M. FLORKIN

UNITY AND DIVERSITY IN BIOCHEMISTRY

An Introduction to Chemical Biology

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by

MARCEL FLORKIN
University of Liège

Translated from the French by

T. WOOD
University of Sydney

LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
DPN, DPNH	Diphosphopyridine nucleotide
DPT	Diphosphothiamine
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
FP	Flavin phosphate
F-6-P	Fructose-6-phosphate
F-1,6-PP	Fructose-1,6-diphosphate
Gal-1-P	Galactose-1-phosphate
G-1-P	Glucose-1-phosphate
G-1,6-PP	Glucose-1,6-diphosphate
G-6-P	Glucose-6-phosphate
HDP	Heptulose diphosphate
HMP	Heptulose monophosphate
LTPP	Lipothiamide-pyrophosphate
PGA	3-Phosphoglyceric acid
PGAD	Phosphoglyceraldehyde dehydrogenase
PRPP	Phosphoribosylpyrophosphate
Ru-P	Ribulose-5-phosphate
Ru-PP	Ribulose diphosphate
TPN, TPNH	Triphosphopyridine nucleotide
UDP	Uridine diphosphate
UDPG	Uridine diphosphate glucose
UMP	Uridine monophosphate
UTP	Uridine triphosphate

TRANSLATOR'S PREFACE

THE translation of Professor Florkin's book has been complicated by the rapid progress of biochemistry in the last few years, necessitating many alterations and additions to the original text, to keep it up to date and to include new material. The subject-matter dealt with in these pages covers a very wide field, and I would crave the indulgence of the reader for any errors due to lack of familiarity with some of the topics discussed. Part of this discussion is at a philosophical level and, although not having had the pleasure of meeting Professor Florkin personally, I have tried to convey as faithfully as possible the ideas and concepts set out in the original text. When in doubt, I have stayed as closely as possible to the phraseology of the original. I have retained the term "biosphere", for example, to describe the total collection of living organisms. Enzyme nomenclature, too, has been a problem, but when in doubt I have used the terms given in Baldwin's *Dynamic Aspects of Biochemistry*, or alternatively, the simplest and most descriptive name for the enzyme. I have endeavoured to write in good concise English and I trust that the result is clear and readable.

TERRY WOOD

INTRODUCTION

IN these pages, the reader will find neither a treatise nor a textbook on biochemistry, but a number of essays grouped around ideas of the unity and diversity of organisms in the biochemical sphere. "The manifold and the one" are eternal preoccupations of the human intellect, and we must not be surprised that, from the time biochemistry has been able to gather together a sufficient number of facts, the search for the lowest common denominator of all organisms or a "unity of biochemical plan" has been confused in many minds with the idea of a comparative biochemistry. The latter is a problem which is perhaps more relevant to natural philosphy than to scientific investigation, for we are becoming more and more aware of the extreme diversity of biochemical function arising during cellular differentiation in a single organism, as well as in the multiplicity of species and even of individuals. The biosphere, by which we understand the total amount of living matter, behaves like a chemist of a very special type. All the organic compounds present in the many regions of the biosphere and resulting from its biosynthetic activities have structures lying within certain definite limits. The first part of this book provides a concise catalogue of these structures but is not coincident with the contents of a textbook of organic chemistry provided that the latter is not defined as it was by Berzelius at the beginning of the 19th century, when he wrote that organic chemistry is that section of physiology describing the composition of living things and the chemical reactions going on therein. This definition of organic chemistry is no longer valid today; beginning with the synthesis of a naturally occurring substance, urea, organic chemistry has extended its domain to the synthesis of a tremendous number of non-natural substances. One of the objectives of biochemistry is to define and understand the nature of the collection of compounds composing living matter and to distinguish them from those originating from non-living sources and human inventiveness, all of which are described by the broad generalizations of chemistry.

The biosphere is not only a chemist of a special type, but also one of great antiquity whose methods have been developed over a long period of time since long before there were laboratories of organic chemistry, and are of an efficiency far from being paralleled in these laboratories. This point is developed further in the two essays which make up Parts 2 and 3 of this book with the intention of demonstrating the originality of this organic chemist who has laboured since the dawn of time and comparing his methods with those of the laboratory chemist. The essays making up

Parts 1, 2 and 3 are devoted to general aspects of the biosphere, i.e. to the biochemical facts common to living beings and which constitute their lowest common denominator, or, their "unity of biochemical plan". The cellular theory, as proposed by Theodor Schwann in 1836, taught that organisms are formed not only of cells, of modified cells and cellular products, but that a multicellular organism has two levels of individuality, one on the cellular level and one at the level of the whole organism. In its final perfected form, the cellular theory recognizes that each cell is derived from a pre-exisiting cell. The results of biochemical research have taught us that the manifestations of an underlying biochemical unity are present in each cell, according to a topochemistry briefly described in Part 4. In this context, the "unity of plan" is simply the cellular theory from a biochemical point of view; in the same way as they are *units* of structure, cells are *units* of metabolism. The unity of a structure and cellular metabolism is only another expression of cellular continuity and the persistence in this continuity of a definite collection of genes controlling the synthesis of the collection of enzymes present in each cell. However, no cell is limited by the underlying biochemical unity, for this is only the canvas on to which the cell can embroider the numerous variations constituting its own biological nature, the "unity of plan" remaining an abstraction.

In Part 5 it is only possible to provide a few very brief examples of biochemical variation and biochemical evolution. The few cases quoted will enable the reader to locate some aspects of biochemical variation at the level of cellular differentiation and at the taxonomic level in the same organism. These examples show that the biochemical manifestations of variation are founded on the extension of the general processes of cellular biochemistry and constitute atypical expressions of general metabolic systems, variations on each theme being more or less pronounced but fitting in with general ideas of variation of genotype and biochemical adaptation of the organism to its surroundings.

Part 6 presents the idea of the metabolism of the biosphere as a whole and illustrates how this metabolism, like that of each organism, consists of an entry and an exit of energy and material, but now situated at the frontier between the biosphere and the inorganic world. The metabolism of the biosphere is conditioned by manifestations of biochemical variation, without which life would disappear. In fact, the unity of the biochemical plan of organisms comes down finally to a continuity of the biosphere in time and space and the accompanying biochemical diversity which has appeared as biochemical evolution has progressed with the extension of the biosphere. This extension has only been possible when, by means of new ecosystems, the exchanges between the inorganic surroundings and the biosphere, which are a condition for the survival of the latter, have been maintained.

Biochemistry has provided explanations in many fields of physiology and we can now perceive the preliminary signs of similar progress in a field that up to now has been outside the scope of biochemical explanation—that of electrophysiology. The demystification of biology will be a long and arduous task which is only just beginning. Much work remains to be done before the natural order, natural selection, adaptation, evolutionary tendencies, orthogenesis, morphogenesis, etc., are replaced by a knowledge of the reality underlying these somewhat poetic terms.

The author is conscious of the imperfections of his book and criticisms which could be levelled at it. Nevertheless, perhaps biochemists will find therein reasons for interesting themselves to advantage in biochemical variation and not solely in the unitarian aspects of biochemistry. Perhaps chemists will find reasons for recognizing that although it is true that chemistry is one, and everywhere obeys the same laws, yet the chemist within the living cell has his own special methods which can only be unravelled by means of the experimental study of living material. Perhaps, also, the essays that follow will assist in convincing certain biologists that they are wrong when they assert that the natural realities which we describe by the concepts of species and taxonomic classes no longer exist at the level of the molecular phenomena which is the study of biochemistry. In addition, perhaps, they will also become convinced that the field of the metabolism, of cells, of organisms, and of the biosphere itself, offers a fruitful region for the study of some of the most fundamental problems of biology.

Although unable to flatter himself with unqualified success in an undertaking as difficult as this, the author feels confident of the indulgence of the reader for any omissions or errors he may have committed.

MARCEL FLORKIN

Contents

THE BIOSPHERE

THE terrestrial globe is surrounded by an *atmosphere* consisting chiefly of nitrogen, oxygen and argon, but containing other elements and such compounds as carbon dioxide.

The distribution of molecules in the atmosphere is of considerable biochemical interest. At altitudes below 20,000 m the average composition shown in Table I is maintained constant by convection currents, but at higher altitudes, due to the different molecular weights of the gases composing the atmosphere, a separation by sedimentation becomes apparent. Beyond 150,000 m, this separation becomes very marked and progressively the atmosphere becomes less dense until finally it fades into the emptiness of interplanetary space. Nearest to the earth is the troposphere, its composition being kept more or less constant at the values shown in Table I, by convection currents. Its thickness varies from 10 to 15,000 m according to the season and the latitude. Above it is the stratosphere, so-called because of the stratification of different gases in order of their molecular weights. The layers of gas are not disturbed by convection currents and there is no appreciable circulation of molecules in a vertical direction. Whilst in the troposphere the temperature decreases with altitude, in the stratosphere it is independent of the latter.

At around 80,000 m is the start of the ionosphere which takes its name from the fact that it is rendered conducting by the ionization phenomena produced by the sun's ultra-violet radiation. The presence of carbon dioxide in the troposphere, as we shall see, is very important despite its low concentration. The presence in the stratosphere of a diffuse layer of ozone, the ozonosphere, is not less important for it prevents the greater part of the sun's ultra-violet radiation from reaching the surface of the earth where it would otherwise soon put an end to all life.

The earth is also surrounded by a *hydrosphere*, a discontinuous layer of water in different physical states which separates the *lithosphere* from the atmosphere and extends into the latter in the form of water vapour.

The hydrosphere is made up of oceans, lakes, rivers, streams, water absorbed by the rocks and by snow and ice. Oceans cover 70% of the earth's surface and their average depth is 3,800 m, and sea water represents approximately 98% of the hydrosphere. The composition of the sea water and its dissolved gases is given in Table II.

TABLE I

Average composition of the troposphere

(from Paneth, completed by Mason, 1952)

	Composition by volume (p.p.m.)	Composition by weight (p.p.m.)	Total mass in geograms (10^{20}g)	Partial pressure (mmHg)
N_2	780,900	755,100	38·648	593·02
O_2	209,500	231,500	11·841	159·52
A	9,300	12,800	0·655	7·144
CO_2	300	460	0·0233	0·228
Ne	18	12·5	0·000636	
He	5·2	0·72	0·000037	
CH_4	2·2	1·2	0·000062	
Kr	1·0	2·9	0·000146	
N_2O	1·0	1·5	0·000077	
H_2	0·5	0·03	0·000002	
Xe	0·08	0·36	0·000018	
O_3[1]	0·01	0·36	0·000031	
				760·00

[1] Variable, increasing with altitude.

TABLE II

Composition of sea water

(mM per litre)

(after Conway, 1943)

Na	478
K	10
Ca	11
Mg	55
Cl	559
SO_4	29
HCO_3	2
PO_4	traces

Gas dissolved in sea water (ml/l)

(after Mason, 1952)

Oxygen	0–9
Nitrogen	8·4–14·5
Total CO_2	34–56
Argon	0·2–0·4

The total of those parts of the lithosphere, the hydrosphere and the atmosphere, in which life is present, is called the *biosphere*. What are its limits? In the direction of interstellar space it is bounded by the ozonosphere, at about 20 km from the ground. Towards the earth's core the boundary is reached very soon at the surface of the continents and is not generally deeper than this by more than 10 m. On the other hand, in the ocean life can be found at depths down to 10,000 m.

No matter to which theory of the earth's origin one subscribes, it seems probable that there was a time when the terrestrial atmosphere consisted almost entirely of nitrogen and carbon dioxide. It is probable that at the beginning of the earth's history the atmosphere just described had been rapidly transformed into a mixture of oxygen and nitrogen, for the study of the rocks does not show the existence of any marked variations, which shows that the composition of the atmosphere has been much as it is now throughout a great part of geological time. For instance, the state of oxidation of pre-Cambrian rocks is not significantly different from that of more recent rocks. The change from an atmosphere of carbon dioxide and nitrogen to one of oxygen and nitrogen is generally considered to be a result of the process of photosynthesis.

In the course of time, considered on a geological timescale, the atmosphere has had various chemical substances added to it: volcanic gases (chiefly CO_2), oxygen resulting from photosynthesis, CO_2 as a result of metabolism and the decay of organic material, helium-4 produced by the radioactivity of uranium and thorium, argon-40 from the decay of potassium-40. (Natural argon contains the isotopes of mass 36, 38 and 40. Only argon-40 is derived from potassium-40. Argon-40 constitutes 99·63% of natural argon. Similarly for helium: only helium-4 is derived from the α rays emitted by several natural isotopes.)

On the other hand, subtraction of certain chemical substances has taken place: loss of oxygen by oxidation of iron, sulphur, manganese, etc., loss of CO_2 through formation of carbon, petroleum and dead organisms, loss of CO_2 in the formation of carbonates, loss of nitrogen by fixation, loss of nitrogen by formation of oxides by electrical or photochemical action, loss of hydrogen and helium due to the weakness of the earth's gravitational field, etc.

The equilibrium between losses and gains which has been obtained is witnessed by the constancy of composition of the atmosphere over extended periods of geological time. In the special case of carbon dioxide the regulatory role of the hydrosphere also plays a part.

Certain geochemists consider that the hydrosphere remained shallow up to the end of the Paleozoic period, so that the oceans as we know them today have a relatively short history. Others say that the volume of the oceans has not changed much since the pre-Cambrian period. Whichever

is the case it is generally agreed today that the oceans have not changed appreciably in composition since the Archaean period.

Together with Conway (1943) we may advance four hypotheses to account for the chemical evolution of the oceans.

1. The water results from the condensation of water vapour from the primitive atmosphere whilst the chlorides have been added gradually over the ages (constant volume, volcanic chlorides).

2. Both water and chlorides are the result of an initial condensation (constant volume, constant chloride).

3. The water and the chlorides have been progressively accumulated (both oceans and chlorides of volcanic origin).

4. The chlorides were initially present (metallic chlorides in the surface of the earth), the water being gradually added as a result of volcanic action (volcanic oceans, constant chloride).

At the present time the third hypothesis is the one favoured by geochemists. According to this view the mass of substances dissolved in the sea arises from erosion of the terrestrial crust. The total of these substances is enormous. If all the sea water were evaporated, it would form a layer of salt 153 m thick covering the surface of the continents. Goldschmidt calculates that for each litre of sea water 600 g of igneous rock have been dissolved. In other words, for each square centimetre of the earth's crust, during the formation of the oceans, erosion has removed 160 kg of igneous rock. The greater part of this has gone to form the sedimentary rocks.

The biosphere is chronologically after the lithosphere, the hydrosphere and the atmosphere. Although occupying a portion of all three regions, it is discontinuous and *comprises the total mass of organisms*. This definition is additional to what has already been said about the vital nature of the biosphere, its location and its discontinuous character. The mass of the biosphere is much less than that of the hydrosphere or the atmosphere. According to Rankama and Sahama (1950), the relative weights are as follows :

Hydrosphere	69,100
Atmosphere	300
Biosphere	1

But if the biosphere is quantitatively insignificant, nevertheless it is the centre of considerable chemical activity and it can be calculated that in the course of the last 500 million years, that is since the appearance of the Trilobites, it has "metabolized" a mass of material equal to the total weight of the globe.

One can obtain an idea of the size of the biosphere by calculating the annual production of organically bound carbon per square kilometre of the earth's surface. Riley obtains a figure of 160 metric tons on land and 340

metric tons in the sea. The total annual production of the continents is $20 \pm 5 \times 10^9$ metric tons and that of the sea is $126 \pm 82 \times 10^9$ metric tons, a total of $146 \pm 83 \times 10^9$ metric tons.

The predominant chemical elements in the biosphere are hydrogen, carbon, nitrogen, oxygen and phosphorous. Sodium, magnesium, calcium, potassium, chlorine, sulphur and iron, in addition, are always present in concentrations ranging from 0·05% to 1%. Further elements, although in smaller quantity, are always found in measurable amounts : boron, aluminium, copper, zinc, silicon, gallium, molybdenum, manganese, cobalt and iodine. On occasion other elements may be found in living organisms.

The normal constituents of the biosphere are, with the exception of iodine, members of the first four periods of the periodic table. These are the lighter elements. Now, water makes up a large proportion of the biosphere and it is natural that the elements present in living organisms are those most widely distributed in the earth's crust and whose derivatives are most soluble. The electronegativity of the elements also plays an important part. In a biosphere of a predominantly aqueous nature it is natural that elements of weak electronegativity forming soluble cations are easily absorbed and assimilated. The same applies to elements of very strong electronegativity which give readily soluble anions.

To characterize the quantitative relations existing between the various elements entering into the composition of living things, one can say that if one adds up the amounts present of the following eleven metals and metalloids :

Carbon	Sulphur	Calcium
Hydrogen	Phosphorous	Magnesium
Oxygen	Chlorine	Potassium
Nitrogen		Sodium

then one accounts for almost the total weight of the organism—99·9% in the case of a man. This is one way of saying that the major part of any organism is made up of water, lipids, polysaccharides and proteins, and by chlorides, bicarbonates, phosphates, and sulphates of sodium, potassium, calcium and magnesium. The elements making up these compounds are the lighter elements, and, as already stated, are the most common in the surface of the lithosphere and hydrosphere. They include the elements of very weak and very strong electronegativity. It is not unexpected that they should have an important place in the composition of organisms.

As far as the minor elements are concerned we must guard against limiting the list too closely. Provided that there is at least one atom per cell of a trace element, then a function may be assigned to that element. As the methods of detecting trace elements improve, so does the number of trace elements increase. As our knowledge of biochemistry increases so does the

number of trace elements having a known function increase proportionately. All the more reason, therefore, to treat warily any such idea as that of an *element of biochemical importance*.

The lithosphere, the hydrosphere and the biosphere are made up, qualitatively, of the same elements and only differ in the relative proportions of these elements.

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CONSTITUENTS OF THE BIOSPHERE

As LONG as chemistry retained its aura of secrecy and magic; that is, up to the seventeenth century, the study of the chemical compounds making up the biosphere had not even been considered. One may attribute the first step to Van Helmont who, in his work *Ortus medicinae*, published in 1652, described carbonic anhydride as being present in intestinal gases, separated an alkaline substance from blood and attempted to resolve human urine into its constituents. It was necessary to await the development and elaboration of the idea of chemical compounds before Scheele, in 1775, succeeded in isolating uric acid from urinary calculi and Poulletier de la Salle, in 1782, extracted cholesterol from gall-stones with the aid of alcohol.

Following this came the most spectacular period of organic chemistry which saw the establishment of the idea of "radicals" (groups of atoms in the molecule which can be considered as remaining intact throughout a series of reactions, and which can be transferred from one molecule to another without undergoing disruption) and the idea of chemical type (that two substances which possess similar chemical properties have a similar arrangement of their constituent atoms in the molecule). The application of methods capable of isolating substances present in living things without any gross chemical changes taking place resulted in the identification of a large number of these constituents and the list of these increases daily. In 1862, Ernest Wagner, professor at Leipzig, in his *Manual of General Pathology*, stated an important generalization—that, of the material of living substances, apart from water, a large percentage can be divided into three simple organic types which we recognize today as fatty acids, sugars and amino acids. A mushroom, a spinach leaf, a sea urchin egg, the flesh of an oyster, a silk-worm, contain respectively 91·3, 92·3, 77·3, 88·3, and 78·4% of water. The dry residue is made up of these three principal types of compounds to the extent of 94·2% in the mushroom, 72·6% in the spinach leaf, 93·5% in sea urchin egg, 90·5% in oyster flesh and 83·9% in the silk-worm. The spinach leaf contains a somewhat smaller proportion of these organic constituents than the others due to the larger amounts of inorganic material which amounts to 27·2% of the dry weight. The sea urchin egg, on the other hand, contains only 1·5% of inorganic matter, mainly chlorides, sulphates, phosphates and bicarbonates of sodium, potassium, calcium and magnesium. Nevertheless, as stated above,

the biosphere contains small amounts of a great number of other elements which are used to good effect.

Organic chemistry began by the study of certain natural carbon derivatives. In the course of its spectacular development during the nineteenth century, it ceased to be a science of naturally occurring compounds and became a science of imaginary molecules in the sense that a molecule not present in nature but synthesized by an organic chemist is a product of his mind and intellect. Nevertheless, a part of organic chemistry forms a whole segment of the natural science of biochemistry—this is the organic chemistry of naturally occurring substances, a field which depends upon the technical skill of the organic chemist, but whose frontiers and content primarily interest the biochemist.

This chapter is only a brief outline, and is not a catalogue of the chemical structures present in the biosphere. In our present state of knowledge it would certainly be premature to think of establishing such a catalogue. It is only necessary for an organic chemist to examine minutely the constituents of such a narrow portion of the biosphere as, for example, the musk gland of the musk-deer (see E. Lederer; Animal odours and perfumes, *Fortschr. Chem. org. Naturstoffe*, 1950, **6**, 87–153), toad parotid gland (V. Deulofeu; The chemistry of the constituents of toad venoms, *Fortschr. Chem. org. Naturstoffe*, 1948, **5**, 241–266), the cell of a mycobacterium (J. Asselineau and E. Lederer; Chimie des lipides bacteriens, *Fortschr. Chem. org. Naturstoffe*, 1953, **10**, 170–273), or the wood of various conifers (H. Erdtman; Chemistry of some heartwood constituents of conifers and their physiological and taxonomic significance, *Prog. org. Chem.*, 1952, **1**, 22–63), for him to obtain an imposing harvest of new molecules. Many molecules among them are merely variations on a general theme and their interest is greater still from the point of view of comparisons between living organisms. At the present time, without stating categorically that they are present in all organisms, we can make a list of types of organic structure most widely and generally distributed.

I. THE THREE PRINCIPAL BIOCHEMICAL STRUCTURES

A. ALIPHATIC ACIDS

Aliphatic organic acids are very widely distributed in living things, particularly in the form of fats, hence the name "fatty acids".

The first member of the straight chain saturated series is formic acid
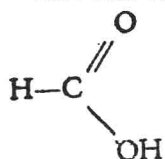
$$H-C\begin{smallmatrix}O\\ \diagup\diagup\\ \diagdown\\ OH\end{smallmatrix}$$ corresponding to methane CH_4, and the first member