A TEXTBOOK OF BIOCHEMISTRY

FOR STUDENTS OF MEDICINE AND SCIENCE

BY

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PREFACE TO THE SIXTH EDITION

Striking results of much new research work published in the past three or four years have necessitated a number of alterations in this edition, involving almost every chapter.

The sections dealing with the vitamins, with diet, and with intracellular respiration, have had to be largely rewritten. Utilisation of radioactive and other isotopes has thrown much light on many phases of intermediate metabolism, and has required frequent mention.

Conceptions of the nature of nucleic acids, and the terminology relating to them, have altered, and the changes have been recorded. New work on creatine has at length solved the puzzle of its formation. Further evidence has been quoted, favouring the view that many viruses are single chemical compounds.

I have attempted to benefit by the constructive criticisms of kindly reviewers of the last edition, and I wish also to thank Professor D. L. Thomson, of McGill University, for a number of helpful suggestions.

Although great distance separates author and publishers and printers, the preparation and printing of this new edition, in these extraordinary times, has been possible without marked delay, thanks to the Royal and American Navies.

A. T. CAMERON.

Winnipeg.
May, 1942.

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 have 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, an ingredient of corn syrup and honey, 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,

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

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*, to excite), 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 similar 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 oxyhaemoglobin ; 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 were first named alphabetically *A*, *B*, *C*, etc., and are being given specific names as their chemical natures are 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. Deuterium and other isotopes are being much used in biochemical studies (*cf.*, for example, Chapter V, p. 118).

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 8·8μ (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, μμ or *m*μ, one-millionth of a millimetre, or one-thousandth of μ. Expressed in terms of this unit a molecule of starch has a diameter of 5 *m*μ. 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 number 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 in them to the extent of 34 per cent. in average adult human blood. Haemoglobin has a molecular weight approximating to 68,000, and thus has a distinctly heavy molecule.

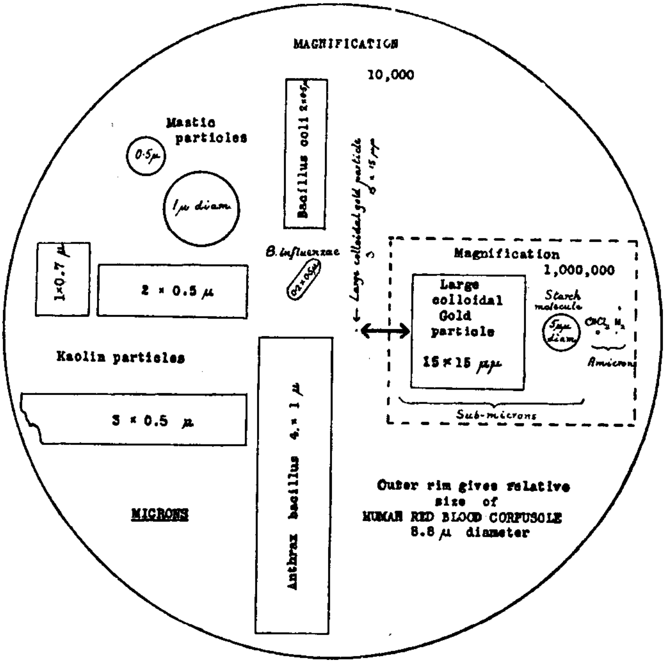

FIG. 1. Scheme illustrating various sizes of particles and molecules. (Modified from W. Ostwald.)

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 $1{\cdot}12 \times 10^{-10}$ gm.

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

$$\frac{\text{Weight of haemoglobin in one cell}}{\text{Weight of one molecule of haemoglobin}} = \frac{3{\cdot}43 \times 10^{-11}}{1{\cdot}12 \times 10^{-10}} = 2{\cdot}88 \times 10^{8}$$

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 " measures 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 at* 100° *C.* is 539·6 (small) calories.

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