

BIOCHEMISTRY IN RELATION TO MEDICINE

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PREFACE TO SECOND EDITION

IN bringing out a second edition of this book we have not attempted to change the main aims which we had before us in writing the First Edition. At the same time, we were of the opinion that recent progress in Biochemistry, even in those aspects of this lively science which are of relevance to medical students, necessitated certain shifts in the balance and emphasis which we had previously adopted. New chapters have been included on the metabolism of the lipides and proteins, while the chapters dealing with Carbohydrate Metabolism, Sterols and Steroid hormones, Plasma proteins, Electrolytes, Vitamins, Haemoglobin and Acid-base equilibrium have been largely re-written. We have not attempted any systematic treatment of endocrinology, but the role of hormones in the regulation of metabolism is discussed in the relevant chapters. We have again included short experimental sections at the end of most of the chapters, although these have not been substantially changed.

We wish to express our thanks to our colleagues and to the reviewers of the First Edition for their helpful suggestions and also for drawing our attention to certain inaccuracies and errors of typography which we had allowed to pass in the First Edition. In particular we wish to thank Dr. D. R. Parsons for reading much of the re-written typescript and for his helpful criticism.

March, 1952

C. W. C.
R. H. S. T.

PREFACE

THE traditional curriculum for the student of Medicine is apt to suffer from the defect that the principles and methods of the experimental sciences of Physiology and Biochemistry are laid aside and forgotten when the student passes from the laboratory to the clinic. Too often the method of the scientist is abandoned in favour of an empirical approach which seeks to elucidate the problems of clinical diagnosis and treatment in terms of the prescribed methods of experience. Occupying, as it does, a borderline position between Chemistry on the one hand and Physiology on the other, the science of Biochemistry may be presented to students either from the chemical or biological standpoint. Our aim in this book has been to present those aspects of biochemistry which are significant in Medicine. The progress which is being made in the medical sciences by virtue of a biochemical approach renders it imperative for the medical graduate to be informed about the dynamic aspects of modern biochemistry. The medical student therefore is faced with this expanding science with which he must equip himself in addition to the more strictly biological knowledge which is an essential part of his training. In the teaching of biochemistry it must be remembered, not only that for the medical student this represents only a part of his training, no matter how important, but also that many medical students of necessity have not the broad background of experience in the physical sciences which a teacher may expect of a student of chemistry.

In this book we have attempted, therefore, to give only a brief and elementary account of the chemistry of carbohydrates, lipides, and proteins, a knowledge of which provides the necessary basis for an understanding of the changes undergone by these substances in the body, and have devoted more attention to a presentation of the biological functions and chemical reactions brought about, or undergone, by these substances in the body. For a more detailed account of the physical chemistry of the proteins or the structural relationships of the steroids, for example, the reader is referred to one of the many excellent text books of biochemistry written more specifically for the chemist than the physiologist, or to the numerous monographs on these subjects. It has been felt that to include in the present book the detailed treatment of these and other aspects of the

subject would succeed only in involving the student of medicine in a maze of difficult chemical problems, many of which are, at present, not completely or clearly linked up with physiological processes.

In the course of teaching in the laboratory, it has been our impression that in many cases the practical work, as carried out by the student, bears a somewhat remote relationship in his mind to the theoretical knowledge which he derives from his reading and lectures. For this reason we have included experimental sections at the end of each chapter, in the hope that by presenting both theoretical and practical aspects of the subject side by side we may be able to bridge this gap which too often exists in the student's mind. The experiments which we have included have been based on those carried out in the medical and Honours Physiology courses at Oxford. No claim is made that in every case the methods used are necessarily the only, or the best, ones which might be employed. They have been selected from a practical course which has been built up over a number of years, and have been chosen primarily for the instruction of students rather than for, say, their suitability as routine methods in a hospital biochemical laboratory.

Owing to the enormous output of biochemical papers in the scientific journals of the world to-day, one useful function which text books or monographs can fulfil is to provide a guide to some, at any rate, of the more important publications, so that the more advanced student or research worker is enabled to read the more detailed aspects of the subject in which he may have a particular interest. For this reason we have, at the risk of reducing the readability of our text, referred to a selection of the original papers and reviews in the literature.

We wish to express our thanks to Professor R. A. Peters, M.C., F.R.S., for permission to include many of the experiments from the practical courses in biochemistry at Oxford. We also owe much to our colleagues, Dr. R. B. Fisher, Mr. J. R. P. O'Brien, Dr. H. M. Sinclair, Dr. A. G. Ogston and the late Dr. E. Walker who have contributed so much to the development of these courses. We are particularly grateful to Dr. V. P. Whittaker for reading the book in proof and for his many valuable suggestions.

OXFORD, *January*, 1949

C. W. C.
R. H. S. T.

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CHAPTER I

HISTORICAL INTRODUCTION

THE science of biochemistry is one of the youngest of the branches of biology. It represents an attempt to analyse the properties and reactions of living tissues in terms of the chemical substances of which they are composed, and the chemical changes which these compounds undergo, as a result of which energy is obtained for the life of the cell.

The advances made by this chemical approach to biological problems, and also its application to such practical aspects of human affairs as medicine, agriculture and industry, are too well known to need stressing in general terms. And so rapidly has the scene changed that it would be premature to attempt to write its history with any hope of an accurate placing of emphasis. On the other hand, in order to view the aims and achievements of biochemistry in their right perspective it is important to realise how it has grown out of the older sciences, and to trace, as far as is possible, the main trends of thought and experimental work that have led to biochemistry in its present form.

A biochemical analysis of cellular function naturally presupposes a knowledge of the chemistry, as opposed to the biochemistry, of the components of living cells. Until the early part of the nineteenth century our approach to this problem from the experimental point of view was gravely hampered by the formidable conception of the "vital action" which was held to be responsible for the formation of the complex substances of which the tissues of animals and plants are made. In 1828, however, Wöhler demonstrated that urea could be synthesized from cyanic acid and ammonia, thereby proving for the first time that not all "organic" compounds are beyond the reach of laboratory preparation.

Gradually, as a result of the work of Mulder, Liebig, Emil Fischer, Thudichum and many others, a knowledge of the chemistry of proteins, carbohydrates and fats was slowly built up, while Thomas Graham in 1850 laid down the principles on which modern colloid chemistry is based—the differentiation

of compounds into small-molecular "crystalloids," forming true homogeneous solutions, and "colloids," of relatively large particle size forming a two-phase system with a surface of separation between the colloidal particles (disperse phase) and the dispersion medium. The nineteenth century also gave us our conception of osmotic pressure, first studied by Pfeffer and van't Hoff, so that by the turn of the century it can be said that physical and organic chemistry had provided the first building-stones for the new science of biochemistry.

The two main roots from which biochemistry has grown were, first, a study of fermentations, leading to knowledge concerning the chemical reactions occurring in biological systems and the ferments or enzymes by which these reactions are brought about, and, secondly, a study of nutrition, leading early on to the discovery of the essential "accessory food factors" or vitamins, and, later, to the role that they play in cell reactions.

Although Spallanzani in 1783 observed that gastric juice was able to liquefy meat, the serious study of fermentative reactions did not begin until the nineteenth century, when the controversy between Liebig and Pasteur took place as to the fundamental nature of these processes. In 1838 Cagniard de Latour had put forward the view that alcoholic fermentation was brought about by a living organism while, later, Pasteur extended and established this conception in connection with the souring of milk and of wines. Liebig, however, held to the opposite view and developed a purely chemical theory of fermentation. The first step towards resolving this controversy had indeed already been taken in 1832 when Payen and Persoz prepared a cell-free extract of barley which was capable, like strong acids, of converting starch into sugar. This extract, which they called *diastase*, became known as a "soluble" or "unorganised" ferment in contrast to yeast, an "organised" ferment. The term *enzyme* for ferments was not introduced until 1878 by Kühne.

It was not until 1897, however, that sufficient evidence was obtained to decide whether any essential difference existed between the actions of "organised" and "unorganised" ferments; in that year Buchner prepared cell-free extracts of yeast which were capable of carrying out all the reactions involved in the alcoholic fermentation, and so opened up the way for the biochemical study of enzyme-catalysed reactions carried out either by living cells or by extracts prepared from

living cells. Nine years later Harden and Young (1906) discovered the existence of co-enzymes in their demonstration of a soluble, dialysable factor, *co-zymase*, necessary in addition to the enzymes required for alcoholic fermentation. In 1909 Sørensen demonstrated the dependence of enzyme action on an appropriate concentration of hydrogen ions, or pH, to use the term introduced by him.

A notable contribution to our knowledge of the nature of enzymes was the isolation by Sumner (1926) of crystalline urease, and later by Northrop (1930) of crystalline pepsin and trypsin (Northrop and Kunitz, 1932). The preparation of an increasing number of enzymes in crystalline form has led to their recognition as specific proteins, the molecular structure of which determines their characteristic specificity. Perhaps the most remarkable development in this field has been the isolation in crystalline form of the virus of tobacco mosaic disease (Stanley, 1937). This giant molecule, with a molecular weight of some 20,000,000, is protein in nature, and yet exhibits many of the qualities, including that of self reproduction, which have hitherto been regarded as the distinguishing mark of a living cell. In the proteins we seem to reach a level in the complexity of molecular structure which divides the living from the non-living.

The microscopists of the seventeenth and eighteenth centuries, Malpighi, Leeuwenhoek and others, laid the foundations for the development of the Cell Theory of Theodor Schwann in 1839. The essential feature of this theory was the recognition that both plants and animals were to be regarded as multicellular organisms. The basic unit of plant and animal tissue was the cell, having a differentiated structure adapted to its own particular specialised function, and possessing its own individual life, but integrated physically and physiologically with the other cells of the body to constitute an organism.

It has been, perhaps, the most fundamental contribution of Biochemistry in the present century to extend this concept of organisation from the cellular to the molecular plane. We owe, in particular, to the work of Hardy, Harkins, Langmuir and Adam a picture of the cellular architecture in terms of an organised molecular structure in which proteins and lipides have a special significance. The enzyme systems of the cell, substrate-specific and spatially orientated, represent an essential element in this structure. They are the primary agents which catalyse the complex array of chemical reactions of the

cell, not at random, but in the co-ordinated sequence which is appropriate to physiological activity, and which in turn are integrated by the extrinsic regulation of hormones and the nervous system.

The principles involved in the early work on fermentations were soon applied first to muscle and later to other tissues, and in the hands of Hopkins, Meyerhof, Embden, Parnas and many others rapidly led to the elucidation of many of the processes of intermediary metabolism in animal tissues. At the same time rapid advances were being made in our understanding of the mechanisms by which metabolites are oxidised. Lavoisier and Laplace in 1780 had shown that "animal heat" is derived from the oxidation of the body's substance, and in the early years of this century the newer knowledge of enzyme catalysis was extended to the field of biological oxidations, principally by the pioneer studies of Bach, Wieland, Thunberg, Warburg and Keilin.

The role of proteins, carbohydrates and fats as the sources of bodily energy and heat was revealed by Pettenkofer and Voit in the latter half of the last century. Nutritional studies, however, early showed that these substances are not by themselves adequate to maintain life. The necessity for other entities in the diet, which make no contribution to the calorie needs of the organism, was demonstrated by Hopkins in 1912. These substances are frequently present only in traces, and so at first eluded detection by the classical methods of chemical analysis. Hopkins fed young rats on a diet of pure caseinogen, fat, carbohydrates and salts. The rats rapidly ceased to grow, even though the energy value of the diet provided was ample for normal growth. When, however, he added a trace of fresh milk to the diet normal growth was resumed. Here, then, was a demonstration of the presence in fresh milk of some new factor or factors necessary for normal growth. The history of the subsequent isolation and recognition of these accessory food factors or vitamins (derived from the word "vitamine" introduced in 1914 by Funk for the "antineuritic" factor present in rice-polishings) is summarised later in this book.

Next, it was necessary to discover the part that these trace factors are playing inside the body, and an important advance was made here by Peters and his colleagues who first defined the biochemical function of a vitamin by demonstrating that vitamin B₁ is an essential component of the enzyme system

responsible for the oxidation of pyruvic acid, a normal intermediary in carbohydrate metabolism. The biochemical functions of many of the vitamins are now known more or less exactly, and a study of them from this point of view has thrown much light on certain of the central enzyme systems of the cells and has provided a rational form of therapy in certain deficiency diseases.

The classical work of Sherrington (1906) elucidated the principles underlying the role of the nervous system in the integration of the multicellular animal. The past fifty years has witnessed the equally far-reaching development of knowledge relating to the part played by chemical factors, operating synergically with the nervous system, in the co-ordination of the organism.

The term "internal secretion" was originally employed by Claude Bernard (1859) to describe the sugar which, as he showed, was passing from the liver to the blood. The discovery of von Mering and Minkowski (1889) that the pancreas possesses an internal secretion concerned in the regulation of carbohydrate metabolism was the forerunner of others which established the existence of an endocrine system of glands whose function is the elaboration of specific chemical regulators of growth, reproduction and metabolism. The proof of the existence of such specific secretions was first provided by the demonstration of the presence of active principles in extracts of these glands; for example the vasopressor effect exhibited by extracts of the posterior lobe of the pituitary by Oliver and Sharpey-Schafer (1894), and the influence of extracts of the Islets of Langerhans in diabetes mellitus by Banting and Best (1922).

One by one, these active principles were isolated; adrenaline by Takamine (1901), thyroxine by Kendall (1919) and insulin by Abel *et al.* (1927). Meanwhile Bayliss and Starling (1902), by their experiments on secretin, had demonstrated that specific chemical regulators were not confined to the secretions of the endocrine glands proper. The term "hormone" was first applied by Starling (1906) to secretin. Later he widened the definition of the term to include "any substance normally produced in the cells of some part of the body, and carried by the blood stream to distant parts which it affects for the good of the body as a whole." Though many would hesitate to accept carbon dioxide as a specific hormone in spite of its influence in the regulation of the respiratory centre, Starling's

concept has been fruitful. The term "neuro-hormone" has been used with reference to the role of acetyl choline and adrenaline in the humoral transmission of nerve impulses, as shown by Loewi, Dale and Cannon (*vide* Dale, 1933).

Even more significant has been the indication of the widespread influence of hormones in the processes of embryonic differentiation and growth. The early embryo contains certain groups of cells which carry within themselves all the factors required for self-differentiation, while other regions of the embryo depend on formative stimuli from elsewhere. Spemann and Mangold (1924) observed that transplantation of a fragment of the dorsal lip of the blastopore into the ventral ectoderm of an early embryo induced in the latter the development of a secondary neural axis. This effect of the dorsal cells is due to the presence within them of a hormone called the primary organiser (Needham, Waddington and Needham, 1934). It seems probable that all the changes involved in growth and differentiation from the relative homogeneity of the fertilised ovum to the fully developed organism, are at every stage controlled by hormones of the organiser type.

The pioneer experiments of Carrel (1912) on the growth of explanted tissues in artificial media have provided the basis for the demonstration that the orderly proliferation and differentiation of cells such as fibroblasts or epithelial cells in the adult organism, as in the embryo, are under the control of local and bloodborne excitatory and inhibitory chemical influences, while abnormal forms of growth stimulus, such as that of the coal-tar hydrocarbons, may lead to a typical carcinogenic type of proliferation.

Meanwhile, amid these spurs to new developments, fundamental work along more strictly chemical lines has produced far-reaching advances in our knowledge of the chemical properties and composition of the proteins, fats and carbohydrates.

Biochemistry, however, is not only a study of the chemistry of substances occurring in living organisms. In so far as it can claim to be an independent science it is much more than this, and its objective is more dynamic and physiological, including, as it does, an understanding of the integration of the multitude of chemical reactions occurring in living matter.

CHAPTER II

PHYSICO-CHEMICAL CONSIDERATIONS

Diffusion and Osmosis

IF water is placed in a cylinder above a concentrated salt solution the original plane of separation between the two liquids gradually becomes obliterated as the salt slowly makes its way through the upper layer of water. Ultimately the dissolved salt is found to be uniformly distributed through the whole volume of fluid. This phenomenon of diffusion was first studied by Graham (1850), who showed that the rate of diffusion of a solute varied with the nature of the substance, and was determined by the size of the diffusing particles. The rate of diffusion is, in fact, inversely proportional to the size of the particle, and directly proportional to the difference in concentration of the solute in the regions between which diffusion is proceeding. Diffusion always occurs from the region of higher to that of lower concentration. In this, as in other respects, we can apply to substances in solution the same kinetic principles as are applicable to gases.

If a vessel of palladium is filled with nitrogen and immersed in an atmosphere of hydrogen, a rise of pressure occurs within the vessel. Palladium allows hydrogen to pass freely through it so that ultimately the pressure of this gas within and without becomes equalised. On the other hand since palladium is impermeable to nitrogen the resulting rise of pressure corresponds to the diffusion pressure exerted by this gas.

Pfeffer (1877) found that substances in solution exhibited an analogous osmotic pressure when the solution is separated from the pure solvent by a membrane impermeable to the solute. A membrane of copper ferrocyanide exhibits a selective permeability since it freely allows the passage of water, but is impermeable to cane sugar and certain electrolytes. In this respect it appears to act as a molecular sieve. If a clay cell, impregnated with copper ferrocyanide and connected to a manometer, is filled with a solution of cane sugar and im-

mersed in water the osmotic pressure which develops in the cell can be recorded, and is found to be directly proportional to the concentration of the solute and to the absolute temperature. The similarity to the behaviour of gases is evident.

Living cells such as the erythrocyte which possess semi-permeable membranes behave in a manner similar to that of the copper ferrocyanide cell. This behaviour accounts for the phenomena of "laking" and "plasmolysis" (*vide infra*).

Laws of Osmosis. van't Hoff's laws of osmotic pressure may be stated as follows:

(1) The osmotic pressure is proportional to the concentration of the solute if the temperature remains constant.

(2) The osmotic pressure is proportional to the absolute temperature if the concentration remains constant.

(3) The osmotic pressure exerted by a solute in dilute solution is identical with that which it would exert if it were present as a gas in the same volume at the same temperature. Thus the gram-molecular weight of cane sugar dissolved in 22.4 litres of water at 0°C exerts an osmotic pressure of one atmosphere.

The above laws only apply strictly to dilute solutions in which the number of solute molecules is so small in proportion to that of the solvent that any effects due to mutual action of the solute molecules, to their actual volume, or to combination by hydration with the molecules of the solvent, may be neglected. In the case of concentrated solutions corrections, similar to those introduced by Van der Waals for gases, must be applied. For fuller treatment of the subject Bayliss, *Principles of General Physiology*, may be consulted.

In the case of electrolytes dissolved in water the osmotic pressure developed is greater than is found for equimolar solutions of substances like cane sugar or urea. This is due to the dissociation of the electrolyte molecules into ions. The resulting osmotic pressure is proportional to the number of solute particles present, irrespective of whether they exist in the form of ions, molecules, or aggregates.

The property of developing an osmotic pressure is also shown by substances existing in the colloidal state which exhibit Brownian movement. Owing to the large size of the individual molecules or particles such as exist in the case of protein sols it is impossible to obtain solutions of any great molar con-