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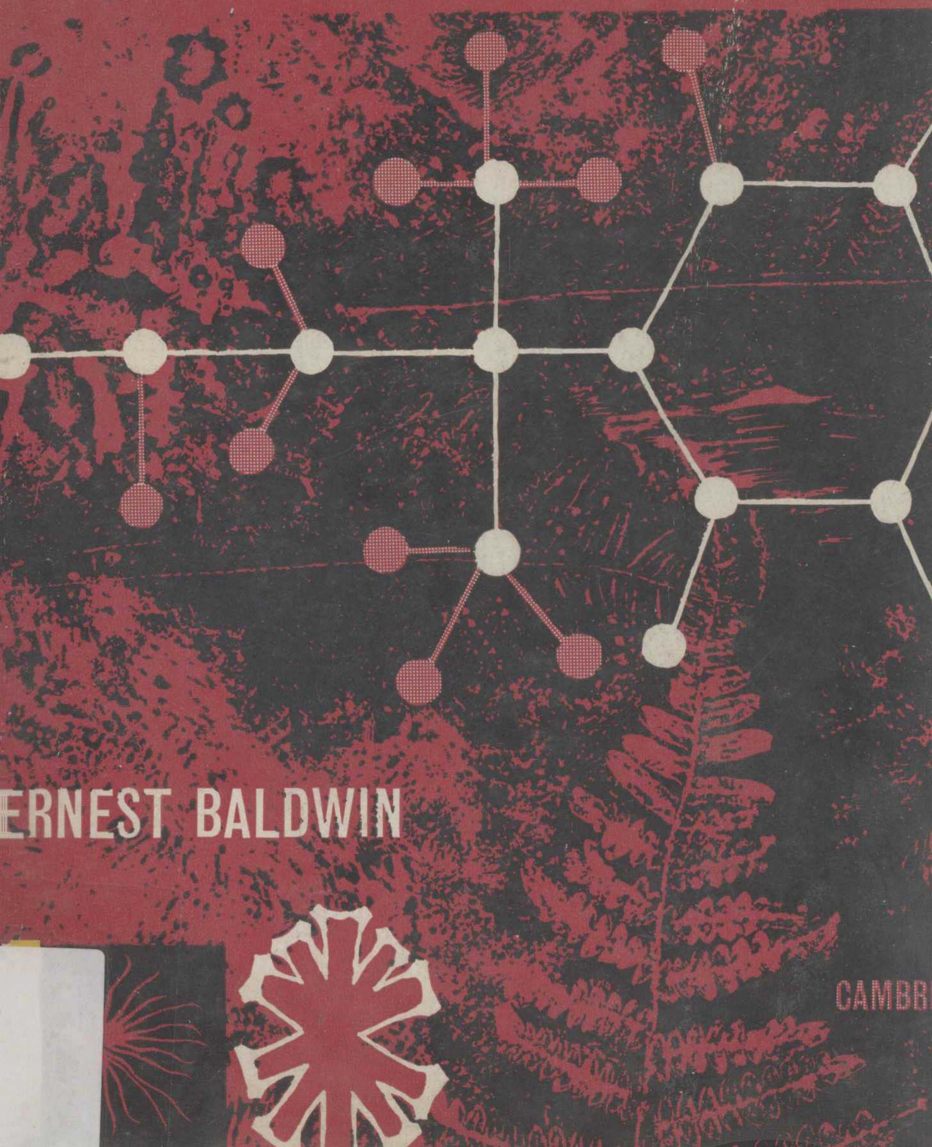
THE NATURE OF BIOCHEMISTRY



ERNEST BALDWIN

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THE NATURE OF BIOCHEMISTRY

BY

ERNEST BALDWIN

B.A., PH.D.

Professor of Biochemistry, University College London
Sometime Fellow of St John's College,
Cambridge

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THE NATURE OF BIOCHEMISTRY

To

PAULINE, NICOLA
AND NIGEL

PREFACE

If it is true that a good wine needs no bush, it may be similarly true that a good book needs no preface. I hope that this may be a good book, for it has been written for a specific purpose. It is meant to be read, not studied.

Degree courses in biochemistry are now offered in a good many colleges and universities. Year by year many school leavers apply for admission to these courses and yet, in my own experience, few of them start with more than a vague idea of what biochemistry is all about. It is for their enlightenment that this book has been made. Possibly there will be others who would find it a useful starting-point for further study.

I have tried to tell a story that is factually accurate with a minimum of oversimplification. If some passages are a little difficult to follow —and biochemistry *is* an intricate subject—I hope the fascination of the story will lighten the reader's task.

It has been difficult to decide what to include and what to omit because, in a book of this size, it is possible to skim over only a part of the whole story. It has been my ambition to keep the book small, for size is apt to be inhibitory to the hard-pressed student of today. In these days of over-early specialization in the schools I have no wish to add to the burden; that is why the book has been written for reading rather than study. It is not intended in any sense to be a supplementary or complementary treatise.

I should like to thank the many friends and colleagues who have given me valuable comment, suggestions and criticism, and special thanks go to Mrs Joan Rosemeyer for preparing the diagrams, and to Mrs P. H. Clarke, whose advice and opinion have been unstintingly given.

E. B.

LONDON
July 1961

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PROLOGUE

The scientific study of living things began with the straightforward observation and description of whole organisms and their visible parts. The only tools needed were the human eye and a few simple instruments—a pair of scissors and a sharp knife, perhaps. The systematic classification of plants and animals was certainly attempted by Aristotle and perhaps even before his time. This classification was based upon observable resemblances and differences between wholes and parts. Only one general property was at that time known to run right through; that all plants and animals are *alive*; they grow and reproduce.

Later, with the invention and development of the optical microscope there came to light a further general feature that is common to all living things, namely that they are all built up from great numbers of microscopic units known as *cells*. The discovery soon afterwards of an immense realm of previously unknown, single-celled organisms—bacteria, protozoa, yeasts and other unicellular fungi—added to the older sciences of botany and zoology the newer science of microbiology.

Many inquiring minds remained unsatisfied with a knowledge only of structure as revealed by the eye, the knife and the microscope, because this provided an answer to only one of two fundamental questions that we all ask, practically from the cradle; we want to know 'What is it made of?' and 'How does it work?'. The problem of how living things work began to be studied fairly early in the history of biology because, once one knew how living things are built and of what parts they are made, it began to be possible to understand some at least of the ways in which they do the things they do. As a result biology began to separate into two main branches, the structural, or *morphological*, and the functional, or *physiological*.

In more recent times, and mainly in the last sixty years, new methods for the study of living things have been added to visual observation, dissection and microscopy, namely methods borrowed from physics and chemistry. Two new biological sciences have been born and are still actively developing, *biochemistry* and *biophysics*. This book is concerned chiefly with biochemistry, a science which aims at answering in chemical terms the questions 'What is it made of?' and 'How does it work?'. Whereas the eye works at the gross level of visible objects, the microscope reaches down to the finer levels of groups of cells, single cells, cellular inclusions as minute as cell nuclei and even smaller particulate materials. Biochemistry, however, works at a finer level still. It operates at a level that cannot be reached even by the most modern optical or even the newer ultraviolet and phase-contrast microscopes.

Biochemistry works, in fact, at the molecular level. Only the electron microscope can reach far down towards this level. It can tell us a good deal about the structure of natural molecules whose molecular weights are of the order of millions, but has the disadvantage that it cannot look at them if any water is present. And water is the most universally distributed of all biological substances. X-radiography again can pick out spatial relationships within large molecules provided they contain regular and more or less crystalline structures. But all these are essentially static observations. Only the application of chemical and physical methods can tell us very much about the dynamic and functional relationships existing between molecules whose molecular weights amount to a few tens, hundreds or thousands. And, water apart, it is of molecules of this order of size that living materials are for the most part composed.

Living organisms of one kind or other can be found in the sea, on the sea shore, in estuaries, swamps and marshes, in rivers, ponds, lakes, on dry land, and even in deserts and hot springs. Even the concentrated salt lakes of California harbour living inhabitants, and that versatile group, the insects, has even contrived an inhabitant for petroleum pools. Yet one of the most striking discoveries of biochemistry has been that, in spite of the immense variety of size, shape, form and structure of living things as a whole, they all have a very great deal in common; far more in fact that any one would have dared to imagine, even after the cell theory had been propounded and had found general acceptance.

In this book we shall attempt to give an account in chemical terms of the materials from which living stuff is made and to give also an account of some of the chemical operations that underlie the manifestations and the maintenance of life itself.

Prologue

Students interested in biology and in chemistry will find in biochemistry a link between these two sciences, and this may help to vitalise and integrate what they have already learned. It may be, perhaps, that this first glimpse of biochemistry will frighten some students, but perhaps it will encourage others to go further and learn more about the fascinating fields that biochemists have already charted and the newer territory that is only now beginning to be explored. To go into new and uncharted territory is always exciting, and in biochemistry we have to explore the innermost molecular territory of life itself.

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1

THE CONSTANCY OF THE INTERNAL ENVIRONMENT

INTRODUCTION

Biochemistry originated as an offshoot from human physiology when it came to be realized that the chemical analysis of urine, blood and other natural fluids can help in the diagnosis of this disease or that. In its early days biochemistry was accordingly known as Chemical Physiology. Now physiology, according to the *Concise Oxford Dictionary* is 'the science of normal functions and phenomena of living things'. Biochemistry is concerned particularly with the chemical aspects of these functions and phenomena and is but one of the many ways in which physiology can be studied. You can learn a lot about physiology by watching the monkeys at the zoo. Or you might take a cat or a dog to bits and study the behaviour of the bits and pieces. And you can go further still by extracting from those bits and pieces a variety of chemical substances, and by studying the chemical composition of those substances and the chemical interactions that take place between them.

If you watch the monkeys in the Zoo you are studying the physiology of behaviour. But if you study the behaviour of animal *molecules* rather than whole animals you could legitimately be called a chemical physiologist, or, in today's language, a biochemist.

THE ORGANISM AND ITS ENVIRONMENT

There are still some people who believe that the earth was originally populated by an act of special creation according to the book of Genesis, but the majority opinion today is that life began many millions of years ago and that from very simple and lowly beginnings the micro-organisms, plants and animals of the present day arose, slowly and step by step, by the painful process of evolution.

It is even possible to argue that life never had a 'beginning'; that life did not suddenly appear in a non-living universe, but was only the result of an increase in the degree of complexity and organization of something that already existed but was not 'living' in the sense that we understand 'life' and 'living' today. One thing is certain however; living things as we know them have changed a lot in the past, are still changing at the present time, and are likely to go on changing in the future.

The great French physiologist, Claude Bernard, was the first to point out that every animal lives in two distinct and separate environments, and not merely in one. There is first of all the outside world, the *external environment*, the properties of which can and do vary widely with the wind and the weather. Secondly, there is the animal's own personal, *internal environment* that it carries around inside it. This internal environment is represented by the animal's blood plasma and tissue fluids. Claude Bernard emphasized that *no matter how the external environment may change, the properties of the internal environment must be kept constant within very narrow limits*; otherwise the animal dies. This is well known; indeed, it is a matter of everyday laboratory experience. For example, if the blood of an animal becomes and is allowed to remain too acid or too alkaline, too dilute or too concentrated, too hot or too cold, death is the result. Most animals possess mechanisms for maintaining this vitally important internal constancy, no matter what happens in the outside world. We, in common with most mammals, maintain a virtually constant body temperature. If it falls we can generate heat and warm ourselves up again by shivering; and if it becomes too high, then we can lose heat by sweating. There are many other examples—for instance the excretion of an unusually alkaline urine after alkaline drinks have been taken.

THE EXTERNAL ENVIRONMENT

The earliest and most primitive of living things can hardly have possessed any of the many regulating devices that are found in modern animals. It follows that if internal constancy was as important then as it certainly is nowadays, these early living beings must have lived in an external environment of which the properties were relatively constant. Such an external medium would enable them to rely upon the constancy of the external world for the internal constancy upon which their own continued existence depended. If such an external environment exists in Nature that, surely, must be the ancestral home of the living things that eventually evolved to populate the world of today.

Terrestrial environments of the kind with which we ourselves are familiar are exceedingly unstable and inconstant in the physico-chemical sense. Environments like these can only be inhabited by animals which, like ourselves, possess devices which allow changes in humidity, temperature and so forth to take place externally without producing corresponding internal changes in the inhabitants. In many ways the terrestrial type of environment is advantageous however, for terrestrial conditions are especially favourable for the growth of plants, which means an abundance of food for herbivorous animals which, in their turn, can feed large numbers of carnivores. Again, an abundant supply of oxygen is available in the atmosphere, but abundance of food and oxygen are only two of the many factors needed if the dry land is to be a paradise for animals in general. These self-same terrestrial conditions also include periods of intense cold and intense heat, and may vary between drought at one time and extreme humidity if not positive flooding at others.

Very much greater physico-chemical stability is to be found in any large body of water. Because of the high viscosity and large specific heat of water, aquatic environments—except where the water is shallow—are relatively free from the wide and often violent thermal and mechanical disturbances met with on the land. Moreover, the free availability of water is a matter of the utmost importance for living things of every kind, because water is their chief constituent. As J. B. S. Haldane once remarked, even the Archbishop of Canterbury is 65 % water. Furthermore, water plays a larger or smaller part in most of the chemical reactions that take place in living organisms and upon which the very life of those organisms depends.

Because of the greater inherent constancy of water, especially when the volume is large, it seems likely that the need for complicated regulatory mechanisms must be less for aquatic than it is for land-living animals. Moreover, because of its enormous bulk and consequent physico-chemical inertia, the sea is probably the only environment that could provide the large degree of physico-chemical constancy that must have been needed to maintain the life of early, primitive and probably delicate living systems which as yet had devised no machinery for combating external changes and climatic conditions.

INTERNAL ENVIRONMENT: IONIC BALANCE

What has been said so far is pretty largely a matter of guesswork, for these early and primitive creatures have long since disappeared, so that whatever we may say or think about their habits, habitats and behaviour must necessarily be a matter mainly of conjecture. We have to try to deduce the nature and properties of these long-vanished organisms from those of the creatures that survived, evolved and are still with us today. But most biologists would agree that the ocean was the most probable cradle of the earliest ancestors from which we all eventually descended.

However, these conjectural hypotheses are not the only arguments we can produce to support the idea of an oceanic origin of life. So far we have considered the external environment for the most part. Now it happens that physiologists, and biochemists too for that matter, often want to study parts of animals and therefore have to be able to keep the parts alive in isolation from the animal itself, and the ability to do this has some important theoretical consequences. All laboratory experience of this kind goes to show that, if the right conditions are provided, the heart or the liver of an animal, even a warm-blooded animal, can be kept alive and functionally normal for longer or shorter periods in complete isolation from the animal from which it came. The author, to give an example, has seen the heart of a snail still beating in a perfectly normal manner no less than a fortnight after removal from its former owner.

Because it is only by studying isolated organs and tissues that we can solve many of our problems, physiologists and biochemists owe what is probably their greatest single debt to Sidney Ringer. It was Ringer who was the first to show, in the early 1880's, that the isolated heart of a frog or a tortoise will survive and continue to beat normally for long periods in a solution containing only the chlorides of Na^+, K^+ and Ca^{2+}. Legend has it that Ringer worked for many months with only NaCl and KCl in different proportions, without finding any satisfactory mixture until one day his laboratory assistant inadvertently made up the solutions with tap water instead of distilled water, thus introducing calcium ions. Without these calcium ions life cannot be maintained. It is necessary only, as Ringer then showed, that the three chlorides should be present in the right proportions and that the total amount of salts should be about right, i.e.

Na^+	K^+	Ca^{2+}	Total
100	1·7	1·0	0·116M

With slight modification and the addition of small amounts of Mg^{2+} these simple solutions can maintain the heart beat of even a warm-blooded animal, but in this case the fluid must be kept neutral, well oxygenated and at the animal's own body temperature. One such solution devised by Tyrode has the following composition:

Na^+	K^+	Ca^{2+}	Mg^{2+}	Total
100	1·8	1·2	0·7	0·155M

All this is interesting and important enough if only as a practical matter, but it has great theoretical importance as well. If we compare the compositions of the bloods of different kinds of animals we get two surprises. First, the proportions of ions present in different bloods are always very similar to each other and to Ringer's and Tyrode's solutions. Secondly, and perhaps more surprising still, the relative proportions of Na^+, K^+ and Ca^{2+} are very similar indeed in animal bloods and in sea water. Some examples are shown in Table 1.

Table 1. *Relative ionic composition of animal tissue fluids*

(The amount of the commonest cation (Na^+) is taken as 100 to facilitate comparison.)

Animal	Na^+	K^+	Ca^{2+}
Sea water	100	2·16	2·27
Coelenterates: *Aurelia* (jelly fish)	100	2·90	2·14
Annelida: *Amphitrite*	100	3·21	2·34
Mollusca: *Eledone* (octopus)	100	2·97	2·73
Mytilus (edible mussel)	100	2·53	2·52
Arthropoda: *Cancer* (edible crab)	100	2·22	2·60
Homarus (lobster)	100	2·04	3·76
Carcinus (shore crab)	100	2·32	2·52
Echinodermata: *Echinus* (sea urchin)	100	2·51	2·42
Asterias (starfish)	100	1·80	1·96
Pisces: *Acanthias* (dog fish)	100	4·61	2·71
Pollachius (pollack)	100	4·33	3·1
Amphibia: *Rana* (common frog)	100	2·41	1·93
Mammalia: *Rattus* (rat)	100	4·27	2·14
Homo (man)	100	3·51	1·73

Although there are large differences in *total* ionic composition and *total* salinity, the *relative proportions* of the most important cations are remarkably similar and certainly far more similar to each other and to those in sea water than would ever have been expected. This, surely, is evidence that life, as we know it today, must have originated in the ocean waters.

From the sea, some animals spread into the estuaries of rivers while others moved up the shore-line to the land, and a few, such as the land crabs and some terrestrial snails, reached dry land in that way. For the majority of animals however the route to land was the more circuitous one:

$$\text{ocean} \rightarrow \text{estuaries} \rightarrow \text{fresh water} \rightarrow \text{land.}$$

Whatever the route, however, every move towards a new external environment confronted the animals with new structural and chemical problems which had to be solved before the move could be accomplished. To take only one example, animals accustomed to breathing in sea water, or in fresh water for that matter, could only live on the dry land when suitably modified respiratory organs—lungs instead of gills—had been developed, and when the blood itself had been so modified as to enable the animal to take the oxygen it needs from air instead of from water. Any animal leaving the security of the relatively constant external environment provided by the sea would need new devices and mechanisms that would ensure internal constancy in the face of unfamiliar external variations.

In spite of their evolution and migrations into different habitats animals as a whole have maintained in their bloods and tissue fluids the old original relative ionic composition of an ancient sea water, and this has evidently been a matter not of choice but of obligation. Even the cells and organs of animals whose ancestors, like our own, became independent of the sea many millions of years ago, cannot tolerate for long any appreciable departure from the normal, sea-water-like composition of the blood as far as Na^+, K^+ and Ca^{2+} are concerned. This necessary internal constancy is something that *has* to be maintained. This is accomplished largely by the excretory organs; indeed, these organs must have been intimately concerned with the maintenance of internal ionic composition long before their functioning as excretory organs proper came into prominence.

TOTAL IONIC CONCENTRATION

Whereas the composition of the bloods of all animals is held constant as far as the *relative* proportions of Na^+, K^+ and Ca^{2+} are concerned, wide variations are found in the *total* ion contents and therefore in the osmotic pressure of the bloods of different animals. As has been mentioned already, the majority of animal migrations towards the dry land proceeded by way of the estuaries of rivers, into fresh water and thence to the land. Now in the estuaries large changes