MOLECULAR BIOPHYSICS

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ACADEMIC PRESS New York • San Francisco • London 1977

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data

Volkenstein, Mikhail Vladimirovich, Date
Molecular biophysics.

Translation of Molekuliarnaia biofizika.
Includes bibliographies and index.
1. Molecular biology. 2. Biological physics.
I. Title. [DNLM: 1. Molecular biology. 2. Biophysics. QT34 V917ma]
QH506.V5813 574.8'8 73-18990
ISBN 0-12-723150-1

PRINTED IN THE UNITED STATES OF AMERICA

Preface

The science of biophysics, currently attracting many disciples, has counted among its practitioners long-established scientists such as Helmholtz, investigating the transmission of nerve impulses, and Maxwell, examining the phenomenon of color vision. The merger of biology and physics has attained consummation in the second half of this century concurrently with the development of sophisticated instrumentation and the molecular approach to the study of life phenomena.

In this volume, the fundamental principles of biophysics and their application to the study of the physical properties of biological macromolecules are presented. In a subsequent volume—"General Biophysics"—a thorough treatment of the thermodynamics of biological systems, an analysis of recognition, selection, and regulatory phenomena, as well as membrane, nerve excitation, mechanochemical, and photobiological processes will be examined.

Biophysics and biology are rapidly developing fields. New findings necessitate revision of old ideas and the introduction of new postulates. In the present book we have attempted to differentiate clearly between established principles and speculative ideas. It is our hope that the factual and the theoretical will be apparent to the reader.

I thank N. G. Esipova, G. V. Gursky, V. I. Ivanov, A. M. Jabotinsky, M. A. Livshits, A. I. Poletayev, Yu. A. Sharonov, and V. G. Tumanian for their valuable help in the preparation of this book.

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Chapter 1

Biology and Physics

1.1 Introduction

Inanimate and animate bodies are both composed of atoms and molecules and thus obey the general laws governing the structure and properties of matter and field. Contemporary physics is turning to the study of life, and the problem of the relationship between physics and biology has assumed particular interest.

That physics and biology are closely interrelated was recognized early in the development of the natural sciences. Later, however, as biologists gained deeper insights into the complexities and pecularities of the life processes, the paths of physics and biology diverged more and more, and the fundamental biological laws, primarily the Darwinian law of natural selection, came to be regarded as totally incompatible with physics.

The interaction of biology and chemistry evolved in a different way. The chemistry of life, organic chemistry, was at first considered completely distinct from inorganic chemistry, for it appeared that the substances present in living organisms could not in principle be obtained *in vitro*: they were amenable to analysis but not to synthesis. The formation of an organic substance was believed to require the participation of a special agent--a vital force. Organic chemistry was looked upon as a reliable basis for vitalism.

Chemistry, however, overcame this dichotomy. In 1777 Lavoisier showed that respiration and combustion were essentially identical processes in that both oxidized organic substances to form water and carbon dioxide. In 1828 Wöhler synthesized, for the first time, an organic substance, urea $CO(NH_2)_2$, from inorganic compounds. From this time on organic chemistry ceased to be the chemistry of life and became the chemistry of carbon compounds. Inspired by this achievement, the more farsighted thinkers of the last century rejected vitalism in favor of materialistic natural sciences. Engels, for example, developed the following ideas [1]: Chemistry brings us close to organic life and it has advanced far enough to guarantee that it alone will explain to us the dialectical transition to an organism;...It is necessary to attain only one more goal: to explain the rise of life from inorganic nature; in the current stage of scientific development this means only the following: to prepare protein bodies from inorganic substances. These ideas led Engels to his formula of life, which says that: Life is the mode of existence of protein bodies, the essential element of which consists in continual metabolic interchange with the natural environment outside them. This formula indicated the fundamental features of life, the knowledge of which thus came down to the understanding of the artificial synthesis of proteins.

We know now that the actual situation is not as simple as that. Some proteins have already been synthesized, yet the problem of life is far from being solved. If an organism is a protein system, we must understand how that system works. As Engels correctly noted, a prerequisite for this understanding is the consideration of exchange with the environment. Therefore we have to deal with an open protein system.

Science has demonstrated that proteins are indeed responsible for the functioning of living organisms. Life, however, requires many other low- and high-molecular substances, primarily nucleic acids, and is impossible without a diversity of interacting substances without chemical heterogeneity. An individual chemical substance of any complexity--whether a protein or a nucleic acid--does not live. It makes no sense to speak of living molecules. A living organism and any functional part thereof invariably represents a complicated heterogeneous system of interacting components, such as large and small molecules, ions, and supramolecular structures.

The advanced ideas of the 19th century were not able to overcome vitalism, proponents of which kept finding new arguments in the development of science itself.

Two great evolutionary theories were developed in the 19th century. The second law of thermodynamics (Clausius, Gibbs, Boltzmann) is suggestive of the evolution of matter in an isolated system toward its most probable state, characterized by maximum disorder, that is, maximum entropy. In contrast to this concept, the theory of biological evolution (Darwin) posits the increased orderliness and complexity of living systems from primitive microorganisms to *Homo sapiens* with his thinking brain. These two theories conflicted with each other because biological evolution, phylogeny, and ontogeny, were quite inconsistent with the equilibrium thermodynamics of isolated systems.

On the other hand, biology had a powerful impact on physics in the 19th century. The law of conservation of energy, the first law of thermodynamics, was discovered by Mayer, Joule, and Helmholtz. It is well known that Mayer based his theory on observations of living human organisms, but it is not so well known that Helmholtz also based his theories on biological phenomena, on a clear cut antivitalistic concept. He wrote that "according to Stahl, the forces acting in a living body are in fact physical and chemical forces of organs and things, but there is some vital soul or force inherent in the body which can tie up or set free their activity. ...I have found that Stahl's theory ascribes to any living body properties of the so-called *perpetuum mobile*. ...So I am confronted with the question of what kind of relations should hold between different forces of nature if we assume that the *perpetuum mobile* is impossible..." [2].

The basic question we have to answer when attempting to develop and study biophysics (i.e., the physics of living nature) is that of the interrelationship between biological and physicochemical phenomena. Two alternatives are apparent: either biology contains something basically foreign to physics and chemistry, or else life is a manifestation of physical and chemical processes occurring in complicated open systems; *tertium non datur*. Either biology contradicts physics or the contradiction is only apparent and the vitalistic theory is untenable whatever its form.

Modern vitalism does not deny the applicability of physics to the study of life processes but, as we will see, urges the creation of a new physics--one that has not yet been formed. On the other hand, physical interpretations of fundamental biological processes are often viewed as impermissible reductionism, striving to reduce the complicated biological laws to simpler physical ones.

However, arguments concerning "reducibility" or "irreducibility" are meaningless, for this approach is not an attempt to subordinate biology to physics, but rather an effort to ascertain the unity of animate and inanimate nature. Physics, as the science of matter and field, is by no means simpler than biology; hence, the notion of reductionism is inappropriate. One should speak not of reductionism but rather of the integration of different domains of knowledge. Thus it is quite clear now that chemical transformations do not involve any phenomena other than physical, and in this sense chemistry is "reducible" to physics. But this awareness in no way jeopardizes the independence or significance of chemistry; rather it provides chemistry with a much more solid and generalized foundation.

Nonetheless, the question whether contemporary physics can assure the knowledge of life phenomena is not devoid of meaning, and in this connection it is appropriate to discuss the concepts put forward by some biologists and physicists.

Bertalanffy developed ideas concerning the so-called general theory of systems (see [3]). He believed that biological phenomena could be understood in terms of the exact sciences and that the imaginary conflict with thermodynamics vanished as soon as it was recognized that organisms are open systems which exchange matter and energy with the external environment. But canonical thermodynamics was concerned with isolated systems, and a thermodynamics of open systems (nonequilibrium thermodynamics) was necessary in order to interpret biological phenomena in physical terms. Bertalanffy considered systems theory as a basis for scientific biology. The system is a totality of interacting objects, and its properties are not reducible to the sum of the properties of its constituent elements. By considering systems it is possible to explore the problems of organization and the integrity of dynamic interaction. These problems are of critical importance for biology.

At a time when the thermodynamics of open systems had not yet been formulated, Bauer discussed the nonequilibrium properties of organisms and devised his fundamental law of biology, which stated that "all living systems are never in equilibrium and use their own free energy to perform continual work against the equilibrium as required by the laws of physics and chemistry under given external conditions" [4]. Bauer's ideas were not understood by his contemporaries, nor for that matter are they appreciated by some present-day commentators (see, e.g., [5]). Although Bauer approached present-day biophysics, his works are of only historical interest today. It is important, however, that he tried to prove that life could be interpreted in atomic-molecular terms, saying that "a nonequilibrium state of living matter and, consequently, its constantly maintained work capacity, are determined ... by the molecular structure of living matter, while the source of the work performed by living systems is eventually the free energy peculiar to that molecular structure, to that state of molecules" [4].

Niels Bohr tackled the problem of the relationship between physics and biology on the basis of the principle of complementarity [6]. He considered the biological laws to be complementary to those obeyed by inanimate bodies and held that it was impossible to determine at one time the physicochemical properties of an organism and life phenomena, because knowledge of the former precludes knowledge of the latter. Life should be considered as the "basic postulate of biology, not susceptible of further analysis, in the same way as the existence of the quantum of action ... forms the elementary basis of atomic physics" [6, p. 21]. Bohr thus considered biological and physico-chemical studies to be complementary, that is, incompatible, though not contradicting each other. This concept has nothing in common with vitalism, for it rejects the existence of any limiting boundary in the application of physics and chemistry to the solution of biological problems. "... no result of biological investigation can be unambiguously described otherwise than in terms of physics and chemistry, just as any account of experience even in atomic physics must ultimately rest on the use of the concepts indispensable for a conscious recording of sense impressions" [6, p. 21].

Using the same complementarity principle, claims were made to the effect that knowledge of morphology and functionality, homology and analogy, environment and internal state, and heredity and adaptation were incompatible. It was thought that the investigation of some one aspect of a biological phenomenon so strongly affects the other aspects that the latter becomes unknowable in principle [7]. Because all noncommutative factors act concurrently in life, the latter is unknowable. One can study the atomic and molecular structure of an organism but one has to kill the organism in order to do so.

This viewpoint was not a new one. As Goethe's Mephistopheles said:

Wer will was Lebendig's erkennen und beschreiben,
Sucht erst den Geist herauszutreiben,
Dann hat er die Teile in seiner Hand,
Fehlt leider! nur das geistige Band.
Encheiresin naturae nennt's die Chemie,
Spottet ihrer selbst, und weiss nicht wie.

Encheiresin naturae is the way of nature, the mode of its action. Goethe thought that an organic creature is so many-sided externally and so diverse and inexhaustible internally that it is impossible to pick out a sufficient number of initial points to survey it or to develop a sufficient number of organs so as to disassemble it without killing it.

Bohr's views changed with the development of contemporary biology. He referred later to a complementarity between "arguments based on the full resources of physical and chemical science, and concepts directly referring to the integrity of the organism transcending the scope of these sciences" [6, p. 76]. The application of the complementarity principle in biology was argued to be justified not by the postulative character of the concept of life but rather by the extreme complexity of the organism as an integral system. In his last lecture on this subject [8] Bohr referred only to the practical rather than the fundamental complementarity involved in the inexhaustible complexity of life. In his letter to the present writer (reproduced in [9]) Bohr in fact abandoned the views contained in his earlier papers, saying "I am well aware that some of my early utterances have caused misconception of my general attitude."

In 1945 Schrödinger published a farsighted book devoted to the relationship between physics and biology [10]; in it he discussed three problems of fundamental importance for biophysics. The first problem concerned the thermodynamic bases of life. The distinction between an organism and an inanimate body consists, he said, in the high orderliness of the organism, which is similar in this respect to an "aperiodic crystal," and in the ability of this orderliness to sustain itself and to produce ordered phenomena. This is a matter of self-regulation and self-reproduction of organisms and cells. Schrödinger attributed this property to the fact that any organism is an open system in a nonequilibrium state owing to the outflow of entropy to the environment. Organisms continuously create "order from order," "extract order from the environment" in the form of "a well ordered state of matter in foodstuffs." He provided an answer to the question of the cause of macroscopicity, the multiatomicity of organisms. In a system consisting of a small number of atoms, fluctuations should destroy any order. It is precisely because of their multiatomicity that organisms can exist in accordance with the laws of thermodynamics.

The second problem is the molecular basis of life, regarding which Schrödinger argued in favor of a materialistic interpretation of the molecular nature of genes and raised the question of the structure of the hereditary substance and the reasons for its stable reproduction in a series of generations. This question was answered by molecular biology, the origin and development of which were much stimulated by the Schrödinger book.

A third problem was that of the quantum-mechanical laws, which are clearly manifested in radiobiological phenomena. In discussing the works of Timofeev-Ressovsky, Delbrück, and others, Schrödinger emphasized that biological processes are consistent with the laws of physics.

Schrödinger's book is very important because in it he not only showed that physics does not contradict biology, but he also correctly outlined the future of biophysics.

Elsässer opposed physics to biology [11]. The store of information contained in the original germ cell, the zygote, is much smaller than that in the adult multicellular organism. In his view, the increase in the amount of information is inexplicable in physical terms because this is a specific "biotonic" regularity. His ideas on this matter will be detailed later (p. 22).

Wigner believed that the self-reproduction of biological molecules and organisms contradicted quantum mechanics [12] and held that the probability of existence of self-reproducing states was practically zero. According to him, the Hamiltonian describing the behavior of a complex system can be represented by a random symmetrical matrix. The state of an organism can be described by a vector v, and an analogous vector w designated for nourishing products. The common vector for the organism plus food is then

$$\phi = v \times w \tag{1.1}$$

After reproduction, we get the vector

$$\psi = v \times v \times r \tag{1.2}$$

where r describes the results of metabolism and the coordinates of two organisms. We have an N-dimensional space for the organism and an R-dimensional space for r. If the "collision matrix" S which represents the final state resulting from interaction between organism and food is a random, stochastic matrix, then

$$v_k v_\lambda r_\mu = \sum_{k',\lambda',\mu'} S_{k\lambda\mu,k'\lambda'\mu'} v_{k'} w_{\lambda'\mu'} \tag{1.3}$$

We get N^2R equations. The number of unknowns (N values of v, R values of r, and NR values of w; i.e., N + R + NR) is much smaller than the number of equations. It would be a miracle if these unknowns were to satisfy the written expression. Wigner followed Elsässer in considering the reduplication of biological macromolecules to be a "biotonic" phenomenon.

In actual fact, as shown by Eigen [13], the matrix S is not a random one. Wigner did not take into account the instructive functions of informational macromolecules. The entire presentation given by Wigner contradicts reality and his conclusion that it is necessary to modify quantum mechanics to make it applicable to biology proved to be untenable. At the same time, the application of quantum mechanics to macroscopic systems requires special consideration.

An important paper by Eigen [13] devoted to the self-organization and evolution of biological macromolecules produced convincing arguments in favor of the sufficiency of contemporary physics for the explanation of biological phenomena.

A living organism is an open, self-regulating, and self-reproducing heterogeneous system, whose most important functional substances are biopolymers--proteins and nucleic acids. Such a system must be investigated physically and chemically. Our knowledge of it should rest on the elucidation of the physical features of life, that is, the physical considerations of the development of nonequilibrium, orderliness, and systematicity in the organism.

1.2 Finalism and Causality

Before discussing the physical basis of life phenomena, we will consider an important feature of life that is usually considered to contradict physics. Biology naturally makes use of a finalistic treatment of the phenomena under study. The development of a zygote into an adult organism can be described using the notion of the goal. The goal of development is the formation of an organism. Its structure is expedient, in that it corresponds to the conditions of existence. Even in the early stages of embryogenesis, definite groups of cells are predestined to develop into some definite organ, and this determines their functionality down to the molecular level. Phylogeny, or evolutionary development, can be described in the same way. This development is directed toward the greatest adaptation of the population (the elementary evolutionary system) to the external conditions.

In this sense an organism is like an engine designed according to some plan for the attainment of a definite goal. Scientific biology does not, of course, consider developmental processes in teleological terms. The attainment of a goal in ontogeny and phylogeny is a result of real causes (natural selection, etc.). Emphasizing the existence of some plan of development, Monod introduced the notion of teleonomy as opposed to teleology [14], having in mind the causality of development. The extraordinary complexity of a living organism (a "living engine") determines its finalistic description, which is not peculiar to conventional physics and chemistry. It is obvious that a statement like: "Sodium and chlorine ions interact for the purpose of forming a cubic crystal" is meaningless. On the contrary, the statement "because sodium and chlorine ions have such and such charges and radii, a NaCl crystal must belong to the cubical crystal system" possesses a clear meaning. Physicists usually ask "why?," whereas biologists often ask "what for?"

The notion of expediency is closely linked with that of optimality. Optimality means attaining some result (goal) through the smallest possible expenditure of energy, the formation of a system which would best execute certain functions, etc.

Biological finalism expresses, on the one hand, the complexity of biological phenomena and structures which prevents their causal explanation at the atomic-molecular level. On the other hand, it characterizes the irreversibility and "antientropicity" of development which implements a plan, a program, the instructing action of information (see p. 25). In actual fact, there is no contradiction between finalism and causality. Finalism arises in physics whenever its principles are formulated as variational. Here are some examples.

A very general formulation of the law of motion of mechanical systems is contained in Hamilton's principle of least action. The Lagrange function $L(q, \dot{q}, t)$, depending on time, coordinates, and velocities, satisfies the condition

$$S = \int_{t_1}^{t_2} L(q, \dot{q}, t)\, dt = \text{Minimum} \quad (1.4)$$

In other words, the variation δS is equal to zero. The action S is minimal, that is, the system moves between two sets of coordinates $q^{(1)}$, $q^{(2)}$ and velocities $\dot{q}^{(1)}$, $\dot{q}^{(2)}$, corresponding to the times t_1, t_2, in such a way that S becomes minimal. *The goal of the mechanical system is its minimal action* and its motion is optimal in this sense.

But expression (1.4) is equivalent to the Lagrange equations of motion

$$\frac{d}{dt}\frac{\partial L}{\partial \dot{q}} - \frac{L}{q} = 0 \quad (1.5)$$

The Lagrange function L expresses the difference between kinetic and potential energy

$$L = \sum_a \frac{m_a v_a^2}{2} - U(r_1, r_2, \cdots) \quad (1.6)$$

where m_a are masses, r_a are radius vectors, and v_a are velocities of material points. Equations (1.4) and (1.5) can be rewritten in terms of Newton's equations of motion

$$m_a \frac{d^2 r_a}{dt^2} \equiv m_a \frac{dv_a}{dt} = - \frac{\partial U}{\partial r_a} \quad (1.7)$$

The finalistic expression (1.4) comes down to the causal equations (1.7) describing motion as a result of the action of forces. Other examples of physical laws formulated in finalistic terms include Fermat's principle in optics, Le Chatelier's principle in thermodynamics, and Lenz's rule in electrodynamics. The number of such examples is in fact unlimited.

The equations of motion (1.8) of mechanics are reversible, since they contain only the second derivatives in time and therefore do not vary if the sign of time is reversed, $t \rightarrow -t$. However, the equations of mechanics have solutions corresponding to either stable or unstable equilibria and motions. Neither state of equilibrium of the pendulum shown in Fig. 1.1 contradicts statics, but state 1 is stable and state 2 is unstable. There always exist small forces and small deviations from the initial state of a material system which perturb equilibria and motions. These perturbations do not change

FIG. 1.1 Stable (1) and unstable (2) equilibrium of a pendulum.

state 1 but do strongly change state 2. Equilibria and motions which are slightly changed by small perturbations are stable and those strongly changed are unstable. But what do "slightly" and "strongly" mean? The general problem of stability of motion was solved in the classical work of Liapounov (1892), who formulated the criteria of stability [15,16]. If any perturbation however small (but not zero), alters the magnitude of some characteristic of motion in such a way that this magnitude deviates more and more from its value in unperturbed motion, then the unperturbed motion is unstable relative to this characteristic. The motions of the pendulum at small deviations from equilibrium state 1 are described by the equation

$$\ddot{\phi}+\omega^2\phi = 0 \tag{1.8}$$

where ϕ is the angle of deviation and ω is the cyclic frequency of oscillation, equal to

$$\omega = 2\pi(g/\ell)^{1/2} \tag{1.9}$$

where g is the gravitational acceleration and ℓ is the pendulum length. The solution of (1.8) is

$$\phi \equiv x = A\cos(\omega t+\alpha), \quad \dot{\phi} \equiv y = -A\omega\sin(\omega t+\alpha) \tag{1.10}$$

in which A is the amplitude of vibration and $A\cos\alpha$ is the initial phase. Excluding t from expressions of x and y, we get a set of trajectories of motion at the phase plane x,y differing by values A:

$$(x^2/A^2)+(y^2/A^2\omega^2) = 1 \tag{1.11}$$