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PHYSICS OF MUSCLE CONTRACTION

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I. INTRODUCTION

THE vigorous growth of biology in recent decades has led it to link up with physics and chemistry. The founding of molecular biology and biocybernetics has brought about a revolution in the scientific world outlook, and we are its witnesses and participants. Modern biophysics starts with the idea that the phenomena and objects of living nature differ from those of non-living nature in being incomparably more complex, but not in obeying any especial "biotonic" laws (e.g., as postulated by Elsasser[1]) that cannot in principle be recognized by the means of exact science. There is no place for vitalism in modern science.

Very large circles of physicists have currently turned their interests to biology. A number of the fundamental phenomena of life are mysterious, and studying them promises great discoveries, both in science and in its applications (in medicine and agriculture). Often an aberration arises—it seems that whatever biological problem a physicist takes up, his theoretical concepts and experimental methods rather soon lead to solving very important problems. The actual situation differs. Thus far, in biology itself, in biochemistry, in cytology, etc., only a few biological problems have been studied well enough to permit a clearcut formulation of the physical problem and effective application of the ideas and methods of theoretical and experimental physics to solve it. Among these problems is muscular contraction, a classical problem of biophysics.

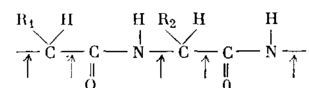
Biophysics is an old science. However, its content has decisively changed in recent years. Previously, biophysics preferentially studied physiological phenomena: the functioning of the sense organs, propagation of nerve excitation, muscular activity, and the general thermomechanical properties of organisms. With the development of molecular biology, cybernetics, and the thermodynamics of open systems, biophysics has turned to study the fundamental phenomena of life: heredity and variability, the function of biological molecules in connection with their structures, the molecular nature of physiological phenomena, and the behavior of an organism as a whole system. Correspondingly, people now usually subdivide biophysics into molecular biology, cell biophysics, and biophysics of complex systems. The problem of muscular contraction is usually assigned to the second division, but, as we shall see, its study has led to discovery of the molecular mechanism of the phenomenon.

The founding of a genuine biophysics requires integration of its three cited divisions, i.e., extending atomic-molecular treatment to processes pertaining to whole systems. The latter are today mainly studied phenomenologically, by physicomathematical, cybernetic model building.

A living organism is a complex self-regulating open system whose most important functional substances are proteins. The self-regulation and self-control of an organism are not effected in the way that they are in the currently existing artificial cybernetic systems. Molecules or ions serve as the direct and feedback signals in an organism. Signal reception amounts to "recognition" of a molecule by a molecule or by a supermolecular structure through various interactions. In this sense, an organism is a chemical, rather than an electromagnetic machine.

Life is impossible without mechanical motion, without movement in space of the organism and its functional parts. Even mitosis (cell division) involves an entire set of motions that culminate in the separation of the paired chromosomes and formation of two new cells. Muscular contraction is the best-studied process of mechanical motion in living nature.

Muscle fibers are composed of proteins, and muscular contraction amounts to a change of state of the protein molecules. Proteins are natural polymers, or compounds of high molecular weight, formed of 20 types of monomeric amino-acid residues (see, e.g.[2,3]). The pattern of chemical structure of a protein chain is as follows:



The R are various radicals, or groups of atoms, occurring in the amino-acid residues. Internal rotations can occur about the C—C and N—C bonds marked by arrows. These give rise to various conformations of the chain that possess differing free energies. Usually certain particular conformations are stabilized in a protein. Hydrogen bonds between the N—H atoms of one link and the C=O atoms of another play an essential role in this stabilization (see[2-4]). Stable conformations have been theoretically and experimentally shown to exist in proteins, even when in solution. Among these conformations, the most important are the so-called α-helix (Fig. 1) and the cross-β form (Fig. 2). In the first stabilizing structure, the hydrogen bonds lie parallel to the axis of the helix, and in the second, they are perpendicular to the chains that they join. However, not nearly all links of protein chains (or amino-acid residues) are ordered in this fashion. Together with the ordered regions, protein molecules contain more labile, disordered regions. Figure 3 shows schematically the structure of the important protein, myoglobin, as established by X-ray structural analysis. The molecule contains α-helical (cross-hatched) regions and disordered regions. We see that the protein molecule as a whole has a highly complex, specific structure.

The free energies are small in the conformational rearrangements of a protein of the type $\alpha \rightleftarrows \beta$, etc.,

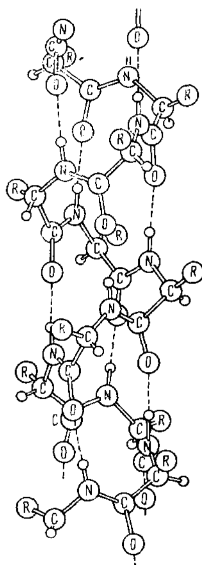


Fig. 1. The α-helix.

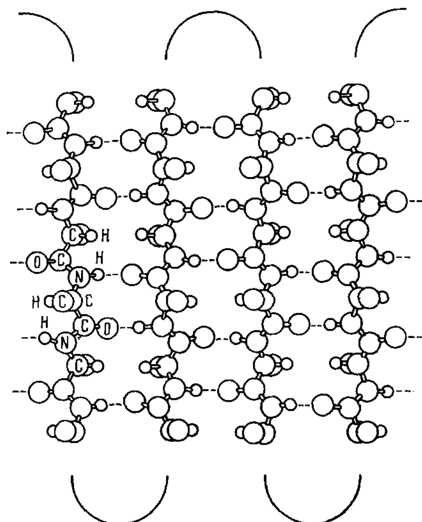


Fig. 2. The cross-β form.

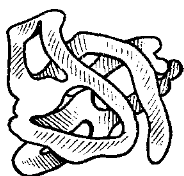


FIG. 3. Diagram of the spatial structure of the myoglobin molecule.

or order ⇄ disorder, that occur by internal rotations about the C—N and C—C single bonds. Hence, such transformations are always possible. In particular, they occur when the protein interacts with other molecules or ions. They alter the state of the protein molecule and its spatial structure. A supermolecular system made of protein molecules undergoing conformational transformation can develop mechanical stress and do mechanical work. As we shall see, this is just how the contractile proteins of muscle or of other biomechanical apparatus function. The contractile proteins serve as the working substance of the biomechanical machine. But what do they consume in working? Where does the energy come from that is transformed into mechanical work?

II. MECHANOCHEMICAL PROCESSES IN MUSCLE

Biological contractile systems function under conditions of constant temperature and pressure. Thus muscle and other biological motile apparatus are not heat machines. There are also no grounds for searching for energy sources in any electric, or a fortiori, magnetic processes. (We note that the mention sometimes found in the literature of the effect of a magnetic field on vital processes rests on no reliable experimental data.)

The function of muscles and other biological motile systems consists in mechanochemical processes. This involves direct transformation of chemical energy into mechanical work (and vice versa), while bypassing a thermal stage. The reagents go from a higher to a lower chemical potential, and the system releases mechanical energy. In the reverse process, mechanical work done on a system increases the chemical potential of the substance.

Before we discuss the concrete biomechanical mechanisms and kinetics of the mechanochemical process in muscle, we need a phenomenological description, the thermodynamics of reversible mechanochemistry. The appropriate cycles were first studied by Katchalsky[5] and by Kuhn and Hargitay.[6] A sufficiently complete review for our purposes is given in the article[7] (see also[2], Chap. 9).

The variation in internal energy of a mechanochemical system can be written in the form

$$dE = T\,dS - dA + \sum_i \mu_i\,dn_i + \psi\,de + \ldots, \tag{1}$$

where dS is the variation in entropy, dA is the work done by the system, dn_i is the amount of substance i introduced from a reservoir into the system at the chemical potential μ_i, and ψ de is the electrical work defined by the change in charge de at the electrostatic potential ψ. For simplicity, we shall restrict ourselves to a system consisting of uniform fibers of length L extended by a force f. In this case

$$dA = p\,dV - f\,dL \tag{2}$$

and expression (1) takes on the form

$$dE = T\,dS - p\,dV + f\,dL + \sum_i \mu_i\,dn_i. \tag{3}$$

If we construct a mechanochemical thermodynamics on this basis, we obtain, as usual, differential relations. Let us write down some of them:

$$\left(\frac{\partial f}{\partial \mu_i}\right)_{L,n_j} = -\left(\frac{\partial n_i}{\partial L}\right)_{\mu_i,n_j}, \quad \left(\frac{\partial f}{\partial \mu_i}\right)_{L,\mu_j} = -\left(\frac{\partial n_i}{\partial L}\right)_{\mu_i,\mu_j},$$
$$\left(\frac{\partial f}{\partial \mu_i}\right)_{n_i,n_j} = -\left(\frac{\partial n_i}{\partial L}\right)_{f,n_j}, \quad \left(\frac{\partial f}{\partial \mu_i}\right)_{n_i,\mu_j} = -\left(\frac{\partial n_i}{\partial L}\right)_{f,\mu_j}, \tag{4}$$

or for short,

$$\frac{\partial f}{\partial \mu_i} = -\frac{\partial n_i}{\partial L}. \tag{4a}$$

On the other hand,

$$\frac{\partial f}{\partial n_i} = \frac{\partial \mu_i}{\partial L}. \tag{5}$$

Let us assume that there is only one reacting chemical component. Thus the system has two degrees of freedom (at constant p and T), let us say f and μ. We can plot the working cycle of the "mechanochemical machine" on the fL plane. Each value of μ will correspond to an L(f) curve that we can call an isopotential (like an isotherm in the pV plane for a thermal engine). All the points of such a curve can be obtained from a mechanical experiment performed at a constant value of μ. On the other hand, an L(f) curve at constant n resembles an adiabatic curve. Such a curve is called an isophore. The isopotentials and isophores are represented on the μn plane by straight lines parallel to the coordinate axes. The $\mu(n)$ curves on this plane can be called isotonic if at constant f, and isometric at constant L.

Figure 4 shows a cycle in the fL plane. The work is given by the expression

$$A = -\oint f\,dL = \int_1 \mu\,dn + \int_2 \mu\,dn = (\mu_1 - \mu_2)\,\Delta n, \quad (6)$$

which resembles the expression for the work in the Carnot cycle:

$$A = \oint p\,dV = (T_1 - T_2)\,\Delta S. \quad (7)$$

The essential difference in these two cases is that a mechanochemical machine cannot be characterized by a value of the efficiency similar to

$$\eta = (T_1 - T_2)/T_1,$$

since there is no absolute zero for the chemical potential. However, a mechanochemical process can be characterized by a coefficient expressing the ratio of the work obtained in the actual cycle to that for an ideal reversible cycle:

$$\eta' = -\oint f\,dL \Big/ \oint \mu\,dn. \quad (8)$$

Model mechanochemical systems have now been realized. A filament made of a polyelectrolyte fiber contracts or stretches, depending on the electrolyte concentration in the surrounding medium. A polyelectrolyte is a polymer, each link of which contains an ionizing group. If these groups are neutral, then the macromolecular chain of the polymer winds up into a random coil (see[2,4,8]). Conversely, electrostatic repulsion between charged groups unwinds the chain, and thus extends the fiber. Katchalsky and Oplatka have built a continuously-operating mechanochemical machine in which the working substance is a polymer fiber alternately immersed in a salt solution and in pure water (at potentials μ_1 and μ_2). The machine stops working when the salt concentration in the two reservoirs becomes the same. Just as a thermal engine acts as a refrigerator when run in reverse by expending work, this machine in the reverse process can be used to extract salt from a solution. In principle, this type of apparatus can be used to desalt sea water.

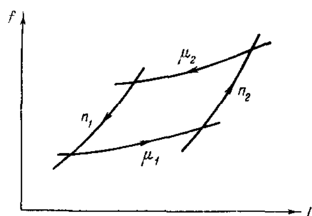
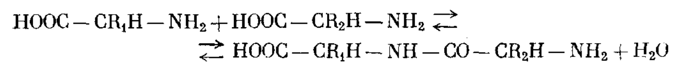


FIG. 4. A mechanochemical cycle.

A number of attempts have been undertaken to explain the action of biological, mechanochemical systems, based on ideas of their polyelectrolyte nature, in analogy with the systems just described. As will be discussed below, these attempts have not culminated in success.

The current state of molecular biophysics makes it possible to formulate two fundamental principles that are natural starting points in explaining the operation of a biological mechanochemical system. Not only motor activity requires an energy source. All fundamental biological processes use up energy. The biosynthesis of protein, or linkage of amino acids into a protein chain, is an endergonic process. That is, it is a process that involves an increase in free energy. This evidently follows from elementary considerations, from the law of mass action. In fact, a reaction of the type



$$HOOC-CR_1H-NH_2 + HOOC-CR_2H-NH_2 \rightleftarrows HOOC-CR_1H-NH-CO-CR_2H-NH_2 + H_2O$$

occurs in the cell in an aqueous medium. Hence, the equilibrium should be shifted far to the left. In biosynthesis, this reaction is coupled with other processes that make up the deficit in free energy, i.e., with exergonic processes.

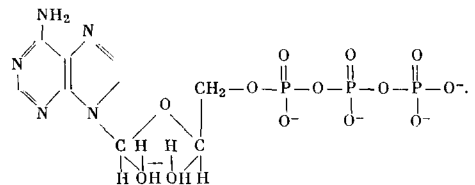
The thermodynamics of a living organism or any of its functional parts is the thermodynamics of an open system that exchanges both matter and energy with the environment. The general phenomenological theory of such systems has been developed in the modern thermodynamics of irreversible processes in a good approximation (for linear processes), and it has already played a very important role in biophysics (see, e.g.[9]).

Free energy is consumed in the active transport of molecules and ions in the cell through the membranes surrounding them. Active transport occurs in the direction opposite to the concentration gradient. Thus, the ion concentrations in the sartorious muscle of the frog and in the surrounding medium are characterized by the following values:

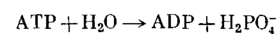
outside: $[Na^+] = 15$ mM, $[K^+] = 125$ mM, $[Cl^-] = 1{,}2$ mM,
inside: $[Na^+] = 110$ mM, $[K^+] = 2{,}6$ mM, $[Cl^-] = 77$ mM.

It takes expenditure of energy to maintain the concentration differences.

In these and other cases, the living cell uses the universal biological energy accumulator, adenosine triphosphate (ATP). The structural formula of this very important compound is



Hydrolysis of ATP, i.e., cleavage of a phosphoric acid residue to form adenosine diphosphate (ADP)



$$ATP + H_2O \rightarrow ADP + H_2PO_4^-$$

involves release of free energy to the extent of

7–10 kcal/mole. It is precisely this free energy that makes possible the processes of vital activity in the cell. As we shall see, this same ATP serves as the source of muscular work. Quantum-mechanical calculations actually explain the elevated amounts of energy in the phosphate bonds of ATP.[10] However, ATP is cleaved during the vital activity of cells, i.e., the accumulator is discharged. It has to be recharged. This results from respiration, in the process of the so-called oxidative phosphorylation (see, e.g.[11,12]). Thus, as we might suppose (and as has actually been shown), the fundamental energy source for the biomechanical process is ATP. However, this situation does not explain at all what is the actual molecular mechanism of conversion into mechanical work of the free energy that is released when phosphate is cleaved from ATP.

A second principle can be formulated as a molecular hypothesis. The working substances of a biomechanical system are proteins. As I have said, these macromolecules can undergo conformational transitions. We can suppose that the pulling or pushing force developed in a supermolecular protein system arises from change in the conformations of protein molecules. Naturally, this assertion demands experimental proof. The hypothesis consists in the idea that a conformational transition of a protein (or proteins) occurs upon cleavage of ATP, and this performs work. The mechanism of transformation of free energy of ATP into energy of conformational rearrangement of a protein must also be studied.

The source data for these ideas come from the classical study of Engel'gardt and Lyubimova.[13] They showed that the muscle protein myosin is an ATPase. That is, myosin acts as an enzyme that catalyzes the hydrolytic splitting of ATP.

Thus, in biological mechanochemistry, the chemical potentials pertain to ATP and to the contractile proteins in various conformational states.

Before we turn to the molecular mechanism of muscle contraction and to its biochemistry and energetics, we must discuss the structure of muscle and its changes during contraction.

III. THE MICROSCOPIC STRUCTURE OF MUSCLE

The vertebrates have three types of muscles: smooth muscles, which are found in the walls of the viscera, the cross-striated muscle of the heart, and the cross-striated skeletal muscles. The discussion to follow will deal mainly with the cross-striated skeletal muscles, for most of the experimental results have been obtained by studying the so-called sartorious muscle of the frog. The fibers of skeletal muscles are characterized by a cross-striated structure that is easily observed under the ordinary microscope. The fibers themselves have diameters from 20 to 80 μ. Each fiber consists of 1000–2000 thinner fibers, or myofibrils, of diameter 1–2 μ. The electron-microscopic studies of Huxley and Hanson[14-17] have shown that the myofibril in turn is built of two types of filaments, thick and thin. It was later shown that the thick filaments are made of the protein myosin, and the thin ones of actin. Fig. 5 shows the microscopic structure of a myofibril, which shows hexagonal symmetry in cross-section (Fig. 6). Figure 7 shows a longitudinal section of muscle fibrils, and Fig. 8 shows the schematic structure of a myofibril. The dark lines in Fig. 7 are the so-called Z-lines. They can also be seen in Fig. 5. The region of a myofibril between two Z-lines is called a sarcomere. It is divided into several zones (see Fig. 8). The central band (A) is anisotropic, and shows birefringence. The isotropic I-bands abut it symmetrically on both sides. When a resting muscle is stretched, an H-zone of lower density appears in the middle of the A-band. All these zones can be observed well in the phase-contrast microscope.

FIG. 5. An electron micrograph of a myofibril.

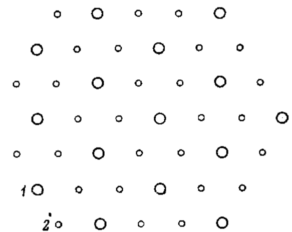


FIG. 6. Diagram of a region of the cross-section of a myofibril. 1–thick filament, 2–thin filament.

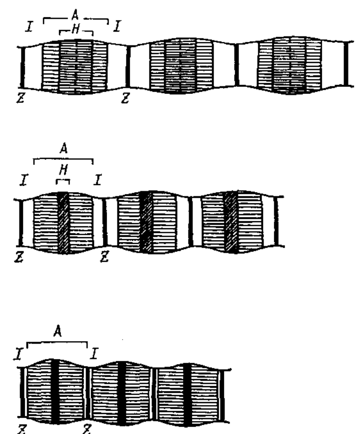


FIG. 7. Longitudinal section of a myofibril at three different lengths.

Figure 9 shows the molecular structure of the sarco-

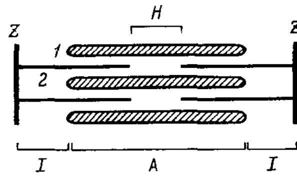


FIG. 8. Diagram of the structure of the sarcomere. 1–thick filament, 2–thin filament.

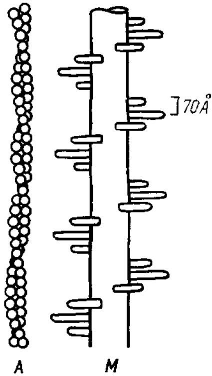


FIG. 9. Diagram of the structures of thick (M) and thin (A) filaments according to electron-microscopic data.

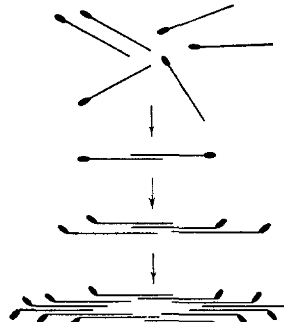
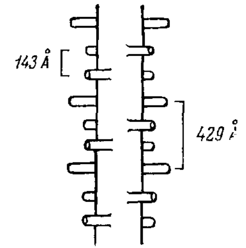



FIG. 11 FIG. 12

FIG. 11. Diagram of the aggregation of myosin molecules into a thick filament.

FIG. 12. Diagram of a thick filament according to x-ray diffraction data.

mere. The sarcomere is made of thick and thin protein filaments. Each thick filament consists of 180–360 longitudinally-oriented myosin molecules, which are responsible for the anisotropy of the dense A-band. The less dense I-band is made of the thin filaments of the protein actin, the molecules of which are double helices (the F-form of actin). The latter arises from polymerization of the globular G-actin. The sarcomere contains about 800 of these G-globules per thin filament.

The structure of the myosin molecule is highly specific. Figure 10 shows a diagram of it, and indicates the molecular weights and longitudinal dimensions of the three regions of the molecule: light meromyosin (LMM), and the two fragments of heavy meromyosin (HMM). The myosin molecules are aggregated in the thick filaments of the sarcomere in such a way that their LMM tails are packed parallel to one another, while the HMM S-1 heads stick out on both sides of the thick filament. One can also obtain this structure in vitro, i.e., prepare artificial thick filaments from a myosin solution. Figure 11 shows a diagram of the aggregation of myosin molecules into a thick filament, as established by electron microscopy. The heads of myosin in the sarcomere stick out of the thick filaments, and can attach to the actin thin filaments to form bridges. Figure 12 shows the structure of the thick filaments of muscle, as established by x-ray diffraction.[18,19]

When muscle contracts, the I-bands shrink, while the A-bands don't change in length. The Z-lines, i.e., Z-disks, which look like lines in a longitudinal section, approach one another. Finally the I-bands disappear altogether, and a denser region appears in the center of the sarcomere. The volume of the sacrcomere changes little upon contraction; consequently, the sarcomere becomes thicker (see Fig. 7).

These phenomena show that the material of the A-band must be more rigid than the material of the I-bands. The observed changes in optical density can be explained only by assuming that matter is displaced in the sarcomere during shortening. Electron microscopy has made it possible to study the contraction of the sarcomere in detail. The results of these studies unequivocally lead to the so-called sliding model of muscle, which is shown schematically in Fig. 13. The actin filaments are attached to the Z-disks (membranes). During contraction, the thick myosin M-filaments move in between the thin actin A-filaments, and the sarcomere contracts like a telescope. This results from the action of the HMM bridges that link the M- and A-filaments. In the normal physiological state of muscle, the M- and A-filaments overlap in such a way that all possible bridges can be formed (Fig. 13a). This is not the case upon stretching (Fig. 13b). Apparently, the A-filaments become deformed at high degrees of contraction (Fig. 13c).

Myosin proves to be responsible for the anisotropy of the A-bands: there is no myosin in the I-bands, and hence, actin in itself does not give rise to anisotropy.

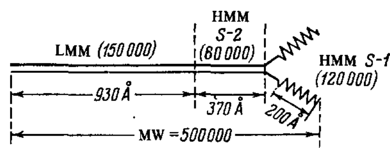


FIG. 10. Diagram of the structure of the myosin molecule. (MW = molecular weight).

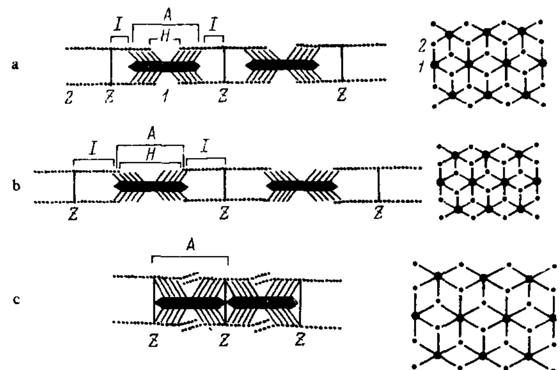


FIG. 13. The sliding model. a) The normal structure of muscle, b) stretched muscle, c) highly shortened muscle.

Study of muscle fibers by small-angle x-ray scattering totally confirms the results of the electron-microscopic studies, and considerably supplements them.[18-20] Since the volume of muscle does not vary upon contraction, one might expect that the distances between the filaments will be inversely proportional to the square root of the sarcomere length. Measurements of the equatorial x-ray reflections give a linear relation between these quantities. This indicates that the bridges lengthen as the muscle contracts. That is, the HMM projections are flexible. On the other hand, small-angle x-ray diffraction confirms the invariability of the lengths of the A- and M-filaments.

In the state of rigor in muscle, the myosin heads form bridges with actin at many places. Helical periodicity (see Fig. 12) is abolished, the 429-Å period disappears, and is replaced by layer lines at 360—380 Å. At the same time, the 143-Å period persists. These phenomena also arise from the flexibility of the bridges, while the fundamental skeleton of the thick M-filaments is preserved.

Thus, the structural studies lead to two fundamental conclusions. First, the sliding model is valid. Second, the myosin is drawn in between the actin filaments and approaches the latter only via the flexible bridges that link actin with myosin. These bridges are made of the HMM projections.

IV. BIOCHEMISTRY OF MUSCLE

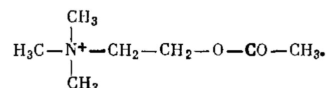
Many years of study by biochemists have now made it possible to define the chemical processes in muscle with great clarity. As I've said, the fundamental biochemistry of muscle consists in the enzymatic activity of myosin discovered by Engel'gardt and Lyubimova: its ability to catalyze the hydrolytic cleavage of ATP.[13] This reaction occurs only in the presence of Ca^{++} ions, and the greatest rate of cleavage here corresponds to equimolar content of Ca^{++} and ATP (see[21]). The effect of ATP on muscle contraction can be demonstrated by an elementary lecture experiment. A muscle fiber extracted in glycerol is put into a vessel of solution containing the ions Mg^{++} and Ca^{++} and univalent ions to give an ionic strength of the order of 0.25 or less. It suffices to drop in a solution of ATP to make the fiber contract visibly.

Experiments with the isolated proteins show that actin actually interacts with myosin to form the complex polymeric protein F-actomyosin. This complex is cleaved by the magnesium salt of adenosine triphosphate, or $MgATP^{2-}$.

Activation of the ATPase by Ca^{++} ions in the absence of Mg^{++} requires one Ca^{++} ion per active center of myosin.

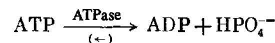
Excitation (activation) of cross-striated skeletal muscle results from transmission of a nerve impulse to the muscle. Contraction can also be initiated artificially by an electrical impulse. In line with what I've said above on the chemical and molecular nature of signal transmission in living organisms, the chemical nature of the innervation of muscle has been deciphered. A very small concentration of Ca^{++} ions in the presence of high concentrations of ATP and Mg^{++} ions activates contraction. The idea has naturally arisen of a "calcium pump,"[21] which operates when acted on by a nerve impulse.

Each fibril is surrounded by a complex system of longitudinal and transverse thin vessels, which as a whole are called the sarcoplasmic reticulum. It has been shown with tagged Ca^{45} atoms that application of a nerve or electrical impulse causes Ca^{++} ions to leave the sarcoplasmic reticulum and enter the liquid bathing the protein filaments, or sarcoplasm. Propagation of a nerve impulse along a nerve fiber or axon means the propagation of a depolarization wave along the membranous envelope of this fiber. The permeability of the membrane is altered: Na^+ ions enter it, while K^+ ions exit. After the pulse has passed, the initial state, which has an excess of K^+ inside and Na^+ outside, is restored again at the expense of ATP energy. When the impulse reaches the nerve ending, or synapse, at the muscle, it releases a specific substance from microscopic vesicles that occur at the synapse. This substance is acetylcholine:

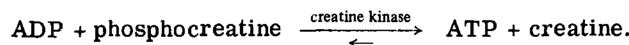


Acetylcholine depolarizes the muscle membrane (certain regions of the Z-membrane), and the final result of these events is release of Ca^{++} ions into the sarcoplasm (see[21,22]). Thus, ATP is necessary both for innervation and for contraction of muscle. Of course, these two processes are stimulated by different ATP molecules.

For a long time, no one could prove by direct experiments that the work performed by a muscle was proportional to the consumption of ATP. The trouble is that ATP is formed again immediately from ADP by cleaving a phosphate group from the so-called phosphocreatine. Concurrently with the reaction



there occurs the Lohmann reaction



Even recently, a number of studies treated ATP only as a plasticizer, or as a substance that is consumed in the relaxation, rather than contraction of muscle. That is, it is consumed upon return to the resting state (see[2]). However, experiments of recent years have directly shown that it is precisely during the contraction of muscle that ATP is cleaved. A reagent has been found that inhibits creatine kinase, and hence stops the Lohmann reaction. At the same time, this reagent, fluorodinitrobenzene, affects neither the contraction of muscle nor the first reaction. Using fluorodinitrobenzene, it could be established that ATP is cleaved both in a single contraction (twitch) of muscle or in a tetanic contraction. On the other hand, the reverse reaction of formation of phosphocreatine from creatine could be blocked with iodoacetate. Then the consumption of phosphocreatine characterizes the amount of ADP. They proved that the amount of ATP cleaved in the contraction of muscle corresponds to the consumption of phosphocreatine in the Lohmann reaction.[21]

Relaxation of muscle (return to the resting state) was

previously ascribed to the action of a certain factor called the Marsh-Bendall factor. It was later found that this factor, whose action has also been observed in vitro, consists of fragments of the endoplasmic reticulum, and relaxation is caused by exit of Ca^{++} ions from the sarcoplasm into the reticulum.

The biochemistry of muscle is an extensive and complex field. Here we shall restrict ourselves only to the bare facts needed for understanding the physics of muscle contraction. We shall return to these processes again in discussing the bioenergetics of muscle.

V. MECHANICAL AND THERMAL PROPERTIES OF MUSCLE

The term "contraction" is used in two senses as applied to muscle. Isometric contraction occurs in muscle when it is fixed in length. Then stress appears in the muscle without shortening. This stress can be measured. Conversely, isotonic contraction means the shortening of muscle under a constant load.

In the classic studies of Hill,[23-26] an experimental methodology was developed for exact measurement of the relation of the rate of isotonic contraction to the load, measurement of the stress developed in isometric contraction, and also of calorimetric measurements of the heat exchange of muscle when it contracts. It has been subsequently refined many times. Figure 14 shows a diagram of a recently-used apparatus.[27] The fundamental device is a lever, to one end of which the muscle M is attached with a light chain. The other end of the muscle is fixed (E denotes the electrodes for stimulating contraction). In studying isotonic twitches, load is applied to the muscle at the point R. The stress is measured with a transducer at the point A. In order to measure isometric stress, the transducer is shifted to the point B. Isotonic shortening is determined by the movement of the other end of the lever, as measured by the photoelement P. The catch C, which is controlled by the electromagnet EM, is used to maintain the muscle at a desired length, or to release an isometrically contracted muscle to a desired extent. Experiments on frog sartorius muscle show that a series of changes occurs in the first 15 msec after excitation of an isometric twitch. They are governed by the process of releasing of Ca^{++} ions from the endoplasmic reticulum. Then the stress begins to increase, and reaches its maximum value in 170 msec (at 0°C). Then it declines, and vanishes totally in more than 1 sec. In isotonic contraction, the shortening declines with increasing load P. It reaches a maximum earlier with heavier loads. Then the muscle relaxes to the initial state, and this takes longer with lighter loads. The development of isotonic strain in muscle follows practically the same time curve as the development of isometric stress.

Hill established empirically the fundamental, characteristic equation in the mechanics of muscle contraction.[23] It has the hyperbolic shape:

$$V = b(P_0 - P)/(P + a) \quad \text{or} \quad P = (bP_0 - aV)/(V + b), \tag{9}$$

where V is the rate of shortening, P is the force (load), P_0 is the maximum isometric tension that the muscle can develop, and b and a are constants.

When P = 0, the rate of isotonic contraction is a maximum:

$$V_m = bP_0/a; \tag{10}$$

Conversely, when $P = P_0$, $V = 0$. When $P > P_0$, the muscle no longer contracts, but stretches. This elongation does not follow Eq. (9).

Ober has proposed another empirical equation:[21]

$$V = B \ln [A/(P + P_1)], \tag{11}$$

where $A = P_0 + P_1$ and B are constants. P_1 is interpreted as being a small internal "frictional force" that hinders shortening.

We shall start with the Hill equation (9), whose very simplicity makes one think that it reflects relatively simple physical patterns underlying contraction.

Experiment has shown that the constant b, which has the dimensions of velocity, increases rapidly with increasing temperature. Thus, b increases by a factor of two upon heating by 10°. The constant a amounts numerically to about $(0.25-0.40)P_0$. Hence, V_m is greater than b by a factor of 2.5–4.

The Hill equation has a limited field of applicability. The point is that the maximum stress P_0 depends on the length of the sarcomere, i.e., on its degree of contraction or elongation. Figure 15 shows the results of measuring the relation of P_0 to the relative length of the sarcomere in an individual muscle fiber.[28,29] This relation is easily explained by the sliding model. P_0 has its maximum value, which corresponds to a certain plateau, at lengths close to the normal physiological length of the muscle. This corresponds to total overlap of the actin filaments and the HMM ends of the myosin filaments, and hence, to the possibility of forming the maximum number of bridges (see Fig. 13a). The overlap and the number of possible bridges decline at greater sarcomere lengths (see Fig. 13b). Correspondingly, P_0 also declines. Finally, the filaments become deformed when the shortening is great (Fig. 13c), and P_0 again falls. The Hill equation is valid in the plateau

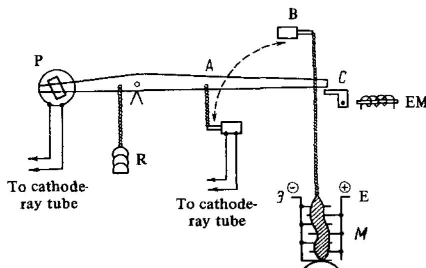


FIG. 14. Diagram of an apparatus for studying muscular contraction (explanation in text).

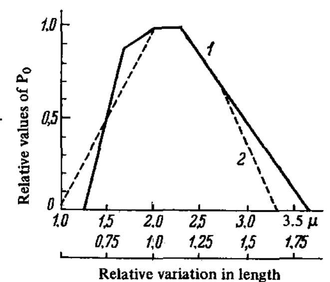


FIG. 15. Relation of tetanic tension to the length of the sarcomere. 1–isolated muscle fiber, 2–frog sartorius.

region, in the region of greatest P_0, i.e., at sarcomere lengths from about 1.8 to 2.3 μ or at relative lengths of 0.9 to 1.25. P_0 depends only slightly on the temperature, apparently increasing slightly with rising temperature.

The mechanical properties of muscle are studied either in twitches or in tetanic contraction. A single nerve or electrical pulse elicits a twitch. When one applies frequent enough pulses in succession, say 15 pulses per second, the twitches merge into a tetanic contraction, since each successive pulse arrives in the so-called refractory period of the preceding one. In other words, the prior excitation hasn't had time to relax.

By using the Hill equation, we can easily calculate the work done by the muscle in a twitch or tetanic contraction. The work is

$$A = PVt = bPt\,(P_0 - P)/(P + a). \qquad (12)$$

The relation of A to P is bell-shaped. A vanishes when $P = 0$ and when $P = P_0$, and reaches its maximum when $P = [a(P_0 + a)]^{1/2} - a$. Since $a = (0.25-0.40)P_0$, this happens when $P = (0.31-0.35)P_0$.

According to the sliding model, the stress developed by a muscle is entirely governed by the actin and myosin filaments and the Z-membranes. All these elements are not completely rigid, but have a certain compliance. The terminal sarcomeres of a muscle fiber are attached to the connective tissue of the tendons, and a compliance, or plasticity, must figure here as well. At the same time, these components introduce a certain elasticity into the movement of the muscle. However, the overall contribution of elastic and plastic deformations does not exceed 3% of the stress developed in the muscle.

Along with doing work, a muscle generates heat. The heat is generated even under isometric conditions, when all the work amounts to stretching the elastic elements without concomitant shortening of the muscle. When an isotonic stress relaxes, work is done on the muscle by the falling load or by the contracting elastic elements. This negative work is degraded into heat.

Precise experiments performed with sensitive thermocouples[24,25] have made it possible to divide the generated heat into a set of components. A certain amount of heat is generated very early, even before stress has arisen. This is the so-called heat of activation, Q_a. Apparently, this quantity is involved with the activation process, i.e., release of Ca^{++} ions into the sarcoplasm and their attachment to the active centers of actin and myosin. Later on, the heat of contraction, Q_c, is released as the muscle contracts. Finally, heat is also released during relaxation, owing to degradation of work. Perhaps the reversal of the chemical process during relaxation also causes small thermal variations. Let us denote them as $+Q_r$. The overall energy balance in shortening is[21]

$$-E = -Q_a - Q_c + A \pm Q_r. \qquad (13)$$

The rate of generation of heat is related to the length of the sarcomere in about the same way as P_0 is, and it reaches a maximum in the region of normal muscle lengths. Hence we can conclude that the generated heat is involved with maintaining tension, and arises from the "bridge" interaction of actin and myosin. This is not true of the entire thermal power, but of its steady-state component h_B. The thermal power generated can be expressed by the equation[21]

$$-dQ/dt = h_A e^{-\gamma t} + h_B. \qquad (14)$$

Under steady-state conditions, the total power expended by the muscle, i.e., the change in energy per unit time, is proportional to the difference $P_0 - P$. Hill derived the equation[23]

$$-dE'/dt = b'\,(P_0 - P), \qquad (15)$$

where $-E = -Q_c + A$, while the constant b' practically coincides with b in Eq. (9). Let us assume, as was found in Hill's[23] early measurements, that the heat of shortening per unit length is a constant. Then, if we denote this quantity by a, we can write

$$-dE'/dt = PV + aV \qquad (16)$$

Then, if we compare (15) and (16), we get the characteristic equation (9), with $b = b'$. This is the usual procedure in the thorough courses in biophysics (see, e.g.[30]). However, 25 years later, after the methodology had been perfected, Hill established that the thermal power generated by a muscle in steady-state contraction per unit length is not constant, but depends on P. This power is empirically expressed by the formula[25]

$$\alpha = (0.16 \pm 0.015)P_0 + (0.18 \pm 0.027)P. \qquad (17)$$

Therefore, Eq. (9) cannot be derived directly from (15) and (16), for the latter equation is false.

In Hill's experiments, the muscle was tetanized (continuously excited) under isometric conditions, and then it was suddenly released and it shortened, raising the load. Here an "extra heat" in addition to the isometric heat, or heat of shortening, was released. Figure 16 shows the results of such an experiment. The arrows indicate the instant of release and the moment that shortening ceased. When shortening ceases, the generation of heat again runs parallel to the isometric curve. In other words, production of heat of contraction ceases. Under constant load, the heat of contraction is proportional to the shortening, but proportional to the load at constant shortening.

All the described results have been obtained during contraction of the muscle. If we consider the entire cycle consisting of a single contraction and relaxation, then the pattern substantially changes.[31,21] No extra heat is observed in the cycle, in contrast to the contraction phase. They measured the work done in this phase and the total amount of heat generated as functions of the load. Subtracting the work of contraction from the latter quantity gave $-Q$, i.e., the heat generated besides the degradation of work upon relaxation. Q proved to be practically independent of the load. The total energy production in the cycle is

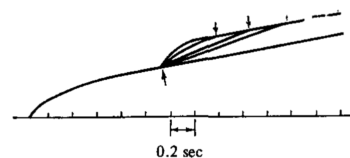


FIG. 16. Heat generation of muscles shortening under different loads.

$$-E = 2.9 + 0.97A \text{ (in mcal/g per twitch).} \quad (18)$$

These results do not agree with Hill's data for contraction (Eq. (17)). They can be formulated as follows: the energy released by a muscle in the overall cycle of a single twitch and relaxation is proportional to the work done plus a constant amount of heat that arises in the conversion of chemical energy into work (see[32]). The discrepancy with Eq. (17) might possibly be explained by absorption of the extra heat upon relaxation of the muscle. However, there are as yet no experiments confirming such an explanation. Thus the problem of the extra heat remains unsolved. Here we need new, very thorough experiments.

The efficiency of muscle can be expressed by the ratio of A to E (45% is the maximum value for frog muscle; values have been determined in the turtle of as much as 80%).

VI. THEORY OF MUSCULAR CONTRACTION

Evidently, the theory of muscular activity must explain without contradiction all three groups of the numerous facts described in the previous sections. However paradoxical this may be, the difficulties of building a physical theory of muscular contraction involve to a considerable degree the very abundance of these facts. The less people knew about muscle, the easier it was to advance physical hypotheses. However, it is not devoid of interest to acquaint ourselves with the attempts to interpret muscular contraction that preceded the firm establishment of the sliding model. These attempts are highly instructive, and sharply characterize the development of ideas in modern biophysics.

A polyelectrolyte model has been studied in a number of theoretical papers (see p. 271). Kirkwood and Risemann[33] suggested that the contractile element of muscle is a protein filament, some of whose amino-acid residues are phosphorylated by reaction with ATP. Then the filament becomes negatively charged and elongates, and this explains relaxation. When the ATP is cleaved, the filament loses its charges and contracts. Conversely, Morales and Botts[34] (see also[35,36]) suggested that the contractile protein fraction is positively charged (by Mg^{++} ions). Adsorption of ATP compensates these charges, and the filament contracts. An "entropic" elastic force acts in muscle, similar to that governing the elasticity of rubber.[2,8] Other hypotheses based on the properties of polyelectrolytes have also been proposed: winding and unwinding of protein chains, owing to variations of charge.[6,3]

Aside from the fact that these theories contradict a number of the described facts, and above all the data of electron microscopy, it is very hard to reconcile electrostatic effects in a medium that amounts to a tenth-normal salt solution with the strong forces of muscle. Skeletal muscle generates stresses of the order of several kg/cm^2—it suffices to recall the feats of weight lifters. The thermomechanical properties of muscle also contradict the polyelectrolyte models.

Astbury considered muscular contraction to arise from conformational transition in myosin owing to its interaction with actin.[37] In this study he made no attempts to explain the biochemical and thermomechanical properties, but the general idea that Astbury advanced is still of value.

Frenkel' has suggested treating a muscle fiber in a way similar to rubber.[38] The fiber contracts because its modulus of elasticity has been increased. The modulus of elasticity of a rubber increases with increasing degree of vulcanization, i.e., as more cross-links are introduced between the polymer chains. Frenkel' considered that the cross-links in muscle are created by ATP molecules. This study[38] is only of historical interest.

Éĭdus considered that muscular contraction arises from surface-tension forces in the capillary system of the mutually-contacting myosin and actin filaments immersed in the sarcoplasm,[39] with ATP only altering the surface tension. The sliding model already figured in this study, but the initial assumption of continuous contacts between actin and myosin is refuted by experiment. These protein filaments interact only via the bridges. Five years later, Gamow, who was apparently unacquainted with[39], advanced the same idea, but in contrast to Éĭdus, in purely qualitative form.[40]

Continuous interactions between actin and myosin also figure in the model of Spencer and Worthington.[41] They assume that electrostatic forces are exerted between actin and myosin. Activation of muscle involves imparting charges to the actin filaments of opposite sign to those on myosin. These charges travel along the actin filaments. The role of ATP is reduced to that of plasticization. The study[41] has no serious physical justification.

Szent-Györgi, one of the most prominent biochemists of our time, has concerned himself greatly as an experimentalist with muscular contraction. He is responsible for a distinctive hypothesis on the mechanism of contraction.[42] He ascribes an especially important role to water. Myosin is maintained in the extended configuration by a specifically expanded water structure. Destruction of this structure causes contraction, while its restoration causes relaxation. The energy required to break down the structure is supplied by ATP. Szent-Györgi suggests that the rapid transition is explained by energy migration through the quasicrystalline water net by resonance energy transitions. This hypothesis is speculative, for there is no evidence for the existence of a special water structure nor for migration of energy through this structure. Szent-Györgi's hypothesis does not agree at all with the results of the electron-microscopic and thermomechanical studies.

This type of hypothesis is highly characteristic of a certain circle of ideas in molecular biophysics. The tendency is natural to interpret biomolecular processes on a quantum-mechanical basis, starting with the special electronic properties of proteins and nucleic acids, say, semiconducting and even superconducting. Following Szent-Györgi, many authors have undertaken attempts along this line. Not once have they been successful. Water, proteins, and nucleic acids are dielectrics. In the absence of quanta of radiation, in dark biology, one has no occasion to deal with their special electronic properties. The specific behavior of biological macromolecules is primarily determined by conformational transformations, rather than electronic phenomena. Quantum mechanics explains the phenomenon of the chemical bond itself, the very existence of molecules, and their distinct conformations, etc., but (dark) biology has no quantum-mechanical features out-

side of ordinary chemistry.[43,2] At the same time, the conformational behavior of biomolecules is regulated by the electronic properties of a series of cofactors that are π-electron systems or atoms of the transition metals.[3] However, this regulation has no relation to fictitious semiconductor properties of proteins.

Szent-Györgi himself later said about his Bioenergetics that it was "hardly more than a dream". Hence he was quite surprised that Bioenergetics had been translated into Russian.[44] Szent-Györgyi's argumentation in favor of his fantastic conceptions is ingenious. "When I moved to Woods Hole and took up fishing, I always took along a huge hook. I knew that I wouldn't catch anything just the same, but, you know, it's nicer not to catch a big fish than a little one."

Let us return to muscle. In 1948, Éngel'gardt advanced an interesting idea on the nature of contraction.[45] He proposed that the protein in resting muscle is in an ordered crystalline state. Contraction of muscle is due to melting of the protein crystals. The active phase is relaxation, rather than contraction. The role of the contractile protein consists in resisting compression, and the role of ATP consists in influencing the assumed phase transition. A similar idea was formulated later by Pryor[46] and by the well-known theoretical physicist T. Hill.[47] In modified form, these ideas have kept their significance even now (see below). Flory, a very prominent scientist in the field of physical chemistry of polymers, has also studied muscular contraction, starting with the idea of phase transitions.[48] Hoeve and Flory have obtained indirect experimental confirmation of these hypotheses. They established that the ATP-induced abrupt shortening of glycerinated muscle fibers immersed in an ethylene glycol-water mixture begins only at a definite concentration of the mixture.[49,50]

Finally, I must mention some attempts to combine in a model the relative sliding of actin and myosin filaments with the coagulation of these filaments.[51,2] These attempts have not led to any convincing results.

VII. THE SLIDING MODEL OF MUSCLE

The situation has substantially changed since the sliding model of muscle (see Chapter III) and the bridge interaction of myosin with actin were established. Any theoretical concepts not in accord with this model have lost their value.

Davies has proposed a pictorial molecular interpretation of contraction.[52] The HMM knob of myosin can exist in two conformations. In the absence of Ca^{++} ions, the protein occurs in a disordered, but extended form, owing to repulsion between the $MgATP^{2-}$ attached to the active center and negative charges distributed in its neighborhood. In activation, a Ca^{++} ion entering from the sarcoplasm attaches to the bound ATP, and forms a chemical or chelate bond with an ADP molecule occurring at the active center of actin. A number of studies have firmly established the presence of ADP in F-actin (see[53-55]). The calcium neutralizes the charge of the ATP and abolishes the repulsion. The HMM knob undergoes a conformational transition, contracts, and releases the potential energy stored in the extended conformation (Fig. 17). This contraction pulls the actin filament one step further along, and simultaneously

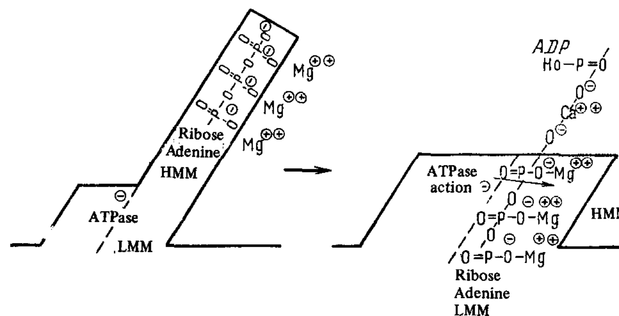


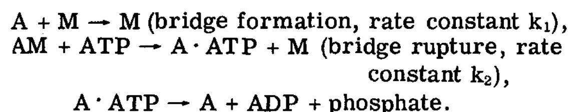
FIG. 17. Diagram of the changes in a bridge according to Davies.

shifts the combined ATP into the region of the hypothetical ATPase active center. The ATP is cleaved, and the bridge created by the Ca^{++} ion is broken. Then the process is repeated anew (Fig. 18). Davies showed that quantitative estimates of the concentrations, work, and heat stemming from this model do not contradict experiment. Tonomura has independently developed similar ideas (see[56]).

Davies' model faces a number of difficulties. It does not explain how the free energy of ATP is realized and utilized. It is not clear how Ca^{++} can form a bridge when Mg^{++} is already attached to the ATP. However, these difficulties can be overcome without any substantial change in the molecular model.[21] In order to confirm it, we need direct evidence of conformational transitions in the protein, similar, we must assume, to phase transitions. There are some indications of this in the literature.[57,58]

It is quite probable that Davies' model will later be replaced by a more realistic one. Possibly the conformational transition occurs in actin rather than in myosin, as the Japanese authors[53] assume. However, it apparently takes place in some way or another.

Huxley[59] is responsible for the first attempt to construct a quantitative physical theory based on the sliding model. He assumes that the active projection M of myosin oscillates about a certain position along the filament, while the active center A of actin is immovably fixed on it. He writes the kinetic equations for the reactions:



$$A + M \rightarrow M \text{ (bridge formation, rate constant } k_1\text{)},$$
$$AM + ATP \rightarrow A\cdot ATP + M \text{ (bridge rupture, rate constant } k_2\text{)},$$
$$A\cdot ATP \rightarrow A + ADP + \text{phosphate}.$$

Certain variations are ascribed to the constants k_1 and k_2 as functions of the distance x between A and M

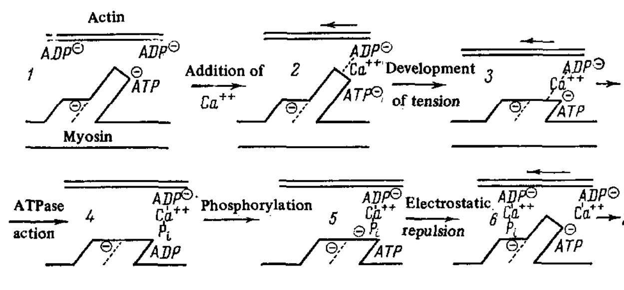


FIG. 18. Molecular model of contraction according to Davies.

along the myofibril; the solution takes account of the relative displacement of A and M as the sarcomere contracts. With a suitable choice of several parameters, Huxley got numerical agreement between the mechanical characteristics of muscle and the results of calculating them from the Hill equations (9) and (15) (see also[60]).

Deshcherevskiĭ[61] has recently decisively simplified and refined Huxley's theory. His study considers three states of the AM bridges: closed bridges that develop tensile force, closed bridges that hinder sliding of the filaments, and open bridges. The bridges close (independently of one another) and pull on the filaments, causing active contraction. Later on, the same bridges begin to resist motion, since the filaments have shifted, and finally, the bridges break. If we denote by n_0 the total number of active regions, by n the number of pulling bridges, and by m the number of resisting bridges, we can write as the kinetic equations

$$\dot{n} = k_1 (n_0 - n - m) - (v/L)\, n, \tag{19}$$

$$\dot{m} = (v/L)\, n - k_2 m; \tag{20}$$

Here v/L is the rate constant for conversion of pulling into resisting bridges. It is equal to the velocity v of relative motion of the filaments divided by the distance L between two consecutive active centers to which a bridge can attach. He supplements these equations with an expression of Newton's second law:

$$M\dot{v} = f_0 (n - m) - f n_0, \tag{21}$$

Here M is the mass being displaced, f_0 is the force developed by one pulling bridge, and f is the external force (load) per bridge. Under steady-state conditions, $\dot{n} = \dot{m} = 0$, and $\dot{v} = 0$ (v = const). If we eliminate n, m, and n_0 from Eqs. (19)–(21), we find

$$(f + a)\, v = b\, (f_0 - f). \tag{22}$$

This equation coincides with Hill's characteristic equation (9). All quantities in (22) are referred to a single bridge, and v is the velocity of shortening in the half-sarcomere. The constants a and b are expressed in terms of k_1, k_2, f_0, and L by:

$$a = k_1 f_0 / (k_1 + k_2), \tag{23}$$

$$b = k_1 k_2 L / (k_1 + k_2). \tag{24}$$

Thus, Hill's empirical equation has been derived theoretically for the first time on the basis of model concepts. Deshcherevskiĭ's theory is essentially macroscopic, since it treats the elementary processes as being irreversible (Fig. 19).

Recently Hill started with the theory that he had previously developed on phase transitions in linear, regular systems,[62] and suggested treating the contraction of the sarcomere as being a first-order phase transition between two states of the protein filaments (with and without bridges).[47] The first state corresponds to an extended sarcomere in which the myosin and actin filaments do not overlap, while the second one corresponds to a shortened sarcomere in which they do. An applied load hinders the phase transition, and the condition for this can be written in the form

$$f_0 L = \Delta F. \tag{25}$$

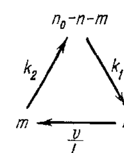


FIG. 19. Kinetic diagram of formation and rupture of MA bridges according to Deshcherevskiĭ.

The chemistry of the process is not taken into account, and the role of ATP is reduced to a small change in ΔF. Hill later rejected this theory, mainly because f_0 (i.e., P_0) is not constant, but depends on the length of the sarcomere (see p. 275).[63]

Evidently, a theory of muscular contraction must be kinetic rather than thermodynamic. Later studies by Hill[64] have been devoted to refining Huxley's theory. However, he has not succeeded in carrying out the treatment in such a simple and clear form as Deshcherevskiĭ has done.

VIII. THE MOLECULAR THEORY OF MUSCULAR CONTRACTION[65]

A molecular-kinetic theory of muscular contraction can be devised by starting with ideas of internal friction, in accordance with the data of biochemical, structural, and thermomechanical measurements.

The bridges do not operate synchronously, and at a given instant in the shortening of a muscle, only a certain effective number n of bridges participates. This number is smaller than the maximum n_0, where n_0 is the number of HMM knobs. Correspondingly, the muscle develops a stress P that is less than P_0. When $P = P_0$, all possible bridges are participating in contraction.

It is natural and reasonable to assume that n depends linearly on f (or P). If $f = f_0$, then $n = n_0$, and if $f = 0$, then $n = n_0 r$, where $r < 1$. Here $n_0 r$ is the number of bridges functioning in the unloaded muscle. However, it differs from zero. Consequently we have

$$n = n_0 r + [n_0 (1 - r)\, f/f_0]. \tag{26}$$

Here n depends on the rate of shortening. One might suppose that when the rate of relative displacement v of the protein filaments is large, the bridges don't have enough time to attach, and n must be small. The ratio n/n_0 characterizes the steady-state probability of attachment of a bridge.

Attachment of the bridges implies the presence of internal friction between the filaments being displaced. Newton's second law for a system having friction has the form

$$M\dot{v} = n f_0 - n_0 f - n\beta v; \tag{27}$$

Here nf_0 is the tension developed by the sarcomere, $n_0 f$ is the applied stress (load), and $n\beta v$ is the frictional force. Under steady-state conditions, $\dot{v} = 0$. Then, if we substitute the expression (26) into (27), we get Hill's equation in the form

$$[r f_0 (1 - r)^{-1} + f]\, v = f_0 r\, (f_0 - f)/\beta\, (1 - r). \tag{28}$$

Thus, the constants a and b have the form

$$a = \frac{r}{1 - r} f_0, \tag{29}$$

$$b = \frac{r}{1 - r} \frac{f_0}{\beta}. \tag{30}$$

The problem consists in determining the frictional coefficient β independently in terms of molecular constants.

Formation of a bridge serves to trigger a conformational transition that is accompanied by performing the work f_0L and releasing the heat q_0. One can represent the change in the state of the trigger system by the curve shown in Fig. 20 (here 1 is the state of the protein undergoing conformational transition before the bridge is formed, and 2 is that after bridge formation). Bridge formation and the subsequent conformational transition require energy of activation, as is directly evident from the marked growth in b with increasing temperature (p. 275). External force exerted on the bridge hinders its formation, i.e., hinders functioning of the trigger system. This force is directed along the actin or myosin filaments, and hence, at a certain angle to the bridge (Fig. 21). Thus the force $f\eta$ acts on the bridge, where $\eta = \cos\varphi$, and l is the length of the chemical or chelate bond formed by the Ca^{++} ion to attach the HMM knob to the active center of actin. The discussed model is very general; it is even compatible with the model of Davies (p. 278).

According to the theory of internal friction of liquids, which is based on the theory of absolute reaction rates, the rate of formation of bridges can be expressed by the formula[66]

$$\nu = (kT/h)\,(e^{-F_+/kT} - e^{-F_-/kT}), \qquad (31)$$

where F_+ and F_- are respectively the free energies of activation of the processes $1 \to 2$ and $2 \to 1$, and h is Planck's constant. Eq. (31) is based on the microscopic reversibility of the process.

The quantities F_+ and F_- for a symmetrical barrier (see Fig. 20), are equal to

$$F_+ = F - (\Delta F/2) + (fl\eta/2), \quad F_- = F + (\Delta F/2) - (fl\eta/2), \qquad (32)$$

and hence

$$\nu = (kT/h)\,e^{-F/kT}\cdot 2\,\mathrm{sh}\,[(\Delta F - fl\eta)/2kT]. \qquad (33)$$

The basic assumption consists in the idea that $\nu = 0$ when $f = f_0$ (f_0 is the critical force that makes the transition possible). This means that

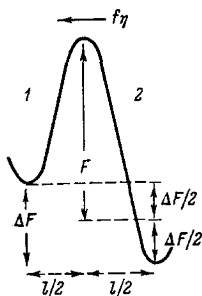


FIG. 20. Energy curve for bridge formation and concomitant events. [65]

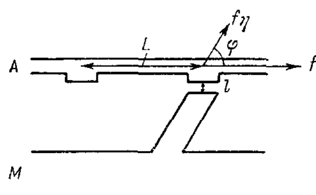


FIG. 21. Diagram of an MA bridge. [65]

$$\nu = (kT/h)\,e^{-F/kT}\cdot 2\,\mathrm{sh}\,[(f_0 - f)\,l\eta/2kT], \qquad (34)$$

and if

$$(f_0 - f)\,l\eta \ll 2kT, \qquad (35)$$

then

$$\nu \approx (l\eta/h)\,e^{-F/kT}\,(f_0 - f). \qquad (36)$$

Each $1 \to 2$ transition causes a relative shift of the filaments by one step of length L. The linear velocity of this transition is νL. The probability w of attachment of a bridge depends on the macroscopic velocity of relative motion, and is expressed by the ratio of νL to v. We have

$$w = n/n_0 = \nu L/v. \qquad (37)$$

If we substitute the expressions (26) for n, and (36) for ν, into (37), we get Hill's equation in the form

$$[rf_0(1-r)^{-1} + f]\,v = [f_0Ll\eta/(1-r)\,h]\,e^{-F/kT}(f_0 - f). \qquad (38)$$

Upon comparing (38) with (28), we find an expression for the frictional coefficient

$$\beta = (rh/l\eta L)\,e^{F/kT}. \qquad (39)$$

The constant b is expressed in terms of molecular parameters:

$$b = [f_0Ll\eta/(1-r)\,h]\,e^{-F/kT}. \qquad (40)$$

Now we must make some numerical estimates. First of all, we must convince ourselves that the condition (35) is valid.

The tension is exerted in each half-sarcomere. The number N of bridges in a volume amounting to 1 cm^2 multiplied by the length of a half-sarcomere (1.1 μ) is 6.5×10^{12}.[16,67] Experiment gives P_0 of the order of 3 $kg/cm^2 = 3 \times 10^6$ $dynes/cm^2$. Each bridge exerts a force $f_0 = P_0/N = 4.6 \times 10^{-7}$ dynes. Let us assume a bond length $l = 2$ Å, and the unknown quantity $\eta < 1$ to be equal to 0.5. We get $f_0 l = 4.6 \times 10^{-15}$ erg $= 2\,kT \cdot 0.055$. Thus the condition (35) is well satisfied. L is of the order of 50 Å (the distance between two active centers of actin). The quantity f_0L amounts to 23×10^{-14} erg.

According to (10), the quantity b equals $2.5\,V_m$–$4\,V_m$. The maximum rate of shortening of frog sartorious muscle at 0°C is 1.33 L_0/sec, where L_0 is the initial length of the muscle.[23,26] In our calculations, we must take $L_0 = 1.1\,\mu$, or half the length of the sarcomere. Hence $V_m = 1.5\,\mu$/sec, and $b = 4 \times 10^{-5}$ cm/sec. We find the parameter r by comparing the expression (29) with the condition $a \approx 0.25\,f_0$ (p. 275): $r \approx 0.23$. If we substitute into (40) the estimated values of f_0L, r, η, and l, as well as of b, we find F = 14 kcal/mole. When the temperature is raised by 10° (from 300°K to 310°K), b must increase by a factor of 2.1. Experiment gives an increase by a factor of 2.05.[23] The presented theory explains well the temperature-dependence of the constant b.

The probability w of attachment of a bridge can also be calculated directly. Let us denote by x the distance between the active elements M and A, as measured along the fibril. The probability that attachment has occurred is w(x). The probability dw of attachment in the next interval dt of time is $(1 - w)k(x)dt$, where $k(x)dt$ is the a priori probability of attachment of a

bridge in the time dt = dx/v. If we solve the equation that we obtain,[68]

$$\dot{w} = (1-w)\,k(x), \tag{41}$$

we find

$$w = 1 - e^{-\lambda/v}, \tag{42}$$

where

$$\lambda = \int_{-\infty}^{+\infty} k(x)\,dx. \tag{43}$$

Approximately, $k(x) = \nu$, and

$$\lambda \approx (l\eta/h)\,e^{-F/kT}(f_0 - f)\,L; \tag{44}$$

When $\lambda/v \ll 1$, again we get (37)

$$w = n/n_0 \approx \lambda/v \approx (l\eta/h)\,e^{-F/kT}(f_0 - f)\,L/v. \tag{45}$$

We can easily see that indeed $v > \lambda$ for all $f < f_0$. If we substitute the value $w = n/n_0$ from (45) into (38), we find

$$w = b/(b+v) \tag{46}$$

If $v = 0$, then $n = n_0$ and $w = 1$. If $v = v_m = b(1-r)/r$, then $n = n_0 r$. The parameter r is to be interpreted as the minimum probability of attachment of a bridge when $v = v_m$:

$$r = w_m = b/(b+v_m). \tag{47}$$

The molecular theory leads to results equivalent to those derived in the Huxley-Deshcherevskiĭ theory (pp. 278 and 279). Let us compare the formulas. First of all, a comparison of (29) with (23) gives

$$k_2/k_1 = (1-2r)/r. \tag{48}$$

Upon substituting the steady state of m from (20) into Eq. (19):

$$m = vn/Lk_2,$$

we find

$$\dot{n} = k_1 n_0 - \{k_1 + [(k_1+k_2)\,v/k_2 L]\}\,n \tag{49}$$

and at $\dot{n} = 0$ we get the steady-state ratio n/n_0. Let us equate it to Eq. (46):

$$k_1\{k_1 + [(k_1+k_2)\,v/k_2 L]\}^{-1} = b/(b+v). \tag{50}$$

From (48) and (50), we get

$$k_1 = \frac{l\eta}{h}\,e^{-F/kT}\,\frac{1}{1-2r}\,f_0 = \frac{b}{L}\,\frac{1-r}{1-2r} = \frac{v_m}{L}\,\frac{b}{v_m - b}\,, \tag{51}$$

$$k_1 = \frac{l\eta}{h}\,e^{-F/kT}\,\frac{1}{r}\,f_0 = \frac{b}{L}\,\frac{1-r}{r} = \frac{v_m}{L}\,. \tag{52}$$

Thus, k_1 and k_2 have been expressed in terms of molecular constants. If we rewrite Eq. (21) for $\dot{v} = 0$ in a form similar to (27), and substitute in the steady-state value of m, we find

$$f_0 n - f n_0 = f_0 m = f_0 v n/Lk_2. \tag{53}$$

By comparing (53) with (27), we convince ourselves that

$$\beta = f_0/Lk_2, \tag{54}$$

and if we substitute in the value of k_2 from (52), again we get (39).

In the molecular theory, friction arises from the attachment and detachment of bridges, since these processes require an activation energy. Naturally, the frictional coefficient proves to depend exponentially on the temperature. In the macroscopic theory, the molecular model is replaced by the formal model of Huxley—a bridge either pulls or it resists, although actually these phenomena must occur simultaneously. An advantage of the microscopic molecular theory consists in the fact that it permits one to explain the kinetics, and as we shall see, the thermodynamics of contraction, by expressing the corresponding constants in terms of the molecular parameters l, η, F, f_0, and L. Also it permits one to study the temperature dependence of b (and f_0). Such a study gives results in accord with experiment. This agreement, along with the equivalence with the macroscopic theory, is evidence in favor of the idea that the molecular theory presented here actually reveals the physical nature of the processes occurring in muscle.

An approximate estimate of the free energy of activation of the process gave 14 kcal/mole. This value is rather close to the experimentally-found free energy of activation of enzymatic cleavage of ATP by actomyosin isolated from muscle. Experiment gives 15.8 kcal/mole.[21] We can assume that the cleavage of ATP that takes place when a bridge is formed is precisely the step that requires the greatest activation energy in the entire process under discussion. At the same time, there are no grounds for expecting complete coincidence between the discussed quantities, since the presented theory refers not to isolated ATPase studied in vitro, but to contracting muscle.

We can estimate the effective viscosity of the relative displacement of the protein filaments. To do this, we must multiply the frictional coefficient β by the distance between the actin and myosin filaments, which is ~100 Å. The effective viscosity is of the order of 2×10^4 poise. For comparison, I point out that the viscosity of polyisobutylene of molecular weight 5×10^5 at 78°C is 7.8×10^7 poise.

In the presented theory, we have assumed that the bridges attach independently of one another. How well grounded is this assumption? Doesn't cooperativity exist here, in the sense that attachment of one bridge influences the attachment of the adjoining ones? The concept of cooperativity arises naturally in the biophysics of proteins. There are many facts indicating interactions between the elementary links of protein chains, and interactions between the globular subunits in polymeric proteins. Conformational transitions in proteins are cooperative processes. This is manifested especially clearly in the so-called helix-coil transitions in the denaturation of a protein. These transitions resemble phase transitions.[2,4] The so-called allosteric proteins, which play a very important role in regulation of biochemical reactions, are polymeric, cooperative systems.[2,3] People have repeatedly advanced in the literature the idea that actin and myosin show cooperative, allosteric properties, including the current author.[3] Deshcherevskiĭ[61] tried to take cooperativity into account in his study.

Let us introduce cooperativity into the kinetics of bridge formation by assuming that the free energy of activation F is in itself a function of the number of bridges that have been formed:

$$F = F_0 - (n\varepsilon/n_0). \tag{55}$$

We have the equation (cf. (37))

$$n/n_0 = (l\eta/h)\, e^{-F_0/kT} e^{n\varepsilon/n_0 kT} (f_0 - f)\, L/v. \tag{56}$$

Using (26), we get the characteristic equation in the form

$$(a + f)\, v = b e^{r\varepsilon/kT} e^{(1-r)f\varepsilon/f_0 kT} (f_0 - f). \tag{57}$$

The v(f) curve at appreciable values of ϵ/kT proves to be convex, rather than concave. For the sake of argu- agreement with experiment, we must assume that $\epsilon \ll kT$. In this case,

$$(a + f)\, v = b \left[1 + \frac{1-r}{f_0}(a + f) \frac{\varepsilon}{kT}\right](f_0 - f). \tag{58}$$

That is to say, cooperativity of the type under discussion introduces only an insignificant correction into the Hill equation, and one of no special interest. However, as a whole, the problem of cooperative properties of the actomyosin system remains open.

The initial idea of the molecular theory of muscular contraction consists in the influence of the external force f on the activation process of bridge attachment and conformational transition of the protein. At one time, Polissar proposed that the rate of some unknown reaction that governs contraction depends exponentially on the external force.[69] Hill also introduced such an exponential relationship in his theory of a phase transition in muscle.[47] However, these studies did not permit them to derive the characteristic equation nor to interpret its constants on a molecular basis.

IX. BIOENERGETICS OF MUSCLE

To digress from the heat of activation, let us represent the change in internal energy in a twitch of a muscle by the quantity (cf. p. 276)

$$-E' = -Q_c + A = -\Delta F - T\Delta S. \tag{59}$$

The change in free energy ΔF characterizes the maximum work that the system can perform. However, in a mechanochemical system, the work is performed at the expense of chemical energy (formula (1)).

The amount of internal energy released per bridge amounts to

$$\Delta E = f_0 L + T\Delta S + \sum_i \mu_i \Delta n_i = f_0 L + q_0 + \sum \mu_i \Delta n_i. \tag{60}$$

The heat of contraction per 1 cm of shortening is 350 $g \cdot cm/cm^2$. That is, $q_0 = 2.6 \times 10^{-14}$ erg.[67] The change in free energy is

$$\Delta F = \Delta E - T\Delta S = f_0 L + \sum_i \mu_i \Delta n_i. \tag{61}$$

In line with the ideas given on p. 280, this change is equal to $f_0 l\eta$. We have

$$f_0 l\eta = f_0 L + \sum_i \mu_i \Delta n_i, \tag{62}$$

Hence,

$$f_0 = -\sum_i \mu_i \Delta n_i / (L - l\eta). \tag{63}$$

The maximum force f_0 must depend weakly on the temperature, varying as μ, i.e., as RT. This agrees with experiment. The cited numerical estimates give ΔS = 1.3 e.u. The entropy of the system decreases when a bridge is formed. In this sense, the process resembles crystallization. Decrease in entropy is natural, since binding of a bridge implies a decrease in the number of degrees of freedom of the system.

In a cyclic mechanochemical process that performs work,

$$\oint \Delta F = 0, \tag{64}$$

or, according to (61)

$$\oint f_0 dL = -\oint \sum_i \mu_i \Delta n_i. \tag{65}$$

The efficiency η' measures the deviation from (65):

$$\eta' = -\oint f_0 dL \Big/ \oint \sum_i \mu_i \Delta n_i. \tag{66}$$

The work is done as the expense of variation of the concentrations of the chemically-transformed components. In essence, we should take into account three processes here that lead to change in free energy: dephosphorylation (splitting) of ATP, the Lohmann reaction, and the protein conformational transition. A free energy of the order of 7–10 kcal/mole is released in the dephosphorylation of ATP, and about 2.5 kcal/mole in the Lohmann reaction.[21] The change in free energy in the conformational transition is not known.

In the estimates that have been made, the efficiency of muscle amounts to about 40%. However, the later results obtained upon detailed study of the entire contraction-relaxation cycle cast doubt on the estimate of q_0. As I have said, the thermal properties of muscle need more study. Davies et al.[70] state that the free energy of the ATP being cleaved cannot be the source of the heat of contraction, although it is the source of the reaction (presumably in the conformational transition in the protein) that is accompanied by a marked decrease in entropy. However, Bendall thinks that the heat of contraction involves degradation of free energy, rather than entropy.[21]

Regardless of the numerical estimates of q_0, the rate of energy release while a single bridge is being attached is

$$-\dot{E}' = \nu \Delta E, \tag{67}$$

where ν is expressed by Eq. (36). If we substitute in this value of ν, we get Hill's equation (15) in the form

$$-\dot{E}' = \Delta E l \eta e^{-F/kT} (f_0 - f)/h. \tag{68}$$

Therefore, the constant b′ in Eq. (15) is equal to

$$b' = \Delta E l \eta e^{-F/kT}/h, \tag{69}$$

Then, upon comparing (69) with (40), we see that

$$b' = b\Delta E (1 - r)/f_0 L. \tag{70}$$

Considering the estimates that have been made of $f_0 L$ and ΔE, these two values differ insignificantly. However, their physical meaning differs: b contains only the mechanical work, but b′ contains the entire energy change.

One can easily derive Hill's characteristic equation by using (68). The relation of $\dot{E}'$ to the rate of shortening v is given by the evident relation

$$-\dot{E}' = \Delta E n v / n_0 L. \tag{71}$$

If we equate (68) and (71), and substitute in the value of

n/n_0 from (26), we again get Hill's equation in the form (38). The heat of shortening can be written in the form

$$q = q_0 n/n_0 = q_0 f_0^{-1} [r f_0 + (1-r) f]. \quad (72)$$

Upon substituting in the estimated values of q_0, f_0, r, and L, we get

$$\alpha = q/L = 0.03 f_0 + 0.10 f \quad (73)$$

Then, if we normalize this expression so as to permit comparing it with (17), we get in place of (17):

$$\alpha = 0.08 P_0 + 0.26 P. \quad (74)$$

The deviation from (17) is substantial. In order to explain it, we must solve the overall problem of heat production in muscle. However, as we have seen, this hasn't yet been done. Hill's equation (17) itself contradicts the results obtained by studying the whole cycle.

Thus, the steady-state mechanical properties of muscle are satisfactorily explained by the molecular theory, but the thermal properties, the bioenergetics, haven't yet been studied enough, and it is premature to speak of interpreting them theoretically.

X. KINETIC PROPERTIES OF MUSCLE

All the prior discussion, both Hill's characteristic equation and the molecular theory that explains its origin, pertains to steady-state motion, i.e., to shortening of muscle at a constant rate v. Non-steady-state processes are of no less interest, if not greater.

The kinetic equation describing approximation to the steady state can be written in the form

$$\dot{n} = \tau^{-1} (n_0 w - n), \quad (75)$$

where τ is some relaxation time. If we substitute the value of w from (46) into (75), we get

$$\dot{n} = \tau^{-1} [n_0 b/(b+v) - n]. \quad (76)$$

In the general case, Eqs. (76) and (27) are nonlinear, since τ, n_0, and v depend on the length of the sarcomere, and therefore on the time.

We can estimate the time τ that it takes to establish a steady state by starting with simple physical considerations. One must express τ in terms of three quantities: the time $\tau_1 = L/b$ needed for bridge attachment, the sliding time $\tau_2 = L/v$, and the sliding time $\tau_3 = L/v_m$ at maximum velocity; τ must approach τ_2 if $\tau_1 \to \infty$, or approach $\tau_1 - \tau_3$ if $\tau_2 \to \infty$. When $\tau_1 = \tau_3$, τ vanishes, since then the steady-state rate of attachment b coincides with the maximum rate of sliding v_m, and exceeds any other rate of sliding $v < v_m$. That is, the process is known to be steady-state, and sliding doesn't hinder bridge attachment. Using dimensional arguments, we can write a very simple expression that satisfies the listed conditions:

$$\tau = \tau_2 (\tau_1 - \tau_3)/(\tau_1 + \tau_2), \quad (77)$$

or

$$\tau = (1 - b v_m^{-1}) L/(b+v). \quad (78)$$

The same expression arises directly from Deshcherevskiĭ's equations (19) and (20) if one assumes in them that $\dot{m} = 0$. In fact, here we get (49), i.e., Eq. (75) having the value

$$\tau = \{k_1 + [(k_1 + k_2) v/k_2 L]\}^{-1}$$

and, according to (50) and (51), we get the expression (78) again.

However, in itself, Eq. (76) still gives us little toward understanding non-steady-state processes in muscle. At least, we must take simultaneous account of the equation of motion of muscle in the Newtonian form of (27):

$$M\dot{v} = n f_0 - n_0 f - n\beta v.$$

If we use (26) to eliminate f_0 from this equation, we get

$$M\dot{v} + n_0 w \beta v = n_0 w_m (1-w) f/(w - w_m), \quad (79)$$

where $w = n/n_0$ (37), and $w_m = r$ (47). This equation does not contain the elasticity, and it describes non-steady-state viscous flow in the presence of an external force. The action of the latter on the system is defined by the coefficient that contains the parameters n_0, w, and w_m. In the non-steady-state case, these parameters must depend on the length of the muscle, or respectively, the time.

Actually muscle is a viscoelastic material. Neither Eqs. (76) and (79), nor Hill's characteristic equation that corresponds to their steady-state solution, describes these properties that it has. The equation of motion (27) lacks a term Ax having the appropriate sign.

It was also Hill[71,23] who proposed for the first time a phenomenological model description of the viscoelastic properties of muscle. He concluded that active muscle contains an undamped elastic element and a damped elastic element linked in series with the undamped one. Resting muscle is characterized by elasticity alone. Later analysis of the viscoelastic properties of muscle has led to the conclusion that there is a second elastic element in parallel with the first two (see[72]). Formal models describing such a system are a combination of the models of Voigt (Fig. 22a) and of Maxwell (Fig. 22b).[72,73] They have been applied to interpret the experimental results of Buchthal and Kaiser.[74] Evidently one could also use equivalent electrical models for these purposes.

Pasechnik and Sarvazyan have made a calculation for a simplified model (Fig. 23), as applied to their experiments on the viscoelastic properties of muscle when subjected to periodic loading.[75,76] Hill's equation refers only to the contractile element z. The calculation is based on the idea that

$$v = -\dot{x} = v_0 - i\omega x_1 e^{i\omega t},$$

since

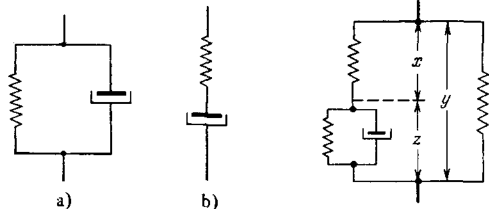


FIG. 22 FIG. 23

FIG. 22. a) The model of Voigt, b) the model of Maxwell. FIG. 23. Simplified model of muscle. [75,76]

$$x = x_0 + x_1 e^{i\omega t}.$$

If one substitutes this value of v into the characteristic equation, and takes into account the fact that (see Fig. 23)

$$x + z = y$$

and

$$P = P(z) = P_{\text{elast}}(x),$$

It proves possible to describe the experimental results, although formally, and to determine the constants of the model from them.

The main difficulty consists in the lack of structural, molecular characteristics of the elastic elements of muscle. The sliding model and the molecular theory based on it do not contain these elements, and they pertain only to the contractile element z. Therefore, all the existing theoretical calculations of the elasticity of muscle are empirical in nature.

Further studies, both experimental and theoretical, must lead to an understanding of the viscoelasticity of muscle (in this regard, see[93], which appeared after I had written this article).

XI. INSECT FLIGHT MUSCLES

All the preceding discussion has pertained to cross-striated skeletal muscles of vertebrates. A topic of extreme interest for biophysics and bionics is the flight muscles of insects (flies, bees, wasps, beetles, etc.), and the timbal muscles of the cicadas, that resemble these muscles in function and structure. They have been the subject of continual attention by a number of investigators, especially Pringle.[77,78]

These muscles are capable of rapid periodic contractions. The frequency of contraction attains the order of 100 Hz. In structure, the insect flight muscles (IFM) greatly resemble the cross-striated vertebrate muscles discussed in this article. Thus it is appropriate to discuss the IFM here. The IFM are also cross-striated, and are constructed hexagonally of thin and thick filaments made of actin and myosin. All the experimental data (the results of biochemical, electron microscopic, and mechanical studies) indicate full applicability to IFM of the sliding model, with bridges formed between actin and myosin, and with cleavage of ATP.

We must take into account two facts in order to understand the nature of the rapid oscillations of IFM.

First, these rapid oscillations require the existence of a directly-functioning elastic element. Microscopy shows that, in contrast to vertebrate muscle, IFM contains a direct viscoelastic connection between the myosin filaments and the Z-membranes. We shall call it the C element. Figure 24 shows the experimentally established arrangement of the actin (A) and myosin (M) filaments, the C-element, and the Z-membrane.[79,78] The C element can undergo large elastic deformations.

Second, the oscillations of IFM have been shown not to be synchronized with the oscillations of potential imposed on them by the nerve endings. Thus, in the fly, the potential has a frequency of 3 Hz, but the wings oscillate at 120 Hz. Hence, the muscle is not activated at every oscillation period. Characteristically, IFM contains little sarcoplasmic reticulum (p. 000). Correspondingly, it is unlikely that the rapid rise and fall of tension in the oscillations involves inflow and outflow of Ca^{++} ions.

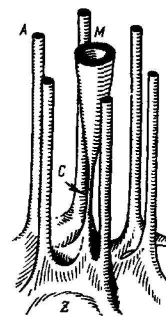


FIG. 24. Diagram of the structure of insect flight muscle.

Thus, the oscillations of IFM are self-oscillations in nature. Figure 25 shows a typical cycle of self-oscillations of the flight muscle of the rhinoceros beetle, i.e., the relation of the stress P to the strain.[78] P and x do not vary in phase, but P lags behind x; the cycle runs counterclockwise. We should note that the forced oscillations of vertebrate muscles are characterized by a cycle running clockwise.

As we know, self-oscillations arise in systems in which friction is produced by forces that depend on the state of motion of the system itself. Here the amplitude of these oscillations is determined by the properties of the system, rather than by the initial conditions.[80] In the simplest case, the equation of motion of a self-oscillatory system with one degree of freedom has the form

$$\ddot{x} + \omega^2 x + \psi(x)\dot{x} = 0, \qquad (80)$$

where $\psi(x)$ is a non-linear function of the displacement x. In the presence of an external force, we can write an overall equation for the oscillations:

$$a\ddot{x} + b\dot{x} + cx + d = P, \qquad (81)$$

Here the coefficients a, b, c, and d are themselves functions of x, or of x and $\dot{x}$. The term cx expresses elasticity.

Evidently, self-oscillations are excited when there is some feedback between the strain and the stress. This relation can be shown schematically by Fig. 26.[78] The interrelation between the stress and the strain varies as a function of the state of activity of the system. The oscillations of IFM satisfy all these conditions. The muscle contains a transducer element that reacts to the mechanical events and controls the state of the contractile system. In particular, this element is localized in the myofibrils. This is shown by the fact that even preparations of IFM that have been washed with glycerol show self-oscillations. Figure 27 shows a

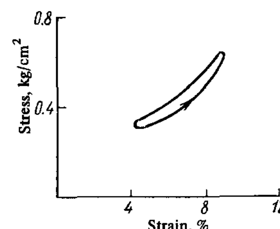


FIG. 25. The cycle of self-oscillations of the flight muscle of the rhinoceros beetle according to Pringle. [78]

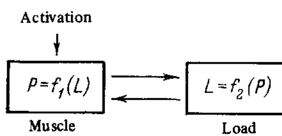


FIG. 26. Analytical diagram of the mechanical phenomena in muscle.

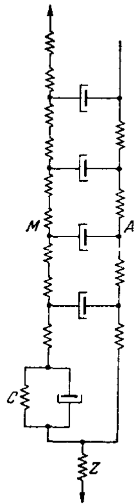


FIG. 27. A formal model of IFM.

formal model of IFM. It does not differ in principle from the models discussed above, but the viscoelastic element C plays an especially important role here.

Pringle[78] has made a detailed qualitative study of the mechanism of self-oscillation of IFM, and he came to conclusions equivalent to a considerable degree to the above-described molecular theory based on the sliding model. Pringle formulates the following eight hypotheses:

1. The transverse MA bridges can undergo a cycle of movements in which they detach from the A filament, undergo certain changes, and then attach again to A and exert an active thrust.

2. The angle that a bridge makes with the filament direction in muscle in the final state (i.e., at the end of the active thrust) is 45°.

3. The active thrust of the bridge takes a definite time.

4. One ATP molecule, or a constant small number of molecules, is hydrolyzed in each cycle of motion of the bridge. The hydrolysis is necessarily implicated with completion of the cycle of motion of the bridge.

5. The cyclic motion of a bridge is initiated by binding a Ca^{++} ion.

6. The angular velocity of a bridge decreases during the active thrust if it is attached to the A-filament and is exerting force.

7. Binding of ADP stabilizes the attachment of the bridge to the A-filament.

8. The number of possible bridges increases when tension has been developed in the M-filaments.

In essence, these hypotheses repeat those in the Davies model (p. 278) and in the molecular theory (p. 279). The existence of a transducer element is expressed by hypotheses 6 and 8. The latter is especially important in IFM. In developing these ideas, Chaplain and his associates have made a detailed cybernetic description of IFM based on the theory of control, and have made a phenomenological analysis of them.[81-83]

It seems evident that one can construct a quantitative molecular theory of the self-oscillations of IFM by starting with the described molecular theory, which explains the steady-state process of contraction. To do this, one must study the non-steady-state solutions of the appropriate equations. Here one must introduce explicitly into the latter elastic elements that have a direct molecular interpretation. Such is the C element, whose characteristic molecular constants must be determined from experiment.

There is thus far no such theory. To devise one is a very interesting and pressing physical problem. To pose and solve it amounts to creating an integrated biophysics, in which regulation phenomena in complex whole systems must be interpreted molecularly.

XII. CONCLUSION

Muscle is an extremely complex system. The treatment in this article has been intentionally simplified in order to point up the major features of the physical mechanism of contraction. Actually, a detailed biochemical picture should include the other proteins of muscle, especially tropomyosin, troponin, and α- and β-actinin. In some as yet unknown way, the first two proteins take part in the interaction of actin with myosin, and the actinins also have a role in the thin filaments.[21,84] However, the existence of these proteins does not change the physical picture of the process. Nevertheless, it is evidently quite essential to elucidate their roles.

The molecular and supermolecular structure of the thick and thin filaments continues to be the object of intensive studies. Electron microscopy and other physical methods show that many more interesting and important things are yet to be discovered in the muscles of different species of animals. Apparently the thick myosin filaments have a highly ordered structure.[85] It is not yet clear what its functionality is. The electron-microscopic studies of Gilëv on crab muscle have established a distinctive symmetric structure of the thick filaments that arises from ordered packing of the myosin molecules.[86] Actin is a more universal component of the thin filaments in muscles of different origins than myosin is in the thick filaments, and deserves no lesser attention. Actin has also been isolated from other contractile systems: from molecular ameboid organisms, and from the mitotic apparatus.

For a real substantiation of the molecular physics of muscle, we need direct studies of the conformational transitions in the contractile proteins, using optical methods and X-ray diffraction. There are as yet no definitive data on conformational transitions in myosin. Conversely, the actin studies have already led to unambiguous results in the studies of some Japanese[53,55] and Soviet[87-89] authors. The presented molecular theory does not localize the conformational transition responsible for the pulling or pushing force (p. 279). Perhaps it occurs in actin. This is favored by the universal occurrence of actin, and by the variety of its structures found experimentally and their mutual trans-

formations. The hypothesis has been advanced that actin is the fundamental mechanochemical "stepping" protein, while myosin regulates the rate and direction of the mechanochemical processes in actin.[90]

Detailed studies of contraction of intact (unharmed) muscle using new methods should refine the picture, and fix the details of the sliding model.

Frank and his associates have developed an original method of optical diffraction that makes it possible to detect changes in the structure of muscle during a twitch at intervals of a millisecond. Using this method, they were able to detect the periodicity, or "ratchet action" of the process. Frank interprets these results as reflecting not only sliding, but also fast processes of shortening of the thick filaments.[91] These studies have not yet succeeded in creating a detailed model picture, but they show that it is more complex than it might seem.

The molecular theory of muscular contraction thus far deals only with steady-state shortening, but not with the stretching of muscle by a load exceeding P_0, nor to relaxation of muscle to the rest state. In the sliding model, relaxation is explained by exit of Ca^{++} ions from the sarcoplasm, and thus by change in $\sum_i \mu_i \Delta n_i$ and decrease in f_0 (p. 282). However, a quantitative theory of this process has not been developed, nor has that of the process of forced elongation (which is apparently characterized by an energy of activation of the elementary event that differs from that which governs shortening).

I have spoken above of the molecular problems involving the viscoelastic properties of muscle. The whole complex of these problems will be solved only by studying the conformational properties of the muscle proteins. The usual structural methods (x-ray diffraction, electron microscopy, spectroscopy, etc.) do not suffice here, since they can't be applied to intact muscle in a dynamic state. Here we need new kinetic, rate methods, one of which has been developed in the laboratory of G. M. Frank.

However, independently of the many problems still awaiting solution, we can state that some of the fundamental assertions of the physics and biochemistry of muscle have already been established. This review has mainly been devoted to them. Muscle is a mechanochemical system that works at the expense of the energy in the high-energy phosphate bonds in ATP. This work is performed by virtue of the bridge interaction of actin with myosin and conformational transition in one or both proteins arising from this interaction. It has been possible on this basis to construct a molecular theory that explains rather well the steady-state dynamics of muscle.

I might add to what I said above on the role of ATP the remarkable fact that the ATPase activity of 16 different types of muscles differing in rate of contraction by a factor of 200 is proportional to this rate.[84] The energy released in the hydrolysis of one ATP molecule can be thought of as a sort of "mechanochemical quantum." Correspondingly, the elementary step in the contraction process is governed by this "quantum."[91]

The physics of muscular contraction also has a more general meaning in modern biophysics. Muscle is the best-studied biomechanical contractile system, but it has much in common with other biological contractile apparatus. The motion of flagella and cilia, the motion of the tails of spermatozoa and bacteriophage particles, the motion of the protoplasm, and the mitotic division of cells involve the functioning of various contractile proteins at the expense of ATP energy.[2,84,92] The sliding model as such applies to the cross-striated muscles of vertebrates and to IFM; it is not yet clear how applicable it is to smooth muscles. However, in the microscopic contractile systems we also encounter structures resembling myofibrils, having complexes of "outer" and "inner" proteins. These systems are highly complex, and the route toward studying them passes through the biophysics of muscle, a macroscopic system.

Even today, the joke hasn't fully lost its meaning: "What is biophysics? It's the work of a doctor with an instrument too complicated for him to understand." However, modern biophysics is gradually ridding itself of pseudoscientific speculation based on ignorance of physics. Of course, the further development of biophysics is the general business of biologists and physicists, and it can't be achieved without their close collaboration. However, the goal of such a development consists in studying biological phenomena on the basis of the ideas and methods of the exact science, physics, of which biophysics must become an inseparable part.

The topic of this review has consisted not only in treating the essence of an important and interesting biological phenomenon, but also in introducing physicist-readers to the circle of ideas in modern biophysics. They will decide how well I have been able to do this.

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