Attacks on Muscle

A. Szent-Györgyi[1]

Marine Biological Laboratory, Woods Hole, Massachusetts; and
Laboratory of Physical Biology, National Institutes of Health, Bethesda, Maryland

UP TO THE PRESENT CENTURY muscle was the favorite object of physiological research. An enormous amount of work carried on by classical methods was repeated later with perfected physical methods. The results of such refinements failed to leave in their wake a deeper understanding than had been obtained before. The reason lies in the wholistic nature of approach. To the physiologist muscle and a muscle twitch are units which cannot be disintegrated.

Attempts to understand muscle by breaking it down to its molecular components date back to the sixties, when W. Kühne (*7*) prepared from muscle a globulin-like protein, myosin, supposedly derived from contractile fibril. Leading contemporaries, Danilevski, (*2*) Halliburton (*5*), and v. Fürth (*11*), corroborated and extended the observation. In the thirties of our century the study of myosin was again taken up by more modern methods (*12*).

Though interesting and specific relations of myosin and ATP were discovered (*4*), the mechanism of contraction and the role of myosin remained obscure.

A third line of attack was taken up in the author's laboratory ten years ago at Szeged and later at Budapest, Hungary. This line was based on the belief that such a complex mechanism as the contracting muscle fibril can be understood neither by keeping it as a whole nor by decomposing it to molecules. It was believed that an understanding could be achieved by breaking the system down gradually, dismantling it step by step, always trying to put together again what we have taken to pieces. It was further believed that no single method of investigation could give the desired information. All methods available had to be employed in one single coordinated effort.

Two years ago the author started organizing such an attempt in this country. Part of this research group is working at the National Institutes of Health at Bethesda, Maryland, and part at the laboratory of the Institute of Muscle Research at the Marine Biological Laboratory in Woods Hole, Massachusetts. In the middle of August the two groups convened at Woods Hole to discuss their problems and methods, the meeting being attended by a few guests interested in muscle. Out of these discussions a new conception of muscular contraction seems to have emerged.

The contractile structure is built of a protein complex, "actomyosin," discovered at Szeged. The "myosin" partner of this complex is in many ways different from earlier myosin and was crystallized by the author. Actin was isolated by F. B. Straub (*12*). In itself, neither actin nor myosin shows any sign of contractility, nor does actomyosin in itself. What makes it work is the master substance of muscle, ATP.

Adenosine triphosphate is a water-soluble nucleotide consisting of small molecules. Its existence was only gradually recognized. Embden (*3*) described adenosine phosphate and Meyerhof and Lohmann (*8, 9*) showed that in muscle adenosine was linked not to one but to three phosphate groups; hence the name ATP, abbreviation for adenosine triphosphate. This ATP contains, in the links holding its phosphate groups together, 11,000 cals of free energy which, according to our present knowledge, is the sole immediate source of the energy of muscular contraction. But ATP is not merely a store of energy. It dominates the whole physical state of muscle. Without it muscle is not only inactive but is also stiff and inelastic. Muscle in *rigor mortis* is actually muscle with no ATP.

Myosin, if not linked to actin, has one striking property, its great affinity to ions. It binds ions very strongly so that its physical state, charge, and solubility depend on the quality and quantity of ions present. Though it is a hydrophylous colloid, myosin is discharged and quantitatively precipitated by 0.025 M KCl or NaCl. It has an especially great affinity to Mg, which is always present in muscle in high concentration. Myosin, by binding Mg and K, develops a high affinity to ATP, with which it links up to a fairly stable complex, "myosin-ATP."

Actin, too, has its striking peculiarities. The most surprising one is that it can exist in two forms, in the form of globules and in the form of long threads.

[1] Public Health Service Special Fellow of the National Institutes of Health.

If extracted by Straub's method, it is in the globular form (G-actin) consisting of small, round molecules of mol. wt 70,000 g. If a small quantity of salt, KCl or NaCl, is added in the presence of traces of Mg, the globules unite to long threads, studied first under the electron microscope by Jakus and Hall (*6*). These relatively thick threads give no information about their finer structure, or the mechanism of the building of threads out of globules. If, however, thread formation is induced under special conditions, as it was by G. Rozsa, the author, and R. W. G. Wyckoff (*10*) at the National Institutes of Health, actin forms very thin molecular threads. If these threads come to lie side by side they develop a cross striation. The distances of this striation can be exactly measured, allowing us to draw some conclusions as to what has happened. What we find is that the small actin globules of mol. wt 70,000 g have united to form bigger units, about twenty of them now forming one particle of mol. wt about 1,500,000 g. These units are slightly elongated, 100 A wide and 300 A long; they associate end-to-end to form long fibers. If several fibers come to lie close to one another the particles of the neighboring threads associate also with their broad side.

Myosin consists of very thin and long molecules. If these are added to fibrous actin the two colloids unite to form a complex. The slender, long myosin filaments attach themselves to the actin filament lengthwise. The reactions of this complex are rather sluggish. If ATP is added it links up with the myosin to form an actomyosin-ATP complex which has the most amazing reactivity. The reactions depend now on the salt concentration. At a high salt concentration the complex dissociates into its components, actin and myosin-ATP. By changing the salt concentration by no more than 0.01 M we can make actin and myosin-ATP unite or dissociate at will. The ionic concentrations of intact muscle are such that the system is balanced on a razor edge and the two proteins just do not unite. The shift in ionic concentration, necessary for their union, is brought about by the wave of excitation and the concomitant electric current.

In the absence of ATP the actomyosin filaments are stable in their straight, stretched condition. Actomyosin-ATP is unstable in this condition. When actin and myosin-ATP unite they lose their charge and therewith they dehydrate and tend to shrink and fold up, going over into a shorter, more stable, and energy-poorer state.

If we do not allow the system to shorten by holding its ends fixed, tension will be developed. Using thermodynamic language we could also say that, by uniting, the two proteins acquire a thermodynamic potential which is dissipated in shortening. Using the language of colloidal chemistry we can also say that contraction is a mutual precipitation of two proteins, or a precipitation of a protein complex by ions, since ions are also involved.

The reaction in which the potential is developed is thermodynamically reversible; it is thus an equilibrium reaction. From the temperature dependence of its constant, free energy changes can be calculated which are in agreement with experiments measuring the work actually done. In these reactions the actin, together with the corresponding quantity of myosin, forms independent units, which react in an all-or-none fashion, that is, either react completely or not at all. Muscular activity is the sum of the independent function of these little units, the size and energy changes of which can be calculated with fair accuracy.

While contraction seems to be clearing up, we still know very little about relaxation and energy coupling. We do not know how the muscle goes back to its resting state and at which point of the cycle the ATP is split and how its energy is utilized by the protein. A recent discovery of F. B. Straub, corroborated and extended by W. Bowen and K. Laki (*1*), together with the author's earlier observations, offers the possibility of a tentative explanation. Straub found that ATP is involved in the polymerization of actin. The author observed that fibrous actin is broken up into globules during contraction of actomyosin, that actin in its globular form dissociates from myosin-ATP much more readily than fibrous actin, and that myosin greatly catalyzes the rearrangement of actin globules to fibers. Hitching all this together the following picture can be made: ATP is instrumental in holding the actin-globules together in fibers. This ATP is split in contraction by the myosin (which is known to have ATP-ase activity). The actin fiber thus falls into globules which dissociate from the the myosin-ATP. The actin globules unite spontaneously to fibers again while myosin-ATP molecules hydrate and stretch out. since myosin-ATP, if not attached to actin, hydrates spontaneously at the ionic concentration of muscle.

In this picture the difficult problem of energy coupling ("energetization") drops out altogether. If the actomyosin-ATP system contracts and performs, say, *X* calories' worth of work, we cannot expect to be able to dissociate it without using up *X* calories' worth of energy in bringing about this dissociation. (If the system dissociates it can go back spontaneously into the relaxed resting state and if we could make it dissociate without using up energy then we

would have a net profit of X calories, that is, we would have a *perpetuum mobile.*) There being no *perpetuum mobile* possible, we can state that if the muscle has done X calories of external work in contraction we need at least X calories' worth of energy to make it dissociate and relax. Since the splitting of the phosphate link of the ATP molecules holding the actin globules together entails dissociation and relaxation, we need energy to bring about its splitting. The problem is solved by nature—by enclosing into the phosphate bond the energy necessary for its later splitting. In this way the link can be split at the right moment in contraction without external help, the energy needed for this splitting having been enclosed in the phosphate bond at its formation. The final source of this energy of the "high energy phosphate bond," which has to pay for all the energy expenditure of contraction, is our food, its oxidation and fermentation.

The reader will have noticed that in developing this concept of contraction the most varied methods were used. A deeper understanding of muscular activity can be arrived at only by coordinated attack on all available lines, be they histology, electron microscopy, preparative chemistry, enzymology, colloidal chemistry, physiology, or even quantum mechanics. The meeting held at Woods Hole represents such an attack.

A deeper understanding of muscle is urgently needed for two reasons. Cardiovascular diseases, which take such a horrid toll, are mostly diseases of muscle, be it the heart muscle itself or muscle cells of the artery wall. It should not be forgotten that the immediate cause of most human death is failure of muscle, and its dysfunctioning causes a great deal of suffering. No rational therapy or prevention can be hoped for without deeper understanding of muscle. Also, we can replace missing, or worn out, parts of a mechanism only if we know about them, and so the analysis of muscle may bring to light substances of therapeutic value. The author has worked out methods for the large scale preparation of ATP making this master substance of the muscle available for medical use, and suggested its clinical trial in all conditions in which dysfunctioning of muscle is involved. Most encouraging results have been reported in various lines.

The fact that ATP is involved in the construction of the bigger functional units of actin indicates that this substance is, so to speak, a mortar of the living edifice. Thus it is imperative to try its clinical application also in degenerative diseases, like arthritis, where the basic biomolecular architecture seems to be impaired.

Relieving human suffering on a big scale may be very gratifying, but all the same, the mainspring of basic research is curiosity, the thirst for new truth and knowledge. Success in therapy is but a natural consequence of such deeper knowledge. Muscle, as a material, holds out the highest promise for those who want to understand the basic principles of life and its architecture.

References

1. Bowen, W. and Laki, K. In press.
2. Danilevsky, A. *Z. physiol. Chem.,* 1881, **5**, 158.
3. Embden, G. and Zimmermann, M. *Z. physiol. Chem.,* 1927, **167**. 114.
4. Englehardt, W. A. and Ljubimowa, M. N. *Nature,* Lond., 1939, **144**, 669; Needham, J., Kleinzeller, A., Miall, M., Dainty, M., Needham, D. M., Lawrence, A. S. C., *Nature,* Lond., 1941, **147**, 766; Needham, J., Shen, S. C., Needham, D. M., Lawrence, A. S. C., *Nature,* Lond., 1941, **147**, 466.
5. Halliburton, W. D. *J. Physiol.,* 1887, **8**, 133.
6. Jakus, M. A. and Hall, C. E. *J. biol. Chem.,* 1947, **167**, 705.
7. Kühne, W. *Physiologische Chemie.* Leipzig, 1868.
8. Lohmann, K. *Naturwiss.,* 1929, p. 624.
9. Meyerhof, O. and Lohmann, K. *Biochem. Z.,* 1932, **253**, 431.
10. Rozsa, G., Szent-Gyorgyi, A., and Wyckoff, R. W. G. *Biochem. Biophys. Acta,* in press.
11. von Fürth, O. *Arch. exp. Path. Pharmak.,* 1895, **36**, 231.
12. Weber, H. H., Edsall, J. T., and von Muralt, A. In *The chemistry of muscular contraction.* New York: Academic Press, 1947.