MOLECULAR BIOPHYSICS

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PREFACE

The subject matter of biophysics is not new. Scientists have always been interested in applying physical ideas and techniques to biological problems. Only recently, however, has biophysics emerged as a formal field of study. The rapid advances of physical science have led many more people than in the past to attempt to explain biological phenomena in terms of physical principles. A Biophysical Society has been organized, and the number of university biophysics departments and of courses in biophysics is increasing at a rapid rate.

Just as the field of physics is so large that all its material cannot possibly be incorporated into one advanced-level textbook, so is biophysics too large for such a comprehensive treatment. Since we cannot cover the entire field, we have focused our attention on the molecular aspects of biophysics. Molecular biophysics attempts to explain the properties of biological systems and biological phenomena in terms of the properties of molecules, both small and large. We have slighted, therefore, those parts of the formal field of biophysics which are closer to physiology. (Previous texts on biophysics, such as that written by Stacy, published by McGraw-Hill, stress the physiological aspects of biophysics.)

The level of the text is suitable for seniors or first-year graduate students. It presupposes some knowledge of mathematics, physics, and chemistry and biology beyond the elementary level. Since most students will not fit into this category, the text, and the course taught from it, has been designed so that students who are strong in at least two of these fields will have no difficulty in getting through the material. Numerical problems have been included at the end of each chapter, and their answers appear at the end of the book.

The book comprises fifteen chapters. The first three chapters, "Physics and Biology," "The Biophysicist's View of the Living Cell," and "Energetic and Statistical Relations in the Lving Cell," provide a general introduction to the characteristics of cells and a description of their constituents, and discuss how the laws of thermodynamics (such as the laws of conservation of energy and the flow of entropy) may be applied to living cells. They include a brief description of information theory and how it is used in describing properties of biological systems. These three chapters introduce the problems which hopefully will be solved by molecular biophysics.

The next four chapters, "Physical Methods of Determining the Sizes and Shapes of Molecules," "X-ray Analysis and Molecular Structures," "Intramolecular and Intermolecular Forces," and "Absorption Spectroscopy and Molecular Structure," describe the various techniques that have been used to analyze molecules. The techniques indicated by the titles cover many experimental fields because the macromolecules making up living systems are so complicated that one method of analysis does not give sufficient information for any clear-cut decisions about structure and function to be made. These four chapters attempt to describe what is known concerning the physics and chemistry of macromolecules associated with biological systems.

The following three chapters, "Enzymes," "Action Spectra and Quantum Yields," and "The Action of Ionizing Radiation on Cellular Constituents," are concerned with the relations between molecular structure and function covered earlier, and discuss how structure and function are influenced by various physical agents such as heat, light, and ionizing radiation, and how the effect of such perturbation may be used to infer something about the nature of the structures involved. To present a well-rounded picture of these subjects, we have found it useful to include descriptions of the various ways of studying enzyme reactions, structures, and the effects of light not only on molecules but on viruses and microorganisms. These chapters represent the beginnings of the synthesis of biological properties in terms of the properties of individual molecules.

The theme is extended throughout the next three chapters, "The Use of Ionizing Radiation to Study Cell Structure," "Microscopes," and "Isotopic Traces in Molecular Biophysics." These chapters are concerned with the various physical techniques which may be used to find relations among the structural and functional elements of cells by use of the classical microscopic techniques as well as by such recent ones as interference microscopy and thin-section electron microscopy, and a description of the use of tracers to determine metabolic rates as well as to determine the distribution of labeled molecules from one generation to another.

The last two chapters, "Molecular Biophysics and Muscle, Nerve, and Eye Studies" and "The Physics of Cellular Processes," represent an attempt to synthesize the information from the previous chapters so as to describe on a molecular basis the properties of muscle, nerve, and eye, and the processes involved in the duplication and transmission of genetic information in cells.

The emphasis throughout the text is twofold: it is both theoretical and experimental. It attempts to describe physical ways of thinking and analyzing biological processes, as well as describing the many and powerful physical techniques which may be used to determine the relations among structures and functions of biological systems.

We are conscious of considerable debt to others. First, we should thank our students in the Biophysics Department, Yale University, who have watched us develop the material, and who have criticized and helped in improving it without, so far, rising up and smiting us. Secondly, our

colleagues in the Yale Biophysics Department, notably Professor Harold J. Morowitz, have continuously helped us, by counsel and correction, over the years. One of the authors is indebted to Dr. L. H. Gray for the invigorating hospitality of the Research Unit in Radiobiology at the Mount Vernon Hospital, where several chapters were written. Mere thanks does not seem to be enough to express to the publishers who brought forth the manuscript into readable letters, but to them we do indeed express gratitude.

New Haven, Conn.

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CONTENTS

CHAPTER 1

PHYSICS AND BIOLOGY

A biophysicist is a person who, for professional reasons, needs both physical and biological insight.

1–1 Introduction. The twofold problem of mankind in which Science has predominantly helped is that of gaining control over Nature, and of understanding Nature. Control is usually achieved first, often with a very limited understanding; as yet probably no part of Nature is completely understood. The problem of gaining control over living processes and of understanding them is the problem of biology, a problem which has proved to be the hardest yet posed by any of the accessible divisions of Nature. The problems of cosmology, and of the atomic nucleus are not solved, of course, but the difficulty there lies in gaining access to the material to be studied rather than in the inherent difficulty of the tasks themselves.

The wonderfully patient and skillful studies of biologists, involving classification, microscopical observation, comparative studies of vision, respiration, nervous and muscular action, plus biochemical discoveries linking many organisms, and above all, the pervading doctrine of organic evolution have brought a systematization to biology that gives modern Science a heritage of inestimable value. The unity of living processes is certainly as simple and single as the unity of geology, and many first-rate biologists believe that it is even more so. For this reason the geneticist works deeply with *Drosophila* or *neurospora* and is impatient of skeptics who doubt the generality of his findings. For this reason the sciatic nerve of the frog serves as a model for neurophysiology, and sea urchin eggs are used for studies of embryology. No biologist hears of a new enzyme system without thought for its generality, and indeed, specialized enzymes, operative only in a limited group of organisms, are remarkable for just that reason.

So it is that today the words "living processes" conjure up no weird range of infinite possibility, but certain broad, clear ideas. One thinks of chromosome division, of enzymatic action, of the travel of electrochemical energy in a nerve, of hormonal growth regulation, and of the physico-chemical energy transfer that underlies metabolism. At present, every one of these processes lacks complete description. In some way the chromosomes grow, divide, and separate, but by what means do they grow? Why do they divide when they do? Why only in pairs? In some way an enzyme combines with its substrate and a reaction proceeds, but how is the reaction effected? Why is it specific? And so on.

Much more complete descriptions of these processes are very nearly on hand, which makes this modern biology almost breathlessly exciting. Nevertheless, "complete" descriptions are more exacting today than they were a century ago. The description of a process must somehow fit with the ideas of atomic physics and chemistry, which means that it may well have to be right, all the way down to atomic detail. When this goal is realized the understanding and control of living processes will be vastly increased.

A tremendous part of the detailed description of biological processes is clearly going to be in terms of chemistry, and with this clear fact in view the biochemists have made one of the most sensational scientific advances of all time. Nevertheless, a moment's thought shows that not *all* the processes will be found to be chemical. For example, it seems certain that the cell has a vital structure and does not simply serve as a reaction vessel, like a beaker. An understanding of the relative positions of the components will be found to be important, and here is where physics takes its place in the study of biology. It is in terms of physics that we are able to describe the physical character of cellular components, how they may interact with one another, how growth and change must alter them, and how these processes play their part in the whole living process. To begin this attack, physics brings to bear one weapon of great power, and an attendant strategic disadvantage that goes with unfamiliarity with the weapon.

The weapon is the newly won knowledge of the laws of Nature: the laws of motion, of electric and magnetic actions, of quantum mechanics, and of molecular statistical behavior. In principle the physicist and physical chemist intuitively feel that these laws should be all that is necessary. They should encompass and interpret biology as they have encompassed and interpreted chemistry and spectroscopy. In practice, however, the laws can be applied only to clearly defined systems, apt for human thought and calculation. The laws *work*, says the physicist, but to figure out how they work, and how to make them work to our will, is something else. And so, the first clear problem of the biophysicist appears: the problem of describing a few important biological systems clearly and simply enough so that the physical laws can be applied.

From time to time elements of biology have become suitable for such physical thought. Our knowledge of the lens of the eye and the way in which an image is formed and with it the elementary facts of optical defects and their remedy by external lenses is a nearly complete branch of biophysics in which the laws applied, those of refraction and of light propagation, are the laws of physical optics. This application is so straightforward and clear that few physicists stop to consider that when they work problems on the eye in elementary physics they are for the moment biophysicists. Physics is so important in this application that a complete course in physical optics, more thorough than that usually given in a general physics course, is necessary before a proper understanding of the behavior of this aspect of the eye can be attained.

This aptness for physical explanation is far from the rule, even though many valuable uses of physics, as in hydrodynamic flow or total energy relations, have been made. The physicist alone will not make the attempt to sort out the proper phases of biology to attack, and the biologist is often all too busy with his own rewarding studies. It thus becomes the task of the biophysicist to study, listen, think, and experiment, with the aim of finding new areas of biology to which physics can be applied. A short account of the history of biophysics, leading up to the present, will help to show that these new areas are being discovered.

1–2 History of biophysics. History is made by people, but it is formulated by historians. Up to the present no adequate historical consideration of biophysics has been written, partly because biophysics itself is not yet organized. A brief glance shows that biophysics has a rich and exciting history.

We can begin by mentioning Galvani, though in the modern sense he was hardly a biophysicist. It is often claimed that Galvani's discovery that the muscles of frogs suspended on copper hooks from a steel wire twitched when chance contact completed the circuit was pure accident. Apparently this is not so; it seems that Galvani had been studying the effect of static electricity on muscle for some time. He correctly interpreted this "galvanic" form of electricity as being due to the dissimilar metals, and he then sought a purely physical interpretation for the flow of electricity out of the muscle through the pair of metals. His discovery, made in 1786, opened two tremendous fields, both still under vigorous research.

It is hardly fair to include Thomas Young in the narrow class of biophysicists, since, among other attainments, he knew seven languages at age 14. He obtained his M.D. at Göttingen in 1796, and in 1801 he was appointed professor of *physics* at the Royal Institution. He seems to have combined almost all possible talents and can be credited with having first proposed the wave theory of light, the theory of color vision, the hydrodynamic nature of heart action, as well as having been among the first to interpret the ancient Egyptian language! His outstanding characteristic was an incisive interest in any form of problem, and he had considerable experimental skill. The explanation of the process of accommodation of the eye and a great deal of the elementary geometrical optics of the eye were discovered by Young.

Perhaps the first real biophysicist was Julius Robert Mayer (1814–1878), who received his training in medicine. While serving as surgeon on a

Dutch ship in the tropics, he noticed that venous blood was of a brighter red color than he had expected from his experience in cooler climates, and he began to speculate whether this might be due in some way to the relationships between heat, work, and the intermediate physiological processes. It seems reasonable to assume he understood that the biological process of metabolism involves a considerable turnover in material from one chemical state to another and that in the process some mechanical work is involved. He seems to have been the first to perceive that the only alternative to a very general validity of the conservation of energy for all forms of energy, including heat, light, chemical energy, and mechanical work, was the operation of a vital force. From this he recoiled. He was astute enough to see that in Regnault's experiments on the specific heats of gases at constant pressure and volume there existed a means of estimating the mechanical equivalent of heat. In 1842 he gave this estimate (4.2 joules per calorie) with devastating accuracy. He went on to state the conservation of energy *as a general principle,* and applied his ideas to a theory of solar heat which invokes as a source of energy a ceaseless shower of meteors entering the sun's gravitational field. This theory was later propounded by Lord Kelvin.

Although Mayer anticipated Joule's careful experimental work on the mechanical equivalent of heat by two years, his claims to the enunciation of the principle of conservation of energy were attacked by physicists, notably Thompson and Tait, and Mayer suffered a great deal as a result. His case was later taken up by two able biophysicists, Tyndall and Helmholtz, and some of the harm was rectified. Even today, a biophysicist must be prepared to defend his discoveries against the skepticism of the more thoroughly entrenched branches of science.

That biology should imperatively demand the conservation of energy undoubtedly occurred first to Mayer. The energy turnover in biological systems still probably exceeds that of any other system on earth, and so only to a mechanical scientist is it remarkable that a biologist should first perceive the convertible nature of energy.

The years following Mayer's discovery were biophysically rich. The greatest biophysicist to date, Hermann Ludwig Ferdinand von Helmholtz (1821–1894), was beginning his work when Mayer made his estimate of the mechanical equivalent of heat in 1842. Helmholtz carried depth of understanding into both biology and physics equally. A list of his contributions alone would fill this chapter. He studied muscular contraction, measured the velocity of nervous impulses, did his share to confirm the law of conservation of energy, invented the ophthalmoscope (which permits observations on the retina), gave a quite reasonable theory of color vision, and aided in the understanding of hearing. The work of Helmholtz will be found to be the starting point of several complete topics considered in more detail later in this book. Some of the credit for the discovery of electromagnetic radiation should go to Helmholtz, for the experiments of Hertz were made in his laboratory and had the benefit of some of his inspiration. Helmholtz was an intellectual giant who purposefully followed scientific aims all his life.

Perhaps the greatest assets of Tyndall, a contemporary of Helmholtz, were his training by Faraday and his ability to give interesting and interpretative lectures. His lectures on "Heat, a mode of motion" are still fascinating to read, and he was clearly a master of experimental exposition. These qualities are essential in any field in which wide and clear understanding is more important than deep dedication to one subject, and they are most helpful in biophysics. Tyndall's curiosity was widespread; for example, his discovery of the scattering of light by submicroscopic objects enabled him to check on Pasteur's ideas of bacterial action. It is interesting to read of the Royal Institution being taken aback by the disastrous invasion of a durable organism, *Bacillus subtilis*, which resisted sterilization by boiling. Tyndall overcame the problem by using a method of alternate heating and cooling that preserved the food and destroyed *B. subtilis*. Tyndall believed that biology was a suitable field for physical study, and he was the first physicist to make a serious contribution to microbiology.

From the time of Tyndall's and Helmholtz' work until about 1930 there seems to have been a lull in contributions to the field of biophysics. This was probably due to the very rapid advances in fundamental physics, which took two first-rate biophysicists, Helmholtz and Gullstrand (who made basic contributions to ophthalmology), into pure physics in the latter years of the 19th century. The tremendous surge forward in atomic physics, which carried on until 1930, provided ample opportunity for the talents of physicists in that field alone. Paralleling this advance was that in the field of biochemistry, a science which proved itself capable of great value in examining some of the most fundamental problems of biology. And so, while atomic physics and quantum mechanics emerged on the one hand and enzymatic, vitaminic, and hormonal biochemistry developed on the other, biophysics was neglected.

By 1930 a change had already begun. A. V. Hill had started his study of thermal and energy effects in muscle, and his series of researches still continues. Astbury had used simple x-ray diffraction apparatus for the study of hair, silk, and wool fibers, and so discovered the three forms of fibrous proteins. Biomolecular x-ray work along these lines is still proceeding rapidly, and our knowledge of insulin, hemoglobin, myoglobin, and thymus nucleic acid is already reasonably precise. About 1930, Gates began a series of studies of biological action spectra, Hecht was involved in the biophysics of the sensitivity of the eye, and Loofbourow and Holiday began work on the absorption spectra of newly purified biological molecules. At the same time, Caspersson used the differential absorption of protein and nucleic acid to show the relation of these in the living cell.

The whole field of electrophysiology took a tremendous step forward with the work of K. S. Cole, Adrian, and Bronk, and the relation of electrical response to vision was actively studied by Hartline. Still more recently, Lea has examined the possibility of using ionizing radiation, with its unique penetrating capability, to study the size and nature of enzymes, viruses, genes, and chromosomes. The method shows promise, and will be increasingly exploited.

This renaissance of biophysics has already proved to be a strong movement, and exciting future contributions from it can be expected.

1–3 Life—order or chaos? One of the first reactions of a student of physics, particularly of experimental physics, is that of surprise at the number of precautions and preliminaries he must observe before the primary rules of Nature seem to apply to the system he is studying. The student of biology instinctively feels that it may prove impossible to reduce biological processes to order and understanding because of their inherent complexity and because they are not controlled systems. It is worth while to consider why this feeling should exist, and whether it is rationally sustained by what we know of living systems.

In the first place, consider the nature of truly chaotic forms of matter. The molecules of a gas are essentially the extreme in chaotic behavior. A statistical analysis of the behavior of a gas based solely on the conservation of energy and of number of molecules, and on the idea that the state of a gas can be described on the basis of probability alone, is quite successful. In such a system there is no continuous energy turnover, nor any ordered growth, nor any reproduction. None of the attributes of life seem to be part of such random, ceaseless, meaningless motion, and it requires a tremendous reach of intuition to explain living processes in terms of so over-randomized a world.

If we go a little further and suppose that a gas has superposed upon it an external field that is directed rather than random, the resulting distribution of gas molecules (as in an exponential atmosphere) is not that which seems to be found in living matter. Concentration gradients and the resulting diffusion processes undoubtedly play a role in the workings of a living cell, but they are not the *only* dominant factors. The gradients in a cell are multiple and changing, and can hardly be both effect and cause at the same time in so active a way. Therefore the extreme idea that primarily randomized processes underlie life is not very plausible.

Let us take exactly the opposite view and suppose that living processes are highly ordered and that they obey the known laws of Nature and