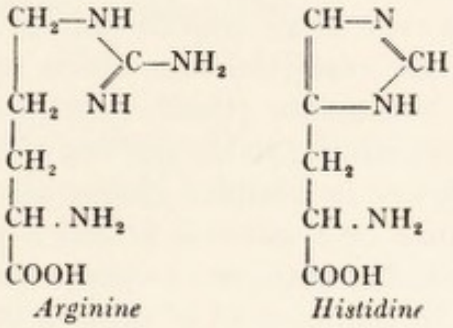
The ten essential acids are lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine, and arginine (*Cf.*, however, p. 362, footnote.)

The twelve non-essential acids are glycine, alanine, serine, norleucine, aspartic, glutamic, and hydroxyglutamic acids, proline and hydroxyproline, citrulline, tyrosine, and cystine.

Arginine can be synthetised by the rat (from histidine) but not at a rate sufficient to keep pace with the demands for normal growth. When it is not fed, however, no loss of weight occurs. Omission of any one of the other nine essential acids leads to loss of weight.

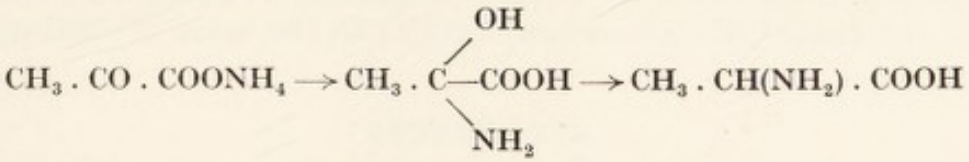
While cystine is not essential, if methionine is fed in sub-optimal dosage addition of cystine stimulates growth.

Tyrosine can be formed in the body from phenylalanine, and citrulline from ornithine and thus from arginine. Arginine can evidently be formed at a slow rate from histidine. Their close chemical relationship is evident from the following formulae.



Arginine *Histidine*

Some idea of the potential mechanisms employed in synthetising many of the twelve non-essential acids in the body is gained from the experimental fact that such acids as alanine can be synthetised by perfusing the surviving liver of an animal with the ammonium salts of the corresponding ketonic acids.



Notes on Diseased Conditions Associated with Abnormal Protein and Amino-acid Metabolism

Proteinuria. In various diseased conditions proteins of the blood plasma leak through the kidneys to give a condition commonly termed *albuminuria*, but more correctly proteinuria,

since the proteins found in the urine include globulin as well as albumin. On heating such urines all stages from a faint opalescence to a heavy precipitate are obtained, depending on the amount of protein present. Such proteinuria is due, not to abnormal protein metabolism, but to increased permeability of kidney membranes, but its existence must be borne in mind in considering Bence-Jones proteinuria.

Bence-Jones Proteinuria. In diseases involving bone-marrow, such as multiple myeloma, a peculiar protein is continuously excreted in the urine. This is named after Bence-Jones, who reported the condition in 1847. This protein, unlike the plasma proteins, is precipitated from urine at temperatures between 40° and 60° C., while when the temperature is raised still higher, it redissolves. Its molecular weight is about 35,000 so that its molecule is approximately the same size and weight as those of egg-albumin and insulin.

Alkaptonuria and **melanuria** have already been mentioned (pp. 169, 171). A still rarer example of aberrant tyrosine metabolism is the condition termed **tyrosinosis** in which *p*-hydroxyphenylpyruvic acid (p. 169) is excreted. In this condition the ability to handle tyrosine is even more limited than in the alkaptonuric.

Cystinuria is a rare condition in which cystine crystals are present in urinary sediments (their microscopic appearance in these sediments is very similar to the picture of cystine in Plate III.). Still more rarely kidney or bladder stones are present, composed of cystine. The cause of cystinuria is still a puzzle, accentuated by the fact that while the amount of cystine excreted in the urine bears some relation to the amount of protein in the diet, yet when pure natural cystine is fed the cystinuric he can catabolise that correctly.

Undue excretion of amino-acids occurs in various conditions, especially in diseases involving marked damage to the liver; in such cases the less soluble acids such as leucine and tyrosine are frequently observable in urinary sediments. These two amino-acids also frequently accompany cystine in the urine of cystinurics.

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CHAPTER VII

THE NUCLEIC ACIDS AND THEIR DERIVATIVES

INTRODUCTION

CELL nuclei contain proteins in combination with nucleic acids, *nucleoproteins*. The nucleoproteins of fish sperms were among the earliest of these compounds studied. These protamine-nucleinates are unions of strongly basic and strongly acid compounds, are, in fact, organic salts. In other nucleoproteins the protein portion is frequently a histone.

The nucleoproteins from cell-nuclei of different tissues differ chiefly in their protein radical. They yield only two nucleic acids, one derivable from animal tissues, the other from plant tissues. The amount of nucleoprotein present in any tissue is proportional chiefly to the richness of that material in cell nuclei. Thymus and yeast are excellent sources, so that " animal " and " plant " nucleic acids are often termed " thymus " and " yeast " nucleic acids. Thymus nucleoprotein is a nucleo-histone.

Nucleoproteins are acid in character, insoluble in water, readily soluble in dilute alkali and in dilute mineral acids. They are precipitated from weak alkaline solutions by acetic acid, and redissolve (with difficulty) in excess of that acid. They are easily split to protein and nucleic acid by treatment with acid or with alkali. The " salt " linkage is easily broken.

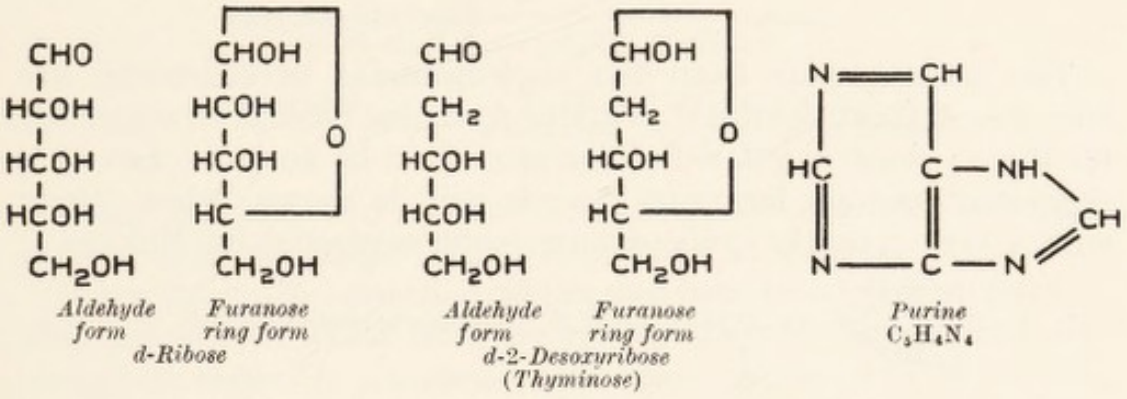
Yeast nucleoprotein can be extracted from yeast by grinding it up with a little water and ether (the latter to kill the yeast cells), extracting with 0·4 per cent. sodium hydroxide, filtering, and adding to the filtrate dilute hydrochloric acid until it is just acid. The nucleoprotein is precipitated. 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.

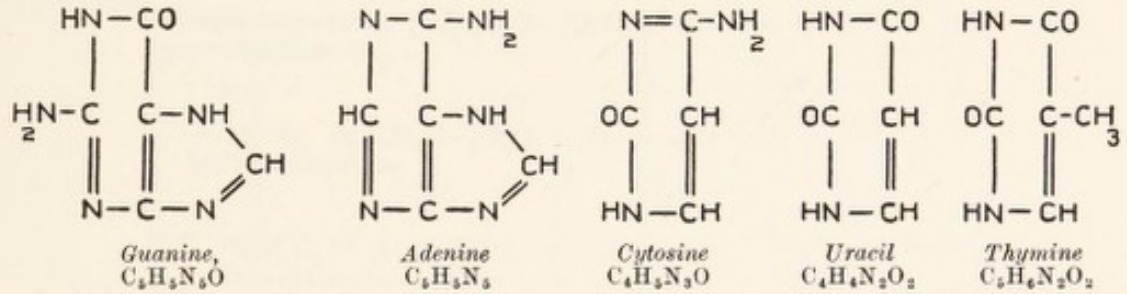
Chemistry of the Nucleic Acids

When yeast nucleic acid is hydrolysed with mineral acids six compounds are set free, phosphoric acid, the pentose *d*-ribose

(*cf.* p. 85), two *purine* bases, guanine and adenine, and two *pyrimidine* bases, cytosine and uracil. Thymus nucleic acid similarly hydrolyses to phosphoric acid, thyminose (*d*-2-desoxyribose), guanine and adenine, and cytosine and thymine.

The only qualitative difference in these products is therefore the substitution of desoxyribose for ribose, and of thymine (methyl-uracil) for uracil. These differences are seen in the following formulae. That of purine is added for contrast, and it will be seen that the six-atom ring is common to purines and pyrimidines and occurs also in vitamin $\mathbf{B_1}$, while the five-atom ring of purines is the iminazole ring of histidine (p. 148), and creatinine (p. 245).





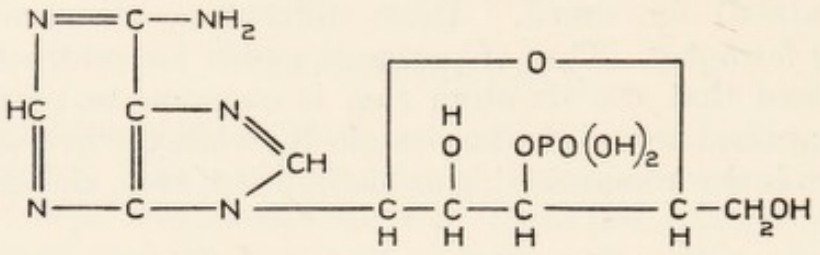
When yeast nucleic acid is hydrolysed, not with mineral acids, but with very dilute ammonia for one hour at 115° C., four *nucleotides* are obtained, adenylic, guanylic, cytidylic, and uridylic acids. Stronger ammonia and longer hydrolysis sets free phosphate and four *nucleosides*, adenosine, guanosine, cytidine, and uridine, the *ribosides* (ribose glucosides) of adenine, guanine, cytosine, and uracil. Acid hydrolysis of the nucleotides liberates ribose phosphate and the purines and pyrimidines. Hence the structure of a nucleotide is indicated by

Purine or pyrimidine radical } —ribose radical—phosphate.

Thymus nucleic acid has not yet been successfully hydrolysed to four nucleotides. The two pyrimidine nucleotides have been

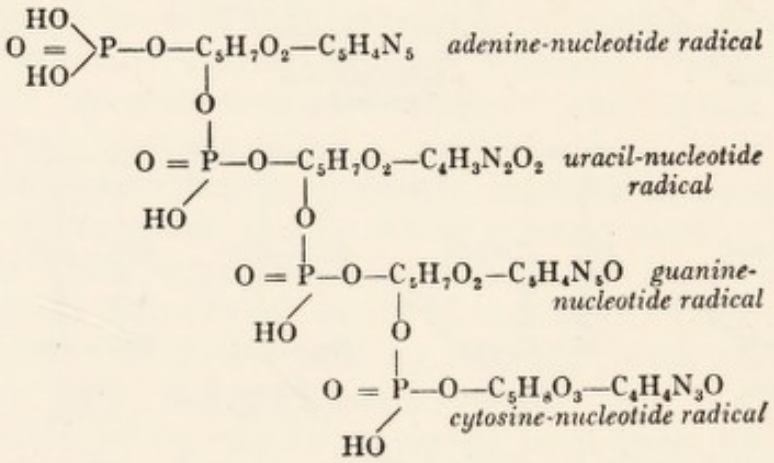
obtained from hydrolysates, and there is sufficient evidence that two purine nucleotides are also linked in the nucleic acid. In these nucleotides the pentose radical is of course that of desoxyribose.

Intensive study has revealed the actual constitutional formulae of some of these nucleotides. Yeast adenylic acid is, for example

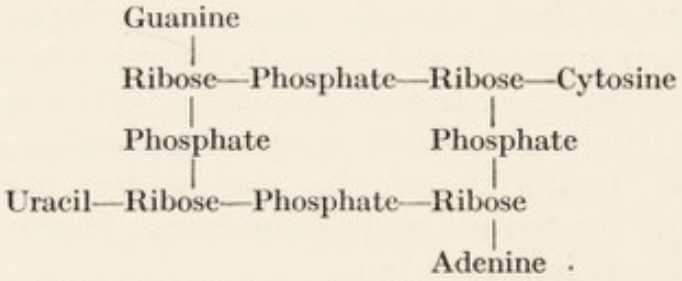


Adenine —— *Ribose Phosphate*

The nucleic acids from the nucleoproteins of cell-nuclei are therefore tetranucleotides. Various formulae have been suggested for them; none is yet definitely proved to be correct. Levene's suggested formula for yeast nucleic acid is shown below, along with a very recently cyclic arrangement suggested by Makino.



Levene's suggested formula for yeast nucleic acid

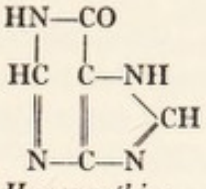


Makino's suggested arrangement for yeast nucleic acid

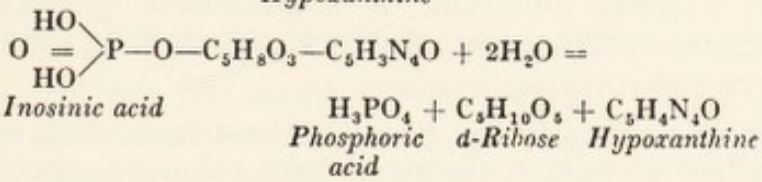
There is a considerable amount of evidence that non-nuclear animal tissues, as for example that in the pancreas, contain a nucleic acid, possibly a pentanucleotide, in which the carbohydrate radical is that of *d*-ribose.

Nucleotides, Nucleosides, and Purines which occur free in Animal and Plant Tissues

Liebig isolated *inosinic acid* from meat extract in 1847. It is a constant and characteristic constituent of muscle tissue. 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



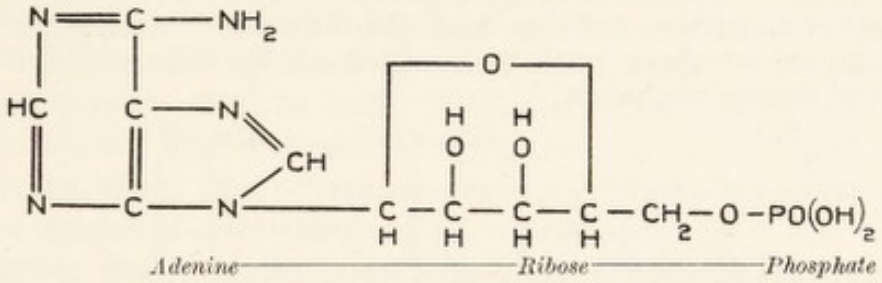
Inosinic acid

$$H_3PO_4 + C_5H_{10}O_5 + C_5H_4N_4O$$

Phosphoric acid — *d-Ribose* — *Hypoxanthine*

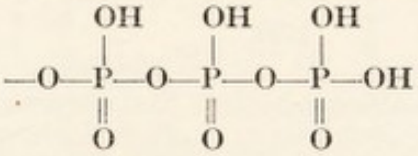
When inosinic acid is heated with ammonia under pressure, *inosine*, a nucleoside, $C_5H_9O_4$—$C_5H_3N_4O$, is formed.

Recently *muscle adenylic acid* has also been isolated from muscle. This differs from yeast adenylic acid in the position at which the phosphate radical is attached to the ribose radical, and its formula is probably



Adenine — *Ribose* — *Phosphate*

Muscle adenylic acid unites with more phosphoric acid to form a di- and a tri-phosphate; the latter, *adenylpyrophosphoric acid*, plays an important *rôle* in muscle respiration (*cf.* p. 292). In it Lohmann has shown that the phosphate radicals are linked together :



Equally important, and still more complex is the adenine-pyridine-nucleotide discovered by Warburg and Christian in the red blood cells of horse blood, and shown to be widely distributed in animal tissues, and to be present in yeast. This compound functions in transporting hydrogen, and can act in conjunction with Warburg's yellow respiratory enzyme (*cf.* p. 288). Its formula is

$C_{21}H_{28}N_7P_3O_{17}$, and it is believed to be nicotinic acid amide—ribose—O . PO (OH) . O . PO (OH) . O—ribose—adenine. Hydrogen is taken up by the pyridine ring in the nicotinic acid amide radical (p. 286).

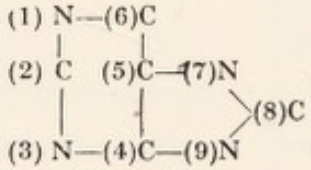
An adenylic acid has been prepared in crystalline form from pig's blood. It appears to be present in normal whole blood to the extent of from 25 to 35 mg. per 100 c.c. Human blood contains amounts of the same order. That of the cat, dog, guinea-pig, and rat, varies from 7 to 12 mg., duck blood contains over 30 mg., and pigeon blood from 60 to 80 mg. per 100 c.c. It is probably identical with muscle adenylic acid, and may play a *rôle* in connection with the adenine pyridine nucleotide.

Vernin, a plant glucoside, is a guanosine (guanine-riboside). *Vicine*, from vetch meal, is a compound of *d*-glucose and 4,6-dioxy-2,5-diaminopyrimidine.

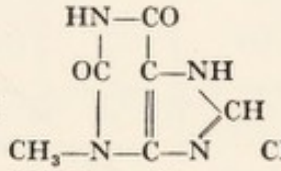
An adenine nucleoside has been prepared from yeast which Suzuki and his collaborators have shown contains sulphur in the sugar radical, the sugar being a *methyl-thio-pentose*.

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.

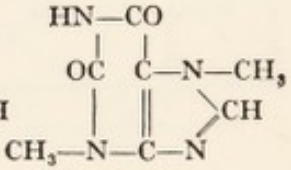
The purines of plants are found, as is to be expected, in such parts of the plants as are richest in nucleoprotein. The three most important are methyl-xanthine, caffeine and theobromine. Assistance in the nomenclature of these purines is obtained by the use of Fischer's numbered purine-nucleus :



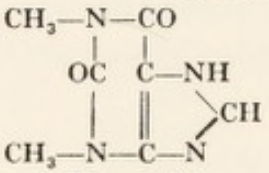
Purine-key



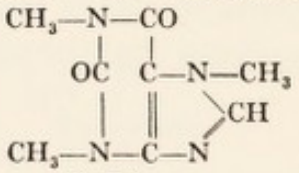
Methyl-xanthine
(3-methyl-
2, 6-dioxypurine)



Theobromine
(3, 7-dimethyl-
2, 6-dioxypurine)

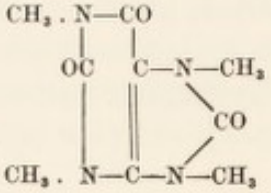


Theophylline
(1, 3-dimethyl-
2, 6-dioxypurine)



Caffeine
(1, 3, 7-trimethyl-
2, 6-dioxypurine)

Johnson has very recently isolated tetramethyl-2, 6, 8-trioxypurine from tea.



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.

Enzymes Concerned in the Hydrolysis of Nucleic Acids

It is probable that the acidity of the gastric juice is sufficient to set nucleic acid free from nucleoprotein. Gastric and pancreatic juices contain no enzyme capable of hydrolysing nucleic acids. The intestinal juice and the cells of the intestinal mucosa contain several enzymes which by successive action split them to nucleosides.

A specific *polynucleotidase* occurs in the intestinal juice of the dog, which hydrolyses thymus nucleic acid, and hence has been termed *thymonucleinase*. Its action is relatively slow.

A non-specific *phosphatase* has been extracted from the intestinal mucosa of the calf and the dog, which hydrolyses nucleotides and other phosphoric esters as well. Nucleotides are rapidly converted to nucleosides. The nucleosides from thymus nucleic acid are all absorbed as such except adenine desoxyriboside. A *deaminase* removes its amino-group before absorption occurs.

A similar series of enzymes has been shown to act on yeast nucleic acid in the rabbit's intestine.

Non-specific enzymes capable of acting on nucleotides are present in various tissues. Thus both kidney and intestinal phosphatases hydrolyse glycerophosphate, hexose diphosphate, and muscle adenylic acid.

Non-specific phosphatases split off all three phosphate groups from adenylpyrophosphate, but Lohmann has found that washed crayfish muscle splits off only one phosphate group, leaving adenosine diphosphoric acid, so that this tissue seems to contain a specific *adenylpyrophosphatase*.

Preparations of nucleotidases, capable of hydrolysing nucleotides, have been prepared from dog and cat pancreas, and from rabbit kidney. Whether these are specific, or are merely non-specific phosphatases, remains to be determined.

Various deaminases are known. Schmidt obtained two from rabbit liver tissue, one of which would act on guanylic acid, the other on guanosine and guanine.

Digestion and Absorption of Nucleic Acids

From what has been written in the preceding section it would seem probable that nucleoprotein of the diet is broken up to its constituents by non-enzymic means. The nucleic acids are then split by specific nucleinases of the intestinal juice to nucleotides, and these are split further to nucleosides by a phosphatase. Part of the nucleosides is absorbed unaltered, and passes to the portal blood. The adenine nucleosides are deaminised before absorption.

At some later stage absorbed nucleosides are split to pentoses and purines or pyrimidines.

Metabolism of Nucleic Acids and their Hydrolysed Products

Anabolism. There is no evidence to show that the animal organism is able to utilise any of the nucleosides, purines, pyrimidines, or pentoses which are absorbed following digestion of nucleic acids. As we have seen (p. 102), pentoses absorbed from the intestine do not appear to be utilisable by the mammalian organism, and if they reach the circulation they are excreted unchanged. The non-identity of yeast and muscle adenylic acids, although they are constructed from identical units, strongly suggests that the animal forms all its own nucleotides.

The animal organism is certainly 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 large amounts of protamine nucleinate for the production 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.

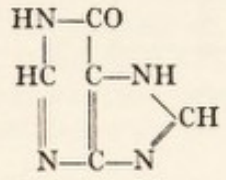
Numerous growth experiments with purified foodstuffs, carried out by Osborne and Mendel, Abderhalden and others, have repeatedly demonstrated that purines are not essential constituents of the diet.

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.

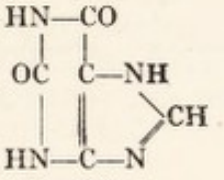
There is a certain amount of experimental evidence that the body can use as precursors of purines the amino-acids arginine and histidine. Histidine seems to be the more probable precursor. Thus Calvery has shown that in the developing hen's egg (in which, of course, purines are being formed) the content of arginine remains stationary, whilst that of histidine markedly decreases.

Catabolism. As already stated, pentoses are probably excreted unchanged. Phosphate is added to the stock of circulating phosphate, and is utilisable. We are therefore only concerned with the catabolic changes of the purines and pyrimidines.

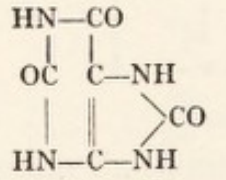
Purines undergo a gradual series of oxidations in the body, through the stages hypoxanthine, xanthine, uric acid and allantoine. For the significance of the chemical names shown below the formulae, see the purine-key on p. 180.



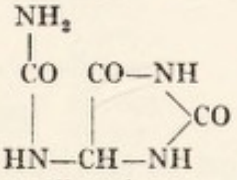
Hypoxanthine
6-oxypurine



Xanthine
2, 6-dioxypurine



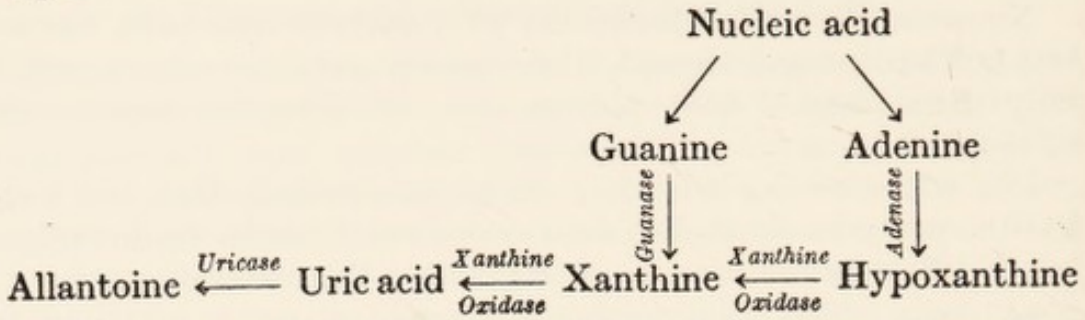
Uric acid
2, 6, 8-trioxypurine



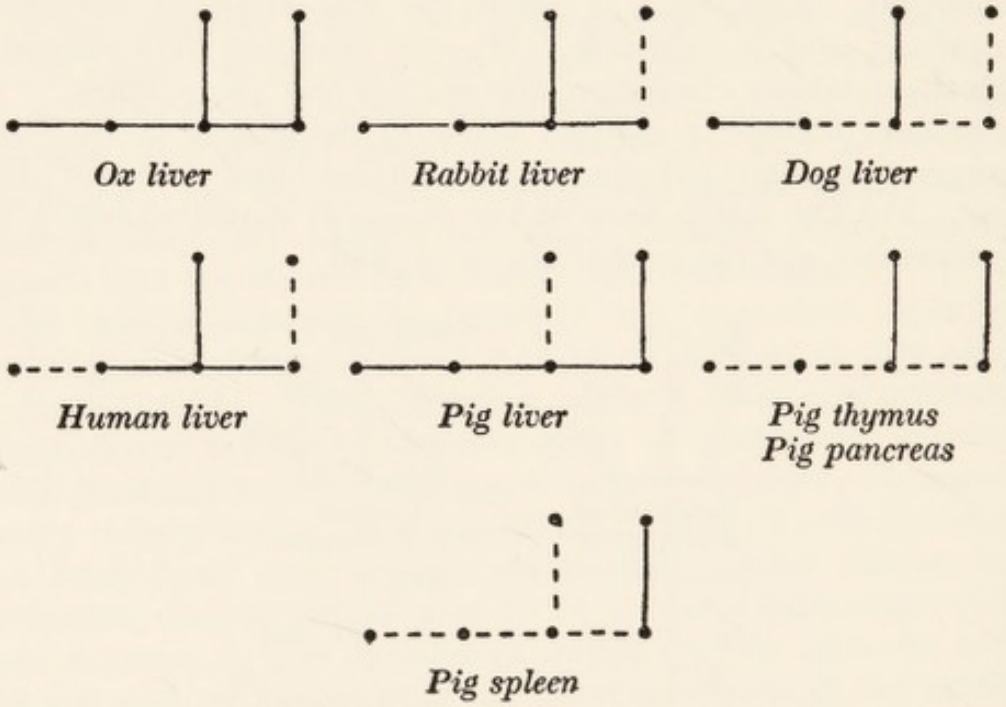
Allantoine

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 below.



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. It is present in relatively large amounts in the human liver, but in no other organ in man.

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." Guanase is present in human kidney, liver and lung, but not in spleen or pancreas. 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.

Thus the animal oxidises its purines in stages, and frequently products are shifted from one organ to another for the next stage.

Man oxidises guanine in various tissues, but the xanthine produced is converted to uric acid solely in the liver; in complete absence of uricase no further oxidation occurs. Although there is no adenase in human tissues, yet very little adenine is excreted. Mechanisms must exist to catabolise combined adenine (adenosine or adenylic acid). It has been seen that deaminisation of adenylic acid can occur in the animal intestine.

Since deaminisation sets free ammonia, some part of the urea excreted by an animal has a purine origin.

Most of the uric acid formed in human tissues is excreted in the urine. A small amount, 30 to 50 mg. daily, is excreted into the intestine *viâ* the gastric juice and the bile, and is then in great part destroyed by bacterial action.

Uricolytic Index. Andrew 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*. Hunter's results are shown in Table XIII.

TABLE XIII. 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.

Endogenous Nucleic Acid Catabolism. The catabolism of nucleic acids from the diet has been discussed. The products (uric acid and allantoine) are of *exogenous* origin (Gk. *exo*, without, *-genes*, born). Tissue destruction or " wear " must also result in similar catabolic changes, and the products are then of *endogenous* origin (Gk. *endo*, within). The excretion of these products during periods of starvation gives a clue to the extent of such endogenous catabolism.

Uric Acid and Allantoine from Other Sources than Nucleic Acids. There is some evidence of the formation of purines from proteins (amino-acids) in mammals. Thus fasting rats, rabbits, dogs, and pigs excrete much more purine-nitrogen, compared to total excreted nitrogen, than corresponds to the ratio of purine-nitrogen to total nitrogen in their tissues. The excess must come from tissue protein. Animals on a non-protein diet excrete less purine nitrogen than when fed a diet containing protein (but not nucleo-protein), and this also suggests formation of purine from protein.

Birds excrete very little urea ; they cannot use the arginine cycle for its formation (*cf.* p. 166). The chief nitrogenous excretory products of birds are uric acid and urates. These must therefore largely be the end products of their amino-acid catabolism. The mechanism of formation of uric acid in the bird is still unknown.

Other Purines Present in Human Urine. Human urine contains traces of adenine, hypoxanthine and xanthine, and still smaller 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.

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, ducks and geese.

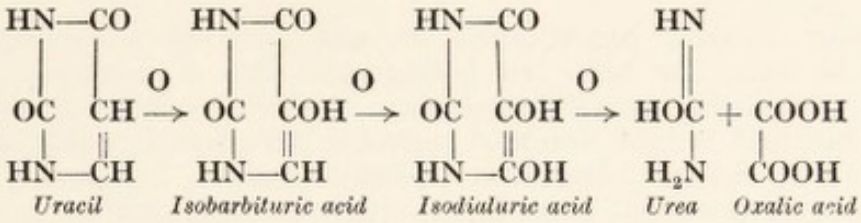
Catabolism of Pyrimidines. There is no evidence that pyrimidines occur free in tissues.

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, for 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.

Cerecedo has obtained some evidence that the catabolism of uracil proceeds through the stages isobarbituric acid, isodialuric acid, urea, and oxalic acid. *In vitro* oxidation of uracil with hydrogen peroxide in presence of charcoal yields these products also.



Diseased Conditions Associated with Nucleic Acid Derivatives

(In the final stages of nephritis there is a marked increase in the uric acid content of blood ; this is merely due to general decreased permeability of the kidneys, and has no relation to nucleic acid catabolism.)

In fevers or wasting diseases associated with much tissue destruction, in leucaemias (through destruction of white blood cells), and in non-gouty arthritis there is some rise in the blood uric acid, due to actual increased formation, and some increased excretion.

Gout is a disease associated with a disturbance of purine metabolism, and with deposition of sodium urate in the tissues

(in the inflamed gouty joints). Whether the abnormal purine metabolism is the result or the cause of gout has still to be determined. During its acute exacerbations blood uric acid is increased, even up to 6 to 10 mg. per 100 c.c., and there is a concomitant increased excretion in the urine.

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CHAPTER VIII

MINERAL ELEMENTS, WATER, ALCOHOL

MINERAL ELEMENTS

Introduction. The number of different elements that are found present in living tissues or in the constituents of food 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 result, its time of onset depending on the animal's store.

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 several 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 all definitely ascertained. The results of deficiency of such elements are shown in various ways. Thus a diet generally poor in minerals 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.

In general it may be stated that the functions of calcium and magnesium (in combination with phosphates) are chiefly concerned with the formation of the mineral framework of bone, while sodium and potassium (with chloride and inorganic phosphate) are the chief mineral ions in the other body tissues and the body fluids.

Excluding bone, tissue cells (and human red blood cells) contain chiefly potassium and phosphate, while body fluids are relatively richer in sodium and chloride.

Importance attaches not only to individual mineral elements in themselves, but also to certain balances between them. Thus in general increase of potassium ions increases the irritability of tissue, while increase of sodium ions decreases irritability; further, a balance exists between sodium and potassium on the one hand, and calcium and magnesium on the other, which especially affects irritability of nerve tissue. Increase of sodium ions increases permeability of animal membranes, but is counteracted by increase in calcium ions.

The essential mineral elements will now be considered in turn; some comments will be added on certain non-essential elements.

Calcium. Normal human blood plasma or serum contains on the average $10{\cdot}5 \pm 0{\cdot}5$ mg. of calcium per 100 c.c. The red blood cells probably contain none—or at most a trace not exceeding 1 mg. The blood serum of infants may contain slightly over 11 mg., while that of women in the late stage of pregnancy and during lactation may be depressed to about 9·5 mg. per 100 c.c.

One-half of the calcium in blood plasma is loosely combined

with protein (functioning as an acid), and is non-diffusible (will not pass through an animal or collodion membrane). The remainder, which will diffuse through such membranes, consists chiefly of calcium ions, with some unionised salts (chiefly bicarbonate and hydrogen phosphate).

The calcium of the cerebrospinal fluid is, under normal conditions, very similar in amount to the diffusible calcium of the plasma; under abnormal conditions when through disease or in the experimental animal the blood calcium is markedly increased or decreased the cerebrospinal fluid calcium is but little altered. It is also of interest that the total calcium of the blood serum of the hen during egg production is increased fourfold, but the diffusible calcium is unaltered.

Blood calcium bears a very constant relationship to blood inorganic phosphate in the normal animal; to this may be related the constancy of composition of bone mineral.

Bone contains about 12 or 13 per cent. of calcium. Other tissues contain amounts of the same order as that in blood plasma. The demands of bone tissue for repair are met from the small amount in the circulating blood; the increased demands for growth in the young animal lead to the but slight increase in the blood plasma already noted, while the drain from the maternal organism for foetal bone growth causes only a very slight lowering.

The height of blood calcium is controlled by two factors, the secretion of the parathyroid glands, and vitamin D.

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.

Calcium is excreted chiefly in the faeces; to less extent in the urine. Adult man excretes 60 per cent. by the intestinal route (slightly in bile, chiefly through the intestinal mucosa), infants still more.

The daily requirement is between 0·4 and 0·8 gm. for adult man, 0·75 and 1·0 gm. for children. Pregnant and lactating women also have an increased need (according to Sherman, 1·6 gm.).

Calcium can be assimilated in almost any form of combination. 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).

Magnesium. This element 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.

In adult man, according to Greenberg, plasma contains 2·4 to 3·0 mg. per 100 c.c., and red blood cells 6·1 to 7·1 mg., the average value for whole blood being 4·5 mg. Eveleth has obtained similar figures for a large number of mammals. In dairy cattle he found that the cell content is less than that of plasma ; ruminants have a lower cell content than other mammals.

A greater proportion of blood plasma magnesium is ultra-filtrable than of plasma calcium. The cerebrospinal fluid content is higher than that of plasma.

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. Magnesium salts are easily absorbed. Fifty to 80 per cent. of magnesium excretion takes place through the bowel, the remainder through the kidneys.

Magnesium plays an essential *rôle* as a co-enzyme in various reactions involving oxidation-reduction and phosphorylation.

When a diet markedly deficient in magnesium is fed white rats McCollum has shown that they develop a syndrome exhibiting vasodilatation (to such an extent that they appear pink), acceleration of heart beat, tetanic convulsions and death. In this condition of " magnesium tetany " the blood magnesium falls to one-tenth its normal value. A corresponding " grass tetany " of cattle has been traced to magnesium deficiency.

Sodium is, as already indicated, a most important constituent of the intercellular fluids of the body ; it is present in tissue cells in much smaller degree. It constitutes 93 per cent. of the metallic ions in human blood plasma ; human red cells contain little or none (although in the red blood cells of carnivora it largely replaces potassium). Its content in muscle and other tissues seems to be largely a function of the amount of intercellular fluid in those tissues.

In the tissue fluids it is almost completely ionised, and balanced by chloride ions. In bone it forms part of a solid mineral complex.

Carnivorous animals obtain in their meat diet comparable

amounts of sodium and potassium. Plants contain relatively much smaller amounts of sodium, averaging less than 1 per cent. of their potassium content. Hence 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.

Statements as to the minimal human daily requirement of sodium chloride vary ; probably adult man requires from 10 to 15 gm. Most of this is either added during the preparation of food or at table.

Over 90 per cent. of sodium excretion is through the kidneys.

There are no sodium depôts in the body ; it is, with the exception of bone mineral, entirely in solution, and can only be conserved during periods of diminished intake by lessening its excretion.

The content of sodium in the blood plasma is maintained at a fairly constant level by the adrenal cortical hormone (*cf.* p. 58). When there is a deficiency of this hormone sodium (and chloride) are excreted in excess, while the blood plasma potassium rises.

Potassium. Most tissues contain more potassium than sodium. Human blood plasma contains only 20 mg. per 100 c.c., while the cells contain about 400 mg. per 100 c.c. Its content in milk exceeds that of sodium.

Man obtains his potassium largely through the vegetables of his diet.

Iron is chiefly required for the building up of haemoglobin, though other tissue compounds are known which contain it. The body conserves its iron well, and the daily requirement is very small. Only a few milligrams are excreted daily and only this loss has to be made good.

The iron requirement of man is chiefly met from the vegetables of his diet. The iron of haematin (from haemoglobin, *cf.* p. 217) is not utilisable. Inorganic iron, when administered, can be absorbed and utilised.

The iron content of plant foods 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. Most of the iron in the diet, as, for example, in egg-yolk and spinach, is in organic combination. It is still uncertain to what

extent such organic sources of iron are decomposed before absorption, but it seems probable that iron is largely absorbed in the ferrous state.

Blood plasma of adult man has an average content of 0·125 mg. per 100 c.c., whole blood 1·7 mg. (due to the iron in the haemoglobin of the red cells). The daily requirement of an adult is estimated to be about 5 mg. (though Sherman suggests 15 mg.), but that of children is considerably greater. A recent estimate suggests that 0·6 mg. per kg. body weight per day is needed for maintenance and growth of normal children of from four to six years of age.

Copper is an essential constituent of crustaceans, whose oxygen-carrier, haemocyanin, is a copper, instead of an iron, compound. Oxidised haemocyanin is blue, the reduced compound is colourless, and we may assume that copper is combined in haemocyanin somewhat similarly to iron in haemoglobin. 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.

Within the past few years it has been shown that copper is necessary in the red-blooded animal for the formation of haemoglobin ; it is therefore an essential adjunct to iron, although haemoglobin contains no copper. It also catalyses the oxidation of ascorbic acid, though whether this is a normal physiological activity of copper is not yet certain.

In normal dogs the liver contains an amount of the order of 2 mg. per cent. (fresh tissue), the kidneys and spleen much less. The blood content of mammals varies from 0·05 to 0·25 mg. per 100 c.c. The total copper present in the tissues of an adult man is stated to be from 100 to 150 mg.

A recent estimate of the copper requirement in diet is, for adult man, 2 mg. per day, and for four- to six-year-old children 0·1 mg. per kg. body weight per day.

Manganese is widely and constantly present in animal and plant tissues, and undoubtedly plays an important *rôle* in some oxidation-reduction system. It is also stated to enhance arginase activity. Human liver has been reported to contain from 0·1 to 0·16 mg. per 100 gm. ; most human tissues contain much smaller amounts.

When female rats are kept for prolonged periods on a diet deficient in manganese, ovulation and therefore reproduction cease. A lesser degree of deficiency causes loss of maternal instinct after birth of young. Males become sterile.

Zinc is an essential element for both plants and animals. Rats

kept on a diet deficient in zinc show an impaired growth rate, their fur growth is affected, and their life span is shortened.

In animal tissues zinc is found in greatest amount in the liver and pancreas; there is very little in lung, brain and testicular material. The blood content varies. Its excretion in man is chiefly in the faeces, which contain about 10 mg. daily; urine contains about 1 mg. per day.

Cow's milk contains amounts of the order of 3 mg. per kg., human milk somewhat less. Zinc does not seem to be present in milk fortuitously, since the zinc content of the active mammary gland is twice that of the inactive gland.

Zinc is possibly associated with insulin action (*cf.* p. 55), and there is some evidence that it may also be associated with the hormones of the anterior pituitary.

Cobalt, if it is essential for animals, is present in their tissues in practically undetectable traces. Yet it has been shown to cure and to prevent a peculiar "enzootic marasmus" ("bush sickness") in sheep in New Zealand and elsewhere, when administered in such small dosage as 8 mg. per week.

Aluminium, arsenic and **lithium** are universal constituents of plant and animal tissues, present in extremely minute traces. There is no evidence to indicate that they are essential elements.

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 inorganic phosphate) in urine and faeces; the amount excreted *viâ* the intestine is largely increased on a vegetable diet, and is probably conditioned by calcium excretion. The faeces 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.

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 and methionine. 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 methionine, which the mammal cannot manufacture for itself.

Iodine is present in blood to the extent of between one and 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 in organic combination (*cf.* p. 53). 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 (the thyroid) seems to occur first in vertebrates. Seawater 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.

A daily intake of 0·002 mg. of iodine per kg. body weight is adequate for human needs.

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. Human blood contains normally from 0·2 to 1·6 mg. 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.

Bromine circulates in the body as bromide, and, like chloride, is excreted almost entirely in the urine. Dibromtyrosine has been isolated from corals, and it is possible that such tissues as thyroid may be able to form this organic derivative.

Fluorine is usually considered to be 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.

It has recently been claimed that fluorine is unnecessary for normal growth and function of the rat, and that, on a fluorine deficient diet, bone and teeth remain normal. There is thus some doubt as to whether or not it is really an essential element.

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 (formed from urea in the kidneys).

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.

DISEASES ASSOCIATED WITH ABNORMAL MINERAL METABOLISM

A number of *deficiency diseases* come under this heading, some due to deficiency of minerals in the diet, others due to deficiency of vitamins or other factors controlling mineral metabolism.

Thus deficiency of vitamin D leads to *rickets*, associated with low blood calcium and phosphate, and inadequate bone mineralisation (*cf.* p. 65). With rickets may be associated *tetany* (*cf.* p. 55) which can also arise from a deficiency of the parathyroid hormone, and is essentially due to a decrease in the ratio of calcium to sodium (and potassium).

Magnesium tetany, due to inadequate magnesium in the diet, has been referred to (p. 192).

In *Addison's disease*, through destruction of adrenal cortical tissue, the sodium/potassium ratio in blood plasma is changed, and sodium is excreted in excess (p. 58).

The *nutritional anaemias*, associated with deficient haemoglobin in the red blood cells, are traceable to a deficiency of iron in the diet, sometimes associated with a deficiency of copper.

A peculiar disease of young chickens, "perosis," a deformity of the tibiometatarsal joint, has been traced to a deficiency of

manganese. A large proportion of chicks raised on a diet deficient in this element have short leg bones, suggesting that, at least for these birds, a small amount of manganese is necessary for normal development of bone.

The peculiar effect of cobalt in preventing " bush sickness " in sheep has been referred to (p. 195).

Although the daily requirement of iodine is so small, yet when a diet is chronically deficient of even this small amount *goitre* (enlarged thyroid) develops in man and animals.

Fluorine affords an illustration of the rarer diseased conditions following chronic ingestion of an excess of a mineral element. (Many mineral elements are of course markedly toxic, but are not normal dietary constituents.) Fluorine in excess is distinctly toxic ; the maximum non-toxic level has not yet been determined. When 8 or 9 mg. of fluorine, as fluoride, per kg. body weight are administered daily to cattle, within a relatively short time there is loss of appetite, and a disturbed metabolism of bone, resulting in thickening and exostoses (bony outgrowths) of the long bones and jaw bones, and flattening of the ribs. Lower dosages produce similar results after a longer period (three years or more may be required). The teeth are affected, with hyperplasia of the enamel.

The treatment of soils over very long periods with rock phosphate and acid phosphate fertilisers (which contain fluorine) can markedly increase their fluorine content and that of the drainage water from them.

Excess of fluorine in drinking water can lead to mottling of teeth in man, and this result is said to have occurred in a number of areas in the United States.

WATER

Introduction. Water is the universal medium for the infinite variety of metabolic processes continually proceeding within animal and plant organisms, both chemical reactions and physical chemical phenomena. In addition, it is one of the most important products of oxidative procedures in the body cells, and itself plays a reactive *rôle* in the hydrolyses which constitute one of the commonest types of biochemical reactions.

This water is, itself, no uniform medium, but a mixture of a number of different types of molecules. Liquid water at blood temperature (38° C.) is stated to consist of a mixture of monohydrol, H.OH, dihydrol $(H_2O)_2$ (either $\begin{matrix}H\\H\end{matrix}\!\!\rangle O : O\langle\!\!\begin{matrix}H\\H\end{matrix}$ or $\begin{matrix}H\\H\end{matrix}\!\!\rangle O\langle\!\!\begin{matrix}H\\OH\end{matrix}$) and trihydrol, $(H_2O)_3$, in the proportion of 29 : 50 :

21. Further, ordinary water, including the water of body fluids, contains a small proportion of " heavy water," deuterium oxide, D_2O (*cf.* p. 130), so that, since deuterium atoms can enter into all the above combinations, many different types of water molecules are possible.

The relative water content of a healthy animal steadily decreases from birth to old age. Indeed, it is probably true to say that it decreases from the time of the fertilising of the egg until death. Thus, according to McQuarrie, the human embryo is, at the sixth week of life, 97·5 per cent. water, at birth of the infant the percentage of water has decreased to just over 70 per cent., while young adult man contains a little over 60 per cent. Throughout life, therefore, we are considerably more than half water.

Water is far from uniformly distributed within the organism, varying from over 99 per cent. in certain fluids such as sweat and cerebrospinal fluid, to less than 10 per cent. in adipose tissue. Leaving out of account the various secretions into the gastro-intestinal tract, and excretions, such as urine and sweat, within the body itself water is found *within* the cells, *intracellular water*, and outside the cells, the water chiefly of the interstitial fluid, the lymph, and of the blood plasma.

The intracellular fluid can be considered a solution of potassium phosphate very rich in colloidal material, while the extracellular fluids can be considered as solutions of sodium chloride, poorer in colloidal material. The cell membranes separating these fluids are in great part impermeable to metallic ions. Intracellular water constitutes about 27 per cent. of the body weight of the dog, intercellular water from 29 (in fat) to 45 per cent. (in lean animals).

The conception of free and bound water has already been explained (p. 34). There is lack of agreement among physiologists and biochemists as to the extent to which bound water occurs in the animal, but it seems probable that intracellular water is in part bound to colloid, while extracellular water is bound to a much less extent, if at all.

Intake and Output of Water. Just as the relative content of water in the tissues decreases from birth to adult life, so the relative need of water lessens. Thus it has been calculated that a three-months' child needs a daily intake of from 140 to 160 c.c. per kg. body weight, while a young adult of eighteen years only requires 40 to 50 c.c. per kg. (McQuarrie). Hence a young adult weighing 70 kg. needs between 2,800 and 3,500 c.c. per day. Such figures apply to persons engaged in normal activities, and living in temperate climates.

The amount required is determined by the amount lost from the body, in urine, sweat, faeces and respiration. When, as in prolonged extreme activity, or with a continuing high external temperature, the amount of water lost in the sweat rises to high figures, dehydration so produced (probably in the salivary glands and mucous membranes of the mouth) continually stimulates the sensation of thirst, so that the intake is correspondingly increased.

In the adult there is a balance between the water intake plus the water formed in tissues through oxidation or synthesis, and the water excreted. In the young growing animal there is retention of water to the extent of almost three parts of water to one part of solid laid down in new tissue.

Somewhat less than half of the intake of water is in " solid " foods. (Again, this statement only holds for persons engaged in moderate activities, and living in temperate climates.) The water content of these " solids " varies between such figures as 15 per cent. for butter, and 90 per cent. for such fruits as oranges and tomatoes. Prepared foods can vary greatly in their water content according to the manner of cooking (*cf.* p. 371). Human and cow's milks have an average content of 87 per cent. of water ; the water content in beverages depends upon what has been added to the water, but averages close to 100 per cent.

Drinking of water between meals is not harmful. Belief otherwise is an ancient fallacy. Drinking of moderate excess of water is without harm. Forced excess administered to animals by stomach tube produces an abnormal condition termed " water intoxication," which ends fatally if the forced intake is continued.

Water Shifts within the Organism. When a large amount of water is drunk on an empty stomach absorption is very rapid. Yet neither this, nor the rapid loss of marked amounts of water from an animal caused by severe experimental bleeding, causes much change in the composition and amount of the circulating fluid, the blood. The body possesses rapid means of adjustment and compensation.

It has been found that in man 1 litre of warm water is completely absorbed from the intestine in from twenty-two to fifty-five minutes after it has been drunk. Absorption from the intestine is much more rapid than excretion through the kidneys, so that although ingestion of much water causes a *diuresis* (literally, an increased production of urine) yet this only accounts for a relatively small part of the *immediate* disposal of that water.

The absorption of water from the intestines is largely through the portal circulation to the liver. The liver appears to act as a

temporary reservoir when excess is drunk, and slowly passes it on to the general circulation; muscle and skin especially can then act as water depôts. The interstitial fluid in such tissues acts, in Cannon's phrase, as a swamp, becoming inundated at such a time of flood, and equally capable of being drained away during periods of drought. Ultimately the excess of intake is got rid of through the kidneys.

The actual shifts of water within the organism are much greater than those which follow ingestion of fluid. The digestive juices pour into the alimentary tract amounts of water in their secretions which considerably exceed the total ingested, and almost all of

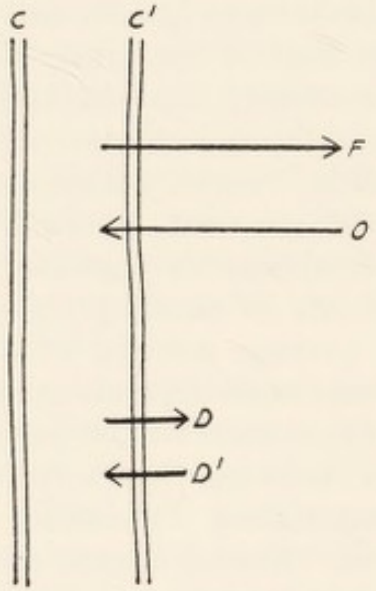

FIG. 16. Diagrammatic representation of the chief factors governing the passage of fluid through a capillary wall. *C*, *C'*, capillary wall; *F*, filtration effect; *O*, osmotic effect; *D*, *D'*, diffusion effects. Arrows have vector significance, indicating, as shown in diagram, a balance.

this water is reabsorbed. Much water, with its solutes, is filtered off from the blood to the tissues, and drained back from the tissues through lymph channels to the blood. Yet these shifts are effected so harmoniously that little change in composition of blood and lymph occurs throughout the twenty-four hours.

Passage of Water Across Membranes. Such a membrane as the capillary wall consists of polygonal or elongated rhomboidal epithelial cells, cemented together at their edges to form a completely closed tube, which is surrounded by a wide-meshed network of muscle tissue that leaves most of the outer part of the tube exposed for passage of material (Krogh). Whenever the pressure within the tube is at all higher than that outside it the tube is dilated, and its cell wall is stretched, facilitating

passage of material across it. Abnormally great pressures can stretch it to the extent that material can pass too easily from the blood within the tube to the tissue spaces outside it; larger molecules than usual can cross the barrier.

In other membranes in the organism comparable conditions exist, but they differ qualitatively. While, for example, the cell membranes of the intestinal wall and of the capillaries are permeable to inorganic ions in general, it has already been pointed out that the membranes of the red blood cells and of many tissue cells are impermeable to cations.

The general conditions causing transference of material across the capillary membrane are illustrated by the diagram in Fig. 16. The pressure within the tube is usually greater than that without it, so that there exists a filtration force, F, pushing out water and such material dissolved in it whose molecules can pass across the wall. Diffusion effects produce migration of such dissolved molecules in both directions (D, D'). The filtration force is almost completely balanced by the osmotic force O (*cf.* p. 14); this is the net difference between the osmotic forces of the fluids, within and outside the capillary tube. Its net effect is to draw water from outside the capillary to within it, since the protein content of the blood plasma is greater than that of the interstitial fluid.

Thus physical forces, controlling the function of the capillary wall, permit diffusion of compounds and ions of small molecular size across it, and tend to force out some water into the tissue spaces (later to be returned through lymph channels).

Chemical Rôles of Water in the Organism. Water is an active agent in all hydrolyses.

$$R \cdot CO \cdot NH \cdot R' + HOH \rightarrow R \cdot COOH + H_2N \cdot R'$$

Water is set free in synthetic processes such as the formation of tissue proteins.

$$R \cdot COOH + H_2N \cdot R' \rightarrow R \cdot CO \cdot NH \cdot R' + HOH$$

Water is also formed in tissue oxidations, as for example in the oxidation of lactic acid in muscle.

$$CH_3 \cdot CHOH \cdot COOH + 3O_2 \longrightarrow 3CO_2 + 3H_2O$$

This formation of water *within* the cell tends to dilute the cell fluid and may, before osmotic adjustment, tend to facilitate diffusion of dissolved material into the cell from the interstitial fluid.

Controllers of Water Exchanges. All metabolic changes which result in changes in the number of molecules in solution, and therefore changes in osmotic pressure, cause local shifts of water.

Such lipides as the lecithins and cholesterol also exercise local

control (*cf.* p. 136), the former being lyophilic (tending to attract water) and the latter lyophobic (tending to repel water).

Inorganic salts and such catabolites as urea can only be excreted in dilute solution, and must therefore carry a considerable amount of water with them through the kidneys.

Marked shifts of water are produced by certain hormones, internal stimuli, and by certain diuretics. Purgatives causing marked excretion of watery faeces also produce such marked effects.

Pitressin of the posterior pituitary, injected in minute dosage, produces some diuretic effect; when it is injected in larger amounts it produces a definite anti-diuretic effect (lessens the output of urine). It is believed that this action is produced through constriction of the efferent vessels of the glomeruli of the kidney.

Cortin of the adrenal cortex controls the volume of blood plasma. This control is perhaps exercised indirectly, through its control of sodium metabolism (*cf.* p. 58). When there is a deficiency of cortin the plasma becomes concentrated—water flows away from the blood to the tissues, while increased excretion of sodium chloride involves increased water excretion.

Excess water intake leads to a diuresis, since it is in large degree ultimately got rid of through the kidneys.

Such compounds as caffeine (in tea and coffee) are specific diuretics, stimulating kidney tissue to excrete.

Functions of Water. In addition to its functions as a universal solvent medium for all the chemical reactions in the living organism, and as a solvent carrier of nutrient and waste material throughout the body, as we have already seen, water plays an active *rôle* in many important biochemical reactions, while through physical chemical mechanisms it permits exchanges of nutrient and waste material between intracellular and intercellular fluids.

It is a most important agent in the maintenance of body temperature. Evaporation of "insensible" and of visible perspiration from the surface of the body is—through the large value of the latent heat of vaporisation of water—one of the chief agencies of heat loss from the body. Aqueous fluids also act as a perfect lubricant between a multitude of surfaces within the body.

Without water there can be no life. Its importance in the life of man is exemplified by the fact that water starvation is fatal within a few days, while complete cessation from solid food is not fatal for several weeks, as long as adequate water is drunk.

Effects of Heavy Water on Life. While the small proportion of heavy water in the fluids of the living animal, equal to that in water from natural sources, is without any known deleterious effect, evidence is accumulating that marked concentration of heavy water is toxic to animals and plants.

Thus the germination of tobacco seeds is greatly retarded in water containing 50 per cent. of deuterium oxide. Frog tadpoles die in 92 per cent. deuterium oxide within a half hour, small fish in two hours, a flatworm in three hours, while paramoecia survive less than forty-eight hours. Water containing only 30 per cent. of the heavy variety does not produce such immediate effects. Injection of heavy water into mice produces hyper-excitability, followed by depression. Injection of a tenth of 1 c.c. through a trephine hole over the brain cortex of the rat produces catalepsy (a kind of auto-hypnotic rigidity) within a few minutes, which lasts several hours. Similar results are obtained by introduction of 1 or 2 c.c. into the spinal fluid of cats.

DISEASED CONDITIONS ASSOCIATED WITH ABNORMAL WATER METABOLISM

Oedema. When dogs are bled repeatedly over a number of days, half a litre of blood being withdrawn at each bleeding, and the corresponding volume of red blood cells suspended in Locke's solution (which contains the inorganic constituents of plasma in correct concentration) is then injected after each bleeding, so that the net result of these bleedings is removal of plasma protein, after five or six days a marked oedema occurs, fluid passing from the blood to the tissue spaces, lymph vessels, and serous cavities of the body. This pathological shift of fluid commences when the plasma proteins have fallen from a normal 8 per cent. to less than 4 per cent. (more particularly when the plasma albumin falls below 2·5 per cent.). Through withdrawal of this large proportion of the plasma proteins the osmotic pressure—the inward pull—of the plasma ceases to balance the outward filtration thrust (maintained by the heart-beat), and ingested fluid, when it reaches the blood, simply flows out to the tissues.

So-called *nutritional oedema* or *famine oedema* or *war dropsy* is due to the same lowering of plasma proteins, traceable to deficient protein intake, while the oedema accompanying *nephritis* is likewise due to such lowering, in this case through excessive excretion of the albumin and globulin of the plasma through the kidneys into the urine.

The opposite condition, *dehydration*, in its extremer phase termed *anhydraemia* (lowered water content of blood), can arise through too small an intake of water (initially counteracted by flow of fluid from muscles and skin), or through excessive vomiting (especially in infants), or marked loss of water in the extreme diarrhoea of Asiatic cholera and the choleriform diarrhoea of infants ; this can lead to such water loss as to produce a fall of 20 per cent. of body weight in forty-eight hours and a rapidly fatal termination. In such conditions the blood plasma is markedly concentrated, while kidney excretion is diminished even to a complete anuria (absence of urine).

Individuals exposed to continuous high temperature, especially when engaged in extremely hard work (such as ships' stokers and miners) not infrequently suffer from *heat-stroke*. This condition is due to marked loss of body salts, rather than of water *per se*, salts lost to the body in solution in the sweat excessively and continuously produced. The drinking of pure water increases its incidence ; drinking a dilute solution of sodium chloride to quench thirst prevents its occurrence.

Diabetes Insipidus is a disease in which there is a continuous excretion of a very dilute urine. Except for its dilution the urine is normal in quality. The condition is essentially one of unbalanced water control, leading to marked loss of water. This leads to increased and continuous thirst, resulting in marked increased intake of water. The condition is associated with some lesion in the posterior pituitary gland, or the adjacent hypothalamic region of the brain ; a large proportion of patients exhibiting the condition obtain benefit by injections of pitressin.

Ethyl 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 rate of absorption is lowered when alcohol 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 faeces 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 XVI.) 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 in about the same proportion as their water contents. 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.

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 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.

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.

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CHAPTER IX

THE BODY FLUIDS

Introduction

In this chapter some account will be given of the digestive juices and of sebum; more detailed consideration will be given to the general vehicle of exchange, the blood, and such local vehicles of exchange as the lymph, the cerebrospinal fluid, ocular fluids, synovial fluid, and amniotic fluid.

The Digestive Juices

A brief account of these fluids was given in Chapter I. Results of typical analyses are shown in Table XIV.

TABLE XIV. 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, about 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

Such analyses reveal little concerning the function of these juices. The enzyme content is so small that ordinary chemical analyses cannot estimate it. Of non-enzymic constituents of importance mucin of the saliva is only present to the extent of 0·2 per cent. or less, and hydrochloric acid of the gastric juice to about 0·4 per cent. Most of the non-enzymic constituents of these juices (excluding bile) can be regarded as compounds passively secreted from blood by the secreting glands. This applies especially to the mineral ions and such easily diffusible compounds as urea.

TABLE XV. ENZYMES PRESENT IN THE DIGESTIVE JUICES.

Fluid	Enzymes
Saliva	Amylase, doubtful trace of maltase
Gastric juice	Pepsin,* rennin, gastric lipase
Pancreatic juice	Trypsin,* chymotrypsin,* carboxypeptidase, pancreatic amylase, pancreatic lipase
Intestinal juice	Various peptidases, maltase, sucrase, lactase, nucleinase, phosphatase, deaminase, etc.

* Present in zymogen form, needing activation.

In order to get a correct conception of the digestive juices, Table XIV should be supplemented by Table XV, summarising their enzymic contents.

The actions of these enzymic constituents have been already considered (pp. 94, 125, 160); it has been pointed out that bile is both secretion and excretion (p. 6).

Table XIV shows 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.

It is difficult to estimate the average amounts of the various secretions produced per twenty-four hours. For example, the amount of saliva will vary widely with the time of chewing, the character of the food, etc. Rowntree has collected the following figures from various sources for the total daily secretion in normal adult man : saliva, 1,500 c.c., gastric juice, 2,000 to 3,000 c.c. ; bile, 300 to 500 c.c. ; pancreatic juice, 500 to 800 c.c. ; intestinal juice, 3,000 c.c. The total secretion indicated by these figures is thus of the order 7,000 to 8,000 c.c.

Sebum

Sebum, the secretion of the sebaceous glands in the skin, 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.

Blood

The average amount of blood in normal man is about 8·8 per cent. of his body weight. It consists of formed elements, the red and white corpuscles, and the platelets, suspended in plasma. The red blood cells normally average 4·5 to 5·5 millions per cubic millimetre, and constitute 40 to 45 per cent. of the blood volume. The white blood cells only number 4,000 to 10,000 under normal conditions, and the much smaller platelets 200,000 or more per cubic millimetre. Excluding consideration at present of the white cells and the platelets, the chemical constituents of blood are

divisible into four groups, although certain constituents belong to more than one group.

(i.) Constituents specific to blood as a separate system (many physiologists regard it as a " tissue "). These include compounds associated with maintenance of its osmotic pressure, others associated with the clotting of blood, and still others required for transport of oxygen and carbon dioxide.

(ii.) Nutrient and mineral materials *en route* to the tissue cells from the alimentary canal, or from depôts to tissues in

TABLE XVI. 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):			
Haemoglobin	39–32 gm.	0 gm.	16–13 gm.
Plasma protein	0	6·7–8·2	—
Plasma albumin	0	4·6–6·7	—
Plasma 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	6–7	2·4–3	4·5
Chloride ion	130–165	350–380	270–300
Phosphorus			
as inorganic phosphate	3–5	1·5–4·5	—
as lipide	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.
Lactic acid	—	—	5–20
Uric acid	1·9	3·9	1–3·5
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	—

need of them, and oxygen on its way from the lungs to tissue cells.

(iii.) Hormones and vitamins in circulation to the tissues needing them from the tissues which form them, or after absorption from the alimentary canal. Enzymes are also present, some probably through leakage from tissues to which they belong, others with intrinsic functions associated with the blood. Traces of immune bodies are present (*cf.* Chapter XIX.).

(iv.) Waste products on their way from the tissues which formed them to the tissues which excrete them. Certain partially oxidised products may be in transit from one tissue to another for further oxidation before excretion.

In addition the portal circulation carries from the intestinal mucosa to the liver various compounds absorbed from the intestine which are then detoxicated in the liver before excretion, *viâ* the general circulation. These will be discussed in Chapter XIII.

Since the red blood corpuscles and the plasma are in constant contact with each other, and co-operate in many functions, it is of interest to contrast their composition ; this has been done in Table XVI.

The data in the Table refer to venous blood.

The specific gravity of plasma is about 1·026, of the red cells 1·09, and of whole blood 1·05 to 1·06.

It should be noted that the chloride content of blood is usually, though inaccurately, 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. This bicarbonate is to be regarded as in part functioning, and not merely as excretory material.

The hormones, vitamins, and enzymes are present in too small amounts to be included in Table XVI, while the immune bodies are present in still smaller amounts. The enzymes present include traces of amylase, proteases and peptidases, lipase, oxidases and catalases. In addition the red cells contain a specific *carbonic anhydrase* which plays an important *rôle* in respiration and will be dealt with in Chapter XI.

The nutrient materials have already been considered (pp. 97, 127, 162). In this section consideration will be given to the nature and function of haemoglobin, the plasma proteins, the maintenance of the osmotic pressure of the blood, and the mechanism of blood

clotting, while some notes will be added on the composition of the white cells and platelets.

Haemoglobin 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 haemoglobin present in human blood is about 15 per cent., equivalent to 13 gm. per kilo. body weight. The average man of 70 kilos. body weight contains therefore about 900 gm. of haemoglobin. It hydrolyses to *globin*, a histone and *haem*, a compound containing iron.

Haemoglobins 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 haemoglobins 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.

The amount of iron in haemoglobins from different species is very constant, 0·33 per cent. Ultracentrifuge and other methods indicate that the molecular weight is about 68,000 (p. 145); this corresponds to four atoms of iron per molecule. *The essential biochemical property of haemoglobin is its power to unite easily with oxygen, and to give up this oxygen easily*, whereby blood is able to carry a large amount of oxygen in chemical combination from lungs to tissues. One gm. of haemoglobin will unite with 1·34 c.c. of oxygen (giving oxy-haemoglobin), and this is in the exact ratio of one atom of iron to one molecule of oxygen, so that an equation may be written

$$HbFe_4 + 4O_2 = Hb(FeO_2)_4.$$

Solutions of haemoglobin are purple-red in colour, corresponding to venous blood; those of oxy-haemoglobin are scarlet-red, corresponding to arterial blood. Oxy-haemoglobin, while readily soluble in water, is insoluble in alcohol, ether and other fat solvents. Haemoglobin 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-haemoglobin.

When potassium ferricyanide is added to a concentrated solution of oxy-haemoglobin, or to blood that has been shaken up with air, the colour changes to brown, through the formation of *methaemoglobin*. 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-haemoglobin is written $Hb(FeO_2)_4$ then methaemoglobin 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 haemoglobin.

When oxy-haemoglobin solutions are exposed to a partial vacuum oxygen is readily liberated. Methaemoglobin is not affected by this treatment. Both are readily converted into (reduced) haemoglobin 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-haemoglobin shows two definite dark bands in the yellow-green region, and through methaemoglobin less marked bands in the yellow-green and a definite band in the red part of the spectrum. Reduced haemoglobin produces a more diffuse single band in the yellow-green. Oxy-haemoglobins 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 haemoglobin or of oxy-haemoglobin the colour of the solution changes to cherry-red, due to the formation of carboxy-haemoglobin. This gives a spectrum very similar to that of oxy-haemoglobin, though each band has been shifted slightly towards the green region. Carboxy-haemoglobin 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 haemoglobin and oxy-haemoglobin 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-haemoglobin 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-haemoglobin are isomorphous (belong exactly to the same crystal system) with those of the corresponding oxy-haemoglobin, 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-haemoglobin, $Hb(FeNO)_4$, is formed.

The blood of all vertebrates contains haemoglobin. 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 larvae 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 *haemocyanin*, a copper- instead of an iron-protein. This, like haemoglobin, contains a high percentage of histidine radicals. Oxy-haemocyanin is blue; animals which contain it are therefore blue-blooded. (Reduced) haemocyanin is colourless. This compound is not so efficient an oxygen-carrier as haemoglobin. Different species contain different haemocyanins. These have undoubtedly very complex molecules; ultracentrifugal methods have given such different molecular weights as 397,000 (haemocyanin from *Pandalus*) and 6,680,000 (from *Helix*). The copper content of haemocyanin 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 haemocyanin is a copper porphyrin.

Certain polychaete 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 haemoglobin, 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 haem in general behaviour.

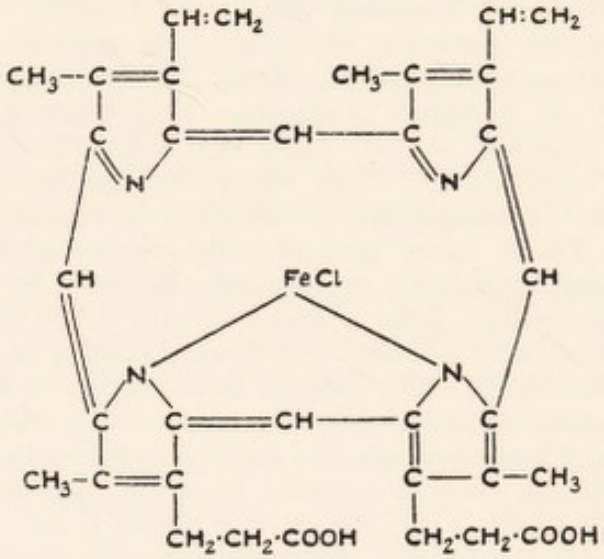
On hydrolysis haemoglobin yields 94 per cent. of *globin*. Globin contains 55 per cent. of carbon, and 16·9 per cent. of nitrogen. It is very easily soluble in acids and alkalies, and behaves essentially like a histone. On hydrolysis it yields unusually large amounts of leucine (29 per cent.) and histidine (11 per cent.). Globin is colourless.

Addition of dilute alkali to haemoglobin leads to the production of *haemochromogen;* addition of acid to oxy-haemoglobin results in the formation of *haematin*. Haemochromogen also has a characteristic absorption spectrum. According to the work of Anson and Mirsky it consists of a molecule of *haem* united to one molecule of " denatured " globin; one molecule of haemoglobin yields four of haemochromogen. On adjustment to neutrality

of the solution containing it haemochromogen polymerises back to haemoglobin.

When haemoglobin is heated with sodium chloride and glacial acetic acid characteristic minute dark brown rhomboid crystals of haemin, $C_{34}H_{32}N_4O_4FeCl$, are produced. The relationship between haem and haemin is expressed by the formulae X . FeOH and X . FeCl. The corresponding bromine and iodine haemins have been prepared.

Haemin is built up from four pyrrole nuclei. It has been synthetised by H. Fischer and K. Zeile (1929), who give it the formula :



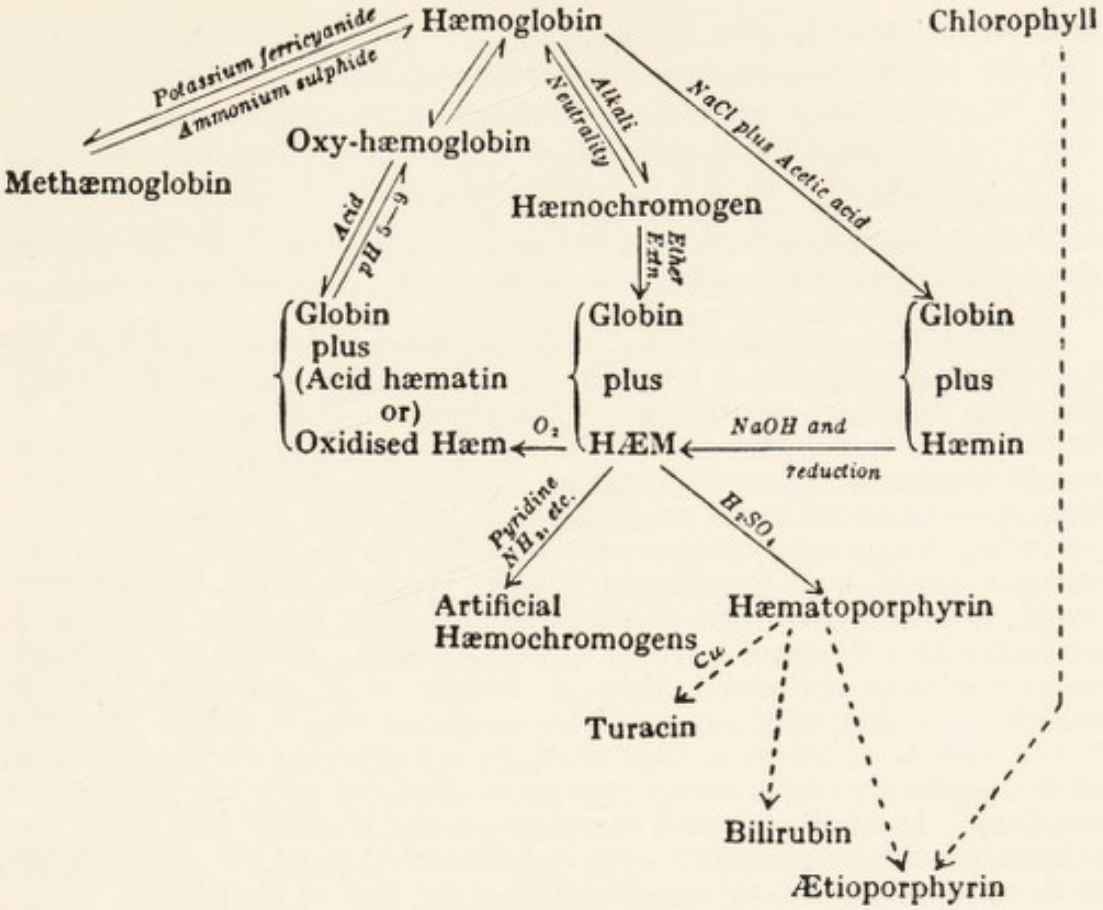
Haemin

Haem can combine with many nitrogen compounds to form 'haemochromogens"; these are named from the particular nitrogen compound concerned. Such are cyanide, ammonia, amine, protein, pyridine, and nicotine haemochromogens. Haem itself is an oxidative catalyst, but such unions render its catalytic action far more effective. The haemochromogens have all very similar absorption spectra, with two sharply defined bands.

When haem or any of its derivatives is dissolved in concentrated sulphuric acid the atom of iron is split off, and *haematoporphyrin*, $C_{34}H_{38}N_4O_6$, 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.

Haematoporphyrin 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 haem compounds is shown in the following scheme :



Plant chlorophyll has the same pyrrol skeleton as haem, with an atom of magnesium replacing the atom of iron.

(There still appears to be some confusion as to the precise nature of haematin, and as to the direct relationship between methaemoglobin and haem.)

Haem 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 haemochromogen. The *actiniohaematin* of certain actiniae is a haem derivative. *Cytochrome* is a mixture of three haemochromogens ; 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 haem :* " Haemoglobin, it now appears, may no longer be regarded as the pre-eminent haem pigment, nor is its haphazard distribution now as mysterious. Haemoglobin is merely an occasional specialised derivative of an iron substance haem, which is much more widely distributed."

Haemoglobin 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 XIV., p. 318). 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-haemoglobin is more acid—will combine with more of the potassium of the red corpuscle —than reduced haemoglobin, 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 haemoglobin is enclosed in the red cells, rather than being simply a dissolved constituent of the plasma. If blood contained its haemoglobin 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 haemoglobin, 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 haemoglobin. Unless the capillary wall were less permeable than it usually is in mammals, and in consequence less efficient, some haemoglobin 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 haemoglobin in its most efficient environment which is not that of the plasma."

Stadie and others have recently revived an old theory and adduced new and strong evidence that haemoglobin assists in the carriage of carbon dioxide in blood by combining directly with it, the free amino-groups in the haemoglobin molecule actually reacting to give a carbamino-linkage, and at the same time binding base

$$-NH_2 + CO_2 + K^+ \rightarrow -NH.COOK + H^+.$$

The formation of *carbhaemoglobin* is a rapid procedure. Stadie believes that the proteins of the plasma also partly function in the same way.

Plasma Proteins. Since *serum* is the liquid exudate from clotted blood, and does not exist as such in the living animal, the terms " serum protein," " serum albumin," and " serum globulin " are inexact and should not be used, except in reference to analyses of serum. Plasma contains albumins, globulins, and fibrinogen. The number of albumins and globulins present is still uncertain.

By " salting-out procedures," such as treatment with ammonium sulphate of different concentrations, two globulin fractions are obtained, *euglobulin*, which separates at one-third saturation, and *pseudo-globulin*, more soluble, and needing half-saturation to precipitate it. Such methods of separation are open to criticism, in that chemical changes of denaturation type may be produced in the protein by the

salt concentration used to precipitate, and so this itself may induce a fractionation. Some of the recent experimental work bearing on the problem may well be mentioned, to indicate its complexity.

Burk has recently claimed to separate plasma globulin into two fractions by dialysis; both fractions had approximately the same molecular weight, 173,000. He concludes that pseudo-globulin cannot be considered a changed or a decomposition product of euglobulin. Elford, Graber, and Fischer have carried out ultrafiltration of serum through graded collodion membranes, and have obtained evidence of the presence of particles corresponding in size to molecules of albumin, and pseudo-globulin, and of others equal to twice the size of molecules of albumin. They think the latter are of a globulin aggregate, corresponding to euglobulin; this complex appears to dissociate to simple molecules on dilution of the serum.

Hewitt has fractionated the crystallised albumin from horse serum into a fraction almost free of carbohydrate radicals, with a very low tryptophane content of the order of one tryptophane radical per molecule of protein, and a second fraction, more soluble and less coagulable, which he names *seroglycoid*, and which contained over 10 per cent. of carbohydrate radicals, and a higher tryptophane content. This fraction represents only about one-tenth of the total serum albumin. He has also claimed to have separated a globulin, *globoglycoid*, rich in carbohydrate radicals, from the albumin fraction.

McFarlane, using ultracentrifuge and other physical methods, found that solutions of re-crystallised albumin from horse serum were monodisperse—*i.e.*, contained molecules uniformly of one size; solutions from reprecipitated globulin from horse serum consisted of molecules of different sizes. "Native serum" showed the presence of two fractions, 80 per cent. of small size, 20 per cent. of larger size, but on gradual dilution of this serum the distribution of the two types changed and ultimately became equal, suggesting capability of change from one type of protein to the other.

Pickering and Block and others have advanced theories suggesting that the total plasma protein constitutes for functional purposes one interrelated complex, whose total integrity is of more importance than variations in its constituents. Variations in the albumin and globulin fractions in disease, and the dependence of oedema on an essential lowering of plasma albumin below 2·5 per cent. rather than on a lowering of total protein below a fixed figure scarcely support such views.

It is evident that at the present time no definite statements can be made concerning the albumins and globulins of blood plasma, and until further work has clarified our knowledge it is safest to speak of the *albumin and globulin fractions* of the plasma.

The place of formation of these proteins is unknown. Their nature would appear to be specific; they differ from the other albumins and globulins present in body tissues. Their function, or one of the their functions, is to maintain a constant osmotic pressure. Their presence also helps to give the blood a definite viscosity (although that viscosity is still more due to the presence of the red cells). Further they may play some part in keeping the hydrogen-ion concentration of the blood constant.

As Table XVI shows, plasma globulin is usually present in much smaller amount than is plasma albumin. In certain infections or toxaemias these ratios may be definitely altered. In streptococcus and staphylococcus infections, and in nephrosis, the globulin may amount to as much as 60 or 70 per cent. of the total plasma protein. Muscular activity increases the plasma protein, chiefly the albumin portion.

Fibrinogen is a globulin differing from other plasma globulins because it is precipitated by half-saturation with sodium chloride and 20 per cent. saturation with ammonium sulphate. 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 septicaemia the blood fibrinogen may markedly increase.

The Osmotic Pressure of the Blood Plasma. This is the sum of the osmotic pressures of all the substances dissolved in the plasma. The fraction due to its proteins is small, but all important, since, under normal conditions of permeability of the capillary walls, proteins cannot pass through them, and since the protein content of the lymph is smaller, this minute difference of osmotic pressure permanently, in health, holds fluid within the capillaries and conserves the concentration of the plasma.

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 Clotting of 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 haemorrhage. Yet the blood circulating in the body does not clot.

The process of clotting and the causes of clotting are still incompletely understood. It has been claimed that clotting can be produced in different ways and by different substances. Howell, one of the authorities on the subject, reviewing all its problems a few years ago, considered that more definite knowledge of the mechanism of clotting cannot be obtained until the individual compounds which function in the process have all been obtained in a state of complete purity. No attempt will be made here to deal with conflicting views, but only an endeavour to

present a classical view of normal blood clotting, based largely on Howell's own researches. It is to be stressed that this view probably includes much of the truth but not the whole truth.

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.

If oxalated blood is centrifuged and an equal amount of saturated solution of sodium chloride is added to the plasma, fibrinogen is precipitated. It can be purified by washing with half saturated sodium chloride solution, redissolving in dilute solution of sodium chloride and reprecipitating, and repeating the whole process again; the pure solution of fibrinogen finally 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, some compound adherent to the fibrin goes into solution. 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 on fibrinogen. Calcium ions may be necessary for this action.

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.

In the presence of calcium ions prothrombin is changed to thrombin which is then able to convert fibrinogen into fibrin.

Since circulating blood contains calcium ions, prothrombin and fibrinogen, why does it not clot under ordinary circumstances? Two additional factors appear to play a *rôle*.

Howell has obtained a compound from liver which he terms *heparin* and which is very active in preventing clotting, both *in vitro* and *in vivo*. He believes that heparin is normally present in blood as an anticoagulant, an *antiprothrombin*. He has also shown that pure *cephalin* aids clotting, though the other phosphatides do not. Cephalin can come into contact with 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. Cephalin possesses all these properties.

Thus it seems probable that the clotting process, as it normally occurs, includes the following steps. When tissues are lacerated and blood leaves its usual channels cephalin is brought into contact with it. Cephalin in some way removes heparin from the blood, whereupon the calcium ions of the plasma react with prothrombin, and thrombin and fibrin are successively formed.

This theory also accounts for the prevention of clotting by injection of heparin. Hirudin, extract of leech heads, probably acts in a similar manner to heparin.

The weakness of the theory at present largely lies in our

ignorance of some of the substances that are reacting. These substances will be considered briefly.

Fibrinogen and cephalin have been dealt with (pp. 222, 114). Fibrin may be a compound of the protein fibrinogen with thrombin, but the ease with which thrombin can be removed from fibrin rather throws doubt on this view.

It has been pointed out (p. 191) that the diffusible calcium of blood is largely in ionised form. It seems rather paradoxical that excess of calcium ions will prevent clotting. The minimum content in blood which will enable it to coagulate is 1·3 to 2·1 mg. per 100 c.c. (whole blood), while the maximum concentration which still permits clotting is 650 mg. per 100 c.c.

Much work has been done in recent years on the nature of heparin. It seems to be present in many tissues. Lung tissue contains more than liver tissue. Its brucine salt has been obtained in crystalline form, and indicates that its empirical formula is $C_{25}H_{65}O_{50}N_2S_5$ so that it has a relatively small molecule. It is possibly a mucoitin polysulphuric ester.

Little definite is known concerning the chemical nature of prothrombin and thrombin. Both have protein-like properties. Prothrombin is precipitated from solution by half saturation with ammonium sulphate, behaving to this extent as a globulin. Many investigators have believed that thrombin behaves as an enzyme, but this is unlikely. It is inactivated by heating it to 60° C. for a few minutes, but, although a small amount will change a much larger amount of fibrinogen to fibrin, the reaction seems quantitative, and not enzymic.*

White blood cells, leucocytes of several types, have such *rôles*

* It is perhaps more dangerous to make dogmatic statements concerning the mechanism of blood coagulation, than concerning any other of the unsolved problems of biochemistry. But it has seemed preferable to present the student with a single theory, admittedly incomplete, and incompletely proved, than to confuse him with an account of numerous theories, too often introducing complex nomenclatures of compounds of questionable reality. Eagle, in a recent critical review (1937) of the literature on this subject, writes: "The very fact that there are now so many contradictory and mutually exclusive theories implies that the experimental data on which they are based are either in error or incomplete." And as a corrective to the straightforward story given in the text, it may be mentioned that Eagle stresses the recent finding that trypsin or certain proteolytic snake venoms can replace calcium and platelets, and, acting alone, can convert prothrombin to thrombin; he concludes therefrom that the calcium-platelet system induces a change in prothrombin analogous to proteolysis. He leans towards the old concept that the action of thrombin on fibrinogen is also enzymic in nature, pointing out that papain, a proteolytic enzyme, can convert fibrinogen to a fibrillar gel resembling fibrin. He considers that at present neither the heparin theory of Howell nor any other theory now in vogue is backed by sufficient experimental evidence to give convincing reasons for the fact that, under normal conditions, the circulating blood does not clot.

as phagocytosis (Gk. *phagein*, to eat), the ingestion of foreign material, including bacteria, and hence these wandering free cells form one of the defensive mechanisms of the body against disease. They have their own chemical economy ; thus they possess their own enzymes, and can store such material as glycogen.

Platelets are essentially associated with clotting, rapidly disintegrating at the site of an injury, and liberating cephalin. The platelets of horse blood have been examined in some detail. Dried material contains in percentages, protein 71, phospholipides 12 (lecithin 7 and cephalin 5), cholesterol 3, with traces of other lipides including glycerides, and ash 5·5.

Notes on Diseases Associated with Abnormal Conditions of the Blood

Nutritional anaemias, due to deficient formation of haemoglobin through lack of iron (and copper) in the diet, have been referred to (p. 198). They are characterised by a low haemoglobin content of the red cell, accompanying a low production of red cells by the bone marrow.

Pernicious anaemia is specifically characterised by a low red cell count in blood, with normal haemoglobin content in the cells that are present. This condition arises from deficiency of some factor present in liver, a haematopoietic (blood-forming) principle formed in the gastric mucosa, set free into the gastric juice (and possibly modified there by some vitamin or other constituent of the gastric juice), absorbed from the intestine and stored in the liver, and apparently needed for correct functioning of the bone marrow.

Polycythaemia (an increase of red blood cells above normal) occurs as a physiological response to prolonged existence at high altitudes (where oxygen pressure in the atmosphere is low, and more haemoglobin is needed to convey sufficient oxygen to the cells) and as a similar physiological response to an impaired or sluggish circulation, as for example in congenital heart disease. *Polycythaemia vera*, however, is associated with some pathological functioning of the bone marrow, whereby either red cell formation is excessive, or the resistance of the cells to destruction is greater than usual, or both factors are involved.

Haemolysis, the destruction of red cells and " loosening " of their haemoglobin content, is easily brought about *in vitro* by considerable dilution of blood with water, and, in consequence, such a change of osmotic pressure as leads to their disintegration (the so-called " laking of blood "). In one form of jaundice, *haemolytic jaundice*, the fragility of the red cells is greater than

usual, and slight changes in the osmotic pressure of blood causes their destruction.

Haemophilia is a rare disease in which clotting is markedly delayed, so that even slight wounds can result in death. Which clotting factor is at fault is not yet known. There is no lack of fibrinogen. The error may lie in an unusual resistance of the platelets to disintegration.

Thrombosis represents the opposite condition, in which clotting occurs too easily; it may arise after an operation under anaesthesia. Slight damage to a vein as for example interference with circulation by tight bandaging may lead to formation of a clot within the vein, a thrombosis. If part of such clot is loosened into the circulation (an embolus), serious results may ensue. It has recently been shown by Best and his colleagues that heparin can be usefully injected as a preventive measure in cases where thrombosis is likely to occur.

The "Middle-men" Fluids

Such fluids as lymph and cerebrospinal fluid can be considered as playing the *rôle* of middle-men between blood and tissues, with the difference that they pass on completely the material supplied to them by blood and by tissue cells. In considering the composition of these fluids it is of predominant interest to contrast them with blood plasma, since the comparison should give some clue to their mechanism of formation, whether by active secretion or passive filtration. For perfect comparison analyses are requisite of samples of blood and fluid obtained from the same animal simultaneously; arterial blood should probably indicate a closer relationship than venous blood. Usually such comparisons have

TABLE XVII

Comparison of Blood Serum and Thoracic Lymph of the Dog

	Blood Serum	Thoracic Lymph
	gm./100 c.c.	gm./100 c.c.
Total solids . . .	8·3	5·2
Protein	5·6	3·6
Non-protein N . . .	0·0272	0·0270
Sugar	0·123	0·124
Chloride Cl . . .	0·392	0·413
Calcium	0·0104	0·0092
Inorganic P . . .	0·0043	0·0036

been made with venous blood, and sometimes only average figures are available for comparison.

Table XVII. is based on data of Arnold and Mendel (1927) for direct comparison of venous blood and thoracic lymph of the dog. They found further that whenever changes are produced in the composition of blood plasma by experimental manipulations, corresponding changes are exhibited by the lymph ; the relationship between the two fluids is constantly maintained.

The figures for human blood plasma and cerebrospinal fluid in Table XVIII. are taken from various sources. Those for the aqueous fluid of the eye of the ox and horse are added for comparison, and are taken from Krause's monograph on the " Biochemistry of the Eye."

TABLE XVIII

Contrasted Composition of Various Fluids

	Human Blood Plasma	Human Cerebro-spinal Fluid	Aqueous Fluid of Eye	
			Ox	Horse
	gm./100 c.c.	gm./100 c.c.	gm./100 c.c.	gm./100 c.c.
Protein . .	7·5	0·07	0·022	0·02
Non-protein N .	0·030	0·021	0·016	0·022
Urea . . .	0·035	0·025	0·017	0·028
Creatinine . .	0·001	0·001	0·0013	0·0016
Glucose . .	0·08	0·06	0·036	0·088
Sodium . .	0·32	0·33	0·32	0·31
Potassium . .	0·020	0·012	0·020	0·020
Calcium . .	0·0105	0·006	0·007	0·007
Magnesium .	0·0026	0·0033	—	0·0022
Chloride . .	0·36	0·44	0·41	0·43
Inorganic P .	0·0030	0·0025	—	0·0032

TABLE XIX

Comparison of Human Blood Plasma and Synovial Fluid

	Blood Plasma	Synovial Fluid
	gm./100 c.c.	gm./100 c.c.
Protein	7·00	4·97
Non-protein N . . .	0·0262	0·0261
Amino-acid N . . .	0·0056	0·0059
Urea	0·0339	0·0339
Chloride Cl . . .	0·339	0·357

Cajori and Pemberton (1928) have furnished figures for synovial fluid from (human) knee joint effusions, sterile, and presumably to be regarded as typical transudates (see below). Their average results, directly contrasted with figures for blood plasma from the same patients, are given in Table XIX.

The amniotic fluid from the sac containing the foetus is at present regarded as a dialysate of maternal blood admixed with foetal urine. Cantarow found the following results (Table XX.) as the average of thirty-six direct comparisons between fluid and maternal blood, mostly obtained from women just prior to birth of the child.

TABLE XX

Comparison of Human Maternal Blood Plasma and Amniotic Fluid

	Blood Plasma	Amniotic Fluid
	gm./100 c.c.	gm./100 c.c.
Protein (excluding fibrinogen) .	6·06	0·53
Non-protein N . . .	0·024	0·024
Uric acid	0·0031	0·0045
Sugar	0·084	0·019 (0·000 to 0·059)
Calcium	0·0098	0·0055
Inorganic P	0·0043	0·0031

When these tables are examined it is seen that the fluids fall into two groups. Lymph and synovial fluid contain about two-thirds as much protein as blood plasma. The other fluids contain only a very small amount. Lymph and synovial fluid are therefore not simply filtrates from blood plasma through an animal membrane.

These two fluids exhibit definite differences from plasma in their chloride and calcium contents. The chloride difference can be explained by a Donnan equilibrium effect (p. 36) produced by difference in protein content. This may also explain the figure for calcium in lymph, since nearly half of the plasma calcium is held by protein, and has been shown to decrease with decrease of plasma protein as in oedema.

Whether the cerebrospinal fluid is a true dialysate from blood plasma or a secretion by the choroid plexuses of the brain (which form it) has long been and still is a matter of controversy. The lack of protein, and the similarity of figures for the other constituents, suggest a dialysate. Closer examination of these figures however reveals definite differences which should not be produced in simple dialysis. That for chloride may be due to a Donnan equilibrium

effect and that for calcium is explained apparently by lack of protein in the fluid. But such differences as those for magnesium and potassium, and the fact that changes in blood plasma calcium are not reflected in the fluid (p. 191) suggest strongly that the fluid is not a mere dialysate and that active secretion plays some part in its formation.

Transudates 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 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. Plasma 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 action of autolytic enzymes (see Chapter XV.).

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CHAPTER X

THE BODY TISSUES

INTRODUCTION

It is quite obvious that when such tissues as that of brain, or muscle, or liver are analysed, in reality the analysis is of a mixture of tissues. Thus, a sample of muscle will include blood vessels and their contents, and intercellular fluids, as well as the muscle cells typifying that tissue. Hence all such analyses need careful scrutiny. For association of a particular compound with a particular function it is all important to determine whether that compound is or is not truly a constituent of the actual tissue cells performing the function, and is not merely present in extracellular material. This is often difficult to determine ; an example already mentioned is the presence or absence of chloride ions within muscle cells. On the other hand there is no doubt about the presence of phosphocreatine within the muscle cells.

In this chapter there will be no attempt to record full chemical analyses of the various tissues. For many of them such data are not yet available. Some examples of such analyses will be given, but more stress will be laid on compounds present in the tissues which have a special relation to their functions ; consideration of these functions is therefore incidentally involved.

For convenience these tissues will be considered in relationship to their embryological origin, although, with present knowledge, this treatment will be found to reveal but little evidence of chemical relationships between tissues of like origin.

To quote then some elementary embryology, the fertilised ovum develops to a mass of cells arranged in three layers enveloping a cavity and constituting the blastoderm. These layers, the external *ectoderm*, the mid *mesoderm*, and the internal *entoderm* are at first composed of undifferentiated embryonic cells, but become modified, structurally and chemically, and gradually are changed to the various tissues making up the animal organism.

The following tissues of ectodermal origin will be considered : epidermal tissue, nerve tissue, certain parts of the eye, and the mammary glands.

The following tissues of mesodermal origin will be considered :

muscle, connective tissue, vascular tissue, lungs, kidneys, and gonads.

Some information is available concerning the pancreas and liver, of entodermal origin.

Of the endocrine glands the pituitary, pineal, adrenal medulla, and accessory chromophil glands are of ectodermal origin, the adrenal cortex and gonads of mesodermal origin, and the thyroid, parathyroids and thymus of entodermal origin. The bulk of our biochemical knowledge of these glands relates to the hormones they elaborate. We know little or nothing of the mechanism of formation of these hormones, or of other compounds specific to the glands and associated with this mechanism.

These hormones have been dealt with in Chapter III. (p. 51). Some additional notes will be added on the gonads.

TISSUES OF ECTODERMAL ORIGIN

Epidermal Tissues

The *skin* constitutes about 18 per cent. of the body weight of adult man; its average area is about 1·6 square metres. It consists of two layers, the *corium*, overlying the musculature, and composed largely of connective tissue, and the outer layer, the *epidermis*. The inner zone of the epidermis is constantly forming new layers of cells, which are pushed outwards, flatten, and die, to become the outer horny layer of the skin. Hair, nails, and in animals and birds, horn, hoof, and feathers, are modified epidermal structures. These all share the common property of consisting largely of insoluble keratins.

The connective tissue composing the corium is of mesodermal origin, and is largely composed of collagen, with some elastin. Ordinary analyses of skin are therefore analyses of a mixture of tissues of different derivation.

An approximation to the composition of this mixed integument can be obtained by collating the figures of various analyses published by different investigators, and is shown in Table XXI. All figures are in percentages. Figures for lipide tend to be misleading, since subcutaneous fat is apparently often incompletely removed.

It has been pointed out that skin acts as a transient reservoir for glucose (p. 102), and, over longer periods, for water (p. 202). Probably the interstitial fluid of the corium is the fluctuating medium.

The compound essential to epidermal tissues and absent from others is keratin. The keratins from different sources show

Table XXI

Composition of Skin

	Man	Dog	Rat	Guinea-pig
Water	61–74	—	58–69	61–73
Total Protein	24	—	30	30
Lipides	1–11	—	1·2	2·6–8·6
Glucose	—	0·67	—	—
Potassium	0·17–0·34*	0·16–0·40*	0·40†	0·23†
Sodium	0·30–0·41*	0·16–0·25*	0·23†	0·38†
Calcium	0·03–0·06*	0·03–0·06*	0·08†	0·10†
Magnesium	0·02–0·04*	0·02–0·04*	0·02†	0·04†
Chloride-Chlorine	0·20–0·46*	—	0·63–1·44†	0·57–1·82†

* Fat-free tissue. † Dried tissue.

certain resemblances in their contents of amino-acid radicals. Reference has been made to Block and Vickery's work (p. 158), which suggests that in keratins the radicals of histidine, lysine, and arginine are present in the molecular ratios of 1 : 4 : 12, indicating some degree of common molecular pattern.

Table XXII shows a marked similarity in the figures for many of the amino-acids of the four keratins of vertebrates, and a most striking contrast between these and the figures for silk-fibroin, from silk, a secretion of the silk-worm. Yet silk-fibroin is regarded as a typical keratin.

Table XXII

Percentages of Amino-acids Derivable from Keratins from Sources Named

	Ox horn	Sheep horn	Sheep wool	Horse hair	Goose feathers	Silk
Glycine	0·30	0·40	0·58	4·70	2·60	40·50
Alanine	1·20	1·60	4·40	4·70	1·80	25·00
Serine	0·70	1·10	0·10	0·60	0·40	1·80
Valine	1·20	4·50	4·50	0·90	0·50	0·00
Leucine	18·30	15·30	11·50	7·10	8·00	2·50
Phenylalanine	—	1·00	—	—	—	1·50
Tyrosine	4·60	3·60	2·90	3·20	3·60	11·00
Aspartic acid	2·50	2·50	2·30	0·30	1·10	0·00
Glutamic acid	3·00	17·20	12·90	3·70	2·30	0·00
Lysine	—	0·02	2·30	—	1·04	0·25
Arginine	4·68	2·70	8·60	7·60	5·00	0·74
Histidine	—	—	0·66	—	0·35	0·08
Cystine	—	7·50	7·30	7·98	6·40	0·00
Proline	3·60	3·70	4·40	3·40	3·50	1·00

Consideration has already been given to possible causes for the great insolubility of the keratins.

According to Marston, in animals which produce hair or wool there is an excessively great demand for cystine for keratin production; 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 is stated to contain only 20 per cent. of water. Of the lipide 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.

Block has shown recently that the cornifying epidermis of human skin gives protein with a histidine/lysine/arginine ratio of 1 : 6 : 7, instead of the 1 : 4 : 12 ratio found by him in true keratins, and he concluded that the process of keratinisation is incomplete. Such a conclusion involved the assumption that during cornification of the dying or dead tissue change of protein from one type to another is possible.

Nerve Tissue

Analysis of nerve tissue is analysis of tissue of ectodermal origin, with the exception that brain tissue includes some vascular tissue, and microglia, both of mesodermal origin, with some blood and interstitial fluid.

There are few recent systematic analyses of nerve tissue in the literature. The work of Palladin and his colleagues on the chemical topography of the nervous system, now in progress of publication,

TABLE XXIII

Percentage Composition of Nerve Tissues

	Grey Matter of Spinal Cord Dog	Grey Matter of Medulla Dog	Cerebellar Cortex Dog	Cerebral Cortex Dog	Coeliac Ganglion Cow	Sympathetic Trunk Cow
Water . .	71·6	81·6	80·7	81·3	75·6	59·3
Proteins . .	9·9	9·8	10·1	10·6	14·9	22·5
Cholesterol . .	4·3	1·8	1·5	1·5	0·3	1·3
Unsaturated phosphatides (as lecithin) . .	6·1	2·9	2·7	2·7	0·9	1·5
Saturated phosphatides and cerebrosides .	4·4	3·1	2·8	3·1	—	—
Creatine . .	0·21	0·23	0·21	0·16	0·06	0·11

permits compilation of certain data, which are shown in Table XXIII. It should be remembered that the grey matter of the brain and cord consists essentially of nerve cells, and the white matter of nerve fibres.

Palladin endeavours from these and similar results to trace a phylogenetic connection in the variations of the chemical constituents of different grey nervous tissues. He stresses the greater lipide content and lower protein in the phylogenetically older tissue of the spinal cord, as contrasted with the more recently developed cortex of the cerebellum and cerebrum, and concludes that the lipides are less important for brain function than the proteins. This type of systematic enquiry into the relationship between chemical distribution and physiological function may well lead to important results, although his present conclusions cannot yet be regarded as established.

Palladin finds further that the anterior and posterior roots of the nerves leaving the spinal cord (of which the former have a greater water content) contain more cholesterol than other parts of the nervous system, while a high protein content, with a median content of cholesterol, is characteristic for the non-medullated nerves.

No recent comparative studies of " grey matter " and " white matter " of brain are available.

The *proteins* of brain include approximately equal amounts of simple proteins and nucleoproteins, and smaller amounts of *neurokeratin*. This material seems peculiar to nervous tissue. It resists gastric and tryptic digestion, and treatment with organic solvents, and dilute acids and alkalies. Block has recently analysed the hydrolysed products of neurokeratin, and found that it yielded 1·8 per cent. of histidine, 3·1 per cent. of lysine, and 4·8 of arginine, giving a molecular ratio of 1 : 2 : 2, instead of the 1 : 4 : 12 considered to hold for true keratins. Hence he considers that neurokeratin cannot properly be classified as a true keratin, in spite of its similar degree of insolubility. The amount of it in different parts of the nervous system varies considerably, between such extremes as 0·3 per cent. in the cerebellar cortex and 0·33 per cent. in cerebral cortex, on the one hand, and 2·6 to 2·9 per cent. in the white matter of the corpus callosum, suggesting that it is especially associated with the structure of nerve fibre rather than that of the nerve cell.

Brain and cord are especially rich in phosphatides and cerebrosides, so that one is led to infer that these compounds are intimately associated with the functioning of the nervous system.

An ancient theory that brain contained a specific constituent

protagon, consisting of phosphatide and cerebroside linked through a sulphate radical, has been discarded.

Creatine, as Palladin's figures indicate, is distributed in small amount throughout nervous tissue ; it is present there as phosphocreatine (creatine phosphoric acid). It will be shown later (p. 253) that this compound is one of the most important constituents of the muscle cell, being intimately associated with the mechanism of muscular contraction. The muscles of the body contain 98 per cent. of its creatine, nerve tissues most of the remainder, but its function in nerve tissue is not known.

Brain tissue contains small amounts of various compounds associated with oxidation-reduction processes, such as adenosine triphosphate (p. 292), and a number of dehydrases. Its glycogen content is small but definite.

Thus Kerr has shown that the average glycogen content in 100 gm. of brain is, for the dog, 0·102 gm., for the cat, 0·086, for the rabbit, 0·082 (and for the sea-turtle, 0·306 gm.). Dog's cerebrum contains per 100 gm., 98 mg. of glycogen, 57 mg. of glucose, and 18 mg. of lactic acid. Brain has a carbohydrate metabolism very similar in type to that of muscle, but has very little capacity for glycogen storage, so that whenever this small supply is depleted and the sugar concentration cannot be maintained from blood plasma, as when hypoglycaemia is induced by injections of insulin, brain suffers from carbohydrate starvation, and this seems to be one of the main causes of the symptoms in hypoglycaemic shock. Further consideration of the oxidation-reduction systems in such tissues as brain is given in Chapter XII.

Brief mention was made in Chapter III. (p. 60) of "local hormones," sympathin (which is probably adrenaline), and acetylcholine, which are responsible for transmission of nerve impulses from nerve endings to receptor structures in other tissues, and probably assist in transference of the impulses across the nerve cells also. These compounds are normally present in brain and nerve, and are probably formed in the tissues which employ them.

It has recently been stated that human brain is relatively rich in copper, 2·5 mg. per cent., and poor in iron, 0·4 to 1·4 mg. per cent. Its mineral content totals just under 1 per cent.

Eye Tissues of Ectodermal Origin

These tissues include the lining epithelium of the front of the eye, the crystalline lens, the lacrymal glands, and the retina. Concerning the first there is little chemical knowledge.

The *crystalline lens* contains 60 to 70 per cent. of water, the amount decreasing with age. The lipides present include phospholipides and cholesterol. Krause reports the presence of six proteins. Analysis of bovine lens from one year old cattle showed the following percentage distribution of protein : nucleoprotein 0·1, mucoprotein 0·8, albumin 1·5, a scleroprotein 12·5, α-crystallin 31·7, and β-crystallin 53·4.

The scleroprotein is not a keratin. The four principal proteins yield no glycine on hydrolysis, but are rich in arginine radicals. The crystallins behave chemically like globulins.

The lens is built up of fibres, of which the tubular portion is composed chiefly of the scleroprotein, while the viscous fluid within is chiefly crystallin. The properties of the lens are probably associated especially with those of the crystallins.

Nothing specifically bearing on their function, the secretion of tears, is known concerning the chemical composition of the *lacrymal glands.*

Tears contain 98·2 to 99·0 per cent. of water, with minute traces of protein 0·07, urea 0·03, glucose 0·065, sodium chloride 0·066 per cent., and of various enzymes. It is frequently claimed that human tears possess bactericidal properties, due to the presence of a specific compound lysozyme.

The *retina* is characterised by a highly organised lipide structure, a complex specific photochemical system, and a vigorous oxidative system (Krause). The lipide content is much lower than that of nervous tissue. At least four proteins are present, including an albumin, a globulin, and a neurokeratin.

Bovine retina is stated to contain, in mg. per cent., anserine 31, carnosine 2, creatine 46·5, creatinine 4·5, adenine nucleotide 4, and a trace of carnitine. The creatine is present as phosphocreatine (*cf.* p. 248). The qualitative resemblance to muscle tissue (p. 242) is interesting.

The rods of the retinae of vertebrates contain the so-called " visual purple " or *rhodopsin*, a yellowish-red to purplish-red solid, easily bleached by light, and differing somewhat in different species. Its colour is capable of regeneration. This compound is non-dialysable and colloidal ; it is soluble in bile salts. Strong oxidation converts it to " visual yellow," and this is converted to fluorescent " visual white " by ultra-violet light.

Visual purple is in some way associated with visual perception. It belongs to the class of carotenoid-proteins, of which other examples are the blue pigment of crustacean shells (p. 125), and ovoverdin, a green compound of astacene and a scleroprotein which is present in the eggs of the lobster.

The Mammary Glands and Milk

The mammary glands are secondary sex-organs of the female, gradually stimulated to full development by her oestrogenic hormones, and stimulated further to complete functional activity by these same hormones, additional stimulus to actual secretion of milk being subsequently furnished by prolactin of the anterior pituitary and the suckling reflex set up by the offspring.

The function of the mammary glands is to secrete milk, and in so doing they perform 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 glands, other probably specific proteins (though milk globulin and plasma globulin from the same animal may be identical), and its fats contain radicals of several of the lower fatty acids not present in blood. It is gradually being demonstrated that the glands contain a large number of active enzymes.

The constituents of milk 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 XXIV.

TABLE XXIV. COMPOSITION OF MILKS

	Human	Cow	Goat
Water (average) .	87·5	87	87
Protein . . .	1·5–0·7	4·0–2·5	3·7
Lipides . . .	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.

Milk proteins consist of casein, 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 protein. (In the British nomenclature *caseinogen* is used for the natural protein in milk, and *casein* for its split product which is precipitated in clotting. Since the naturally occurring compound actually secreted into milk seems the more important, the American usage has been followed throughout in this text, and the term "casein" employed for the protein of milk, "paracasein" for its split product functioning in milk-clotting.)

Milk fat consists largely of oleates and palmitates, with smaller amounts of the glycerides of the lower fatty acids (*cf.* p. 134). Small amounts of lecithin, cephalin, and cholesterol are present.

It has long been thought that blood phospholipides were the source of milk fat, but it has recently been shown that during milk secretion the phospholipide content of blood does not diminish in amount during passage through the mammary glands of cows; it is therefore almost certain that milk fat is formed from the neutral fat and cholesterol esters of blood.

It is usually considered that the mammary glands form the specific carbohydrate of milk, lactose, from glucose, and evidence is available from experiments with cows that the drainage of glucose from blood passing through the glands roughly corresponds with the amount of lactose formed. Grant has demonstrated the production of lactose from glucose *in vitro* by tissue slices of mammary gland, and the non-production of lactose from galactose, fructose, and mannose.

This synthesis necessitates the conversion of glucose to galactose, a configurational change of possible difficulty. Some recent work has suggested that the gland forms its galactose from lactic acid, rather than directly from glucose.

The mineral constituents of milk include potassium, sodium, chloride, 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 diffused 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.

TISSUES OF MESODERMAL ORIGIN

Muscle

Introduction. Muscle tissue does all or almost all the work of the body, and in so doing produces much of the body's heat. In this section some attempt will be made to indicate the chemical

mechanisms by which work is done by muscle. The concomitant problem of heat production will be dealt with later.

Muscle is able to do work because of the inherent contractility of its tissue. To relate this to its chemical composition some reference must be made to muscle structure. Only skeletal muscle will be considered here. Maximow's description is chiefly followed.

FIG. 17. Fibrils of the wing-muscles of a wasp, highly magnified (Schäfer). A. A contracted fibril. B. A stretched fibril. C. An uncontracted fibril.

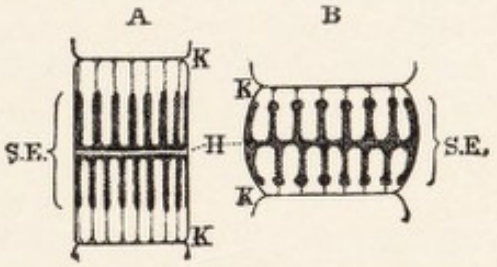


FIG. 18. Diagram of a sarcomere in moderately extended condition (A), and in contracted condition (B). K, K., Membranes of Krause. H. Line or plane of Hensen. SE. Dark portion (Schäfer).

Skeletal muscle of mammals is made up of long cylindrical *fibres*, held together by connective tissue. Each fibre is a large multinucleated cell, varying in length from 1 to 41 mm., and in thickness from 0·01 to 0·1 mm., depending upon the particular muscle and the species of animal. Each fibre is an independent unit covered by a membrane, the *sarcolemma*, of 0·001 mm. thickness. The cell is cross-striated in a regular manner (whence the term " striated muscle "), and is composed of a protoplasmic mass, the *sarcoplasm*, and very thin cross-striated *fibrils*. The thick-

ness of the latter varies from 0·002 mm. to one-tenth of that figure or even less. They are distributed in compact bundles and separated by sarcoplasm.

Each myofibril is composed of a series of contractile units, *sarcomeres*, divided from each other by narrow discs (the Z-discs, or Krause's membranes). The central portion of each sarcomere appears darker under the microscope, and is *anisotropic*, while the lighter portion on each side of it is *isotropic*. Anisotropic material is double refractive when examined in a polarising microscope, permitting light to pass through crossed Nicol prisms and so appearing bright. Double refraction is usually associated with systematic arrangement of molecules, while single refraction as in isotropic material indicates absence of such systematic arrangement. The outer parts of each sarcomere are lighter in appearance, but isotropic, and dark under the polarising microscope. According to Schäfer, in the act of contraction the lighter material flows into tubular pores in the central, darker material.

The arrangement can be best seen in such highly differentiated tissues as the wing-muscle of the wasp, and Schäfer's representation of the arrangement there is shown in Figs. 17 and 18.

There is further evidence that the anisotropic portions of the sarcomeres largely consist of rod-like structures.

The General Composition of Striated Muscle. The approximate composition of muscle is shown in Table XXV.

TABLE XXV. 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	17
Phospholipides	4·5		
Cholesterol	0·3		
Creatine	0·37–0·60	0·35–0·57	0·2– 0·7
Creatinine	0·01		
Carnosine	0·2–0·3		0·25
Carnitine	0·02–0·03		
Purine compounds	0·08–0·18	0·08–0·16	0·05–0·09
Inositol	0·003		
Glutathione	0·06		
Glycogen	0·1– 3·7		0·7

Many other compounds are present in traces. The chief constituents will be considered in turn.

Proteins of Muscle. Muscle contains insoluble proteins from the incorporated connective tissue, and nucleo-proteins from the cell-nuclei. Neglecting these, the muscle cells contain at least four proteins, two globulins, *myosin*, and " globulin X," and two albumins, myogen and myo-albumin. These four are present in the proportions of 68, 21, 10, 1. The two albumins are quite soluble in water, and are not easily separable. Globulin X is soluble in more dilute salt solutions than is myosin.

The sarcoplasm of muscle, 30 per cent. of its weight, can be obtained as muscle " press-juice." Analysis of this press-juice indicates that the sarcoplasm contains all the myogen and myo-albumin, and probably all the globulin X. *The myosin is present in the myofibrils.*

Myosin can be extracted from ground mammalian muscle by cold dilute alkaline salt solution (potassium chloride and phosphate) of *p*H 7·85, and can be purified by repeated precipitation (by dilution, or by lowered *p*H) and re-solution. It has unusual and characteristic properties. Its solutions set to gels; shaking transforms them to sols. On standing gels form again. This property is termed *thixotrophy*.

Solutions of myosin at rest are isotropic; they do not affect polarised light. When set in motion they become doubly refractive, thus exhibiting the phenomenon termed *fluxional birefringence*. This indicates that rod- or needle-shaped structures must be present; thus the myosin molecules are needle-shaped structures, and probably constitute the rod-like structural elements of the anisotropic bands of the sarcomere.

The property of birefringence is irreversibly lost when the myosin is treated with agents producing denaturation, such as acids and alkalies. The other proteins of muscle do not exhibit thixotrophy or fluxional birefringence.

Myosin threads have been made by squirting myosin solutions into water. When stretched and dried they become strongly birefringent. Soaked in dilute solutions of potassium phosphate at *p*H 7·4 (corresponding to that of muscle) they take up water to the extent of 80 to 84 per cent., *i.e.*, the protein content is of the same order as that in muscle. X-ray photographs of these threads are similar to those of muscle. Quantitative studies of their birefringent properties have led to the conclusion that *the anisotropic bands of the fibrils are made up of myosin molecules or micellae, arranged in some degree of order, with the long axis of the molecular needles parallel to those of the fibrils.* The isotropic

bands are not completely devoid of birefringence (possessing about one-tenth of that of the anisotropic bands) and are considered to be made up of myosin micellae in a less ordered arrangement.

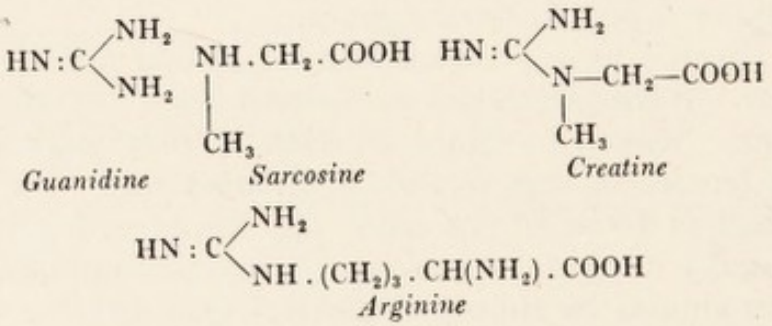
When muscle contracts the anisotropic bands are believed to shorten, and a decrease in the double refraction occurs. In this contraction there would appear to be a shortening and thickening of the myosin micellae, and at the same time a denaturation of dehydration type. It is also known that small amounts of phosphate ions specifically increase the solubility of myosin, and as will be seen later (pp. 248, 294) during contraction there is an increase of phosphate ions in the muscle cell.

Thus it would appear possible that contraction of muscle is causally associated with change from myosin gels, with an ordered arrangement of molecule-needles, to myosin sols, with less ordered arrangement, this change involving shortening of fibrils, and being initiated by an increased concentration of phosphate in the neighbourhood of the myosin molecules.

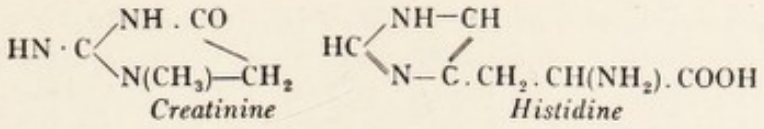
Cytochrome, a mixture of haemochromogens, (*cf.* p. 218), is present in the muscle cell in traces, and plays a *rôle* in its oxidation-reduction processes (p. 287).

Phospho-creatine, Creatine, and Creatinine. Phospho-creatine (or phosphagen or creatine-phosphate) and myosin are the compounds intrinsically associated with muscle contraction, and therefore with muscle function. Creatinine is the degradation product of creatine.

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.



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 still more rapid change of creatinine into creatine. Both compounds are decomposed more slowly to urea and sarcosine, and thereafter to ammonia, methyl-hydantoic acid and methyl-hydantoin.

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.
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 creatine is concerned with muscle function.

Rapidity of contraction seems to be correlated with creatine 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 foetuses 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 (occurrence of creatine in urine) 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 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 no unusual occurrence. It is an important constituent in the urine of birds.

Creatinine 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 mg. per 100 gm.—than other tissues and than blood—1 to 2 mg. The urine of all vertebrates constantly contains creatinine. In man the daily excretion is from 1 to $1\frac{1}{2}$ gm.

Folin's Creatinine Coefficient and Harding's Creatine Coefficient. Creatinine excretion is functionally significant. On diets 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 mg. per kg. body-weight per twenty-four hours, averaging about 24 to 25 mg. 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 mg., and from five to thirteen years 9 to 13 mg. per kg.

The rate of creatinine excretion is definitely lower during sleep.

Folin regarded the creatinine output as " an index or measure of the total normal tissue metabolism." Shaffer related it to one special catabolic process which takes place largely, if not wholly, in muscle tissue ; 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 mg. 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 precursors of creatine in the body, and *the site of its formation* are still undetermined. It can be considered as formed from one or more amino-acids ; its close relationship to arginine and to histidine suggests that one or other of these is the probable precursor. (Guanidino-acetic acid may be an intermediate stage in its formation, and is normally excreted in traces in urine.)

Two methods have been used in attempts to determine its precursors. Possible precursors have been fed animals, and then the creatine content of their muscles has been compared with that of controls. Since muscle can be regarded as almost saturated with creatine this method has limitations.

Many animals continually excrete creatine (suggesting an excess of supply over demand). Possible precursors have been fed such animals in an attempt to find those which would lead to increased excretion of creatine as an indication of its increased formation from the compounds fed. This method also is open to sources of error. Thus we do not know what controls creatine formation, and it is quite probable that in absence of increased demand for it no further amounts will be produced from its precursors even if they are fed. As Hunter has pointed out, the production of creatine is probably regulated by functional demand, and there is no more reason to believe that increasing the supply of its precursor will increase its formation than that administration of a dose of tyrosine will increase the production of thyroxine or adrenaline.

It is usually considered that creatine is formed in muscle, but this logical view cannot be considered proved. 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.

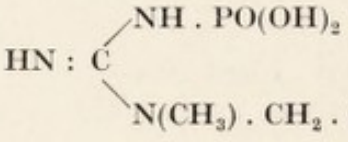
Harding showed that, provided sufficient protein material be fed, there is a constant creatine production at all ages, proportional to the body-weight, and therefore to the total number of body cells. Harding concluded from this constancy that, since there is very different muscular development in children 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 muscle or some other tissue, from specific amino-acids ; if formed in non-muscular tissue, then it circulates in the blood, 98 per cent. being absorbed by muscle and the other 2 per cent. by other tissue. Muscle becomes almost completely saturated. Men 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.

Creatine-phosphate, phospho-creatine, or phosphagen. 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. Our knowledge of its form of combination in muscle is recent, and is due to Fiske and Subbarow, and Eggleton and Eggleton.

All of the 400 to 500 mg. of creatine in each 100 gm. of muscle, except a possible 30 mg. 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 much 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 Nature of Creatine-Phosphoric Acid. The constitution of "phospho-creatine" (Fiske and Subbarow), or "phosphagen" (Eggleton and Eggleton), or creatine-phosphoric acid is :



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.

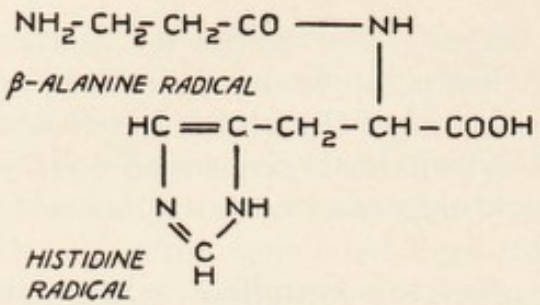
The hydrolysis and the synthesis of creatine-phosphoric acid seem to vary with the *p*H of the medium containing it. In alkaline solutions synthesis prevails, while with a *p*H of 6 hydrolysis is rapid. The reaction is associated with the glycogen-lactic acid changes, but is not dependent on them, and is actually more essential to muscular contraction than the breakdown of glycogen (*cf.* Chapter XII.).

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 metabolism of muscle.*

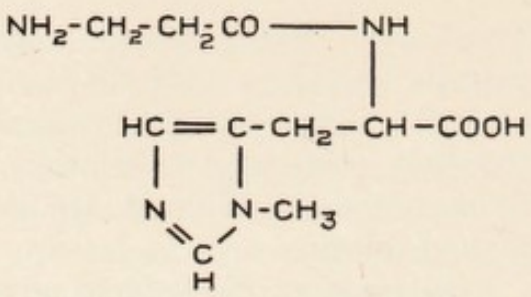
The creatine of heart and smooth muscle, and also of testes and spleen, in both mammals and birds, is present as creatine-phosphate ; in fact this represents the active or effective form of creatine in all tissues.

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. Phospho-arginine is not attacked by arginase.

Carnosine and Anserine. Carnosine is β-alanyl histidine. Anserine is methyl-carnosine.



CARNOSINE



ANSERINE

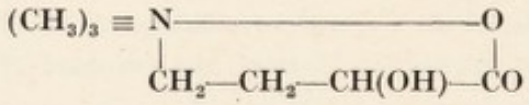
β-Alanine has not been found to occur naturally except when linked in the carnosine molecule. Although muscle contains an amount of carnosine which must be considered far from negligible, its function is not yet known. It is soluble in water and dextro-rotatory. Carnosine cannot diffuse from the muscle cell during the life of that cell.

Starvation lowers the carnosine content of the 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.

Anserine was first obtained from goose-muscle, whence its name (L. *anser*, goose). The maximum amount found was 0·12 per cent. It is absent from other tissues of the goose.

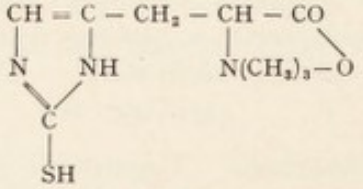
Anserine is also present in the muscle of fowl, pigeon and crow, and appears to replace carnosine in these birds. It is present along with carnosine in crocodile muscle, but is absent from the muscle of the dog-fish and beef muscle, although stated to be present in dog muscle. Carnosine is present in 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 :

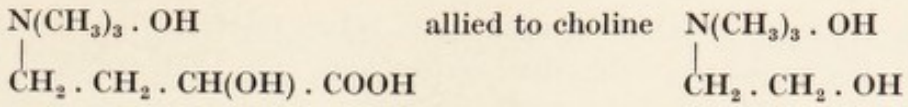


When it is hydrolysed with baryta trimethylamine is liberated. Carnitine can be regarded as the anhydride of

* A curious histidine derivative containing sulphur, *ergothioneine* (also termed in the literature thioneine, thiasine and sympectothione), which was first isolated from ergot in 1908, has been found in the red cells of blood, in amount corresponding to 0·15 per cent. of pig's whole blood, and one-tenth this quantity in human blood. Its significance is not known.

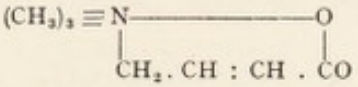


Ergothioneine

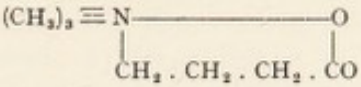


Nothing is as yet known of the function of carnitine.

The related croton betaïne



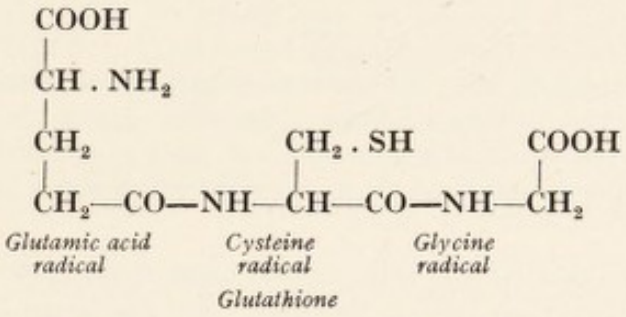
has recently been isolated from beef muscle. Feeding experiments have shown that some γ-butyrobetaïne



and less croton betaïne are oxidised to carnitine in the organism. These have a slight curare-like action, but carnitine is innocuous.

Fish muscle contains trimethylene oxide, $(CH_3)_3N : O$, which perhaps corresponds to carnitine in fishes.

Glutathione was first isolated from muscle, by Hopkins, in 1921. It occurs in two forms, "reduced" and "oxidised" glutathione. The reduced form is a tripeptide, glutamyl-cysteyl-glycine :



In the "oxidised" form the cysteine radical has been "oxidised" to cystine by removal of hydrogen, so that, if the reduced form is written G . SH, the oxidised compound is G . S . S . G.

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.

The changes in which glutathione takes part will be dealt with in the discussion on biological oxidations (see Chapter XII.).

Adenylic and inosinic acids and the corresponding **adenosine pyrophosphate** (pp. 179, 292) are important constituents of muscle, whose functions are concerned with its oxidative changes. These functions will be discussed in Chapter XII.

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. 302), 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. 303). 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.

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.

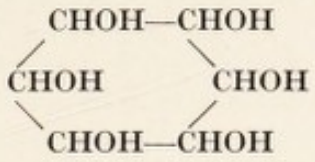
It has already been pointed out (p. 101) that following contraction of muscle some of its glycogen breaks down to lactic acid through the stage of a *hexose diphosphate* (the "lactic acid precursor," or *lactacidogen* of Embden). A fraction of the lactic acid is oxidised, and this process provides the energy for muscular contraction, and for reconversion of most of the lactic acid to glycogen.

Experiments on the frog have demonstrated that glycogen is distributed in resting muscle throughout the sarcoplasm, but is present in greater concentration in the doubly-refracting bands of the fibrils. In fatigued muscle there is a decrease of glycogen in the isotropic bands; it is conserved principally in the anisotropic bands. During the recovery of the muscle from fatigue the distri-

bution of glycogen gradually returns, with its re-formation, to that seen in the resting tissue.

Various statements have been made as to the presence of reducing sugar in muscle. One of the most recent claims that skeletal muscle contains fermentable sugar in amount corresponding to 15 per cent. of that in blood plasma, while heart muscle contains about twice this concentration. Such statements will need verification.

Inositol, of which a trace is present in muscle, is a slightly sweet hexahydric alcohol, $C_6H_{12}O_6$, with the constitution :



It is widely distributed in plants, free, and combined as *phytin*, the calcium magnesium salt of inositol phosphoric acid. The plant enzyme phytase specifically splits phytin to inositol and phosphate.

Its presence in animal tissues may arise accidentally from its presence in vegetable foods. There is some evidence, however, that it is formed in the egg-embryo of the fowl, and that the embryo of the dog-fish *Acanthias* forms the isomeric compound *scyllitol*, present in fish tissues.

The function of such compounds as inositol and scyllitol is not known.

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 Significance of the Compounds Present in Muscle. Of those compounds concerning whose function we are commencing to know a little, myosin is the substance which brings about actual contraction through changes in its physical state, creatine-phosphate is probably the controller of that physical state, and is perhaps the immediate recipient of the nerve message which leads to contraction (it might be compared with the detonator of an explosive charge), glycogen is the source of energy (for the next, rather than for the immediately preceding contraction), and the nucleotides, glutathione, and cytochrome are concerned with facilitation of the oxidations liberating that energy.

Carnosine and carnitine almost certainly have definite functions specific to muscle, while inositol perhaps has a function.

More detailed consideration will be given to some aspects of the oxidative processes in Chapter XII.

Abnormal Creatine Metabolism in Certain Pathological Conditions

Febrile conditions result in creatinuria, associated with increased creatinine excretion.

Creatinuria, accompanied by diminished creatinine excretion, occurs in a number of conditions involving muscle wasting (muscle atrophies and dystrophies). When glycine is administered to patients exhibiting these conditions, it appears to produce an increase of the creatinuria at least transiently. The cause of this effect has not been ascertained. It can scarcely be traced to a lack of glycine, since these patients are normally able to provide sufficient of this amino-acid to detoxicate administered benzoate to hippuric acid (*cf.* p. 313).

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 section of the nerve supply to a muscle the creatine content of this muscle remains constant until its degeneration commences.)

Connective Tissues

The connective tissues of the body include fibrous and elastic tissue, cartilage, bone, and dentine of teeth, but not enamel (which is of ectodermal origin). Certain of these tissues normally undergo calcification, so that there is superposition of mineral on organic structure. Comparison of non-mineralised and mineralised tissues is therefore of interest. Knowledge of the nature of bone mineral and the process of calcification is still in the stage of theory, although intensive work on these problems has been carried on for a long period. Abnormal and pathological calcification is also of importance, both from a theoretical and a clinical viewpoint. But while bone and the process of calcification have been much studied, there is little recent work on non-mineralised connective tissue, which merits more attention than it has received.

Non-mineralised Connective Tissue. The chief organic constituents of such tissues are scleroproteins, and of these the most widely distributed is *collagen*, present in white fibrous tissue such as the Achilles tendon to the extent of 32 per cent., and in smaller amounts in elastic tissue, cartilage, and bone. Probably

different collagens exist, with similar close chemical relationships to those exhibited by the keratins of different tissues of the same species of animal. Collagens contain less sulphur (and therefore presumably fewer cystine radicals) than keratins.

The *Tendo Achillis* of the ox contains, according to old analyses of Buerger and Gies, in percentage figures, water 63, inorganic material 0·5, organic material 36·5, including 31·6 of collagen, 1·3 of a mucoprotein, traces of other proteins, and 1 per cent. of lipide material.

The *Ligamentum nuchae* (a strong ligamentous band from the back of the neck, consisting chiefly of yellow connective tissue) can be taken as typifying elastic tissue. That from the ox, also according to old analyses of Gies, is composed of 57·5 per cent. of water, 0·5 of inorganic material, and 42 per cent. of organic material, including the scleroprotein *elastin* 32 per cent., collagen 7, mucoprotein 0·5, albumin and globulin 0·6, and 1 per cent. of lipide material.

Cartilage, the parent tissue of bone, consists of mesodermal cells embedded in intercellular substance which is regarded as formed by the cells. This matrix is largely composed of a mucoprotein, *chondromucoid*, collagen, and another scleroprotein (chondro-albumoid).

When collagen is boiled for several hours with water it is converted to *gelatin*. The process involves definite chemical changes and marked changes in physical properties. Thus while collagen gives a distinct Millon's test, indicating presence of tyrosine radicals (p. 151), gelatin gives the test only faintly, indicating that the tyrosine radicals have been split off or altered. While collagen is a typical insoluble scleroprotein, gelatin is very soluble in water, from which it sets to the typical gel. On account of its characteristic properties gelatin has been very thoroughly studied. Table XI. (p. 153) gives the amino-acid radical content. It is usually but incorrectly classified with the scleroproteins. It might almost be termed a "false protein." The chemistry and physical chemistry of collagen, much more important from the viewpoint of functional significance, have been almost ignored.

Elastin also merits more study. No recent examination of its hydrolytic products seems to have been made, and the older analyses are incomplete. Since undoubtedly the characteristic properties of elastic tissue are associable with this compound, further knowledge of it should prove highly instructive.

The chondromucoid of cartilage hydrolyses to protein and *chondroitin sulphuric acid*, which successively hydrolyses to

chondroitin and sulphuric acid, chondrosine and acetic acid, and chondrosamine (p. 83) and glycuronic acid (p. 83).* Chondroitin sulphuric acid is also obtained from other mucoproteins of connective tissue. Most mucoproteins yield the similar mucoitin sulphuric acid, in which radicals of glucosamine (chitosamine, p. 83) replace those of galactosamine (chondrosamine). To these is related chitin, a polymerised acetylated glucosamine which forms the exoskeleton of crustacea.

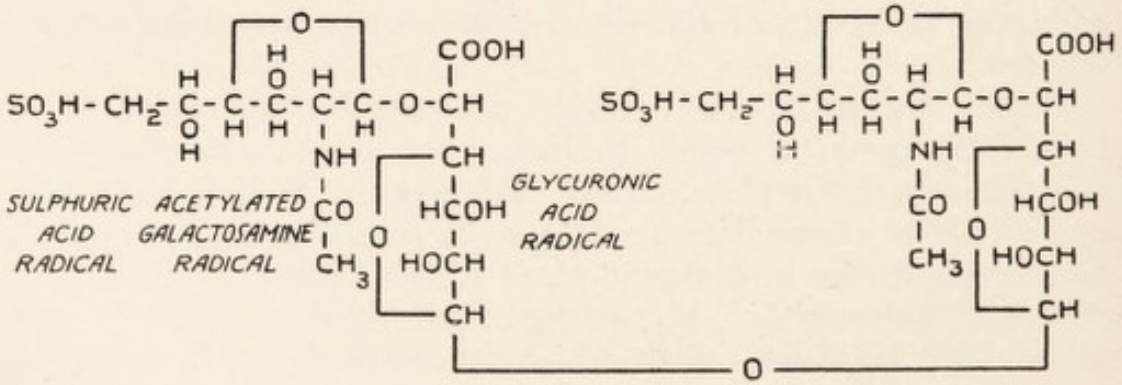
Bone, the supporting structure of vertebrates, is connective tissue in which the matrix is impregnated with mineral compounds laid down in crystalline or semi-crystalline arrangement. Many bones contain cavities filled with *bone-marrow*, a mixture of connective tissue elements and lipides (yellow marrow), or myeloid, blood forming tissue (red marrow). In early life the red variety predominates ; it is slowly replaced by the yellow marrow. In the adult red marrow is found in the vertebrae, ribs, sternum, and towards the ends of the long bones.

Bone contains less than 50 per cent. of water and a variable amount of fat. Dried, fat-free bone consists of 30 to 40 per cent. of organic material, the remainder being inorganic. The organic material consists chiefly of *ossein*, a collagen. A mucoprotein is present, which yields chondroitin sulphuric acid.

Bone, like muscle, is anisotropic, giving a striking picture of Maltese crosses in polarised light. This is due to the presence and arrangement of doubly refractive collaginous fibres, since it persists in carefully decalcified bone.

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.

* The constitution of chondroitin sulphuric acid is believed to be



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.

Huggins, in a recent review, considers that the known facts are best fitted by the view that bone salts are mainly a mixture of hydroxylapatite and carbonate apatite, in which other difficultly soluble compounds of magnesium and fluorine, etc., tend to accumulate.

Formation of Bone. Robison has shown that bone and teeth contain 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). It is not known whether the phosphatase of bone is specific to that tissue.

Kay and others have developed methods for the estimation of phosphatase 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.

Bone is under control of the parathyroid hormone, and directly or indirectly, the growth hormone of the pituitary. Vitamins **D**, **A** and **C** exercise some control on its structure and growth. The mechanism of these controls is not yet known.

The *dentine* of teeth possesses 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) and only 3·5 per cent. organic material. Its protein is, according to Gies, of keratin, and not of collagen type, indicating the ectodermal origin of enamel. It contains relatively less magnesium and carbonate 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 and phosphate 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 ; it has been stated that an increase in magnesium content makes certain marine shell structures more compact.

Pathological Calcification. Wherever there is necrosed or diseased tissue there may be a possibility of calcification. This is well exemplified by the calcification of old tuberculous lesions. Calcification of the kidney and other tissues can result from overdosage of irradiated ergosterol, and also from deficiency of vitamin **A**. Apparently the concentration of calcium and phosphate in blood plasma is so close to that in saturated solutions of calcium phosphate that many slight changes in plasma equilibria can lead to deposition of that compound.

The composition of such solid deposits tends to approximate very closely to that of the solid matter of bone. They, also, contain some magnesium and some carbonate.

Vascular Tissue

This is composed of a mixture, varying in proportions in capillaries, arteries, veins, and lymph vessels, of endothelial cells,

originating from embryonic connective tissue, connective tissue cells, layers of thin collagenous or reticular fibres, elastic fibres, and smooth muscle cells. Little or nothing is known of any compounds of collagen or elastin type specific to vascular tissue.

Lungs

The functional portion of the lungs consists essentially of collagenous and elastic fibres and smooth muscle cells, and, in the alveolar sacs, cells which may be of mesodermal or endodermal origin. Again, nothing is known of the degree of specificity, if any, of the proteins of the collagenous and elastic fibres of this tissue.

Kidneys

Little specifically chemical is known concerning the compounds present in kidney tissue. The precise mechanism of the separation of urine from blood plasma in the secretory unit—the *nephron*—consisting of glomerulus and tubules is still uncertain, though it is usually considered that the glomerular process is largely or wholly the filtration of a very dilute urine from blood plasma, this dilute fluid being subsequently concentrated during passage through the tubules, by reabsorption of water and useful compounds such as glucose.

The kidney, however, is more than a mere mechanism for secretion of waste products. Certain chemical reactions are essentially associated with kidney tissue. It shares with the liver the power of detoxicating benzoates by formation of hippuric acid (*cf.* p. 313). It possesses specifically the power of forming ammonia from urea of the blood plasma, with the functional purpose of conserving blood bases (p. 167), while it is much more powerful than liver tissue in oxidising acetoacetic acid, and thus carrying out the final stage of the oxidation of fatty acids (p. 133).

The Gonads

In recent years most of the experimental biochemistry on the gonads has been directed towards the isolation and study of their specific hormones. These have been dealt with in Chapters III. and V. (pp. 59, 119, 122).

It is perhaps pertinent here to consider a little further the functions of these hormones, and the products of the gonads which they are designed to assist.

Under the stimulus of the gonadotropic hormones of the anterior pituitary the testes develop, and themselves secrete, perhaps in the so-called " interstitial tissue " lying between the

spermatogenetic elements, the hormone testosterone, which stimulates the development of, and controls the secondary sex organs of the male (penis, seminal vesicles, prostate) and his secondary sex characters, while the spermatogenetic elements, under pituitary stimulus, elaborate the spermatozoa.

The gonadotropic hormones of the pituitary stimulate the development of the ovaries, so that ultimately many follicles enlarge considerably and, in woman and certain other of the higher mammals, one follicle ruptures, discharging an ovum. (In many mammals a number of follicles discharge their ova at each ovulation period.) The enlarged follicles produce oestradiol, which stimulates and controls development of the secondary sex organs and characters of the female, and, in woman, the first phase of the changes in the uterus during the menstrual cycle, a proliferation of its basal epithelium.

Following discharge of the ovum the ruptured follicle fills with blood, becoming thus a " haemorrhagic follicle," and then rapidly changes to a corpus luteum, which secretes progesterone. Progesterone causes the uterus to develop to the second phase, so that its epithelium becomes glandular in character and fit to receive a fertilised ovum. If the ovum does not become fertilised it passes down the adjacent Fallopian tube and is lost, and shortly afterwards the corpus luteum atrophies, the unruptured enlarged follicles also, there is thus a rapid decrease in production of oestradiol and progesterone, and menstruation follows.

If, immediately following ovulation, the ovum is fertilised by a spermatozoon, it rapidly alters in character, and following passage down the Fallopian tube after eight to ten days it burrows into the prepared wall of the uterus—*nidation*—whereupon the precursor of what becomes the chorionic membrane of the placenta (tissue elements derived from the ovum) elaborates a specific hormone which passes to the maternal organism, and stimulates the corpus luteum so that this tissue persists and progesterone continues to be produced, a process apparently essential for the early stage of normal pregnancy.

This primordial hormone, essentially associated with the ovum itself, strongly resembles in its action that one of the gonadotropic hormones of the pituitary which is usually termed the luteinising hormone, and so is termed the *anterior-pituitary-like hormone of the placenta*, or, simply, APL.

Little or nothing is known so far of the chemistry of the mammalian *ovum*. The corresponding *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 sperms at different periods of the year Kossel and Schenck showed that in the unripe stages of these sperms there are present different peptone-like complexes containing radicals of arginine, or arginine and lysine, or arginine and histidine, or 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 process 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. Spermine has 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, prostate, pancreas, and also from yeast. The maximum yield from semen is 0·24 per cent., prostate contains over 0·1 per cent., pancreas contains about 0·02 per cent., 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 faeces 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

$$NH_2 . (CH_2)_3 . NH . (CH_2)_4 . NH . (CH_2)_3 . NH_2.$$

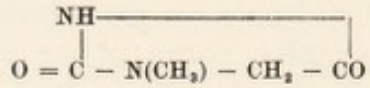
Its function is still unknown. It is accompanied, in much smaller amount, by *spermidine*,

$$NH_2 . (CH_2)_3 . NH . (CH_2)_4 . NH_2$$

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-methyl-hydantoin,



Its significance is not known, but, since testes contain an appreciable amount of creatine (*cf.* p. 245), it is interesting to note that it can be obtained from creatine by the action of alkali (*cf.* p. 245).

In mammals the fertilised *egg* develops into the foetus within the body of the mother and derives filtered nourishment 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.* p. 258). 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—*ovo-mucoid*—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 haemoglobin, and which has been named *haematogen*.

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 fatty acid radicals of yolk fats are chiefly those of palmitic and oleic acids, with less stearic, 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. But 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. *The milk of the mother is the perfect food of the nursling, but we must not stress it as giving too great a clue to the perfect food of the adult.* The composition of milk has already been dealt with (p. 239).

TISSUES OF ENTODERMAL ORIGIN

The Pancreas

The pancreas produces both an external secretion, the pancreatic juice, and an internal secretion, insulin, and is thus an exocrine and endocrine gland, made up of two different tissues, acinar tissue producing the juice, and the islets of Langerhans, producing insulin.

Human pancreas contains about 28 per cent. of solid material and 72 per cent. of water. Of the solids 10 per cent. is lipide, and 15·5 per cent. protein. The pancreas is rich in nucleo-proteins, one of which is peculiar in that it is present in cell cytoplasm, while the nucleic acid derived from it yields *d*-ribose, and not desoxy-ribose (p. 178).

The pancreas seems to contain only traces of albumins and globulins, most of its protein content being insoluble in water and neutral salt solutions. It contains numerous zymogens, as is to be expected from the composition of pancreatic juice.

The 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. lipide 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. lipide, 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 much higher figures have been recorded. From experiments in which 10 per cent. glucose was injected intravenously into dogs it would appear that 20 per cent. of its fresh weight is the maximum amount of glycogen which the liver can store. The glycogens of the livers of rats, rabbits and fish are chemically identical.

A number of proteins are present ; the amount depends to some extent on diet. The peculiar brown colouring pigment of the liver has not been thoroughly investigated.

About half the fatty content of normal beef livers consists of phospholipides. In fatty degeneration of the liver, produced experimentally by such means as phosphorus poisoning, and occurring in various pathological conditions, the lipide 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.

Of all glands the liver presents the greatest uniformity of its functioning cells. Maximow states " In spite of the manifold functions which the liver cells perform there is a marked similarity in appearance in all of them. . . . It would appear that all of the liver cells are equally endowed with the same functional capacities, but that their active participation in these processes depends on the location of the cell in the (liver) lobule." The cytoplasm of the cells presents an appearance varying with the functioning state. Sometimes the cells seem completely filled with glycogen, while at other times they contain large numbers of fat droplets.

The essential uniformity of the liver cells is the more striking when the many functions of this organ are recapitulated. A partial list of such functions includes (i.) glycogen formation, storage, and hydrolysis to glucose ; (ii.) saturation and unsaturation of fatty acids, their elaboration from other compounds, and their oxidation to acetoacetic acid ; (iii.) formation of its own special proteins, and of others such as fibrinogen ; (iv.) probable elaboration of many of the amino-acids which are not essential diet constituents ; (v.) deamination of amino-acids and conversion of ammonia to urea ; (vi.) elaboration, or partial elaboration, and storage of a specific haematopoetic compound ; (vii.) production of bile pigments from their porphyrin precursors ; (viii.) production of bile salts, this involving production of cholic acids from cholesterol, and production of taurine from cysteine ; (ix.) pro-

duction of vitamin A from carotene ; (x.) numerous detoxication mechanisms (*cf.* Chapter XIII.).

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CHAPTER XI

EXTRACELLULAR RESPIRATION

Introduction. *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. Tissue respiration and the biological oxidations which are involved in it will be considered in the next Chapter.

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. are employed in filling the " dead space " of the trachea and bronchioles, while the lung space to which the other 350 c.c. are 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 XXVI., 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 XXVI. 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. 342).

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 haemoglobin to oxy-haemoglobin. 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-aerotonometer* 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 XXVI. (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.

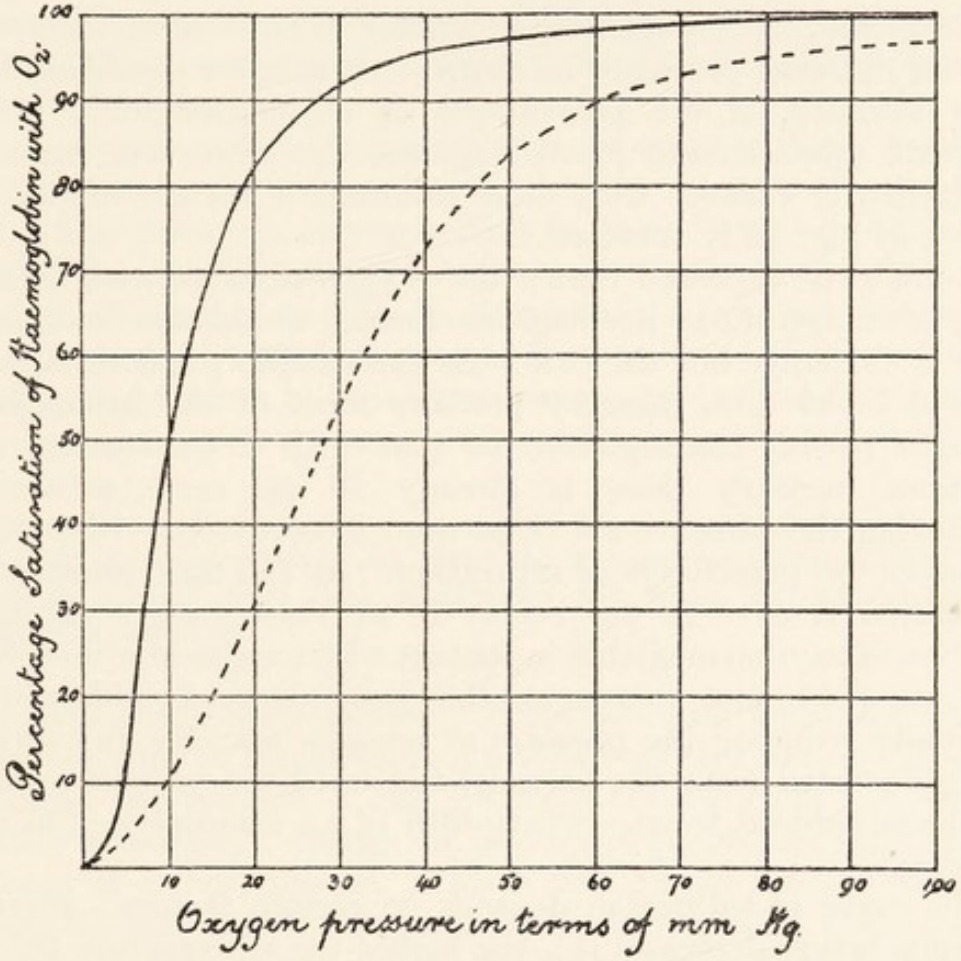

FIG. 19. Dissociation curves of haemoglobin. *Continuous line:* Haemoglobin in contact with oxygen only. *Dotted line:* In presence of carbon dioxide at 40 mm. Hg pressure. (After Barcroft and Poulton, *J. Physiol.*, 1913, xlvi., Proc. v.; and Christiansen, Douglas, and Haldane, *ibid.*, 1914, xlviii., 262.)

Addition to water of salts such as are present in blood *decreases* the amount of gas that can be held in solution. But Van Slyke has shown that the presence of haemoglobin increases the solubility of nitrogen to such an extent that whole blood will dissolve slightly more than pure water. 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 haemoglobin

which can unite with oxygen, and that while venous blood contains reduced haemoglobin, arterial blood contains chiefly oxy-haemoglobin. Here, then, is obviously the chemical mechanism for carriage of oxygen.

When solutions of haemoglobin take up oxygen to form oxy-haemoglobin 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 haemoglobin, is brought into contact with atmospheres containing different pressures of oxygen, then, after equilibria have been attained, if the percentages of oxy-haemoglobin 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. 19 is obtained.

It might be expected that if the oxygen pressure were doubled the percentage of oxy-haemoglobin 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 haemoglobin changes to oxy-haemoglobin, 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-haemoglobin 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 haemoglobin 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-haemoglobin is formed for the same oxygen pressure. Increase of the saline content of the solution also diminishes the percentage of oxy-haemoglobin 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 haemoglobin. This is shown in Fig. 19, 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 13 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 haemoglobin is between 72 and 80 per cent. saturated. Arterial blood yields about 18 volumes of oxygen, its oxygen pressure is 91 mm., and its carbon dioxide pressure 40 mm. The haemoglobin 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. 19 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 haemoglobin 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—

$$H_2CO_3 + K_2HPO_4 \rightleftharpoons KH_2PO_4 + KHCO_3$$
$$2H_2CO_3 + 2KHbO_2 \rightleftharpoons 2KHCO_3 + 2Hb + HOH + 3O.$$

An additional important mechanism in the carriage of carbon dioxide is the formation of carbhaemoglobin by reaction between the gas and the free amino-groups of haemoglobin. This has been briefly discussed in Chapter IX. (p. 220).

Carbonic Anhydrase. Studies in the rate of liberation of carbon dioxide from blood and from bicarbonate solutions exposed to a vacuum showed that the speed with which blood loses its carbon dioxide is much greater than simple dissociation and diffusion from solution can account for. Henriques calculated that only about 17 per cent. of the gas actually evolved from whole blood in a given time can be accounted for by these physical mechanisms, although that lost from blood serum can be fully accounted for.

The greater speed of liberation is brought about by two mechanisms, (i.) the dissociation of carbhaemoglobin—both formation and decomposition of this compound take place very rapidly—and (ii.) the catalytic action of a specific enzyme, *carbonic anhydrase*, which is present in the red blood cells.

The preparation of this enzyme in very active form is largely the work of Roughton. Highly active preparations can be made by shaking red blood cells diluted with an equal volume of water and ethyl alcohol (in ratio 6 to 4) with chloroform until the haemoglobin is coagulated, and then centrifuging. Three layers separate, denatured protein above, and chloroform below, while the middle layer contains the carbonic anhydrase. Further treatment of this solution by dialysis, adsorption of impurities on calcium phosphate, and evaporation of the solution *in vacuo*, has given a preparation 2,000 times more active than whole blood in causing dissociation of bicarbonate solutions.

The enzyme is stable in this solid preparation, is readily soluble in water and salts solutions, and shows all the properties of a typical enzyme, being relatively undialysable, and destroyed by heating to 65° C. for thirty minutes. It is stable over a range of *p*H 4 to 12, but rapidly loses its enzymic properties in more acid or alkaline solutions.

When one part by weight of this solid preparation is dissolved in seven million parts of water, it doubles the rate of evolution of carbon dioxide from a bicarbonate-phosphate mixture. It also catalyses the reverse reaction $CO_2 \rightarrow HCO_3^-$.

This enzyme, therefore, during the passage of blood through the lungs, is very largely responsible for the speedy decomposition of bicarbonate :

$$HCO_3^- \rightarrow OH^- + CO_2.$$

while during passage of blood through the tissues it facilitates the reverse change.

The distribution of the enzyme seems fairly specific. The red blood cells contain a rich supply. A trace is said to be present in sperm, and muscle (after perfusion to remove blood) appears to contain a trace. The pancreas contains more, but pancreatic juice has none. Traces are said to be present in liver, spleen, and the central nervous system, but it is quite possible that these may be due to incomplete removal of blood. It is absent from lungs, heart, kidney, gall-bladder, intestinal tissue, and peripheral nervous tissue, and from milk, bile, and urine.

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. The approximate average oxygen and carbon dioxide tensions in the different media under discussion are shown in Table XXVII.

TABLE XXVII. 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-haemoglobin dissociates ; carbon dioxide entering from the tissues facilitates the dissociation.

The series of changes in lungs, blood, and tissues is shown graphically in Fig. 20.

It can easily be demonstrated that oxidation is almost entirely confined to the tissue cells. 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

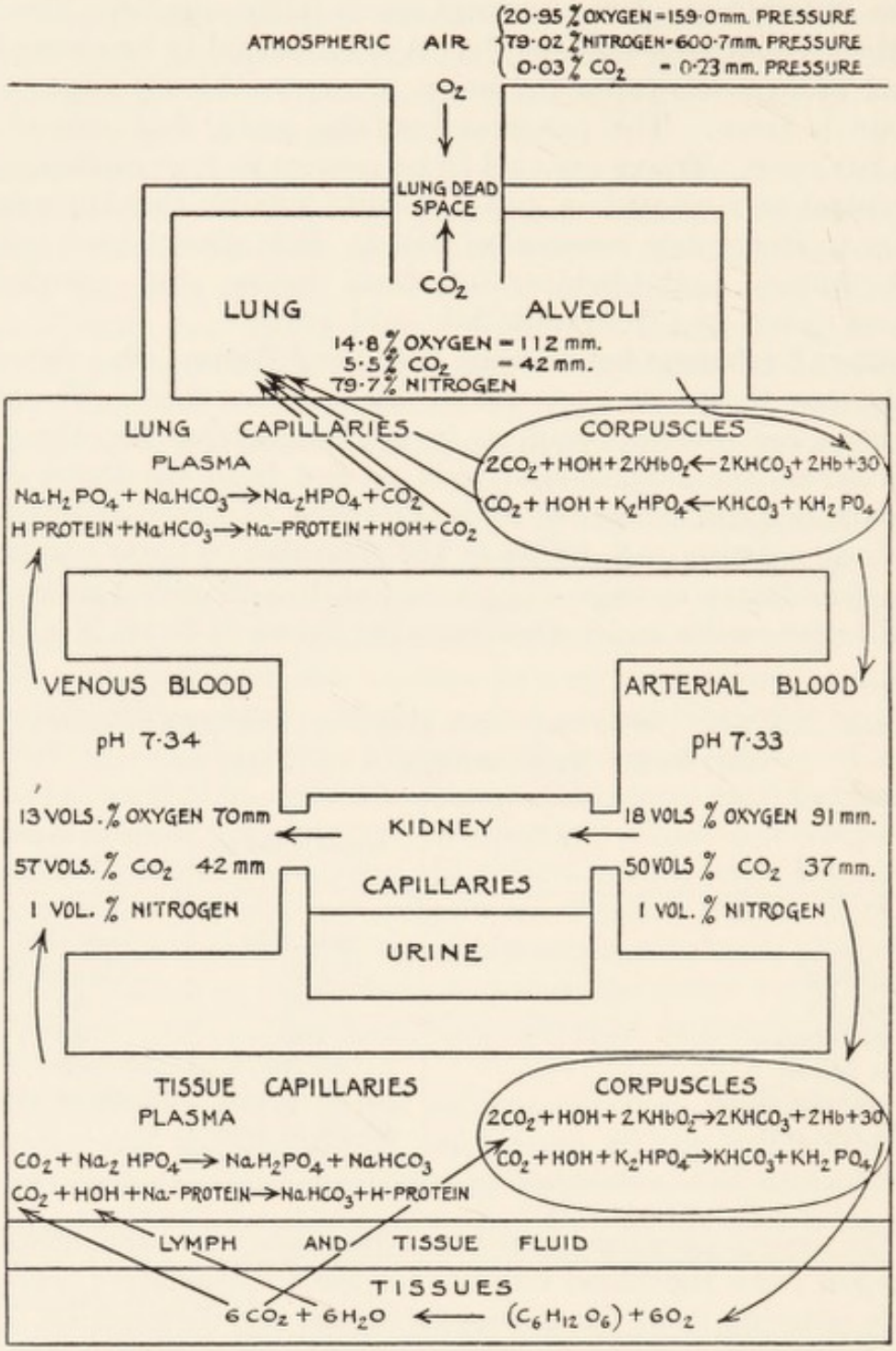

FIG. 20. Graphic representation of respiratory exchanges in lungs and tissues. Gas in volumes per cent. Pressures in mm. Hg.

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.

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 exercise.

The high degree of constancy of the *p*H of blood is governed by the blood-salts, especially the carbonates, and the blood proteins, which act as a complex system of *buffers* (*cf.* p. 30), 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.

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.

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 :

$$HX + NaHCO_3 = NaX + H_2CO_3\,(= NaX + HOH + CO_2),$$

or

$$H^+ + X^- + HCO_3^- = X^- + H_2CO_3.$$

The net result will be a slight decrease in HCO_3^- ions, resulting in further ionisation of $NaHCO_3$, 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. This has an important bearing on acidosis.

Phosphates and proteins of blood produce similar effects (*cf.* p. 31).

Acidosis and Alkalosis. The conditions indicated by these terms require further definition. In acidosis the body is less alkaline than normally; alkalosis is the opposite condition in which it is more alkaline. A transient acidosis exists in marked fatigue through accumulation of lactic acid (p. 101) and a more permanent acidosis in untreated diabetes mellitus through perversion of fat metabolism and undue accumulation of acetoacetic acid, β-hydroxybutyric acid and acetone leading to *ketosis*, and *ketonuria* (*cf.* pp. 138, 139).

An alkalosis can be induced in man by continued rapid breathing, which unduly depletes blood and tissues of carbon dioxide. The net result of this depletion can be represented—

$$HCO_3^- - CO_2 = OH^-.$$

Continued vomiting can cause an alkalosis through marked loss of hydrochloric acid in the gastric secretion.

Control of pulmonary respiration is centred in certain small areas of cells in the floor of the fourth ventricle of the brain. These cells are peculiarly susceptible to increased concentration of carbon dioxide in this tissue itself or in the blood circulating to it, with the lowered *p*H accompanying such increase.

The slightest degree of acidosis sets up a stimulus to these respiratory centres, as a result of which the rate of pulmonary breathing is increased, and, if the cause of the change is transient, this helps to restore a normal condition.

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 haemoglobin 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, haemoglobin, in addition to all its other properties, is found to act as a polyvalent acid to such an extent that oxy-haemoglobin can neutralise almost half the base present in the cell (which, in man, can be considered as almost entirely potassium). Reduced haemoglobin is not quite so strongly acidic. The rest of the base is neutralised almost entirely by chloride and bicarbonate.

The acidic property of haemoglobin 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-haemoglobin and reduced haemoglobin 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 II. is applied to the conditions within and without the red cell, and if in the first place we consider only hydrogen, chloride and haemoglobin, we can write :

Cell	*Membrane*	*Plasma*
Hb^-		
Cl^-		Cl^-
H^+		H^+

and from the theory of the equilibrium we have—

$$(H^+)\text{cell} \times (Cl^-)\text{cell} = (H^+)\text{plasma} \times (Cl^-)\text{plasma},$$

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. 21 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 as potassium, gives $0{\cdot}163 \times 39{\cdot}1$ gm. of potassium, *i.e.*, 6·4 gm. (Table XVI. 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 haemoglobin. The haemoglobin figures refer to the equivalent concentration of base found by actual experiment to be neutralised by the haemoglobin.

Similarly, columns 3 and 4 give the corresponding concentrations of chloride, bicarbonate and plasma protein (expressed similarly in

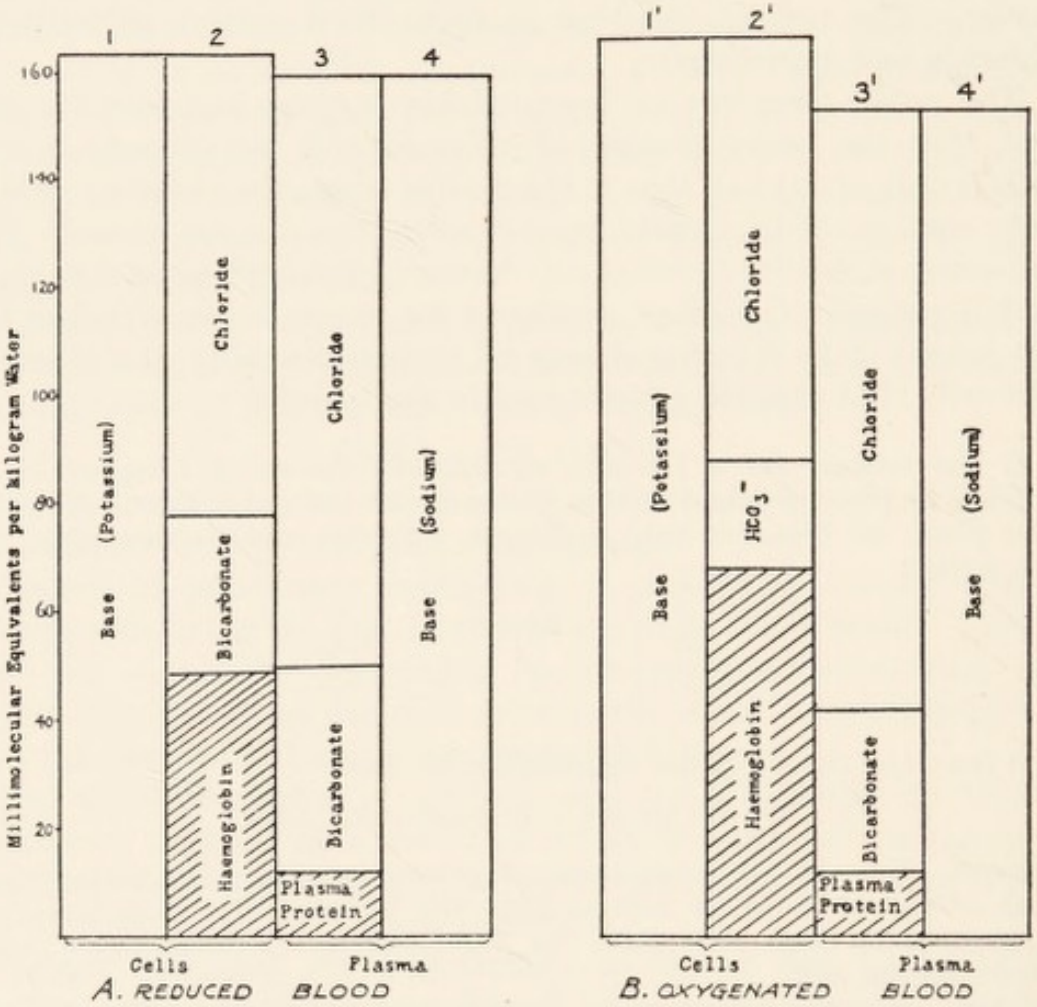

FIG. 21. Distribution of base, bicarbonate and chloride in reduced and oxygenated blood. (After Van Slyke.)

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 haemoglobin 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.

Notes on Abnormal Conditions Associated with Extracellular Respiration

Lowered atmospheric pressure, such as that encountered at high altitudes, since it involves lowered oxygen pressure, if it be sufficient to lower appreciably the degree of formation of oxy-haemoglobin during passage of blood through the lungs, leads to oxygen-lack in the blood and tissues, an *anoxaemia*. As a result of this various symptoms of " mountain sickness " may develop. Continued exposure to the stimulus of such low oxygen pressure leads to a polycythaemia (*cf.* p. 226).

Increased atmospheric pressure is only dangerous when it is suddenly reduced, as when caisson workers or divers after being subjected to high pressure for some time are suddenly exposed to normal atmospheric pressure. During the period of high pressure much more nitrogen is taken up by blood and tissue fluids than normally (since its solubility is proportional to pressure), and sudden drop of pressure causes release of minute bubbles of nitrogen gas from body fluids and tissues, causing acute local pain, and in more extreme cases vomiting and (if brain tissue is affected) partial paralysis and even death.

Cyanosis, a condition in which various tissues show a purplish tinge, is due to presence of unduly large amounts of reduced haemoglobin in the capillaries, and even in the venules and arterioles. It can arise through various causes interfering with oxygen uptake, including lowered oxygen pressure in the inspired air, and diminished rate of circulation as in congenital heart disease.

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CHAPTER XII

INTRACELLULAR RESPIRATION

INTRODUCTION

THIS chapter will deal essentially with oxidation-reduction mechanisms within the cells of the mammalian organism. Some other cellular mechanisms involved with these, or furnishing illuminative contrasts, will be mentioned.

Progress in the study of oxidation-reduction mechanisms has to date chiefly revealed their extraordinary complexity; it is doubtful whether a complete account can yet be given of any single oxidation as it occurs within the living cell. Most of the studies of these problems have of necessity been made with tissue extracts or tissue slices *in vitro*, in the presence of artificial oxygen-carriers such as methylene blue (which easily reduces to a colourless leuko-base which again absorbs molecular oxygen to form the original compound, typifying the perfect " carrier "), or in the presence of unnatural inhibitors, such as cyanide or fluoride.

Any detailed account of such experiments, on which present knowledge largely rests, would be altogether too extensive for this volume. An attempt will only be made to give some account of the more important compounds which take part in oxidation-reduction mechanisms, and the types of reaction which they assist.

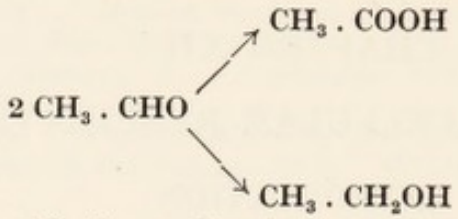
Oxidation, in the strictest sense of the word, involves combination with oxygen, but the term has gradually become used more and more loosely, implying at first either combination with oxygen or loss of hydrogen, and finally, the loss of an electron (e) such as occurs, for example, when a ferrous ion (Fe^{++}) is oxidised to a ferric ion (Fe^{+++}).

$$Fe^{++} = Fe^{+++} + e$$

Two theories of biological oxidations have been formulated, peroxide formation, in which specific enzymes (oxidases and peroxidases) activate oxygen, and dehydrogenation, in which specific dehydrogenases activate hydrogen so that it can react with a hydrogen-acceptor, or with oxygen. Actually most oxidation-reduction systems appear to employ a combination of the two procedures.

A special type of procedure (perhaps also a combination of

the two) is illustrated by the Cannizzaro reaction, in which an aldehyde suffers simultaneous change to acid and alcohol. For example, acetaldehyde yields acetic acid and ethyl alcohol.



Biological oxidation-reduction may occur under aerobic conditions, molecular oxygen being present, or under anaerobic conditions, in absence of molecular oxygen.

Many enzymes aid the catalysis of oxidation-reduction processes ; in fact the ease with which tissue juices yield enzymes capable of carrying out a great variety of different actions makes one wonder whether all these enzymes are specifically different.

Most of the enzymes are either oxidases, with the power of activating molecular oxygen, or dehydrogenases, with the power of activating hydrogen. Present knowledge does not always permit a statement as to whether a particular enzyme is oxidase or dehydrogenase. In addition, the enzyme catalase is widely distributed in tissues ; its action is protective, the prevention of accumulation of hydrogen peroxide.

Certain specific compounds, co-enzymes, are involved in many oxidation-reductions. In addition, numerous carriers (of oxygen or of hydrogen) play important *rôles*. Some of these are very complex, such as the haem compounds termed cytochromes, while others are simple compounds, such as glutathione, ascorbic acid, and thiamin phosphate. It is sometimes difficult, with present knowledge, to distinguish between enzymes proper and carriers of protein complexity.

Some account will be given of these enzymes, carriers, and co-enzymes, and then some typical reactions will be discussed. Many of these involve phosphorylation. This, itself in no sense an oxidation, must also be considered, since it is essentially involved in many oxidation-reduction mechanisms.

Throughout the following account the term " substrate " will be used to indicate the metabolic product which is being oxidised or reduced, as the case may be, the actual cause for the complex devices nature has elaborated as oxidation-reduction systems.

Enzymes

Green and Brosteaux (1936) have suggested a systematic classification of dehydrogenases, which can be used as a basis

for a general grouping of most of the enzymes associated with oxidations and reductions, such as the following :—

(i.) *Aerobic Oxidases.* These can react directly with molecular oxygen, producing hydrogen peroxide. They do not require a co-enzyme. Examples are uricase and xanthine oxidase, and an amino-acid oxidase derived from kidney tissue.

(ii.) *Cytochrome Oxidases* (*or Dehydrogenases*). These do not react directly with molecular oxygen. They can react with oxygen through cytochrome, but not through flavoprotein (the *yellow* respiratory ferment). They do not require a co-enzyme.

An example of this class is Warburg's " respiratory ferment." This is widely distributed in plant and animal tissues, and is probably identical with Keilin's " indophenol oxidase." On account of an absorption spectrum closely resembling that of known haemochromogens, this compound is also considered to be a haem-protein. It can only act as a transporter of oxygen in presence of (reduced) cytochrome.

(iii.) *Co-enzyme Dehydrogenases.* These cannot react directly with molecular oxygen. They can react through flavoprotein, but not through cytochrome. They require a co-enzyme. Warburg terms this group the " Zwischenfermente," and Euler the " apodehydrogenases." According to their views these " apodehydrogenases " loosely combine with co-enzyme to form the " holodehydrogenases " which actually function as enzymes.

This group include the lactic and malic dehydrogenases of mammals (which may be identical), and the triosephosphate and alcohol dehydrogenases of yeast.

Certain enzymes do not appear to fall in the above groups.

Aldehydemutase, a tissue enzyme, activates the dismutation of the Cannizzaro reaction :

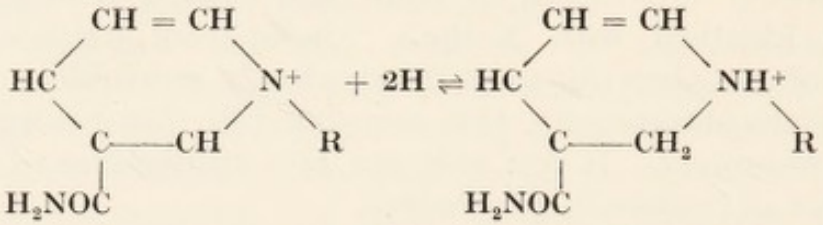
$$2\ RCHO + HOH \rightleftharpoons RCH_2OH + RCOOH$$

Catalase (*cf.* p. 48) has been crystallised, and shown to be a compound of a protein and a haem derivative, neither of which alone possesses the properties of the enzyme. Catalase is a widely distributed cell enzyme which accelerates the decomposition of hydrogen peroxide to water and molecular oxygen. It is assumed (but not definitely proved) that this action is protective, designed to prevent a dangerous concentration of peroxide. There is some evidence that the function of catalase may not be confined to this decomposition. Results of Keilin and Hartree suggest, for example, that ethyl alcohol is oxidised to aldehyde by catalase and nascent hydrogen peroxide.

Co-enzymes

Phosphopyridine nucleotide catalysts are *co-zymase* of yeast, and Warburg and Christian's *co-ferment* of mammalian tissues.

Co-zymase, diphosphopyridine nucleotide, can be represented as (Nicotinic acid amide)—(ribose)—(phosphate)—(phosphate)—(ribose)—(adenine). The co-ferment is a triphosphopyridine nucleotide, with a similar constitution, but an extra phosphate radical. Both act through the substituted pyridine group, which transfers two atoms of hydrogen from one compound (the donator) to another (the acceptor). This is shown in the following scheme, where R represents the rest of the co-enzyme molecule.



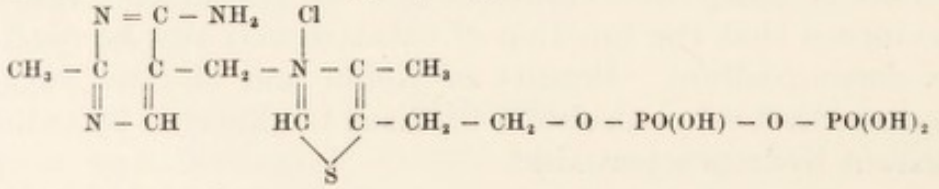
Evidence is available that the co-ferment probably catalyses the oxidation of the aldehyde group of a phosphorylated polyhydroxyaldehyde. Neither co-zymase nor co-ferment, alone, can activate the fermentation or the glycolysis of glucose.

Both must be carefully differentiated from adenylic acid of muscle (*cf.* p. 179), which does not activate oxidation-reduction processes.

Co-carboxylase, though its name suggests that it is chiefly concerned with decarboxylation, is intimately associated with oxidation-reduction processes in some way that is not yet understood. It has been isolated in crystalline form from bottom yeast by Lohmann and Schuster in 1937, and proved to be *thiamin* (vitamin $\mathbf{B_1}$) *pyrophosphate.** Stern and Hofer obtained a 2 per cent. yield of it by treating thiamin with phosphorus oxychloride, $POCl_3$, while still more recently Tauber prepared it in almost 100 per cent. yield by treating the vitamin in orthophosphate solution with an enzyme prepared either from dried yeast, or from the duodenal mucosa of the pig, so that it can obviously be readily formed in the mammalian organism and is probably the form in which the vitamin functions in mammals.

Washed yeast cells contain some protein-enzyme which functions

* The formula of thiamin pyrophosphate is



as a carboxylase. When such cells are suspended in a pyruvate-phosphate medium the addition of thiamin pyrophosphate strongly promotes the evolution of carbon dioxide. The reaction so catalysed is believed to be

$$CH_3 . CO . COOH \rightarrow CH_3 . CHO + CO_2$$

Peters has shown that when polyneuritis develops in pigeons on a diet deficient in thiamin, the brain loses its power to respire, and pyruvate accumulates in brain tissue. Administration of thiamin corrects this abnormality. The apparent association of the vitamin with pyruvic acid metabolism is strengthened by the finding of Platt and Lu that the blood of patients with beri-beri contains considerable amounts of pyruvate. It has been suggested that thiamin pyrophosphate (co-carboxylase) may form the prosthetic group of the actual functioning carboxylase enzyme.

It seems unlikely that the function of vitamin $\mathbf{B_1}$ is merely to cause destruction of pyruvate; this effect on pyruvate is more probably associated with the utilisation of the vitamin in some metabolic process associated with oxidation. Thiamin not only destroys pyruvic acid *in vitro*, but when slices of brain tissue are placed in pyruvate solution, addition of thiamin promotes oxygen-uptake, that is, it promotes the tissue respiration of brain. The pyrophosphate (co-carboxylase) also does so. It has recently been reported by Westenbrink and Pollak (1937) that there is a time-lag of some ten minutes before actual increase of oxygen-uptake can be detected after addition of thiamin; this strongly suggests that the vitamin is phosphorylated to the co-carboxylase before action commences.

Furthermore, it seems unlikely that the action of vitamin $\mathbf{B_1}$ is solely connected with nerve-tissue in mammals. There is evidence for example that pyruvic acid accumulates in other tissues in the polyneuritic animal. Further, the vitamin has been shown to play certain essential *rôles* in the development of the plant embryo and in influencing root production, while it apparently has a definite place in the metabolic processes of bacteria and insects as well as of the higher plants and mammals. Williams, in a recent paper, summed up a discussion on its function: "It is difficult to believe that its action in the higher forms of life is restricted to specialised tissues or to a narrow function."

Carriers

Cytochromes a, *b*, and *c* are three haemochromogens characterised by specific absorption spectra, which appear to be universally

distributed in tissue cells where aerobic oxidation occurs, but which are absent from anaerobic organisms. These cytochromes, according to Keilin, act as successive intermediate carriers of oxygen between the oxidase-oxygen system and the dehydrogenase-substrate system. Cytochromes also apparently possess peroxidase activity. Cytochrome *b* is slightly autoxidisable. The other two are not.

Cytochrome *c* has been obtained in a pure or almost pure condition by Keilin and Hartree (it has not yet been crystallised). They obtained 1 gm. from 6 kg. of ox heart and found by diffusion procedures that it has a molecular weight of 16,500. It contains 0·34 per cent. of iron, being one atom per molecule. When treated with a platinum catalyst and hydrogen, it is reduced, one atom of hydrogen being absorbed per atom of iron.

When cytochrome *c* is mixed with hexosemonophosphate and triphosphopyridine nucleotide, the cytochrome is not reduced. Addition of flavoprotein leads to its easy reduction, suggesting that flavoprotein can act as a link between co-ferment and cytochrome in the cell at reduced oxygen pressures.

Warburg's yellow respiratory enzyme is a specific protein with riboflavin (vitamin $\mathbf{B_2}$) phosphate as prosthetic group (*cf.* p. 70), and is frequently termed *flavoprotein*. It seems to be especially associated with dehydrogenase systems, though it cannot act as a hydrogen carrier for such systems when they are isolated from others. It has been shown (Euler, Green) to function as an efficient oxygen carrier for the glucose-dehydrogenase system of liver and for the hexosediphosphate systems of blood and yeast.

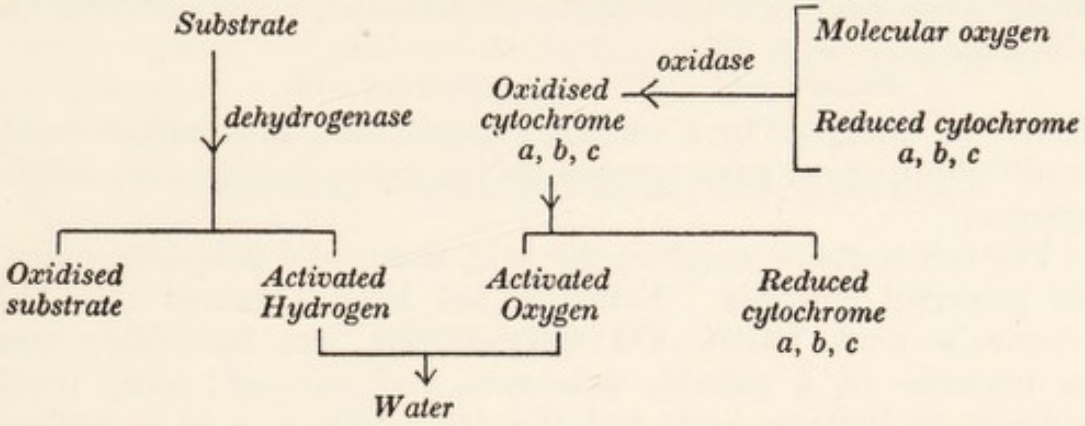
Glutathione (p. 251) and *ascorbic acid* (p. 72) are both hydrogen carriers, both being converted easily to " oxidised " form by loss of hydrogen, both being widely distributed in tissues, while there is evidence that in some instances their actions are interconnected.

In presence of co-ferment the hexosemonophosphate system of yeast or of red blood cells reduces glutathione very rapidly (the hexose thereby becoming oxidised). In presence of oxygen the limiting factor in the speed of this reaction is the speed of autoxidation of reduced glutathione.

Ascorbic acid is oxidised by removal of hydrogen to dehydroascorbic acid (p. 72). This change is catalysed by a trace of copper or by haemochromogen. Hopkins and Morgan have shown that in presence of ascorbic acid oxidase and dehydroascorbic acid glutathione is rapidly oxidised, while the acid is reduced to ascorbic acid, which can therefore be regarded as a co-enzyme of the oxidase enabling it to oxidise glutathione.

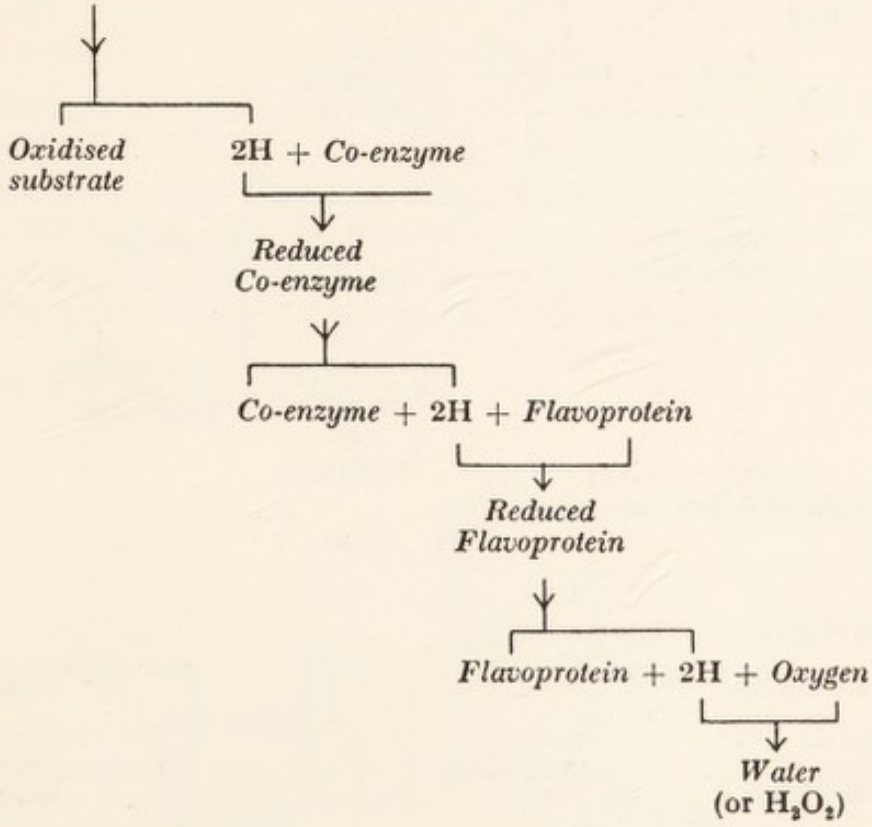
Typical Systems Concerned with Oxidation-Reduction

General Systems. Keilin has brought together the conception of Warburg concerned with oxygen transfer with that of Wieland concerned with hydrogen transfer, to a unified system, of which the following can be taken as an example of a typical oxidation occurring in such a system as muscle. The cytochromes appear to hand over oxygen from one to the other until finally it is available in an active form for reaction with hydrogen.



Warburg's ideas on the transfer of hydrogen in the left-hand side of the above scheme are indicated in the following arrangement, which suggests how hydrogen is gradually handed on from one compound to another, until finally it is able to react with oxygen.

Substrate (acted on by dehydrogenase in presence of co-enzyme and flavoprotein).



Such actions are *interlocked*. They cannot proceed in part.

There is evidence of even greater interlocking between the two systems than is indicated in the above schemes, as for example that the changes of flavoprotein and cytochrome *c* are interconnected, in so far that the reduced form of flavoprotein may be oxidised by cytochrome *c*.

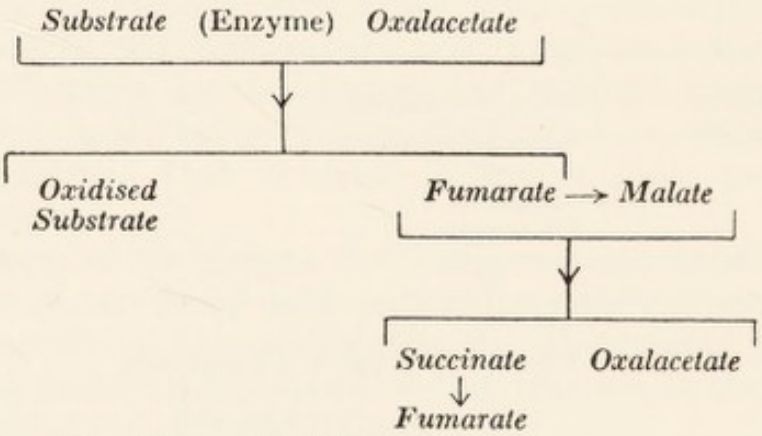
Fumarate-succinate System. This system is being studied by Szent-Györgyi, Green, and others. The reaction between fumaric acid * and succinic acid may be represented, *e* representing an electron,

$$\textit{Fumaric acid} + 2H^+ + 2e = \textit{Succinic acid}.$$

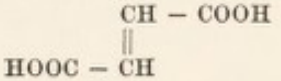
It can be catalysed by a suitable enzyme, but, to establish rapid equilibrium, one or more carriers are needed to provide a reversible system.

The cell contains succinic, fumaric, and malic dehydrogenases, all powerful enzymes. Evidence has been obtained that (i.) oxalacetic acid, HOOC . CO . CH_2 . COOH, can furnish oxygen in presence of a suitable substrate and enzyme, being itself reduced to fumaric acid, and (ii.) that fumaric acid is further dehydrogenated, being converted in part to malic acid, HOOC . CHOH . CH_2 . COOH, which then (in presence of the three hydrogenases, co-ferment, and a suitable carrier) reacts with it to yield succinic acid and oxalacetic acid,† the succinic acid being again reduced to fumaric acid.

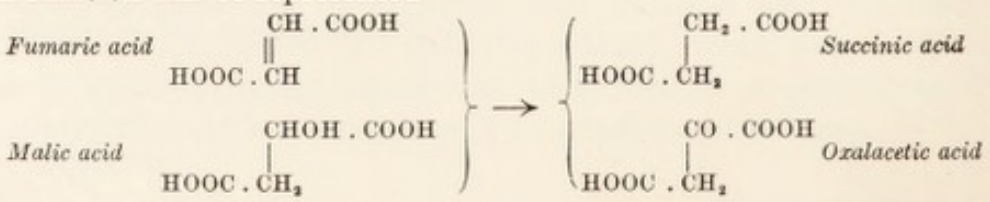
This involved system for oxidising a substrate can be partially represented—



* Fumaric acid has the constitution



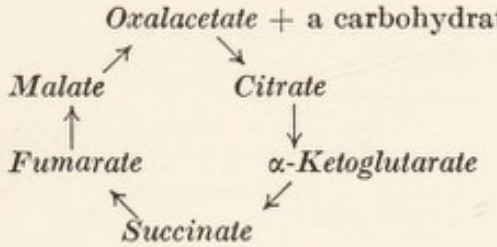
† This reaction can be represented



Further, Stolz and Hastings (1937) have shown that both an oxidase and a dehydrogenase system are essential for the succinate-fumarate interchanges.

Citric Acid Cycle. Krebs, from work on isolated tissue, has obtained evidence of another cycle involving fumarate. It is not at present possible to state whether this, or that suggested by Szent-Györgyi, or both, are important cycles in mammalian tissue oxidations, although the known presence of citric acid in many tissues, and in secretions such as milk, suggests its metabolic importance.

Krebs' cycle can be represented :

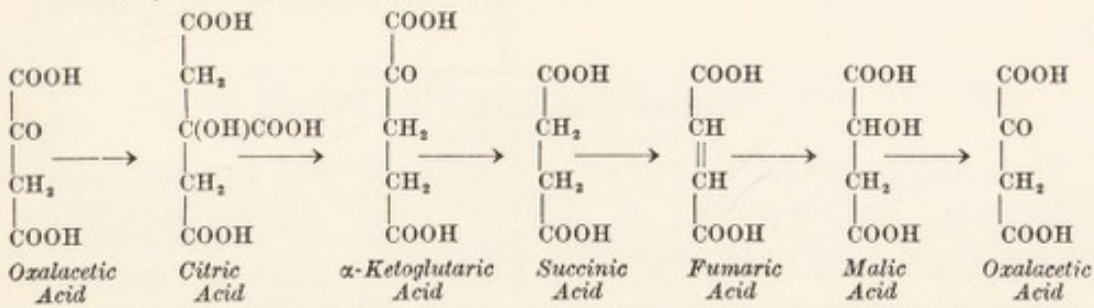


Recently, in support of his views, he has shown that when succinic, fumaric, malic, or oxalacetic acid is injected intravenously into rabbits, their urine subsequently contains considerable amounts of citric and α-ketoglutaric acids, while if citric acid is injected, α-ketoglutaric and succinic acids are excreted. Such facts accord with the above cycle. *

Phosphorylation

Perusal of the preceding pages, and consideration, for example, of the marked differences in properties between phosphocreatine and creatine, and hexosephosphate and glucose, must have suggested that increased capability of reaction is conferred on a compound by phosphorylation, and that combination with phosphate is one of the commonest of biological reactions. Hence

* This cycle can be represented



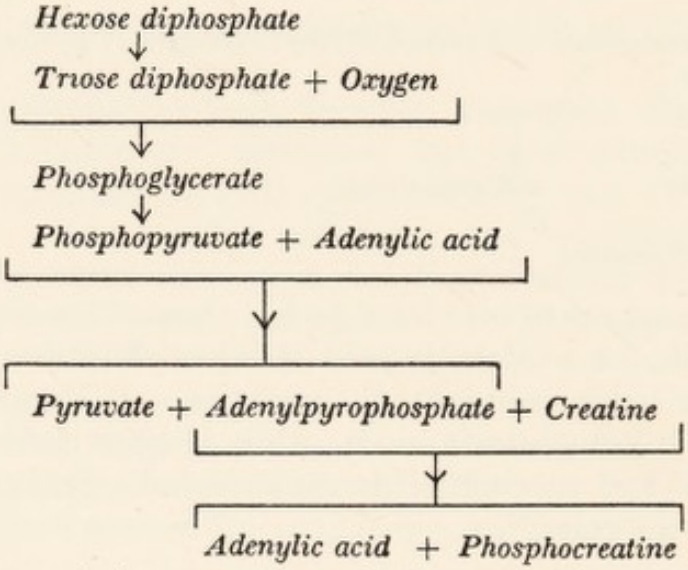
Knoop and Martins have shown that in alkaline solution, in presence of hydrogen peroxide, oxalacetate and pyruvate slowly react to form citrate, suggesting a potential mechanism for the above scheme.

some account must be given of the mechanisms by which phosphorylation occurs in the cell.

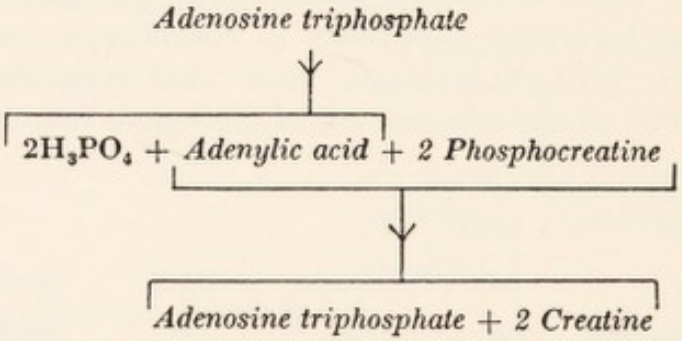
The reaction between creatine and phosphocreatine, written simply *

Creatine + K_2HPO_4 = *Potassium phosphocreatine* + *Water*

actually involves a complex system of exchanges, which is intertwined with the catabolism of carbohydrate. Recent work by Innes, following studies by Ostern, Needham, and others, suggests that under aerobic conditions, such as occur in muscle, phosphocreatine is formed from creatine in accordance with the following scheme :

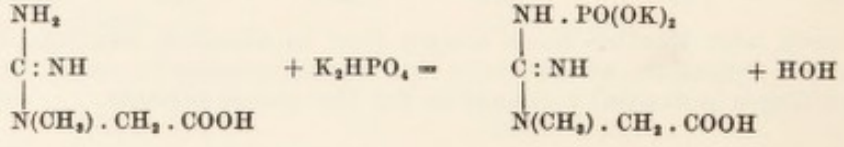


According to Lohmann the splitting of phosphocreatine can be brought about by a special phosphatase (phosphocreatinase) in the presence of adenosine triphosphate, the free phosphate really being set free from the latter.

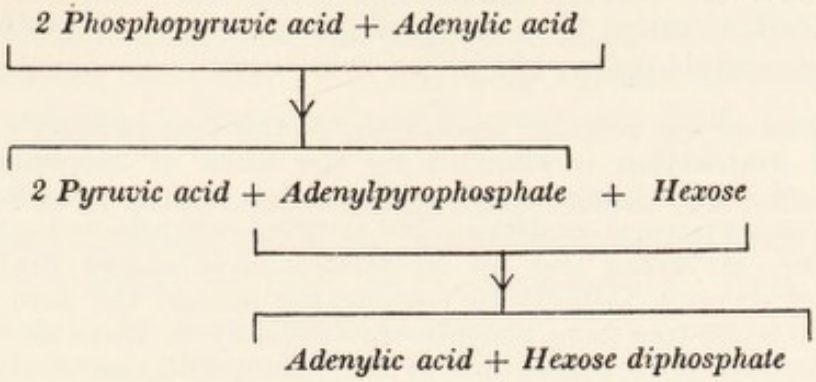


Thus phosphate needs carriers, of which adenyl pyrophosphate (adenosine triphosphate) of muscle, and diphosphopyridine nucleotide of yeast, are important examples.

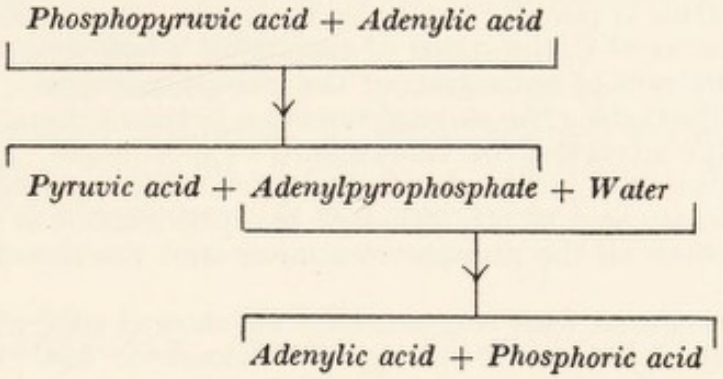
* Thus:



Another typical chain of processes, concerned in glycolysis, is—



Still another process suggests a way in which inorganic phosphate can be liberated.



Such reactions are catalysed by a number of phosphatases (some of which perhaps should be termed *phosphorylases*). It seems possible that there may be the same relationship between individual phosphatases and specific carriers as exists, for example, between dehydrogenases and their carriers.

As the above examples indicate, many phosphorylations are coupled with oxidation-reduction simultaneously proceeding in other systems.

At least one (pyro)phosphatase, concerned with yeast fermentation, needs the presence of magnesium ions. Adenylpyrophosphatase is not activated by magnesium. Bauer has suggested that when magnesium does function it acts as a link between phosphatase and co-zymase.

Typical Metabolic Processes Associated with Oxidation-Reduction Systems

Lactic Acid Production (Muscle) and Alcohol Production (Yeast). In muscle the two most important reactions associated with the function of that tissue are the splitting of phosphocreatine associated with the actual contraction, and the conversion of glycogen to lactic acid, with oxidation of a small proportion of

the latter to provide energy for the whole system. As has been pointed out already, the change from glycogen to lactic acid is largely reversible (*cf.* p. 101).

The proof of the relative association of the two processes to actual muscular contraction is afforded by the work of Meyerhof and of Lundsgaard. The change in phosphocreatine 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 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 phosphocreatine.

Further illustrating the closer connection between change in phosphocreatine and 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 all the phosphocreatine is split the muscle passes into rigor.

He experimented with frog muscle. He showed that when frogs are poisoned by injection of 40 mg. of iodoacetic 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, sixty to a hundred muscle twitches can be obtained, and these are apparently 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, and the muscles become distinctly alkaline in reaction. His work has been fully confirmed.

Some idea has been given of the complex changes involved in the splitting and re-formation of phosphocreatine (p. 248). The changes from glycogen to lactic acid are even more complex. They are paralleled to some extent by the complex series of changes by which the yeast cell ferments glucose, changes summed up in the simple equation

$$C_6H_{12}O_6 \Rightarrow 2CO_2 + 2CH_3 . CH_2OH$$

The yeast cell also provides itself with energy from this change, the difference between the potential energy of glucose and that of the alcohol produced furnishing the cells with 0·117 Cals. per gram glucose fermented (*cf.* p. 308).

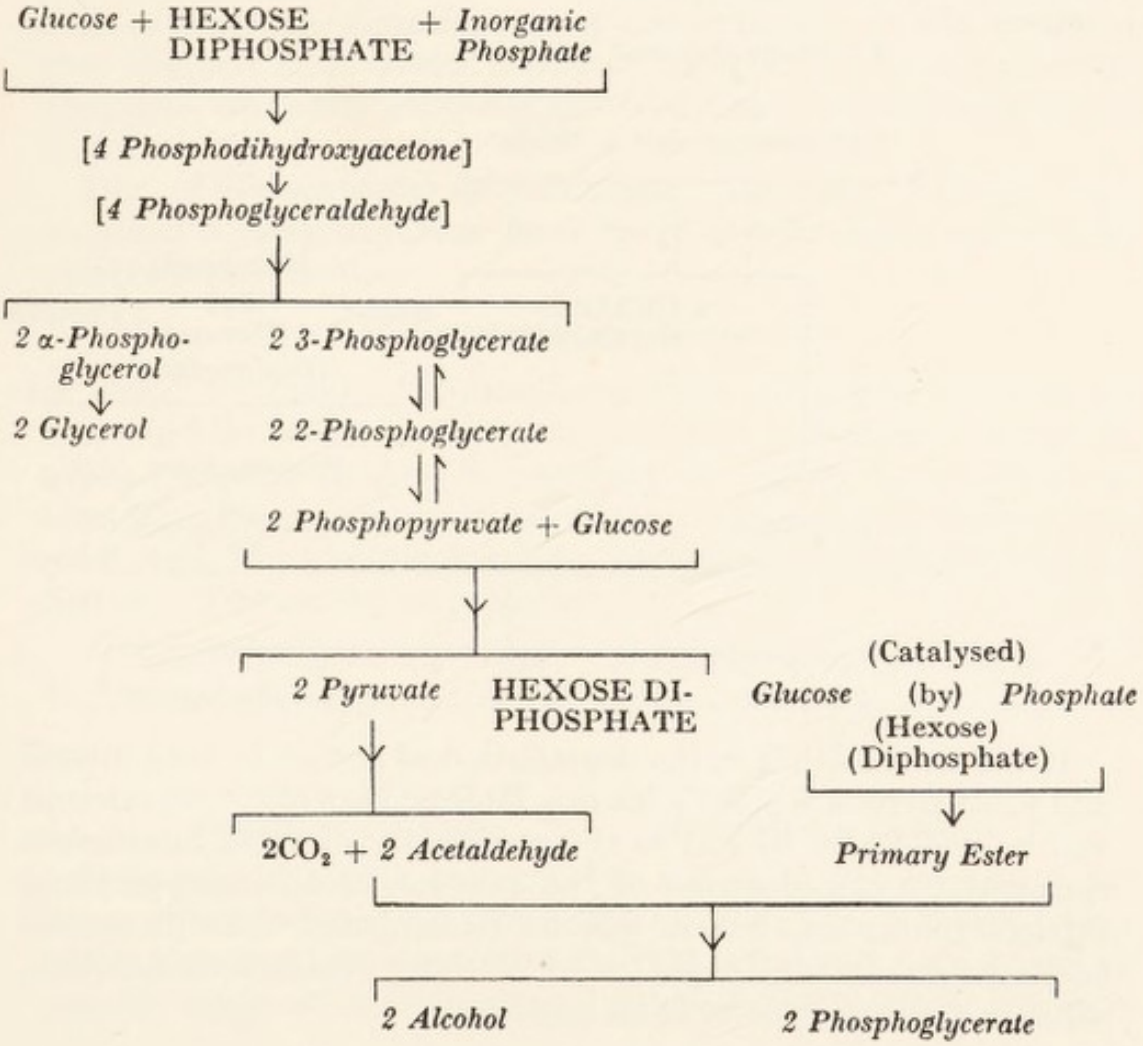
In spite of the constant attack, which may be said to have commenced over thirty years ago with Harden's demonstration of the important *rôle* of phosphate, and has been continued ever

since by Embden, Meyerhof, and many others, the whole story of yeast fermentation, and the formation of lactic acid in muscle is still far from complete. The following schemes are taken from those postulated by Meyerhof and Kiesseling in 1935, as reported by Robison.

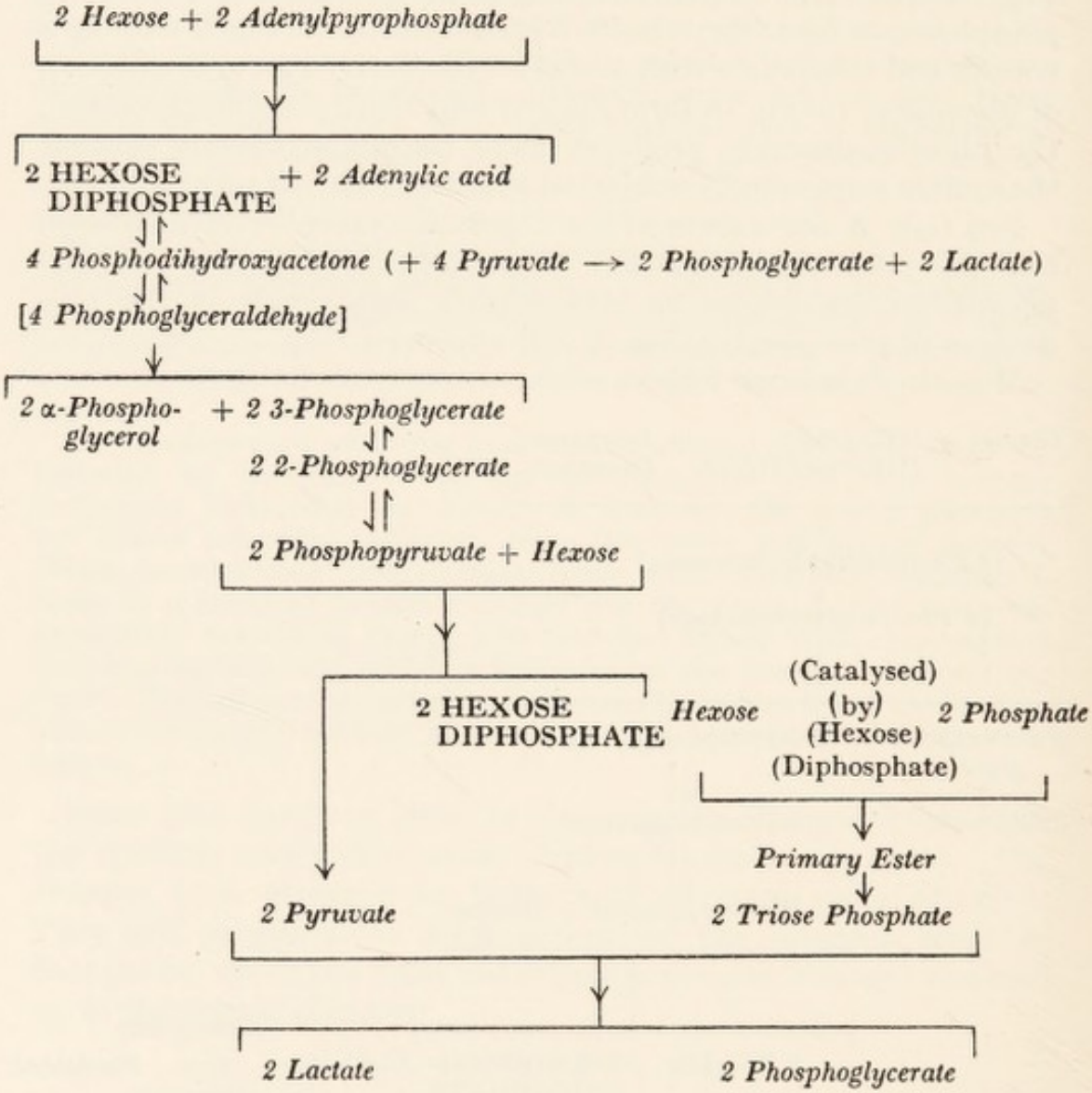
In alcoholic fermentation phosphopyruvate is first formed from glucose (through a stage of phosphoglycerate) and, reacting with more glucose, produces hexosediphosphate. This in some way catalyses the breakdown of glucose as it reacts with more phosphate, to form Meyerhof's " primary ester " (which may be a triose), and this reacts with acetaldehyde derived from breakdown of phosphopyruvate to form alcohol and more phosphoglycerate ; the phosphoglycerate produces more phosphopyruvate, so that the system is repeatedly subjected to catalysis by its own members.

Not only is the nature of the " primary ester " still unknown, but the presumed formation of phosphodihydroxyacetone and phosphoglyceraldehyde as intermediate compounds in the production of phosphoglycerate is still unproved.

Meyerhof's scheme follows :



Neither the precise path of glycogen formation from glucose in muscle, nor that of its initial breakdown, is known, but one can assume that phosphorylation plays an essential *rôle* here as in most phases of carbohydrate metabolism, and that, in the breakdown of glycogen a hexosephosphate (the lactacidogen of Embden) is an important intermediate stage. From that point onwards comparison and contrast can be made with the yeast fermentation of glucose, as in the following scheme.



It will be seen that in the degradation of hexose in both muscle and yeast pyruvate plays a leading intermediate *rôle*. No attempt will be made here to add to the complexity of these interlocked reactions by consideration of the enzymes and co-enzymes and carriers concerned, but it will be remembered that in muscle adenosine tri- and diphosphate and Warburg's co-ferment function, while in yeast co-zymase is all important.

Oxidation of Amino-acids. Krebs has established, by the systematic study of tissue slices from different organs, that both liver and kidney can deaminate amino-acids, forming keto-acids and ammonia. The oxidation is believed to take place in accordance with Knoop and Neubauer's scheme, which involves initial dehydrogenation. The oxidation is aerobic.

$$\begin{aligned} R \cdot CH(NH_2) \cdot COOH &\longrightarrow R \cdot C(:NH) \cdot COOH + 2H \\ &\longrightarrow R \cdot CO \cdot COOH + NH_3 \end{aligned}$$

Kidney contains two oxido-aminases which appear to induce this action. One acts on the naturally occurring amino-acids, the other (which is extractable from kidney tissue) acts on their optical isomers. Since these do not usually come in contact with kidney tissue, the reason for the existence of the second enzyme has not been ascertained.

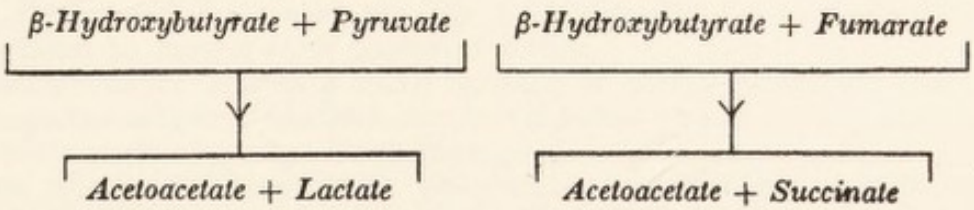
It is extremely probable that this type of oxidation also needs carriers and perhaps co-enzymes ; what these are has not yet been determined.

Neber, confirming the findings of London, finds that the liver, then the kidneys, and next the intestinal mucosa are the important sites of oxidative deamination of amino-acids. It will be noted that the first can convert ammonia to urea, the second excretes ammonia, while any ammonia absorbed from the third is conveyed to the liver.

The β-Hydroxybutyrate-acetoacetate Reaction. Green has obtained a dehydrogenase from heart muscle which specifically catalyses the reaction

$$\beta\text{-Hydroxybutyrate} \rightleftharpoons \text{Acetoacetate}$$

in either direction. Triphosphopyridine nucleotide (co-ferment) is an indispensable component, acting as a hydrogen acceptor, while a specific oxidase is necessary to restore the reduced co-ferment under aerobic conditions. Various metabolites can react with the hydroxybutyrate (or acetoacetate) in this linked system. Two examples are given.



It is probable that this chapter indicates the complexity of the problems related to intracellular respiration, without giving much clue to their solution. To that extent it represents the present stage of investigation. Nevertheless, within the past

two or three years a much more accurate conception has been obtained of the agents governing these processes, and of the potential steps involved in any oxidation-reduction process. The next few years may yield a clear picture.

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CHAPTER XIII

BACTERIAL ACTIONS AND DETOXICATION MECHANISMS

INTRODUCTION

In this chapter some account will be given of the chemical actions produced by bacteria infesting the intestines of man and other mammals, and of the detoxication mechanisms which have been developed to prevent harmful results to the host from the products of these actions, many of which are toxic. Some notes will be added concerning actions produced by bacteria not present in the intestinal flora, and by some of the other forms of plant life.

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 faeces 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 Caesarean 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 weight of dried faeces. One mg. of faeces has been found to contain four thousand million bacteria. They are present in the gut from close below the pylorus down 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 faecal nitrogen. Osborne and Mendel report that 70 per cent. of the nitrogen of the rat's faeces is present in bacteria.

As the faeces 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.

Bacterial Actions in the Intestine

Intestinal bacteria attack carbohydrates and protein decomposition products but 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:

$$\underset{\textit{Cysteine}}{SH \cdot CH_2 \cdot CH(NH_2) \cdot COOH} \longrightarrow CO_2 + \underset{\textit{Thioethylamine}}{SH \cdot CH_2 \cdot CH_2 \cdot NH_2}$$

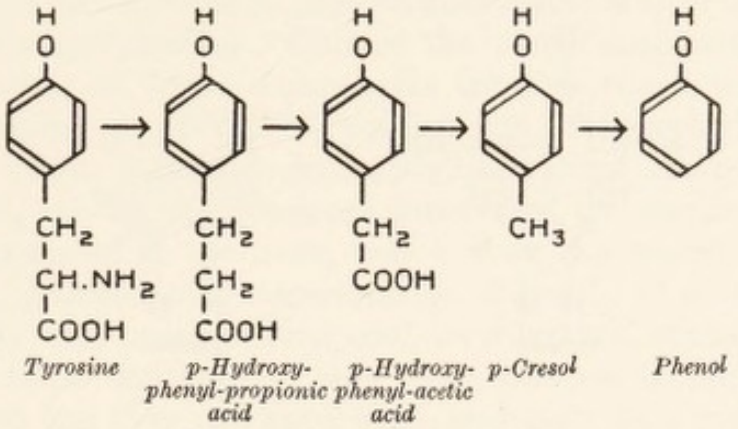
$$\longrightarrow \underset{\textit{Ethyl mercaptan}}{SH \cdot CH_2 \cdot CH_3} + NH_3$$

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.

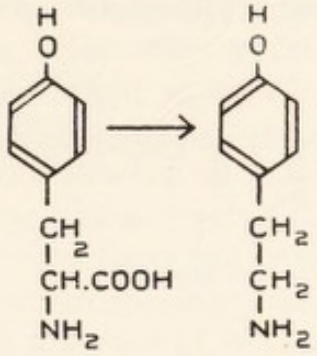
These actions can be illustrated with tyrosine. By the first

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.

(Raistrick has found that parahydroxy-phenyl-acrylic acid, HO . C_6H_4 . CH : CH . COOH, is the intermediate stage in the deamination of tyrosine, yielding by reduction the parahydroxy-propionic acid ; he believes that this is the usual type of initial stage of deamination in bacterial actions.)

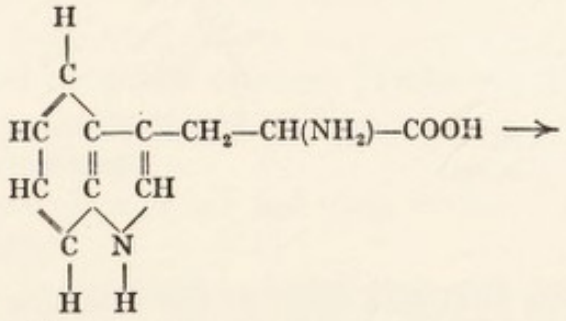


Tyrosine *p-Hydroxy-phenyl-propionic acid* *p-Hydroxy-phenyl-acetic acid* *p-Cresol* *Phenol*

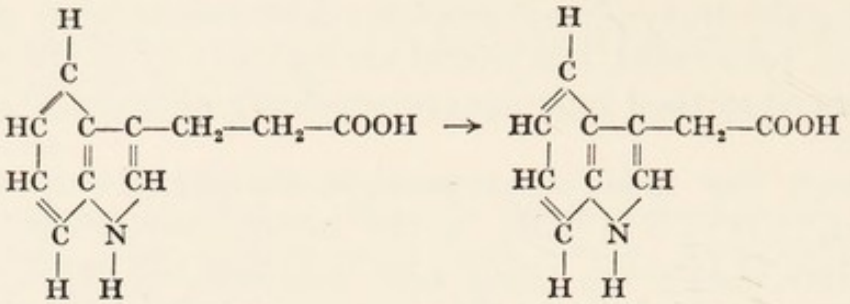


Tyrosine *Tyramine*

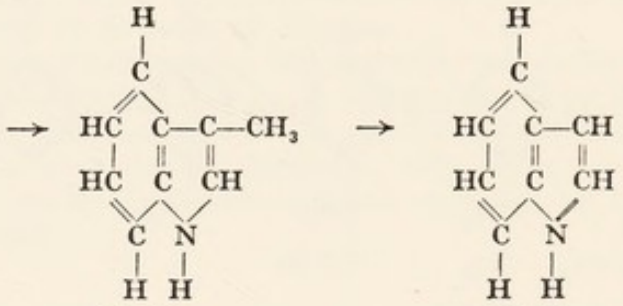
By the first method tryptophane gives rise to a very interesting series of compounds—



Tryptophane

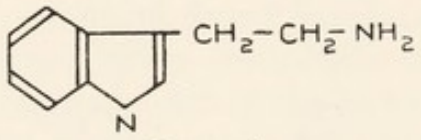


Indole-propionic acid *Indole-acetic acid*



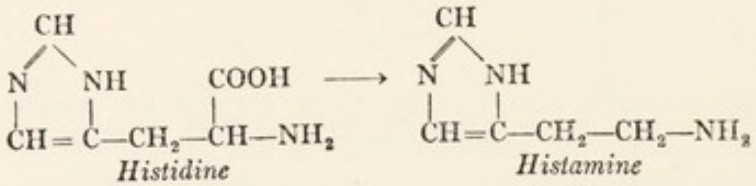
Skatole *Indole*

By the second method tryptophane gives rise to tryptamine (indole-ethylamine)—



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 casein, 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 in 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 adrenaline, a normal product of the adrenal glands, also 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 ; the liver combines them with sulphate.

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 faeces is due in large part to indole and skatole. The liver oxidises indole, and the product is excreted as a combined sulphate, indican, 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 proteins and 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. diphtheriae.*

According to very recent work of Pappenheimer (1937) diphtheria toxin is a heat coagulable protein, extremely sensitive to denaturation by a moderately high temperature, and in acid medium below *p*H 6. It has been obtained in such degree of purity that 0·0001 mg. of dried material is sufficient to kill a guinea-pig in five days.

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. diphtheriae*, in absence of carbohydrate, and having therefore to act on protein derivatives to derive material for its energy requirements, produces as a bye-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 attack carbohydrates rather than 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 diarrhoea.

Bacterial action in the intestine is, as far as man is concerned, an almost unmixed curse.

Chemical Actions brought about by the Lower Forms of Plant Life

A brief account has just 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 both, 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 for intestinal bacteria 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 XXVIII. 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 XXVIII. DECOMPOSITION OF AMINO-ACIDS BY SAPROPHYTIC AND PARASITIC PLANTS

Amino-acid	Decarboxylation Product	Deaminisation Products	
		Acids	Other than acids
Glycine . .	Methylamine (B.)	*Acetic acid* (B.Y.)	*Methane* (B.)
Alanine . .	Ethylamine (Mi.)	*Propionic acid* (B.)	*Acetaldehyde* (Y.)
		Acetic acid (B.)	
Serine . .	(Cholamine)	*Propionic acid* (B.)	*Ethylene-glycol* (Y.)
		Formic acid (B.)	
Cysteine . .	(Thioethylamine)	—	*Methyl-Mercaptan* (B.)
			Ethyl sulphide (B.)
			Hydrogen sulphide (B.)
			Sulphate (B.)
(α-Amino-butyric acid)	—	*Butyric acid* (B.)	—
Valine . .	*Isobutylamine* (Mi.)	*Isovaleric acid* (Mi.)	
		Isobutyric acid (Mi)	
		Acetic acid (Mi.)	
		Formic acid (Mi.)	
Leucine . .	Isoamylamine (B.)	*Isovaleric acid* (B.)	*Isomylalcohol* (M.F.Y.)
		Butyric acid (B.)	
		Isobutyric acid (B.)	
Isoleucine .	—	*Methyl-ethyl-propionic acid* (Mi.)	*Active Amylalcohol* (Y.)
Phenylalanine .	Phenylethylamine (Mi.)	Phenylpropionic acid (B.)	*Phenylethylalcohol* (Y.)
		Phenylacetic acid (B.)	
		Phenyl-lactic acid (Y.)	
Tyrosine .	Tyramine (Mi.)	*p-Hydroxyphenyl-propionic*, and *-acetic acids* (B.)	*Tyrosol* (Y.)
			p-Cresol and Phenol (Mi.)
		p-Hydroxy-phenyl-lactic acid (Y.)	
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.)	(Glutaric acid)	
		Succinic acid (Mi. Y.)	
	γ-Butyro-betaine (? Mi.)	*Butyric*, *Propionic*, *Acetic* and *Formic acids* (Mi.)	
Lysine . .	*Cadaverine* (B.)	*ε-Aminocaproic acid* (Mi.)	
Arginine . .	Agmatine (B.)	*δ-Aminovalerianic acid* (Mi.)	*Urea* (B.)
	Putrescine (B.)		
Histidine . .	*Histamine* (B.)	*β-Iminazolylpropionic acid* (B.)	*β-Iminazolylethyl alcohol* (Y.)
Tryptophane .	*Tryptamine* (B.	*Indolepropionic* and *Indole-acetic acids* (B.)	*Tryptophol* (Y.)
		Indole-lactic acid (Y.)	*Skatole* and *Indole* (B.)
Proline .	(Pyrrolidene)	(γ-Aminovalerianic acid)	—
		n-Valerianic acid (B.)	

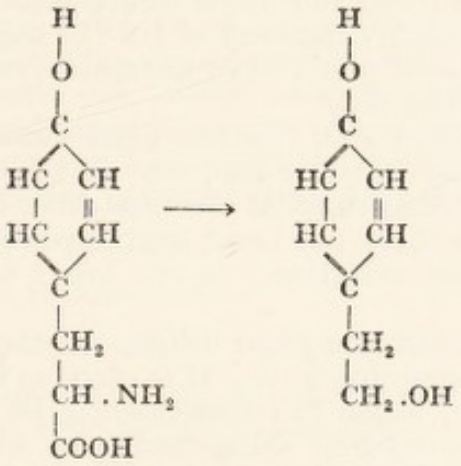
Whilst many of these agents, especially amongst the bacteria, may bring about the same reaction, a certain degree of specificity of action is by no means absent.

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*

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 p. 166). 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 p. 323). 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 (p. 303) 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 (*cf.* p. 249). *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, It has been shown 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 ; 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—

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \qquad (1)$$
$$C_6H_{12}O_6 = 6CO_2 + 2C_2H_5OH \qquad (2)$$

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 XVI., Table XXX.), 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. *B. coli* decomposes formic acid to carbon dioxide and hydrogen, and, under appropriate conditions, can reverse the reaction.

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.

" *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 laevo-form more easily, while *B. aceti laevolactici* 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. kuetzingianum*, 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 linoleic.

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 laevulan, 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 that intestinal bacteria have no action, or a negligibly small action, on fats (p. 300). It has been found that *B. typhi*, *B. coli*, *B. dysenteriae* Shiga, *B. proteus*, and *Spirillium cholerae* 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 gelatin, 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 Polyporeae, Agaricaceae 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.

Detoxication Mechanisms

Introduction. The body possesses two lines of resistance to cope with the invasion of harmful chemical compounds ; both are chemical defence mechanisms. Bacterial toxins, of protein or similar complexity, are rendered harmless by " antibodies." These will be dealt with in Chapter XIX. Simpler toxic compounds ingested in food and capable of absorption from the gut, or formed by bacterial action in the intestine, are dealt with largely by the liver, and chemically changed to less toxic or non-toxic compounds which are then excreted through the kidneys.

Quick speaks of the strategic position of the liver between the route of entry of most toxic compounds, the portal circulation, and the body proper ; there is a considerable amount of evidence that ability to detoxicate decreases when the liver is markedly damaged by poison or disease.

The two chief methods of detoxication are oxidation and conjugation. Oxidation is of course a normal physiological process, and evidence is accumulating that the conjugating procedures are also not designed specifically for the treatment of foreign poisons but are utilised for routine physiological changes. The formation of detoxicated products by the same mechanisms

are, at least in a number of cases, probably incidental rather than specific.

One of the striking peculiarities in detoxication mechanisms is the different way in which different species of animals often deal with the same compound. This may ultimately give a clue to fundamental differences in their metabolism.

Many of the facts concerning detoxication have been elicited by the feeding of toxic compounds which would not normally be ingested or formed in the intestine. Nevertheless, in view of the light possibly thrown by the results on the potential capabilities of the organism in physiological processes, it seems desirable to refer to some of these experimental facts.

Oxidations. Generally speaking, the organism oxidises a toxic compound as far as possible, and then, if necessary, detoxicates the product by some other process.

Primary and secondary alcohols are oxidised to acids, but tertiary alcohols and halogen-substituted alcohols are resistant to oxidation, and are conjugated. Examples of such oxidations are the conversion of methyl alcohol to formic acid, and of isopropyl alcohol, $(CH_3)_2 . CHOH$, to acetone, $(CH_3)_2 . CO$.

Benzene derivatives of fatty acids are oxidised by the successive etching away of two carbon atoms of the side chain at a time, in a manner analogous to the oxidation of fatty acids themselves (*cf.* p. 132). If the side chain contains an odd number of carbon atoms, benzoic acid results, if an even number, phenylacetic acid. These are then conjugated.

Reduction is a rarer procedure. Chloral, $CCl_3 . CHO$, is reduced to trichlorethyl alcohol, which is subsequently conjugated.

Deaminisation is an intermediate step with toxic amines such as those produced by bacterial action in the intestine. Tyramine (from tyrosine) is converted to tyrosol (*cf.* p. 307), phenylethylamine (from phenylalanine) to phenylacetic acid, and amylamine (from leucine) to isovalerianic acid.

Conjugation is known with glycine, glutamine, ornithine, cysteine, glycuronic acid, and sulphuric acid. The conjugated product is less toxic or non-toxic, or, where the process is used as a normal physiological one, the product is less physiologically active.

Dakin showed many years ago that while such compounds as phenylpropionic acid and cinnamic acid are more or less toxic to animals, when they are conjugated to glycine and fed, the product is non-toxic. Thus he demonstrated that phenylpropionic acid administered to cats in dosage of 0·8 gm. per kg. body-weight killed them in forty to sixty hours, while phenylpropionylglycine, in dosage of 1·5 gm. per kg., was innocuous.

After such preliminary oxidation as is possible, if an alcohol result it is usually conjugated with sulphuric or glycuronic acid, and if an acid, with glycine, glutamine, or ornithine.

Glycine, Glutamine, and Ornithine. Benzoic acid is conjugated with glycine and excreted as hippuric acid in man, horse, cow, dog, cat, and other mammals, but not in birds.

Phenylacetic acid is conjugated with glycine to form phenylaceturic acid in the dog and cat, but not in man, or in birds.

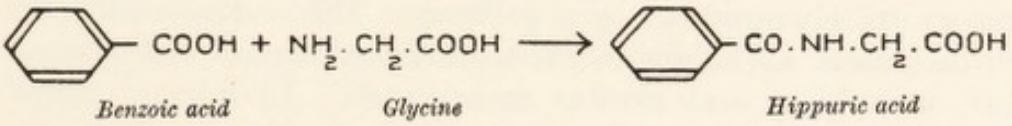
In man and the chimpanzee, phenylacetic acid is conjugated with glutamine, and excreted as phenylacetylglutamine (which is in part combined with urea).

Birds conjugate both benzoic acid and phenylacetic acid with ornithine, of which each amino-group unites in a peptide linkage with a molecule of the toxic acid. Only birds employ ornithine, and only man and (presumably) the higher apes employ glutamine.*

If surviving dog's, pig's, or sheep's kidneys are perfused with a solution containing benzoic acid, hippuric acid is found in the

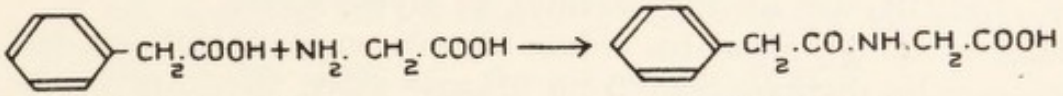
* The changes are therefore as follows :

Mammals :



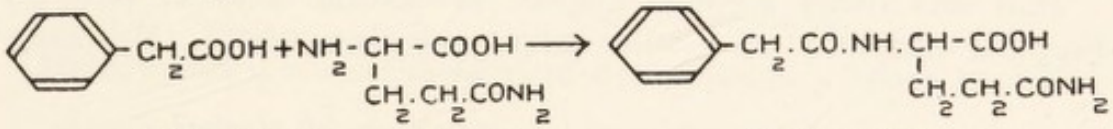
Benzoic acid *Glycine* *Hippuric acid*

Dog and cat :



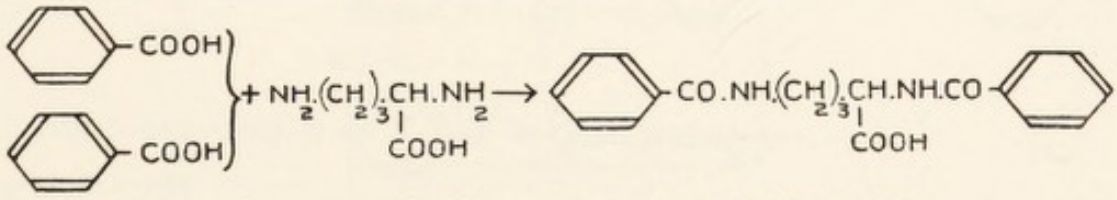
Phenylacetic acid *Phenylaceturic acid*

Man and chimpanzee :



Phenylacetic acid *Glutamine* *Phenylacetylglutamine*

The fowl :



Benzoic acid *Ornithine* *Ornithuric acid*

liquid leaving the kidneys. Man's kidneys also appear to possess the power of bringing about this change. Kidneys possess an enzyme *hippuricase* which will hydrolyse hippuric acid to benzoic acid and glycine. This enzyme, acting under different conditions, probably also effects the synthesis.

Quick has adduced evidence that in the dog only kidney tissue has the power to form hippuric acid. This is not true for the rabbit, and it is generally considered that liver tissue also possesses this power in many mammals.

Cysteine. When brombenzene, chlorbenzene, or iodobenzene is fed to dogs, a *mercapturic acid* is excreted. It is possible that initially a parahydroxy compound is formed, while, subsequently, acetylation takes place.*

Rats and rabbits also possess this power. Naphthalene, when fed to rabbits, yields naphthylmercapturic acid. The power to form these mercapturic acids is lessened when diets are fed which yield little cysteine.

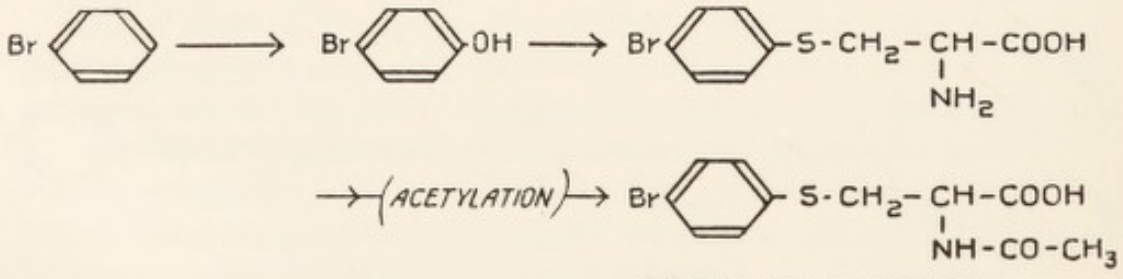
Glycuronic acid is probably one of the most widely used detoxicating agents. Its relationship to glucose has been discussed (p. 83) and it is probably formed from glucose in the liver.

The majority of detoxicated glycuronates are formed by glucosidic union through the aldehydic group. In normal urine only traces of glycuronates are present. The output may be 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. Tertiary alcohols and halogen substituted alcohols as trichlorethyl alcohol, $CCl_3 . CH_2OH$, are usually excreted as glycuronates.

Most of the glycuronates reduce alkaline copper solutions, but they can be distinguished from glucose since they are laevo-rotatory and are not fermented by yeast (although the free acid is dextro-rotatory).

Man and other animals dispose of benzoic acid in part as hippuric acid, and to a smaller extent as a glycuronate. Man only

* For brombenzene the series of changes probably is :



p-Bromphenylmercapturic acid

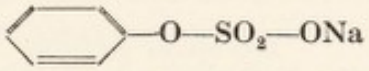
slightly conjugates salicylic acid with glycine (to salicyluric acid), and converts most of it to a glycuronate.

Benzoic and salicylic acids do not yield glucosidic compounds, but *esters* of glycuronic acid. According to Quick benzoic acid yields glycuronic acid monobenzoate,* though Pryde and Williams believe that a benzoylglycuronic acid is formed.

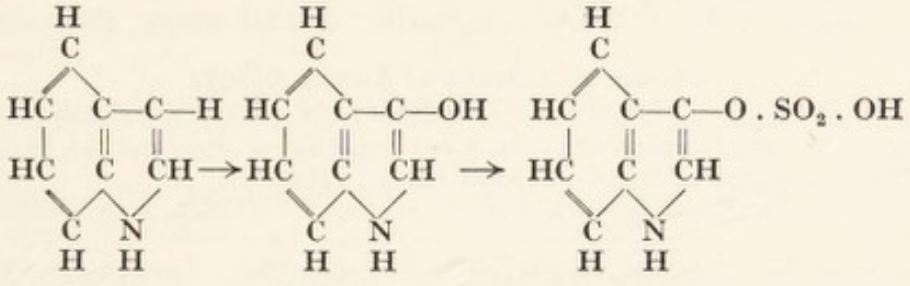
While glycuronic acid can only be oxidised in the body to a slight extent, many conjugated glycuronates can be in large part oxidised, so that the degree of glycuronate excretion is not a clue to the amount formed. The slight degree of oxidation of the acid suggests that it is only formed as needed.

Oestrogenic compounds, such as oestrone and oestriol, are excreted as glycuronates, the conjugation being glucosidic. Progesterone is converted to pregnanediol before conjugation with the acid.

Sulphuric acid. Most of the alcohols formed by intestinal bacterial action are (if they cannot be oxidised further) conjugated with sulphate in the liver, and excreted to constitute the *ethereal sulphates* of the urine. Thus phenol and cresol are excreted as sodium or potassium phenol and cresol sulphates :

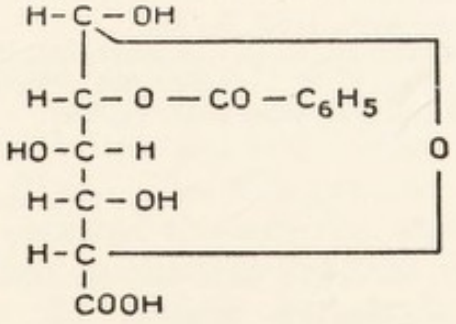


Sometimes, as in the case of indole, initial oxidation to indoxyl occurs before conversion to indican. Skatole is similarly dealt with.



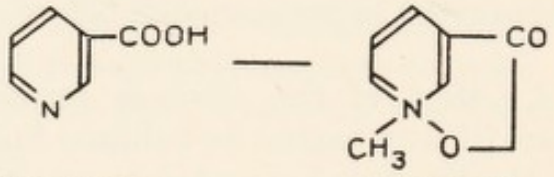
Methylation is a rare method of detoxication. When pyridine

* Quick's formula for glycuronic acid monobenzoate is :



is administered to dogs, pigs, goats, and chickens (but not rabbits), methylhydroxypyridine is formed in the urine.

In view of the importance of nicotinic acid, both as the possible pellagra-preventing vitamin or its precursor (p. 70), and, in its amide form, as a radical of co-zymase (p. 286), it is interesting to note that Ackermann found trigonelline in the urine of dogs, after administering nicotinic acid, although rabbits conjugate it with glycine to form nicotinuric acid.



Nicotinic acid *Trigonelline*

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CHAPTER XIV

THE CHEMISTRY OF THE EXCRETA

INTRODUCTION

THE blood carries excretory products received from various tissues throughout the body 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*.

Bile

Bile has already been dealt with (Chapters I. and V.) 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.

Bilirubin is a crystalline, golden-red compound, having the formula $C_{33}H_{36}N_4O_4$, and derived, through haematoporphyrin, $C_{34}H_{38}N_4O_6$, from haem, $C_{34}H_{33}N_4O_5Fe$ (p. 218). It is easily oxidised to green biliverdin, $C_{33}H_{36}N_4O_8$, while further oxidation gives a series of coloured compounds, including a blue pigment, bilicyanin.

Bilirubin is reduced in the intestine by bacterial action to *urobilin* (*stercobilin*), $C_{33}H_{42}N_4O_6$, a brown pigment to which is due the colour of normal faeces, and still further reduction produces *urobilinogen*, $C_{33}H_{44}N_4O_6$. Urobilinogen can be absorbed from the intestine and excreted through the kidneys in urine, and subsequently oxidised to urobilin (whence the names of these two compounds). In certain pathological conditions the

amounts of uribilinogen appearing in urine are considerably increased.*

When the skin is bruised the series of colour changes are due to the breaking down of haemoglobin and the resulting conversion of haematin 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.

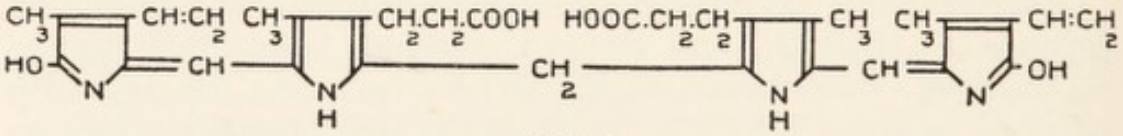
Liver extracts and skin extracts have been found to be equally potent in breaking down haemoglobin, but a mixture of extracts of liver and spleen is still more potent.

The other important excretory compound in the bile is *cholesterol* (pp. 118, 120, 211). 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.

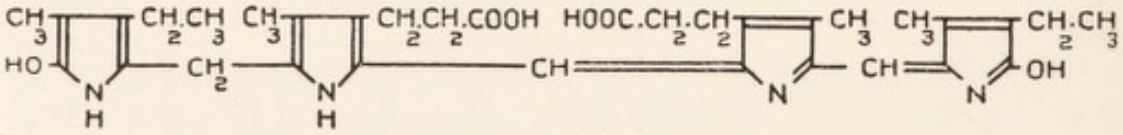
Cholesterol is found in the sterile faeces of the new-born and in faeces during starvation (when the bacterial content of the gut is greatly diminished), but normally in the intestine, through bacterial action, it is partly converted by reduction into *coprosterol*.

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*, $C_{24}H_{36}O_3$.

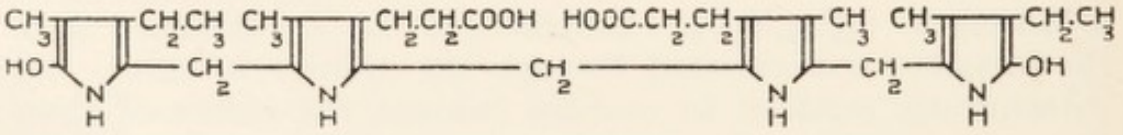
* In the process of splitting off iron and converting haem to bilirubin, the complex structure of the former (p. 218) is ruptured. The constitutional formulae ascribed to bilirubin and its derivatives are shown below.



Bilirubin



Urobilin



Urobilinogen

The Intestinal Mucus

During fasting the faeces 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 chiefly excreted as phosphate.

(Naturally the faeces will contain traces of protein and enzymes from the various digestive secretions poured into the intestine.)

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 faeces, 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 kg. of carbon dioxide and 600 gm. of water.

The Skin and Sweat

Sweat, the secretion of the sweat glands of the skin, is never quite free from epidermal cells and lipides of the sebum (*cf.* p. 212). 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, 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 uraemia, 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 a peptidase having been identified.

Stale sweat is found to contain traces of formic, acetic, propionic, butyric, isovaleric and caprylic acids. 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 gm., equivalent to 185 c.c. per hour, while at 38·5° it is 1·2 gm. 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.

The Urine

The mechanism of the secretion of urine is still a puzzle (*cf.* p. 259), and we do not know exactly how a combination of glomerular filtration and selective reabsorption in the tubules effects the alteration in composition from blood plasma to urine exemplified by such typical figures as those shown in Table XXIX. (modified from Starling's "Principles of Human Physiology").

Examination of the figures in this Table shows that a marked concentration of urea, sulphate, inorganic phosphate, and uric acid is effected, and a distinct concentration of potassium, while proteins and glucose are completely held back (or regained) by the plasma. The apparently marked concentration of ammonia is illusory, since this is actually formed by kidney tissue from urea (pp. 166, 259). It must of course be remembered that different urines vary markedly in concentration and in the relative amounts of certain constituents, and that the figures quoted are only those for a typical normal urine.

The Reaction of Normal Urine. The reaction of a twenty-four

TABLE XXIX. TYPICAL COMPOSITIONS OF BLOOD PLASMA AND URINE, AND CONCENTRATION EFFECTED DURING SECRETION

Constituent	Plasma	Urine	Concentration
	Per cent.	Per cent.	
Water	92	95	—
Proteins	7·9	0	—
Glucose	0·1	0	—
Urea	0·03	2	67
Uric acid	0·002	0·05	25
Sodium	0·3	0·3	1
Potassium	0·02	0·15	7·5
Ammonium	0·0001	0·04	(400)
Calcium	0·010	0·015	1·5
Magnesium	0·0025	0·006	2
Chloride	0·37	0·6	1·5
Inorganic phosphate	0·009	0·27	30
Inorganic sulphate	0·003	0·18	60

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, during 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 (*cf.* p. 246). The urine of most animals, other than man, contains allantoine, which largely replaces uric acid (*cf.* p. 185). Human urine contains but a trace of allantoine. In the urine of birds uric acid largely replaces urea (*cf.* p. 186).

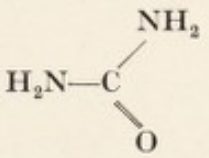
Many of these compounds contain nitrogen, and because of this the *total nitrogen* of the urine, and the *nitrogen partition* furnish important data in many metabolic studies and in many diseased conditions. The following figures may be taken as typical of a normal partition :

Urea-N, 84 per cent. ; ammonia-N, 3 ; creatinine-N, 7 ; uric acid-N, 4 ; *rest*-N, 2 ; total, 100 per cent.

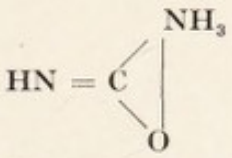
The sites of formation of most of the organic constituents and

their precursors have already been dealt with. Some additional notes concerning the properties of these compounds are given here.

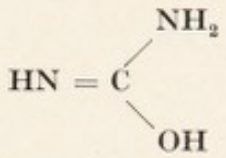
Urea, though discovered in 1773, first analysed in 1817, and synthetised in 1825, still has to have its constitution definitely determined. Of the three formulae usually ascribed to it the third, a modification of the ring formula frequently referred to as the " open " formula, seems to fit most of the facts with reasonable adequacy, and has been used in this text. The three formulae are shown :



Carbamide formula of urea (Dumas)



Ring formula of urea (Chattaway, Werner)



" Open " formula of urea (Werner)

When dry urea is heated biuret, $NH_2 . CO . NH . CO . NH_2$, is formed. Urea forms compounds with nitric and oxalic acids which are only slightly soluble and crystallise to forms easily recognisable under the microscope. It is decomposed by nitrous acid, in presence of a stronger acid, to liberate nitrogen and carbon dioxide. It reacts characteristically and almost quantitatively with sodium hypobromite, a marked effervescence indicating the liberation of carbon dioxide and nitrogen :

$$CON_2H_4 + 3NaOBr \rightleftharpoons CO_2 + N_2 + 3NaBr + 2H_2O.$$

Urea unites with two molecules of xanthydrol, $CHOH:(C_6H_4)_2:O$, to form the extremely insoluble dixanthylurea. On this reaction is based a very accurate method for its estimation.

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 ureae*, 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 Lusk states that he has obtained very active preparations of the enzyme from the gastric mucosa of carnivores.

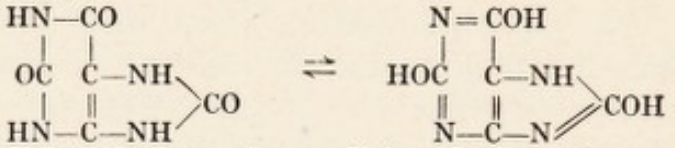
Mack and Villars have shown that urease acts on concentrated solutions of ammonium carbonate with formation of some urea. Nevertheless the process is almost quantitative in the direction of

ammonia formation, and this reaction therefore is employed in the quantitative 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 Plate I.

Uric acid, $C_5H_4N_4O_3$, the end product of purine catabolism in man (*cf.* p. 183), exists in solution in two forms, *lactam*, and *lactim*, in equilibrium 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. 326) 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.

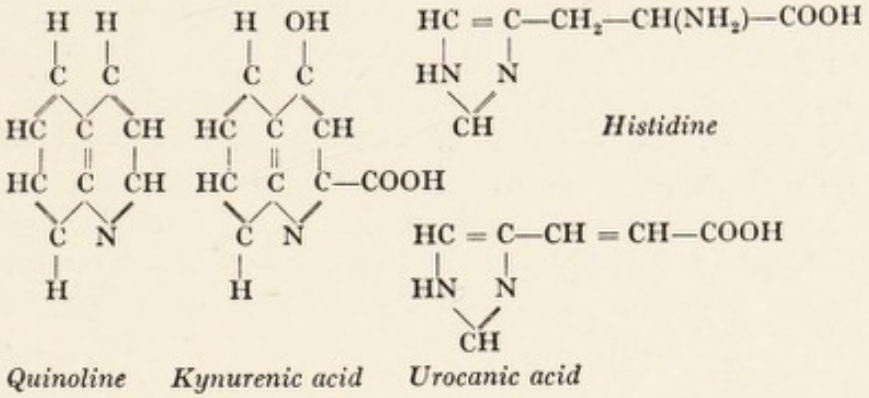
Allantoine, the end product of purine catabolism in most mammals (p. 183), is much more soluble than uric acid (to the extent of somewhat less than one part to 100 of water at 20° C.).

Creatinine and *creatine* have already been dealt with in detail (p. 244). The existence of free creatinine in blood is still a matter of dispute, but the evidence at present available favours the view that blood contains either free creatinine or a creatinine derivative capable of giving the same coloured compound with picric acid as creatinine itself. A rational explanation of the actual presence of creatinine in urine, its derivation from creatine of muscle (p. 249), the relationship between degree of creatinine excretion and degree of muscularity of the individual expressed in Folin's coefficient (p. 246), and the increased " creatinine " of blood in the late stages of nephritis where catabolites are dammed back into

the blood by a failing kidney, seems to depend on the existence of creatinine in blood in some form.

Amino-acids are always present in small amount in urine; man excretes 0·4 to 1·0 gm. per day.

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, closely related to quinoline, is produced from tryptophane (*cf.* p. 170).



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 20 to 40 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.

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 gm. 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.

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 (see p. 318).

Uroerythrin is frequently present in small amount and the red colour of urinary pigments is due to it. Little is known of its composition.

Products of Bacterial Actions and of Ingested Poisons. Certain products of bacterial action in the intestines reach the urine unchanged, and are presumably of a non-toxic nature. Others, and also toxic compounds from food (as described in Chapter XIII.) have their toxicity removed by conjugating them with glycine, glutamine, glycuronic acid, and sulphuric acid.

Hydroxy-acids. Parahydroxyphenylacetic acid, $HO . C_6H_4 . CH_2 . COOH$, and parahydroxyphenylpropionic 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.

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. 302), 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.

Glycine derivatives are typified by *hippuric acid*, $C_6H_5 . CO . NH . CH_2 . COOH$, which crystallises in long, well-defined needles. When hydrolysed by heating with dilute acid it yields benzoic acid and glycine. Its formation is the organism's chief method of disposing of benzoic acid and certain related compounds such as quinic acid, $(HO)_4 . C_6H_7 . COOH$, when these are ingested with fruits and other foods (*cf.* p. 313). Horses' urine is especially rich in hippuric acid, whence its name (Gk. *hippos*, horse).

Glutamine is employed by man and the higher apes to dispose of phenylacetic acid, the product, phenylacetylglutamine, being excreted in human urine.

Glycuronates were discussed in some detail in Chapter XIII. Normally, human urine only contains a few milligrams (p. 314).

Sulphuric acid is used especially to detoxicate compounds with

properties of alcohols, or others which can be oxidised to alcohols, the products constituting the *ethereal sulphates* of the urine.

*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 :

$$HO \cdot SO_2 \cdot OR + HOH = H_2SO_4 + ROH$$

where *R* is an organic radical.

The total output of ethereal sulphates, calculated as SO_3, in normal urine does not exceed 0·25 gm. per day.

The most important of these compounds present in urine are phenol- and *p*-cresol-sulphuric acid, and indoxyl- and skatoxyl-sulphuric acid.

The amount of indican (indoxyl sulphuric acid) 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 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*.†

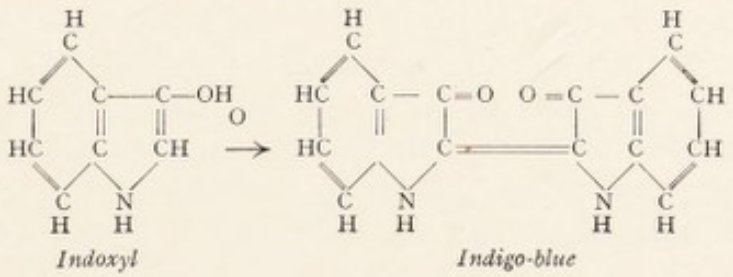
Pathological Constituents of Urine

Glucose is usually considered to be present in normal urine, but only in minute traces of the order 0·01 per cent. or less, 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

* The term "ethereal" is here used in the old meaning of "esterified," a meaning now discarded by organic chemists.

† The reaction is :



Indoxyl *Indigo-blue*

acetoacetic acid, are not detectable in normal urine, but are found present in many conditions in which an acidosis is present (*cf.* p. 278).

Lactic acid 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.

Numerous other abnormal and pathological constituents, *e.g.*, Bence Jones protein, and homogentisic acid, have been discussed elsewhere (*cf.* pp. 174, 169).

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CHAPTER XV

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).

Autolytic 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 autodigestive 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*, now usually termed *kathepsins* (*cf.* p. 161). 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. Kathepsins, therefore, act best in a very slightly acid medium. 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-aminovaleric 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 (*cf.* Chapter VI., p. 162) 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 kathepsins 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.

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 plasma-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 plasma proteins.

Rabbit leucocytes do not contain leucoprotease, but a peptidase. Leucoprotease is absent from the leucocytes of the fowl.

Leucoprotease is much less active than trypsin.

The resistance of living bacteria to such enzymes may be due to anti-enzymes (the spleen also seems to contain 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 a peptidase.

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 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 glycerol extract of clam liver, complete hydrolysis resulting. The digest was concentrated, and then more of the glycerol 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 from the same organ.

Further, since, as has already been mentioned, these intracellular kathepsins 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 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 XVI

QUANTITATIVE METABOLISM

Potential Energy of Foods

In Chapter II. the laws of conservation of Matter and of Energy were stated and their application to living processes (pp. 11, 13), the large and small Calories and Joule's equivalent were defined (p 13), and the important concept of potential energy was set forth (p. 15). 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 XXX.

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* (or *caloric*) *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 ;

TABLE XXX. 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	,, ,,
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	,, ,,

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

TABLE XXXI. AVERAGE ENERGY VALUES OF FOODSTUFFS

	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

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 XXXI.

The Energy Exchanges of the Body

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, faeces, 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 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. 22.

It consists of two precisely similar chambers with double walls. The space between the walls is air-tight, and the two air-spaces

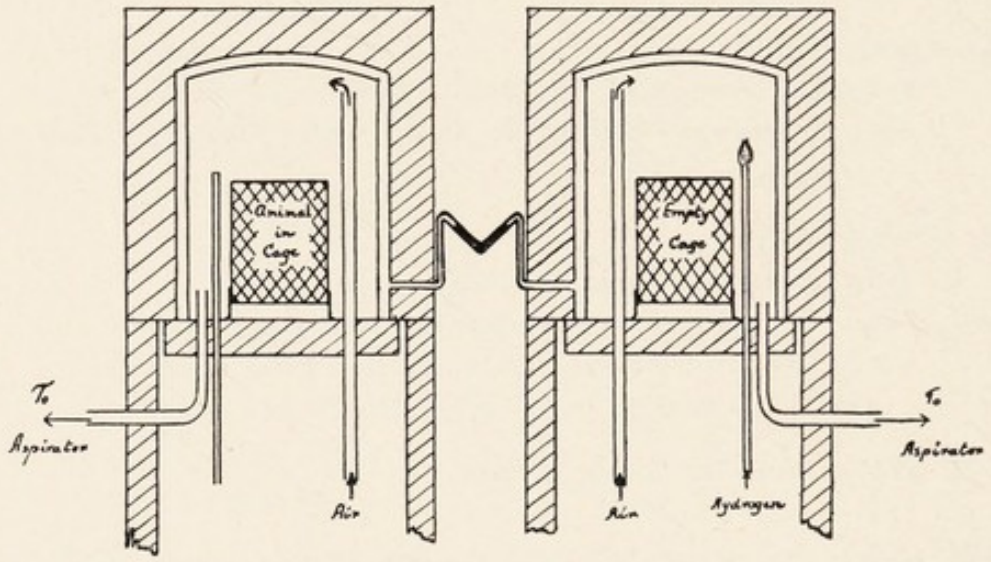

FIG. 22. Calorimeter for small animals, whose heat production is balanced by that from a jet of burning hydrogen. (After Hale White, *Lancet*, 1897, II., 2.)

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

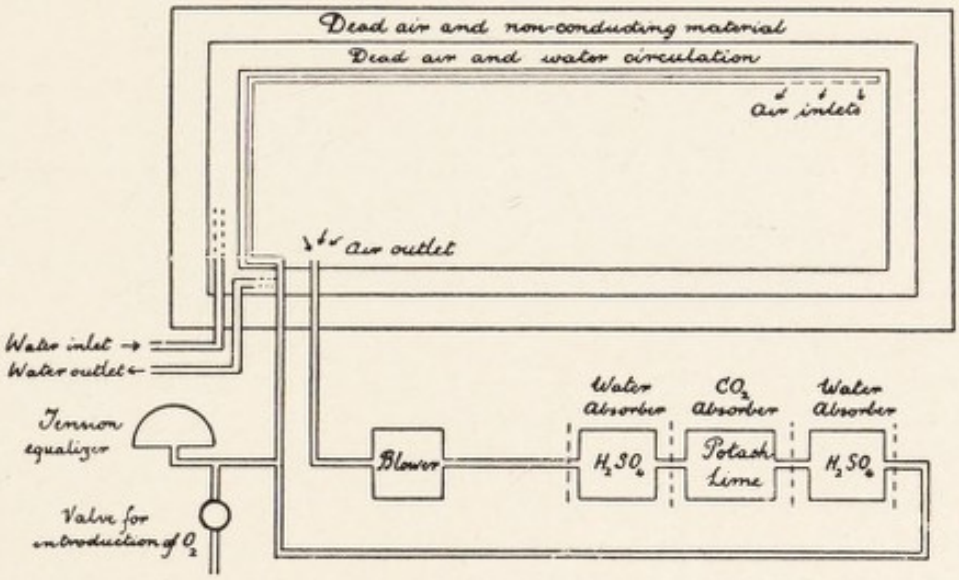

FIG. 23. Diagram of a bed-calorimeter. After diagrams in Benedict and Carpenter's "Respiration Calorimeters," *Carnegie Institution Publication*, No. 123.

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. A schematic sketch of such an apparatus is shown in Fig. 23, and photographs of an actual apparatus in Figs. 24 and 25.

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.

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 :

$$C_{644}H_{1064}N_{166}S_8O_{214} + 690\tfrac{1}{2}\, O_2 = 83\, CON_2H_4 + 561\, CO_2 + 358\, H_2O + 8\, H_2SO_4$$

$$\text{R.Q.} = \frac{561\, CO_2}{690\tfrac{1}{2}\, O_2} = 0{\cdot}81$$

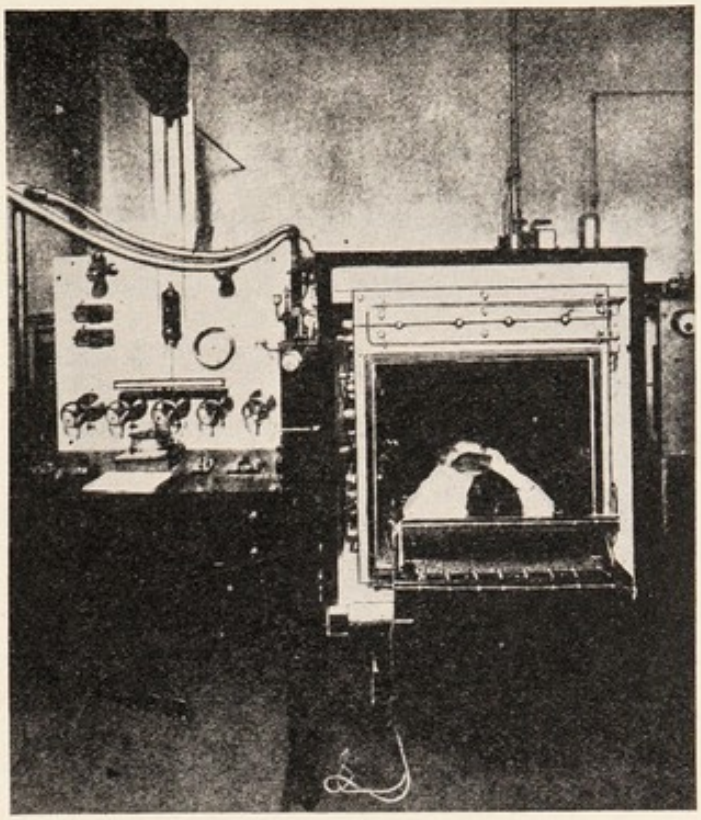

FIG. 24. Photograph of the respiration calorimeter for metabolism determinations of the Russell Sage Institute of Pathology in Bellevue Hospital, New York. The calorimeter is open, and a patient is on the canvas bed partly in the chamber. The rubber pipes lead to the absorbing apparatus. (From Riche and Siderstrom, *Arch. Int. Med.*, 1915, xv., 816.)

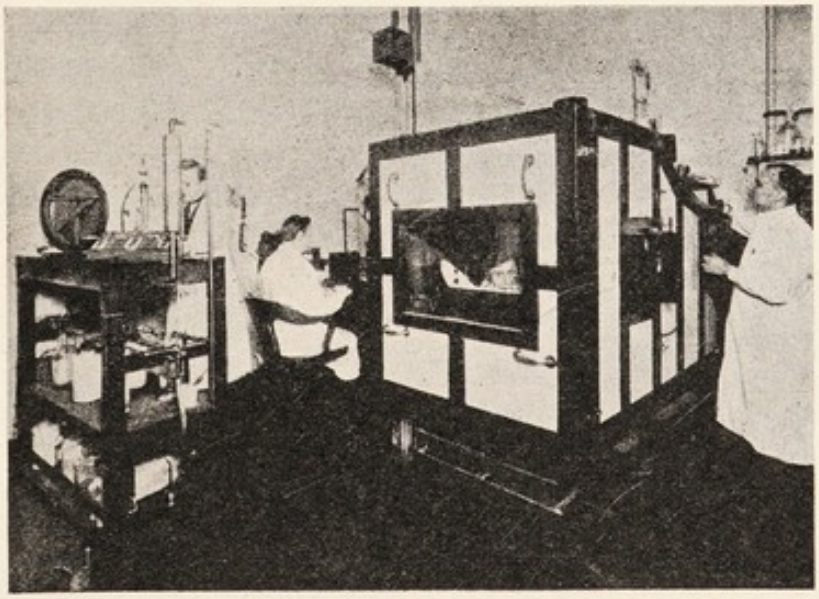

FIG. 25. Photograph of the Russell Sage Calorimeter during an actual test. The absorbers are shown on the left. (From E. F. DuBois, "Basal Metabolism in Health and Disease," Lea and Febiger, Phila. and New York, 1924.)

The respiratory quotient during oxidation of mixtures of carbohydrates, fats, and proteins 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 XXX.), 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, has a very

TABLE XXXII. 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

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 XXXII., 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 consider-

able 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 :

$$19C_6H_{12}O_6 = 2C_3H_5(O \cdot CO \cdot C_{17}H_{33})_3 + 4H_2O + 49O_2.$$

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 " 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 XXXIII.

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

TABLE XXXIII. 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.

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 XXXIV. gives the results of analyses on the different foods ingested, applied to the amounts actually eaten. Table XXXV. 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

TABLE XXXIV. CHEMICAL COMPOSITION OF THE FOOD INGESTED AND ITS HEAT VALUE

Food per day.	Weight per day.	Water.	Protein (N × 6·25).	Fat.	Carbohydrate.	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

figures for protein are obtained. The assumption is approximately correct.

Table XXXIV. shows that there was, during the four days, a total nitrogen intake of 67·1 gm. During the same period nitrogen

TABLE XXXV. CHEMICAL ANALYSES AND ENERGY VALUES OF THE FOUR DAYS' EXCRETA

Material.	Weight.	Protein (N × 6·25).	Fat.	Carbo-hydrate.	Water.	CO_2	N	C	H (in dry excreta).	Heat value.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Cals.
Faeces . .	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

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 XXXIV. shows that the total intake of carbon was 1,465·3 gm., and the total loss (Table XXXV.) 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.

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 XXXVI.),

TABLE XXXVI. 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.

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 XXXIV. the total intake of water was 11,008 gm., and from Table XXXV. 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 kg. 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
Faeces .	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.

The authors calculated the next 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 :

Protein	=	6·0 N
Fat	=	0·005 C + 9·693 H − 1·221 O − 2·476 N
Carbohydrate	=	2·243 C − 16·613 H + 2·093 O − 2·892 N
Water	=	− 1·248 C + 7·920 H + 0·128 O + 0·460 N

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 × − 4·86, equals − 29·16, indicating a loss of 29·16 gm. 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.

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 (faeces)	40·48	—	—	4·53	39·95	—
Solids (faeces)	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

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 were 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 an extent that during hard work the body temperature is actually raised for a period above the normal value. If the amount of work done is accurately determined, and the additional heat produced during the accomplishment of this work is also measured accurately, it is found that the sum of the two forms of energy is about eight times the actual work done (Atwater).

Measurements of the respiratory quotient made during the performance of moderate work approximate to unity, indicating that carbohydrate is being used to provide the energy for the work done.

Calorigenic Action (Specific Dynamic Action)

Specific dynamic action is a somewhat cumbersome and indefinite term coined long ago by Rubner to describe the fact that ingestion of foods increases heat production. The term *calorigenic action of foodstuffs* has recently been suggested as an alternative (Mitchell, Lusk), and has the advantage of giving some clue by its meaning to the process to which it refers.

It has long been known that ingestion of protein stimulates metabolism. As a result more heat is produced than would be derived from oxidation of the actual amount of protein ingested. This is usually termed the *specific dynamic action of proteins.* Lusk showed that it is not due to proteins as such, but to a few

amino-acids. Five (perhaps six) only are concerned. These are, in decreasing order of effect, phenylalanine, tyrosine, glycine, alanine, leucine. Glutamic acid perhaps should be included. The others, as far as they have been tested, are without effect.

The liver is essentially concerned in the action of these amino-acids, since hepatectomised dogs do not exhibit any specific dynamic action of protein. Since more heat is produced, extra 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 deaminisation and 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 may play some intermediate part, since Baumann and Hunt have stated that the effect is not produced after thyroidectomy ; nevertheless cretins and hyperthyroid patients are said to exhibit the usual effect following protein ingestion.

When fats are fed *in excess* a somewhat similar phenomenon occurs, to a slighter extent. 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.

The slight calorigenic action of carbohydrate is believed to be due to excess energy set free as heat during conversion of glucose to glycogen. Curiously enough, it is said to be greater after hepatectomy.

Calorigenic action is not limited to foodstuffs. Such compounds as ammonium salts and dinitrophenol produce marked effects, and such action is also said to be associated with certain hormones and vitamins.

Energetics of Muscle

In the previous part of this chapter quantitative metabolism has been dealt with in so far as it concerns the whole organism. It is of interest to consider some aspects of energy studies of a single tissue, muscle. The two types of study might almost be referred to as relatively macroscopic and microscopic, and in studying energy changes in muscle the small calorie is employed, which it

will be remembered 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 XXXVI. 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 depending to some extent upon the condition of the muscle.

TABLE XXXVII.

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

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 180*n*. 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. 277).

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 for muscle to include both work and heat.

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CHAPTER XVII

DIET

INTRODUCTION

In order to be adequate a diet must be both qualitatively and quantitatively correct. It must contain a sufficiency of all the compounds and mineral elements which the body needs and cannot form for itself, and a sufficiency of all precursors of those compounds which it can form for itself; in addition the potential heat value of the ingested food must be sufficient for the energy requirements of the body. But a correct diet should furnish little or no excess energy; otherwise one of the chief factors of obesity, excess of food, is present.

Furthermore, in considering the material ingested as food, it is important to take into account the preliminary treatment of the food before it is eaten. For example such a term as "potatoes" in a diet list is misleading, if there is any possibility that "boiled potatoes" and "fried potatoes" can be confused. The chemical composition and energy values of these two preparations are very different. It is also necessary in discussing diet to consider the needs of the growing child as compared with adults, the relative needs of man and woman (and the pregnant and lactating woman), and the relation of dietary requirements to body-weight.

A correct diet is made up of water, certain mineral constituents, certain vitamins, an adequate amount of proteins (of such types as will provide a number of essential amino-acids), carbohydrates, fats (including glycerides of certain unsaturated fatty acids) and some amount of "roughage."

The Essential Constituents of a Correct Diet

Water. The predominant importance of water in the diet and the amount needed have been dealt with in Chapter VIII.

Mineral Elements. These have been fairly fully dealt with in Chapter VIII., where evidence has been adduced that calcium, magnesium, sodium, potassium, iron, copper, manganese, zinc, chlorine, phosphorus, sulphur and iodine are all essential for mammals (including man), there being some possible doubt about fluorine, and greater doubt about cobalt (though cobalt

seems essential for sheep). The daily human requirements of a number of these elements have also been stated in Chapter VIII. Some idea of the *total* mineral content in common foods is given by the figures for ash in Table XXXVIII.

TABLE XXXVIII. 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

Vitamins. These have been dealt with in Chapter III. It can be considered as proved that man needs **A**, $\mathbf{B_1}$ (thiamin), $\mathbf{B_2}$ (riboflavine), **C** (ascorbic acid), $\mathbf{D_3}$ (or artificial $\mathbf{D_2}$), **E**, and the pellagra-preventing vitamin. Further work is needed to determine whether $\mathbf{B_3}$, $\mathbf{B_4}$, $\mathbf{B_5}$ and $\mathbf{B_6}$ are needed by man, if indeed all of these truly exist. It is not yet possible to express our daily needs of all the vitamins in terms of ordinary weights and measures.

Standardisation of Vitamins. This has been carried out on small animals, the rat, the guinea-pig, the chick, the pigeon, and usually has been defined in amounts needed to correct an avitaminosis. International units (established by the League of Nations Health Organisation) based on such methods give most misleading and cumbersome figures when used to express the amounts of vitamins present in various food, and the daily requirements of human beings. It is far better to express such quantities by an ordinary system of weights which conveys some definite meaning. Fortunately, this is now possible for **A**, $\mathbf{B_1}$ and **C**, and should soon be possible for $\mathbf{B_2}$ and $\mathbf{D_3}$.

The League of Nations Health Organisation selected carotene as a provisional international standard of reference for determination of the strength of preparations of **A**, and defined the international unit as the potency in **A** of 0·001 mg. of a certain sample of carotene, of which 0·003 to 0·005 mg. was sufficient to restore growth or cure xerophthalmia in rats in which a condition of **A**-avitaminosis had been induced.

Unfortunately this original standard of carotene proved to be contaminated with non-carotene material. Rigid comparison against the purest β-carotene has shown that the international unit really corresponds to 0·0006 mg. of β-carotene. In considering the vitamin **A** content of foods, such as the values given in Table XXXIX., the figures, as far as plants are concerned, are for the carotenes (expressed in terms of β-carotene), while for animal tissues they indicate **A**, or a mixture of **A** and carotene, expressed in terms of β-carotene.

For the international standard of $\mathbf{B_1}$ a special product was selected, prepared by adsorbing a sulphuric acid extract of rice polishings on Fuller's earth. One unit is defined as the anti-neuritic 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.

Now that crystalline $\mathbf{B_1}$ hydrochloride is available for comparison, two different tests have indicated that 0·002 and 0·0027 mg. of this hydrochloride are respectively equivalent to 1 international unit (I.U.). It will be therefore a close approximation to state that 1 I.U. is equal to 0·002 mg. of $\mathbf{B_1}$ (the base itself).

The international unit of vitamin **C** is now defined as 0·05 mg. of pure crystalline *l*-ascorbic acid. This is about one-tenth the amount required daily to prevent development of macroscopic scorbutic lesions in young guinea-pigs that are being fed a scorbutic diet.

The "Line Test." A delicate test is employed to measure the amount of **D**. Rats are rendered rachitic by special **D**-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 breadth of the "line" so produced indicates the extent of healing and therefore the potency of the preparation.

The international standard 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 gm.
Butter contains . . .	0 to 1·0	,, ,,
Cod liver oil contains . .	60 to 150	,, ,,
Viosterol (250 **D**) contains .	15,000	,, ,,
Pure calciferol ($\mathbf{D_2}$) contains.	40,000,000	,, ,,

Obviously the international standard requires to be re-defined in terms of the natural vitamin, $\mathbf{D_3}$. Moreover, since $\mathbf{D_2}$ and $\mathbf{D_3}$ do not produce precisely the same actions in all species of animals, food materials and drugs will need consideration according to their separate contents of the natural and artificial vitamins.

The Vitamin Content of Foods. The **A**, $\mathbf{B_1}$ and **C** contents of a number of the commoner foodstuffs are shown in Table XXXIX. The figures in international units (I.U.) are re-calculated from Eddy and Dalldorf's Tables, and, on the basis of the equivalent values stated above, have also been expressed in milligrams. It will be seen that the latter values are much more easily grasped.

The Daily Requirements of Vitamins. No final authoritative data are yet available on which can be based statements of the optimum human requirements of the different vitamins. These are relatively greater in the infant and growing child than in the adult.

Stiebeling, of the United States Department of Agriculture, in a report made in 1936, estimates that children under four years of age need 4,200 I.U. of **A**, and adults 5,300 I.U. The corresponding amounts of β-carotene are 2·5 and 3·2 mg. The Committee on Nutrition of the League of Nations in a report published in 1935 suggested diets which would provide infants from one to two years of age with 5,000 I.U. of **A**, corresponding to 3·0 mg. of β-carotene. The older child should be given slightly more. This Committee recommended further that the pregnant woman and nursing mother should have a daily intake of 8,700 I.U. (equivalent to 5·2 mg. of β-carotene).

It may be noted that a good cod liver oil contains **A** equivalent to about 36 mg. β-carotene per 100 c.c.

Stiebeling recommends 60–150 I.U. of $\mathbf{B_1}$ for young children, 140–350 I.U. for adults. The corresponding values in terms of

TABLE XXXIX. VITAMIN CONTENTS OF CERTAIN FOODSTUFFS, PER 100 GM., IN INTERNATIONAL UNITS AND IN MILLIGRAMS. VALUES FOR **A** ARE FOR CAROTENE OR **A** IN TERMS OF β-CAROTENE

	International Units			Milligrams		
	A	**B_1**	**C**	**A**	**B_1**	**C**
Bacon	25	85	0	0·015	0·17	0
Beef, lean	100	25–50	0	0·06	0·05–0·10	0
Beef kidney	700–850	40	0	0·4–0·5	0·08	0
Beef liver	14,000	150	0	8·4	0·3	0
Haddock	7	25–50	—	0·004	0·05–0·10	—
Roe	4,200	75	—	2·5	0·15	—
Salmon	100–800	14–50	—	0·06–0·5	0·03–0·10	—
Butter	350–7,500	40	—	0·2–0·5	0·08	—
Milk, fresh whole	110–350	10–25	10–50	0·07–0·2	0·02–0·05	0·5–2·5
Milk, human	—	10–15	—	—	0·02–0·03	5·5–8·0
Eggs	2,750–4,000	25–30	—	1·7–2·4	0·05–0·06	—
Egg-yoke	850–8,500	50–150	—	0·5–5·0	0·10–0·30	—
Bread, whole wheat	present	75	0	present	0·15	0
Bread, white wheat	0	10	0	0	0·02	0
Wheat germ	abundant	600–1,800	0	abundant	1·2–3·6	0
Oats	0–25	65–115	0	0–0·015	0·13–0·23	0
Rice, brown	50–100	abundant	0	0·03–0·06	abundant	0
Rice, white	trace	7	0	trace	0·014	0
Rice polishings	0	280–400	0	0	0·56–0·8	0
Asparagus, green	180–1,000	100–150	200	0·11–0·6	0·20–0·30	10
Carrots, raw	3,400–9,000	15–25	75	2·0–5·4	0·03–0·05	3·75
Cabbage, raw	50–85	20–25	600–650	0·03–0·05	0·04–0·05	30–32·5
Lettuce, green	250–7,000	20–25	70–75	0·15–4·2	0·04–0·05	3·5–3·75
Peas	900–1,500	25–100	300–500	0·54–0·90	0·05–0·20	15–25
Red peppers	7,700	7–10	1,250	4·6	0·01–0·02	62·5
Potatoes	35–50	20	100–150	0·02–0·03	0·04	5–7·5
Spinach	9,000–35,000	30–50	1,000–2,000	5·4–21·0	0·06–0·10	50–100
Tomatoes	850–2,100	15–20	275–500	0·5–1·25	0·03–0·04	14–25
Turnips	25	15	150	0·015	0·03	7·5
Apricots, fresh	3,500–7,000	10	70–100	2·1–4·2	0·02	3·5–5·0
Bananas	280–500	15	200	0·17–0·30	0·03	10
Grape fruit	20	10	650–700	0·012	0·02	32·5–35
Oranges	100–700	30	800–840	0·06–0·62	0·06	40–42
Strawberries	25–125	present	400–500	0·01–0·075	present	20–25
Yeast, dried baker's	—	240–700	—	—	0·48–1·4	—
Yeast, dried brewer's	—	175–1,500	—	—	0·35–3·0	—
Olive oil	50	—	—	0·03	—	—

One International Unit of **A** corresponds to 0·0006 mg. β-carotene; one I.U. of B_1, equals 0·002 mg. crystalline B_1; one I.U. of **C** equals 0·05 mg. *l*-ascorbic acid.

pure thiamin are 0·12–0·30 mg. and 0·28–0·70 mg. The Council on Pharmacy of the American Medical Association recommends 50 I.U. for infants and 200 for adults (0·10 and 0·40 mg.).

Stiebeling recommends 100–250 I.U. of **C** for young children, and 150–375 for adults (equal to 5–12·5 and 7·5 to 18·75 mg. of pure *l*-ascorbic acid). Eddy and Dalldorf consider that 2·5 mg. of ascorbic acid will prevent scurvy in an infant, and 7·5 mg. in an adult.

Using the dichlorphenolindophenol titration method of

estimating **C**, which is fairly reliable, it has been shown that the " saturation point " for **C**, at which any increased intake is merely excreted quantitatively, is reached only for much higher intakes than those just quoted. It has further been shown, for example, that the average adult in Holland has a daily intake varying from 55 to 124 mg. and it has been calculated that the " saturation amount " for an adult weighing 70 kg. is 60 mg. The amounts required to restore to normal the tissue contents of a " depleted " individual are much higher ; scorbutic individuals may require a total intake of 7 to 14 gm. before saturation is reached.

Hence claims are being made that to ensure health much larger intakes than those suggested above are desirable. Probably the truth lies between these extremes.

It is not serviceable to attempt to make statements concerning requirements of $\mathbf{B_2}$, $\mathbf{D_3}$ and **E**, until they can also be expressed in mg. of pure compounds. But the guess may be hazarded that for these also the optimal daily dose will be of the order of a few milligrams.

Proteins. There are two desiderata to be considered concerning the total protein requirement of the diet, the amino-acids provided by the ingested proteins, and the minimal amount of protein needed.

It was shown in Chapter VI. that ingested protein must provide the rat with ten different amino-acids for its normal growth, lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. If sufficient of these ten is provided, the rat can form from them the others which it needs.*

Pending further investigations, it is assumed that this also applies to man. If this assumption is true then it is obvious that the protein in man's food must at least provide the ten named acids. This is of practical dietary importance. For example the protein gelatin contains no isoleucine, no tryptophane, and no valine (*cf.* p. 153), and therefore cannot successfully be used as a principal protein of diet.

The minimum amount of protein necessary in a diet is measured by determining the *nitrogenous equilibrium*.

The earlier students of metabolism found that it was possible to maintain an animal such as the dog in an equilibrium at

* In a recent review Mitchell has summarised evidence that cystine may be an essential amino-acid as well as methionine, quoting such observations as that of Marston, that the cystine content of the dietary protein is the determining factor of the wool-growth of sheep. Rose's conclusions concerning the ten essential amino-acids relate to rats ; rigid application of these conclusions to other mammals may or may not prove to be justifiable.

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 XL.

TABLE XL. 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.

Standard dietaries suggested by the physiologists of the nineteenth century, based upon observations of what the average person actually ate, showed amounts of protein usually well over 100 gm. per day.

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.

The first advocate of the 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 individuals, 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 kg. 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-kg. 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 gm. of nitrogen. A 64-kg. woman maintained a similar balance for the same period on an intake of 3·8 gm. of nitrogen per day.

The self-studies of Röse add further support. He could maintain for a period of some days nitrogenous equilibrium and excellent physical efficiency on a daily intake of 24 to 29 gm. of protein, and lived for fifteen years on an average daily intake of about 40 gm. protein.

Sherman, from consideration of all such studies, draws the conclusion that the protein requirement of a 70 kg. man is 44 gm., but recommends 70 gm.

While it is evident that the average person consumes much more than the " 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 kg., 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.

Recent studies have laid stress on desirability of a definite amount of " animal " protein in a diet (as contrasted with protein from plant sources).

Thus the Advisory Committee of the British Ministry of Health recommends 37 gm. of animal protein out of a total of 100 gm. protein.

It is also desirable to remember that the protein needs of the growing child are relatively considerably greater than those of adult man. Thus Hawley, in 1927, suggested that youths, fifteen

to seventeen years of age, had a 50 per cent. greater requirement than adults, and children of six to nine an equal need.

It is interesting also to remember that a definite deficiency of protein in the diet leads to a pathological condition, famine oedema (*cf.* p. 205).

Carbohydrates and Fats. 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.

Starch, cane sugar, lactose, glucose and fructose are all completely utilisable as carbohydrates. Cellulose and other complex carbohydrates, and the pentoses are not utilisable by man (*cf.*, however, p. 305). Apparently the fat-intake must provide certain unsaturated fatty acids (*cf.* p. 137), but diets have to be most drastically treated to free them from a sufficiency of these unsaturated acids.

Roughage. Actual " bulk " of material assists in promoting intestinal peristalsis. Hence indigestible residues, such as cellulose and similar compounds, contributed chiefly by the vegetables and fruits of a diet, are of indirect value.

Quantitative Requirements of a Correct Diet

Early computations of dietary needs were based on measurements of what normal people actually ate. 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 XXXIII., 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 kg.) 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.

Becker and Hämäläinen also obtained the following figures for women engaged in different occupations: stenographer 1925 calories, bookbinder 2,100 calories, sempstress (machinist) 2,200 calories, servant 2,400 calories, washerwoman 2,700–3,500 calories.

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.

The figure 3,000 calories is now generally accepted as a sound average figure for the average man. Based on this figure as unity a Committee of the Health Organisation of the League of Nations published in 1932 a scale to act as a *rough* guide in computing the needs of a family or a population, and this scale has been widely adopted.

Age	*Male*	*Both Sexes*	*Female*
0–2	—	0·2	—
2–3	—	0·3	—
4–5	—	0·4	—
6–7	—	0·5	—
8–9	—	0·6	—
10–11	—	0·7	—
12–13	—	0·8	—
14–59	1·0	—	0·8
over 60	—	0·8	—

Bigwood and Jacquemyns, dealing particularly with the working population of Brussels, selected the housewife's requirements as unity, and obtained the adult male coefficient (for a working man) as 1·14 to 1·2, and, when unemployed, 0·9.

Many dietaries have been proposed for the average adult man. Some of these are shown in Table XLI. The first three in this table are now chiefly of historical interest; the last two are more definitely based on modern knowledge of actual requirements.

TABLE XLI. STANDARD DIETARIES

	Protein	Fat	Carbohydrate	Calories
Voit (Germany) . .	118	56	500	3,055
Rubner (Germany) . .	127	52	509	3,092
Atwater (United States) .	125	125	450	3,520
Advisory Committee of the British Ministry of Health . . .	100	100	400	3,000
Tyszka (Germany) . .	80–100	60–80	500	3,000

From what has been stated above it may be concluded that the average man of 70 kg. weight has a daily requirement of 3,000 calories, while an allowance of 70 gm. of protein (1 gm. per kg.) is adequate. Dietaries and individuals show great variations in the amount of fat ingested, but fat contents as low as 50 gm. per day give diets which are not too appetising; an intake of 100 gm. to 150 gm. is more usual, and more easily prepared from ordinary foods.

In contrasting optimal values for food intake with those usually ingested, a quotation from a recent report by Burnet and Aykroyd is apposite: "For children, for pregnant and lactating women, for those engaged in hard manual work, abundance is essential. On the other hand the idea of frugality, of a 'moderate ascetism,' is more appropriate in the case of middle-aged and elderly men and women leading a sedentary life."

Calculation of a Diet. If a diet is fixed at 70 gm. protein, 120 gm. fat, and the rest carbohydrate, to yield 3,000 calories, the amount of carbohydrate can easily be calculated from the energy value of these foods (p. 336).

Protein $70 \times 4{\cdot}1 = 287$ cals.
Fat $120 \times 9{\cdot}3 = 1{,}116$ cals. a total of 1,403, leaving 1,597 cals. to be provided from $1{,}587/4{\cdot}1 = 390$ gm. carbohydrate.

If any two of the three is fixed the third can be at once determined.

If a diet be called for to meet the following specific requirements—

Protein . . .	85 gm., equivalent to	348·5 Cals.
Carbohydrate . .	300 gm., equivalent to	1,230 ,,
Fat	100 gm., equivalent to	930 ,,
	Total	2,508·5 Cals.

then, utilising tables of food values, the meal-values shown in Table XLII. 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 meals given in Table XLII. probably do not contain sufficient vitamins.

Alcohol has been sufficiently dealt with in Chapter VIII. 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.

TABLE XLII. 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

The Preparation of Food

It is essential, in planning meals to conform to a specific diet, to take in account the changes in energy value and chemical composition frequently introduced in the preparation of food, while cooking is also, to some extent, a stage of pre-digestion.

Becker and Hämäläinen's figures, as quoted, indicate the type

of correction for energy value which must be applied to food as purchased, in contrasting it with the same food as eaten.

In cooking, starch is partly changed into soluble starch and dextrins. Hard connective tissue is changed, at least in part,

TABLE XLIII. 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

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

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).

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 and vitamins.

Previous auto-digestive changes 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.)

As just stated, 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 XLIII.

Starvation and Deficiency Diseases

Starvation. 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 kg. 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 gm. of glycogen and eliminated 5·84 gm. of nitrogen in the first twenty-four hours, in the second similar period the respective figures being 29·7 gm. of glycogen and 11·04 gm. of nitrogen. A second subject, who only catabolised 64·9 gm. of glycogen in the first and 23·1 gm. in the second period, excreted respectively 12·24 and 12·45 gm. 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 XLIV.

TABLE XLIV. 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

The initial body-weight was about 65 kg. 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.

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 XLV.

TABLE XLV. 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

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.

Deficiency diseases, specific in kind, are definitely associated with lack of one or more vitamins (*cf.* Chapter III.), lack of some essential mineral constituent (*cf.* Chapter VIII.), lack of essential amino-acids (*cf.* Chapter VI.), and, at least in the rat, with lack of essential unsaturated fatty acids (*cf.* Chapter V.).

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 faeces, 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 XVIII

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 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 sequelae 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 caesium, 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 caesium are concerned. That of sodium is practically without action, the salts of lithium and caesium 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. Caesium salts cause somewhat similar effects in the alimentary tract. Lithium, caesium 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 anaesthesia ; 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 haemoglobin 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 anaesthetics, producing anaesthesia 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 anaesthesia 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 anaesthetics, penetrating the cell through its solubility in lipide com-

pounds, 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.

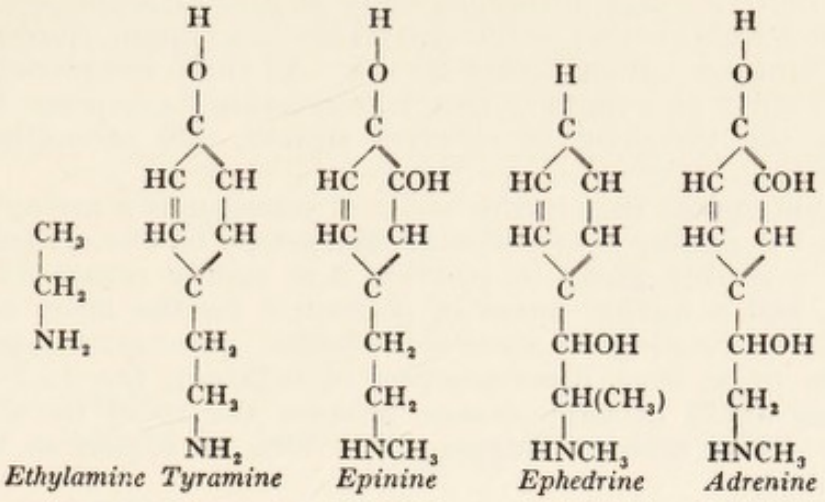
Methyl alcohol, CH_3OH, while an intoxicant, produces toxic results which are in part specifically different from those produced by ethyl alcohol, $CH_3 . CH_2OH$. Single doses of methyl alcohol are less but longer toxic to animals than corresponding doses of ethyl alcohol. More marked symptoms of gastric 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 VIII.). 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 anaesthesia comparable in most respects with that produced by chloroform.

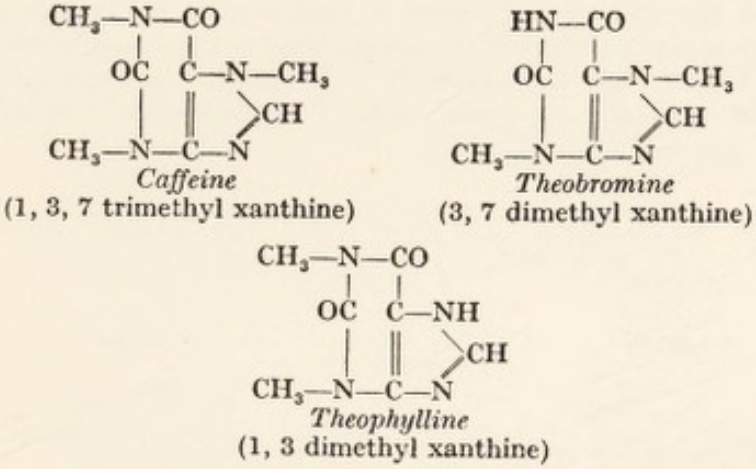
The oxidation products of these alcohols produce effects of quite another kind. Formaldehyde, $H . CHO$, is one of the most powerful germicides, which, however, while irritant, is not very toxic to higher animals. In large doses it leads to nausea and vomiting, narcosis, coma and death. It is rapidly 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 sequelae. 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.



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.

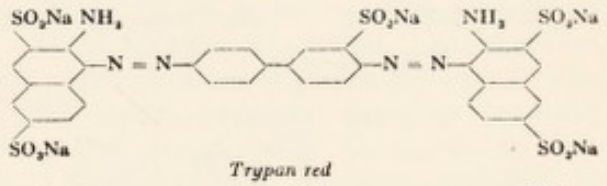
Theobromine, with two methyl groups (3, 7), produces the two latter effects, but has practically no action on the central nervous system. Theophylline, also with two methyl groups (1, 3), has a very

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.

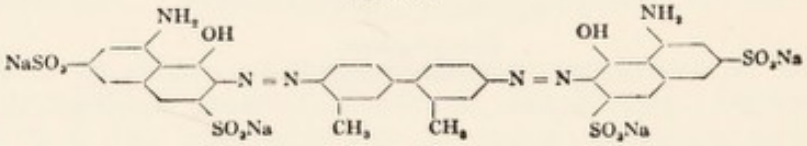
It would appear that in this series of compounds a methyl group in position 1 is mainly responsible for the action on the central nervous system, a methyl group in position 3 is mainly responsible for the diuresis, and a methyl group in position 7 for the heart action. It would be interesting to ascertain 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 benzidine derivative "trypan red" produced some toxic action, while "trypan blue" was distinctly more toxic.



Trypan red



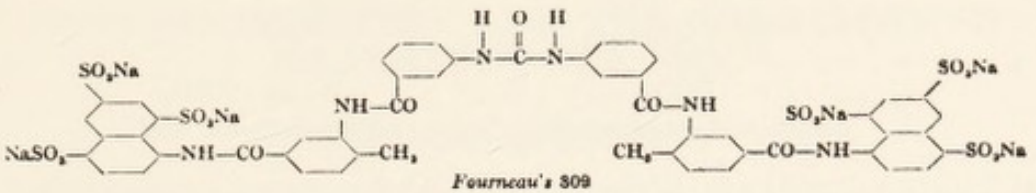
Trypan blue

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 subsequently the firm of Bäyer 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

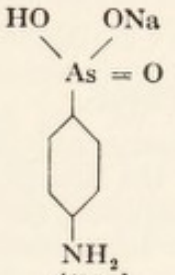
from trypanosomes within a few days, and confers marked immunity for weeks or even months.

Afridol violet

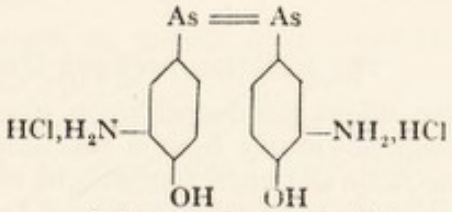


Fourneau's 309

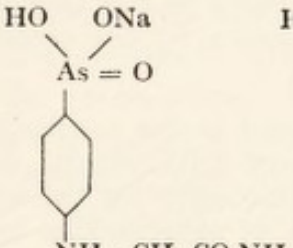
The similar successful results that have been obtained by Ehrlich and others with arsenic derivatives may be exemplified by comparison of atoxyl, toxic to trypanosomes, arsphenamine or salvarsan, still more toxic to spirochaetes and trypanosomes, and tryparsamide (of Jacobs and Heidelberger), with which good results are being obtained in the conquest of African sleeping sickness and the treatment of neurosyphilis.



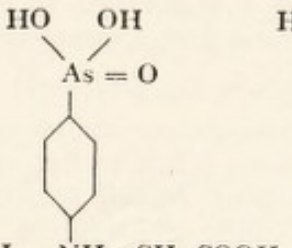
Atoxyl



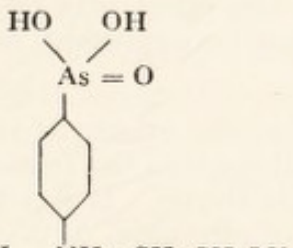
Salvarsan (hydrochloride)



Tryparsamide
(Atoxyl-acetamide)



Phenyl-glycine p-arsonic acid

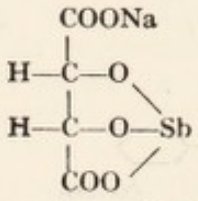


Etharsanol

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 spirochaetal 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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CHAPTER XIX

IMMUNOCHEMISTRY AND THE CHEMISTRY OF FILTERABLE VIRUSES

IMMUNOCHEMISTRY

IMMUNOCHEMISTRY can be defined as the chemical study of the mechanisms which set up resistance and produce immunity to diseases of bacterial or similar origin. Immunology, the science of immunity, has, until very recently, been studied almost entirely by methods involving the technique of the bacteriologist, who has coined a terminology specific to this 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 (Chapter XIII.) that these are absorbed to the circulation, pass to the liver, and, being molecules of small size, they then can and do pass within the liver cells, where they are chemically acted upon so that non-toxic compounds are formed (such as indican and other ethereal sulphates, and conjugated glycuronates).

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 very slowly being ascertained. There is now, for example, good ground for belief that diphtheria toxin is a heat-coagulable protein (*cf.* Chapter XIII., p. 304).

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. In at least one case, Pneumococcus Type I., Heidelberger and Kabat have adduced quantitative proof that the agglutinins and precipitins are identical.

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 resynthetised " plasteins " (compare Chapter XV.) 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 anti-enzyme, a protein, which is digested by pepsin and by papain. 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. Just as with protein-antigens, chemical alterations in these polysaccharides change their serological properties. 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. A possible explanation is that they act on body protein *in vivo* by conjugation or otherwise to form " foreign protein." *Cf.* p. 388.)

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 plasma 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 spacial arrangement, and so leads to the formation of a new globulin, an antibody which, through the specific modification induced by the antigen, can react specifically with it. The antigen does not itself form part of the antibody. The protein nature of antibodies is well exemplified by the digestion of antiurease by pepsin, already referred to.

So far there is little definite evidence to distinguish antibodies from normal plasma globulins by chemical methods. Some data are available, however, that a concentrated Pneumococcus Type I. antibody from horse serum has a slightly greater lysine content and lower nitrogen content than that of normal horse plasma globulin, and, unlike it, is soluble in 5 per cent. urea solutions, while ultrafiltration experiments suggest that the antibodies are among the type of plasma globulins of largest molecular size.

The term *hapten* has been coined to indicate that chemical group of the antigen on which its specificity depends. It is probable that the distinction between the hapten and the antigen itself is not absolute.

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.

Haemoglobin, of most species, shows sufficient crystallographical differences to indicate chemically distinct compounds. Immunological tests indicate that the haemoglobins of most species are different. The haemoglobins of the horse and donkey are crystallographically 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 plasma globulin of the same animal, but the albumin is distinct from the corresponding plasma albumin. The caseins of milks of different species of animals are, at any rate, very closely related. But the antibody to human casein does not precipitate cow casein.

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.

Roepke and Busnell have shown by serological tests that the blood plasma of the laying hen contains a phosphoprotein similar to or identical with vitellin of egg-yolk (*cf.* p. 263), while the plasma of the male fowl contains no detectable amount.

Further instances of this method of chemical analysis are the evidence obtained by Harris and Eagle for the existence of two serologically different globulins in blood plasma, and—as instancing the difficulties associated with the study of plasma proteins (*cf.* p. 220)—the proof by Goldsworthy and Rudd that thrice recrystallised horse plasma albumin still contained about 2 per cent. of globulin.

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.

The amount of antibody produced is much greater than that of the antigen causing its production. For example, the equivalence ratio for ovalbumin-nitrogen to antiovalbumin-nitrogen is stated to be between 1 : 10 and 1 : 11.

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. Various types of such *allergic responses* are known.

Landsteiner and Jacobs have produced positive and constant sensitisation effects in guinea-pigs by repeated intracutaneous injections of very small quantities of such chemical compounds as *p*-nitrosodimethylaniline and 1.2.4-chlorodinitrobenzene (a frequent excitant of allergy in industrial workers). Only those compounds are capable of sensitising which can easily form substitution compounds with bases, and it seems probable that sensitisation produced by such simple compounds is really due to the formation of conjugated antigens (and thus foreign proteins) produced within the organism. Thus acyl and benzyl chloride sensitise the skin and produce an anaphylactic state which can be demonstrated by intravenous injection of the corresponding acylated and benzylated protein. In the same way prontosil (2.4-diaminoazobenzene-4-sulphonamide hydrochloride) produces definite sensitisation in guinea-pigs.

Mention must finally be made of the phenomenon of *phago-*

cytosis, 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.

Bacteriophages, lytic agents acting on bacteria, are believed to be non-living, but their chemical nature is still unknown. They seem to possess definite antigenic properties, suggesting a protein nature. Schlesinger has prepared a much purified anti-*B. coli*-phage, which seems to have the chemical properties of a nucleoprotein. In some of its inactivation and re-activation properties, when treated with cyanide and mercuric salts, " phage " behaves somewhat similarly to certain enzymes.

The Chemistry of Filterable Viruses

Stedman's Dictionary defines " virus " as a collective term for extremely minute living organisms, varying in estimated size from the limit of microscopic vision to the diameter of a molecule of haemoglobin. In the fifth edition of Price's " Medicine " (1937) it is stated that the filterable viruses of such human and animal diseases as smallpox, yellow fever, measles, and epidemic poliomyelitis measure less than 250$\mu\mu$, and some much less, that of the foot and mouth disease of cattle measuring about 12$\mu\mu$; that with few exceptions these viruses are ultramicroscopic, and will pass through porcelain filters, and that " most of the diseases caused by filtrable viruses in man and animals are highly infectious. . . . Some . . . are insect borne. . . . Most of the viruses produce an active immunity which is very lasting."

Until quite recently, therefore, the general conception of filterable viruses has been that they are living organisms of extremely minute size, capable of passing through filters which retain bacteria. There is now strong evidence that some viruses are non-living ; many of them may be simply immense protein molecules, which have the power to cause the formation of like molecules under appropriate conditions. If this should prove to be correct, the mechanism of this vicarious propagation becomes a problem of intense interest, while the dividing line between the living and non-living becomes more difficult to define.

W. M. Stanley, in 1935–36, isolated from Turkish tobacco plants infected with mosaic disease a protein, apparently crystalline, which seemed to possess all the properties of the virus causing the disease. His method consisted essentially of extracting macerated diseased plants with a dilute solution of sodium diphosphate, and then repeatedly precipitating and redissolving the globulin fraction with alternate ammonium sulphate and sodium phosphate treatment, removing impurities in various specific ways.

Subsequently he inoculated several young plants with a solution containing in all 2 mg. of the " crystalline " protein (of which only a trace could actually have been absorbed into the plant cells), and, four weeks later, obtained from these plants 2·3 gm. of " crystalline " protein virus, illustrating the extraordinary rapidity of its propagation.

Serological examination strongly supported the identity of the protein " crystals " with the virus. The protein causes the production of a precipitin which is active for the protein itself, for the juice of diseased plants, and for the " crystalline " protein obtained by similar procedures from tomato plants infected with the same virus.

When the protein " crystals " are subjected to peptic digestion, the rate of digestion and the rate of loss of virus activity correspond. Chemical treatment, as with hydrogen peroxide or with formaldehyde, or treatment with ultra-violet light, causes the protein to lose its infectivity, but its immunological properties are retained. Thus, after inactivation with ultra-violet light, it can still cause production of an antiserum to the active virus.

Wyckoff has applied the air-centrifuge to such studies, and thereby more rapid progress is being made. With Stanley he has obtained the " crystalline " mosaic virus by ultracentrifuging the juice of infected plants. The molecular weight of this protein is estimated to be about 17,000,000.

Thornberry, using very similar methods, has found that the degree of infectivity of the protein " crystals " is not markedly altered by repeated " recrystallisation," while corresponding treatment of tobacco juice from healthy plants yields no trace of a crystalline protein.

Wyckoff, Stanley, and others have prepared several other plant viruses in what appears to be crystalline protein form. Beard and Wyckoff in 1937 reported the isolation of a similar heavy protein which is the responsible agent for the infectious warty-tissue of virus-induced rabbit-papilloma (Shope). They showed by ultracentrifugal studies that the disintegration of the

large molecule of this virus into smaller fragments is paralleled by its inactivation.

Wyckoff has concentrated a homogeneous heavy component from tissues diseased with horse encephalomyelitis (Eastern strain), using a field 50,000 times gravity for from one to one and one-half hours. His results indicated presence of large molecules of uniform size ; if these are spherical then the molecular weight is about 25,000,000, and the diameter of the molecule about $40\mu\mu$.*

It is, of course, possible to advance the hypothesis that the large protein molecule is simply a carrier of an extremely minute living agent, and not the actual agent itself. Examining the available evidence, Wyckoff points out that such a view can scarcely be upheld.

Thus, in the tobacco mosaic protein the infectivity is a property of the protein itself, and cannot be separated from it. Virus proteins are many times more infectious, per unit weight, than the material from which they are prepared. During ultracentrifuging the infectiousness of the product increases just as long as the heavy protein is being concentrated, but it cannot be increased by ultracentrifuging the pure protein. Virus and protein sediment at the same rate.

Horse encephalomyelitis protein spontaneously disintegrates in the ultracentrifuge at about the same rate as its infectivity disappears. Rabbit papilloma protein splits up at those *p*H values at which its activity suddenly disappears.

Bawden and Pirie, in Cambridge, have confirmed the non-living character of the tobacco mosaic virus. They consider that it is a nucleo-protein, and that it does not truly crystallise, but forms "liquid crystals," in "wet-gel" condition, containing 50 per cent. of water. They believe that when recrystallisation is applied to such material, it is not effective in removing impurities.

Bernal, from X-ray studies of the material, believes that it may be possible to obtain it in true crystal form, and it has been pointed out by Smith that over thirty years ago flat plate-like crystals were observed by Iwanowski in the cells of plants infected with the tobacco mosaic disease, crystals which are absent from cells of the healthy plant, and which may be Stanley's crystals formed more perfectly under natural conditions.

* It may be pointed out that while a molecular weight of 25,000,000 appears enormous, such a mass is relatively small as contrasted with that of an ordinary bacterium. Thus it can be calculated that the average weight of a single *Bacillus coli* is $1{\cdot}6 \times 10^{-12}$ gm., and since that of the hydrogen atom is $1{\cdot}66 \times 10^{-24}$ gm. (*cf.* p. 11), if the bacterium consisted of a single molecule its molecular weight would be approximately one million million.

While the final absolute proof is still wanting that Stanley's non-living protein is the actual virus of the tobacco mosaic disease, the balance of evidence most strongly favours that view. If the existence of one virus of this type is admitted, that of many others is rendered probable. While the evidence for non-living character of animal viruses is not so strong, it would be dangerous to admit that plant and animal viruses are essentially different.

It is somewhat amusing to look back ten years and remember the difficulty with which the preparation of the first pure crystalline enzyme, urease, was accepted as fact by scientists in general. There may well be the same difficulty in the acceptance of the protein non-living nature of many of the filterable viruses. Yet in another ten years the acceptance of this conception may be just as general as that of crystalline enzymes is now.

Wyckoff has pointed out that this new conception opens up a new field of research into the mechanism of disease control, stressing in this connection Stanley's results in destroying the infectivity of mosaic virus by slight chemical changes which do not remove its immunological properties.

If the protein (non-living) character of at least some of the viruses be accepted, then their extraordinarily rapid multiplication must be due to abnormal processes within infected cells, catabolised by the virus molecules themselves, which must therefore act autocatalytically. In Chapter II. a brief account of autocatalysis was given, and the case was discussed in which the autocatalyst was a product of the reaction, smaller than the parent substance. In the multiplication of virus molecules the reverse seems more likely. Such an extension of autocatalytic conceptions has already been suggested for bacteriophage by Bordet, while Northrop has pointed out that there is evidence that such enzymes as pepsin and trypsin can increase in amount, producing themselves from other material, under appropriate conditions. Proteases, catalysing synthetic processes, build up proteins from peptide fragments. Can they catalyse their own synthesis ?

Such a concept is well expressed by Bergmann and Niemann : " If the proteinases themselves are proteins and at the same time have the ability to synthetise other individual proteins, then there must exist proteinases which have the ability to synthetise replicas of their own structural pattern and which therefore are able to ' multiply ' in suitable surroundings."

There still remains the fundamental problem : at what stage of complexity and accumulated functional power does non-living matter cease to be non-living ? What is " life," and what constitutes " living " ?

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