

PRINCIPLES OF BIOCHEMISTRY

A Biological Approach

by

M. V. TRACEY, M.A.

Rothamsted Experimental Station, Harpenden, Herts.

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PREFACE

BIOCHEMISTRY is a rapidly growing subject. In the last ten years membership of the Biochemical Society has more than doubled. Growth has been mainly by recruitment from other disciplines, for the ten-year recruitment of about a thousand members is far greater than the output of biochemists from the universities during that period. Botanists, zoologists, chemists, and physicists have all taken up work normally classified as biochemistry. Naturally much of the output of biochemical work remains essentially within the ambit of the older disciplines. The brilliant work of the chemists in elucidating the structure and working out the synthesis of products occurring in living things remains on the borderline between chemistry and biochemistry even though it is essential to the progress of biochemistry. At heart, biochemistry is one of the biological sciences and when substances are examined in isolation their study becomes part of the "exact sciences" of chemistry and physics where they overlap with biochemistry. The core of biochemistry is in the study of complex dynamic systems which are sometimes stigmatized by the exact scientists as "filthy messes" and by the biologists as hopelessly disorganized remnants of what were once organisms. As individual substances are characterized and isolated from these complex systems so their study becomes the concern of the peripheral fields of biochemistry and provide, for example, the basis of new fields of organic chemistry or biological systematics.

This book has been written primarily to survey the biological core of biochemistry in the hope that it will provide a useful means of orientation for those more concerned with specialized parts of the subject. The treatment is uneven—an inevitable result of the uneven development of the subject and of the gaps in the author's knowledge of it. Uneven and incomplete as it necessarily is, it is hoped that it will do something to oppose the present fragmentary tendency in books on biochemistry which tend more and more to deal with restricted fields with little emphasis on the underlying unity of the subject.

It is a great pleasure to acknowledge my debt to Mr. N. W. Pirie, F.R.S., without whose stimulating comments and suggestions the book would have lost much, to Dr. E. M. Crook, University College,

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London, and to my colleagues Miss M. Holden and Dr. G. H. Wiltshire who read it in manuscript and made many helpful suggestions. Naturally the responsibility for all sins of commission and omission is mine.

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PART I

FUNDAMENTALS

CHAPTER I

INTRODUCTORY

THE elucidation of the chemical changes that occur in living organisms is the chief aim of biochemistry. The principles of biochemistry must be derived from experiments done with living organisms or with systems but recently dead. Its subject is therefore life as we know it today; the end product of a thousand million years of evolutionary process during which all but the most successful and most fortunate have been swept away, leaving no trace apart from impressions in the rocks and fossilized remnants of a very few of the substances of which they were formed. We can study only the successful organisms and have no knowledge of what kind of life might be found in our present physical environment if these organisms did not exist.

There are chemical likenesses between all the organisms so far studied. The most abundant sugar in a potato, a shipworm, an elephant, and a bacterium is likely to be glucose. Moreover, the glucose from these organisms will be found to have the same optical configuration. Many of the other sugars and the twenty or so amino acids that make up proteins are similarly ubiquitous. The end products of the digestive processes of animals are sugars, fats, and amino acids and it would be expected that the sugars, fats, and amino acids of animals would be the same as those of plants. The fact that the building stones, from which the materials making up the components of green plants are formed, are essentially the same may be taken as evidence that these organisms are the descendants of a common ancestral form of life. It is probable that organisms other than green plants share their ancestry but still possible that they had a different origin and, being compelled to use the products elaborated by the green plants, have consequent likenesses in composition. Competition between early forms of life would certainly result in the dominance of one, which as it became established would eliminate other forms that had arisen and other forms that might arise in the future. It would be a mistake, therefore, to use our knowledge of life as it exists today to argue that this is the only form in which life can occur and to draw conclusions such as the widely quoted

observation of Engels, "Life is the mode of existence of protein bodies." Mulder in 1845 in stating this for the first time was more cautious, and by avoiding dogmatism produced a formulation still acceptable after more than a century. "Without it (protein) no life appears possible on our planet. Through its means the chief phenomena of life are produced." The polysaccharides seem to have as many possibilities of exhibiting specificity and reactivity as the proteins, though they do not appear to play the central role that proteins play in the organisms we have opportunity to study. It might well be a matter of chance, so far as is known now, that life as we know it is protein-based rather than polysaccharide-based. Systems based on other large molecules may equally well have had a transitory existence. The conclusions drawn from biochemical evidence tell us how one successful variety of living things work but do not tell us how living things *must* work.

Our knowledge of biochemistry today has been severely conditioned by the domination of the subject by older disciplines. It has developed largely as a result of work done with a view to its application in other fields. These have been physiological medicine, nutrition, and the control of harmful organisms whether pests of man's stored products, his domestic animals and plants, or himself. Physiological medicine involves the study of one living thing, man, and the others emphasize the differences between organisms. It is the exploitation of differences in the biochemistry of organisms that is the basis of all attempts to kill one type of organism without killing another, whether in the control of disease or in a search for an insecticide. This intensive investigation of the differences between organisms has tended to obscure the similarities that exist. Together with the fact that different techniques are often needed in dealing with widely different types of organisms it has led to the apparent fission of biochemistry into subjects such as bacterial biochemistry, agricultural biochemistry, and the biochemistry of man (this is usually called simply "Biochemistry"). Consequently biochemistry when compared with the older biological sciences such as botany and zoology is very unevenly developed. It is a fortunate fact that in the search for differences in the biochemistry of organisms there have been many failures, for these failures reveal similarities. In this book it is the similarities between organisms that will be emphasized in the hope that if the subject is viewed as a whole some general principles of biochemistry will become apparent.

The most obvious and longest known similarity between living

things is energetic. It is difficult to conceive of life without death, of growth without decay. Equally old is the observation that life depends on water; without it no plant or animal long survives. In the early part of the nineteenth century, chemistry was applied to living things, and, owing in part to the crudeness of the techniques available, it was thought that living things were almost indistinguishable chemically and the concepts of isomorphism and polymorphism were invoked to explain differences obvious to the eye but not detectable analytically. Speaking of the fats, carbohydrates, and proteins Lehmann said, "There are only three groups of organic substances through which all the vital phenomena are manifested, and even these groups exhibit the most important internal co-relations . . . And do not the members of the individual groups present such uniformity and analogy in their composition, and even in their properties, that the diversity of the processes to which they give rise is perfectly incomprehensible? We are thus obliged to have recourse to isomerism and polymorphism as a prop to our ignorance, . . ." (1853, *Physiological Chemistry*, Vol. III). At about the same time a beginning was made in the study of hydrolytic enzymes, particularly the digestive enzymes of animals. It was found that there was a remarkable similarity between the enzymes from different organisms, so much so that the "trypsins" of snails or pitcher plants were referred to as though they were identical with the corresponding mammalian enzymes. The remainder of the nineteenth and the early part of the twentieth century were occupied in the consolidation of fields already explored and were notable for the advances made in the methods for the determination of the composition of compounds of high molecular weight in terms of their constituent amino acids and sugars. This work began to show that the apparent great similarities in the composition of proteins and polysaccharides concealed the possibility of considerable diversities. Work on nutrition gave especial point to differences between some proteins' content of a few essential amino acids. Largely under the influence of Hopkins the next great advance began in the third decade of the present century with the study of dynamic changes in the cell and the discovery of a large number of enzymic actions other than hydrolytic. The paths of hydrogen transfer and of the metabolic breakdown of glucose were explored with signal success and it became apparent that the enzymes of the cell were organized into systems which, by the co-operation of the enzymes concerned, were capable of carrying out far-reaching transformations. In the fifth decade the importance of certain

phosphorus compounds, known to be intimately concerned with the enzyme systems responsible for metabolism, was emphasized by Lipmann who pointed out their function in the transfer of energy. This energy appeared to be associated with the link between a phosphate group and the remainder of some molecules in which it occurred. Energy could only be transferred from one compound to another if the phosphate group were not freed from combination. This emphasis on the importance of the transfer of groups from one molecule to another rather than the hydrolytic or other alterations in molecules led to the present interest in enzyme systems that mediate the transfer of the most varied chemical groupings from one compound to another without appreciable loss of chemical energy occurring in the process. The application of these discoveries to the usual subjects of biochemical investigation—pathogenic micro-organisms, some fungi and protozoa, a few green plants, and man and his proxies the rat and guinea pig—have shown that they are applicable throughout the organisms that have been investigated and appear to be characteristic of life as we know it.

The differences in chemical composition between different organisms depend on differences in composition of the polymers of high molecular weight (fats, proteins, and carbohydrates) and on specialized constituents such as pigments or alkaloids. In general the differences to be found in the soluble compounds of low molecular weight which are of importance in metabolism is not so marked. The differences in the polymers are striking. Pepsin, the protein-hydrolysing enzyme from the stomach of animals, exists in as many varieties as the species of animals from which it is isolated, yet the action and general properties of each pepsin are nearly the same. It is a common-place of biochemistry that enzymes are specific and though at first sight it might appear that separate enzymes might be necessary to break down, for example, each different pepsin this is not so. The hundreds of different proteins in an animal's diet are all broken down by a few protein-splitting enzymes. Similar considerations apply to the polysaccharides and fats. The reconciliation between the statement that enzymes are generally specific in their action and the fact that the enzymes breaking down polymers can act on a wide range of different polymers lies in the fact that the monomers from which the polymers are built are restricted in number, and the specificity of the hydrolytic enzymes is largely related to the properties of the monomers and the links between them rather than those of the

polymers. Among the very similar range of simple soluble compounds found in all living cells are the monomers from which the widely different polymers are synthesized. Thus from the basic similarity of the small molecular components of the cell very marked differences in the properties of the polymers can be produced. This is a matter first, of which of the alternative monomers that may form a particular class of polymers are used in its construction, secondly, of the manner

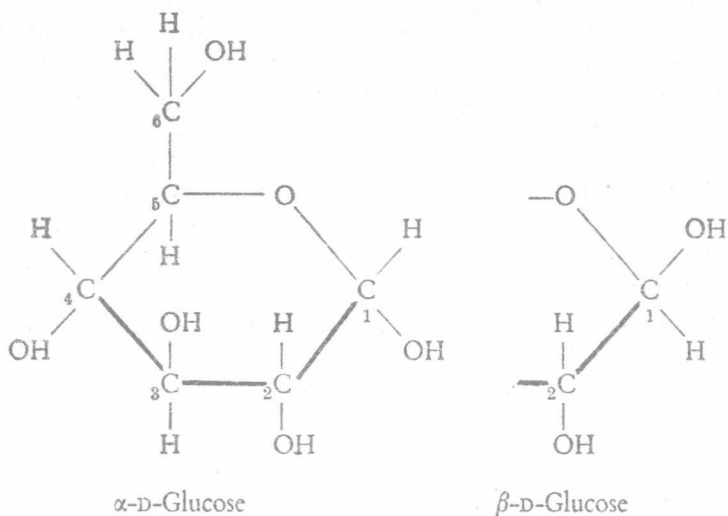


FIG. I. D-GLUCOSE

Showing the alternative configurations at carbon atom 1 and the numbering of the carbon atoms

in which they are combined, and thirdly, of the order in which they are combined.

Glucose is one of the commonest of the monomers used in the construction of polysaccharides. If glucose alone is employed there is no opportunity for variation in the first manner. Glucose has the formula shown in Fig. 1.

The two forms α - and β -glucose exist in equilibrium and attainment of this equilibrium may be hastened enzymically. Glucose is frequently polymerized by the elimination of the elements of water between the hydroxyl groups on carbon atoms 1 and 4. Since there are two possible configurations at carbon atom 1, two forms of pure glucose polymer are possible. Both are widespread. The polymerization of

α -glucose forms one of the components of starch and of β -glucose yields cellulose. These two polysaccharides with strikingly different properties are formed merely by the use of alternative configurations at the carbon atom 1. By joining such chains together by substitution at carbon atom 6 of a unit in one chain, a branched structure is formed. This occurs in starch and in glycogen. Glucose polymers are also known in which the linkage is 1 : 3 or 1 : 6. Alteration of the configuration of the hydroxyl groups other than that on carbon atoms 1 and 6 results in the formation of other commonly occurring sugars, such as galactose (inversion at 4) and mannose (inversion at 2). From these, pure or mixed polymers may be formed with the same possibilities for variation in the linkage and branching as those in the glucose polymers. Sugars with five carbon atoms are also widespread and these pentoses (Fig. 2) may also form polymers among themselves, alone, or together with the six-carbon-atom hexoses. Hexoses with a different structure from that of glucose and its relatives (the aldoses) occur with them. The commonest of these ketoses is fructose (Fig. 2) the other ketoses occurring rarely. Among these simple sugars there is one further opportunity for variation. The ring may be either five- or six-membered. In the aldoses it is normally six-membered and in fructose five-membered. Arabinose, however, may be found in either ring form, the five-membered ring being commonly found in polymers. Small alterations in the groups attached to the ring atoms of the sugars result in the formation of new sugars with different properties. The replacement of a hydroxyl group by a hydrogen atom results in the formation of a deoxy-sugar some of which are widespread. If the replacement occurs at carbon atom 6 the so-called methyl-pentoses are formed while replacement at carbon atom 2 of ribose results in the formation of 2-deoxyribose, a constituent of some nucleic acids. Replacement of a hydroxyl group by a charged group results in sugar derivatives capable of salt formation and, in polymers formed from them, the possibility of interchain links that are easily broken and reformed. Oxidation at carbon atom 6 results in the formation of a series of uronic acids of which those derived from glucose, galactose, and mannose are common. Esterification with a polyvalent acid such as phosphoric or sulphuric also occurs. The sulphate esters are found in many polysaccharides, and phosphate esters are of the greatest importance in cell metabolism and also occur in the polymeric nucleic acids. The replacement of a hydroxyl group by NH_2 results in the formation of amino-sugars of which 2-amino

glucose and 2-amino galactose are found. Normally these basic groups are acetylated (Fig. 2).

Obviously from a selection of these sugars a very large number of polymers can be formed. The linkages between the sugar units will, however, be restricted in their variety, and since the enzymes breaking down polysaccharides have their specific properties related to linkages rather than to the whole polymer, the number of these enzymes is

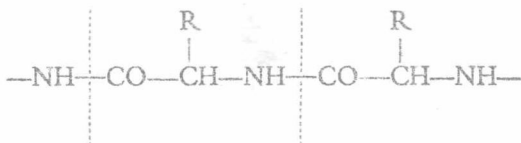


FIG. 3. POLYMERIZATION OF AMINO ACIDS

relatively few though they are able to break down the most diverse polysaccharides originating in any part of the living kingdom.

The proteins similarly depend for their specific properties on arrangements of a limited number of types of unit into a complex polymer in which the arrangement and selection of the simple units (Table I) determine the properties of the polymer as a whole. While polysaccharides may contain only a few different units, usually fewer

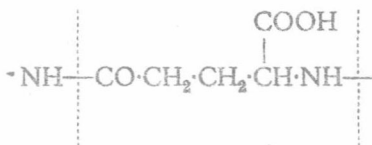
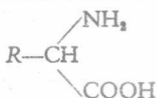


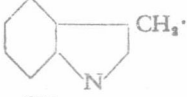


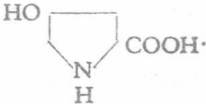


FIG. 4. ALTERNATIVE RARE POLYMERIZATION OF GLUTAMIC ACID BY USE OF THE γ -COOH GROUP

than six and frequently only one, the presence of a large number of the possible units without great preponderance of any single unit is the rule in proteins. Again in contrast to the polysaccharides there is only one usual method of linkage between the units (Fig. 3). (But see Fig. 4 for an alternative polymerization of glutamic acid.) While the sugars may be linked between a number of alternative carbon atoms and a single unit may be joined to three others producing a branched chain, there is little opportunity for this among the amino acids.

TABLE I
PROTEIN MONOMERS



Amino Acid	R
Glycine	H·
Alanine	CH ₃ ·
Valine	(CH ₃) ₂ CH·
Leucine	(CH ₃) ₂ CH·CH ₂ ·
<i>iso</i> Leucine	CH ₃ ·CH ₂ ·CH(CH ₃)·
Cysteine*	HS·CH ₂ ·
Methionine	CH ₃ ·S·CH ₂ ·CH ₂ ·
Serine	HO·CH ₂ ·
Threonine	CH ₃ ·CHOH·
Phenylalanine	
Tyrosine	
Tryptophan	
Histidine	
Lysine	NH ₂ (CH ₂) ₃ ·CH ₂ ·
Hydroxylysine	NH ₂ ·CH ₂ ·CHOH·CH ₂ ·CH ₂ ·
Arginine	NH C·NH·CH ₂ ·CH ₂ ·CH ₂ · NH ₂
Aspartic acid	COOH·CH ₂ ·
Asparagine	NH ₂ ·CO·CH ₂ ·
Glutamic acid†	COOH·CH ₂ ·CH ₂ ·
Glutamine	NH ₂ ·CO·CH ₂ ·CH ₂ ·
Proline	
Hydroxyproline	

* Cysteine is readily oxidized to cystine H₂NCH₂·S·S·CH₂·CH(NH₂)COOH; both compounds occur naturally. See also Fig. 5.

† See also Fig. 4.

Cross linkages between chains of polymerized amino acids may occur through the use of the amino acid cysteine, the free $-SH$ groups of two units being oxidized to form an $-SS-$ linkage (Fig. 5). Other interchain linkages are probably confined to salt formation between acid and basic groups or to hydrogen bonding. Many polysaccharides are exclusively composed of neutral units but proteins normally contain a proportion of acidic and basic units. This means that protein molecules will bear an overall electric charge in solution and that alterations in the pH of the environment will alter the distribution of the individual charges, often with profound effect on their solubility. The surface of a protein will also have a characteristic distribution of charged regions that will be a function of the particular

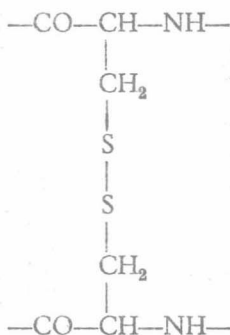


FIG. 5. CROSS LINKAGES

Chains of polymerized amino acids may be cross linked through the $-SH$ groups of cysteine becoming oxidized to an $-SS$ linkage.

arrangement of the units in the polymer and hence unique for each protein. The fact that portions of the surfaces of proteins have uniquely determined charge patterns is believed to be of the greatest importance in explaining their ability to combine specifically with other molecules, such a combination being an essential preliminary to enzyme action. About thirty amino acids have been identified as occurring in living things and of these about twenty commonly occur in proteins (Table I).

The fats are not polymers in the same sense that carbohydrates and proteins are, that is they are not formed by the linkage of an indefinite number of units. They are formed by the esterification of the three hydroxyl groups of glycerol by fatty acids. The number of fatty acids that may take part in this esterification is large and consequently