The Institute of Biology
Studies in Biology no. 11

Muscle

Second edition

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D. R. Wilkie

F.R.S., F.R.C.P.,
M.B., B.S. (London), M.D. (Yale)

Jodrell Professor of Physiology,
University College, University of London

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General Preface to the Series

It is no longer possible for one textbook to cover the whole field of Biology and to remain sufficiently up to date. At the same time students at school, and indeed those in their first year at universities, must be contemporary in their biological outlook and know where the most important developments are taking place.

The Biological Education Committee, set up jointly by the Royal Society and the Institute of Biology, is sponsoring, therefore, the production of a series of booklets dealing with limited biological topics in which recent progress has been most rapid and important.

A feature of the series is that the booklets indicate as clearly as possible the methods that have been employed in elucidating the problems with which they deal. There are suggestions for practical work for the student which should form a sound scientific basis for his understanding.

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INSTITUTE OF BIOLOGY
41 Queen's Gate
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Preface

Muscles are fascinating to work with because they so obviously *do* something. As our knowledge of the mechanism by which they operate is advancing by leaps and bounds, it seems likely that muscle may be the first tissue whose function is completely understood in terms of ordinary physics and chemistry. It is hoped that this book, by creating interest and providing a little information, may help to accelerate that result.

London, 1968 D.R.W.

Preface to Second Edition

The 'leaps and bounds' mentioned above have been healthy ones and it has been necessary to rewrite completely large sections of this book.

None of the fundamental bases described in the First Edition has been overthrown, but much new knowledge has been acquired, especially about the nature and function of the muscle proteins, of their interactions with ATP and other chemical sources of energy, of their control by calcium and of their widespread occurrence in tissues other than muscle. However, enough mysteries remain to ensure that the study of muscle should remain a lively topic for some time to come. Incidentally, this new edition has been changed over to the SI system of units.

London, 1976 D.R.W.

Contents

1 Introduction

It is only by the use of our muscles that we are able to act on our environment—to exert forces and to move objects, including ourselves, around the world. The muscles are biological machines which convert chemical energy, derived ultimately from the reaction between food and oxygen, into force and mechanical work; it is the purpose of this book to explain what is now known about the manner in which this machine works. It is useless to attack such a problem from one narrow point of view, and you will find that it is necessary to apply ideas and experimental techniques derived from mechanics, biochemistry, microscopy, molecular biology, electronics and thermodynamics, in order to find out what is going on. It will be assumed that you already have a background knowledge of these subjects and, more important, a genuine interest in them.

Even unicellular animals, such as the amoeba, can move, though they have no specialized 'muscles' that can be identified under the microscope: however, in most multicellular organisms some of the cells have become specialized for this particular form of energy conversion. In the higher animals muscle constitutes a large fraction of the body, roughly 40 per cent in the case of a man. The 'meat' of the body is almost pure muscle, so is the heart; and the intestines and some other viscera such as the uterus and bladder contain a large fraction of muscle cells too. In spite of the apparent differences in motile mechanism found throughout the animal kingdom, it nevertheless appears that the essential biochemical mechanism is everywhere the same—the machinery is composed of protein, which can usually be identified as actin and myosin (see p. 10); and the fuel is almost always adenosine triphosphate, called ATP for short.

In examining different types of muscle we are therefore concerned to find out how this mechanism has been adapted to suit the different situations that have arisen in the course of evolution. One basic fact about the contractile mechanism is that, in order to exert a force, chemical energy must be continuously expended. In this respect it is unlike a rubber band, for example, which can exert force continually without cost. Secondly, the slower the contraction of the muscle, the more economically is this force maintained, but the mechanical power that can be produced is correspondingly reduced. A compromise must therefore be made to suit each situation. In our arm muscles a high power production is required; this can only be achieved by making the muscles uneconomical at exerting a force, as we soon discover when we try to hold

up a heavy suitcase. In contrast, the folded hind limb of many quadrupeds—such as the cat—would collapse if the muscle within it did not exert force continuously; these postural muscles are accordingly found to be slow in action, but more economical. An extreme example of force being maintained for a long period of time is provided by bivalves such as the oyster and mussel. These can hold their shells shut tight for many hours because they have evolved muscles that can maintain tension very economically, yet they have also evolved a special mechanism to switch the tension on and off again reasonably rapidly.

Other variations on the theme are shown by heart muscle, which has a built-in mechanism to maintain rhythmic contraction of the whole muscle, quite independently of any nervous connections; and the fibrillar muscle that actuates the wings of many insects. This does not shorten like an ordinary muscle, but if it is suitably loaded it generates rapid vibrations.

These functional differences are to a large extent expressed as structural differences, which will be discussed in more detail in Chapter 3. Skeletal, cardiac and fibrillar muscle fibres all have a striking pattern of

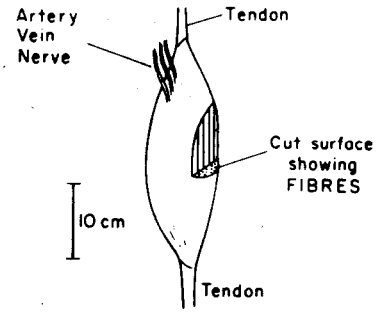


Fig. 1–1 Whole anatomical muscle.

cross-striation. Other types of muscle are called *smooth* but it must be clearly realized that the smooth muscles comprise a very heterogenous group. The smooth muscles of vertebrate viscera are composed of small spindle-shaped cells with very little internal structure, while many invertebrate smooth muscles contain quite highly differentiated systems of protein filaments.

In some contractile systems, such as that in the blood platelets (which accumulate when a blood vessel is cut, and by their contraction arrest bleeding) the actin and myosin assemble themselves into filaments only when required. Vertebrate smooth muscles may operate in a similar way.

In muscle, the relation between structure and function is very close, so in order to present the subject on a concrete basis, let us first consider the detailed structure of a vertebrate skeletal muscle, as shown in Figs. 1–1 to 1–8. This type of muscle is shown because more research has been done on it than on the other types. We are concerned with eight orders of

structure of diminishing size—from gross anatomical down to interatomic dimensions, as indicated roughly by the scales.

Whole anatomical muscle (Fig. 1–1). As mentioned above, the muscles are the meaty parts of our bodies. There are more than 150 different anatomical muscles and almost all of them are attached to the skeleton at both ends, frequently through a strong tendon.

Muscles can only pull—they cannot push—and in order to produce the complex movements of the body the muscles have to act together, but in changing patterns, on the lever system provided by the skeleton, as will be explained in Chapter 7.

The muscle needs an artery and vein so that during exercise it may be abundantly supplied with oxygen, which is required in order to release energy from the oxidation of carbohydrate and fat.

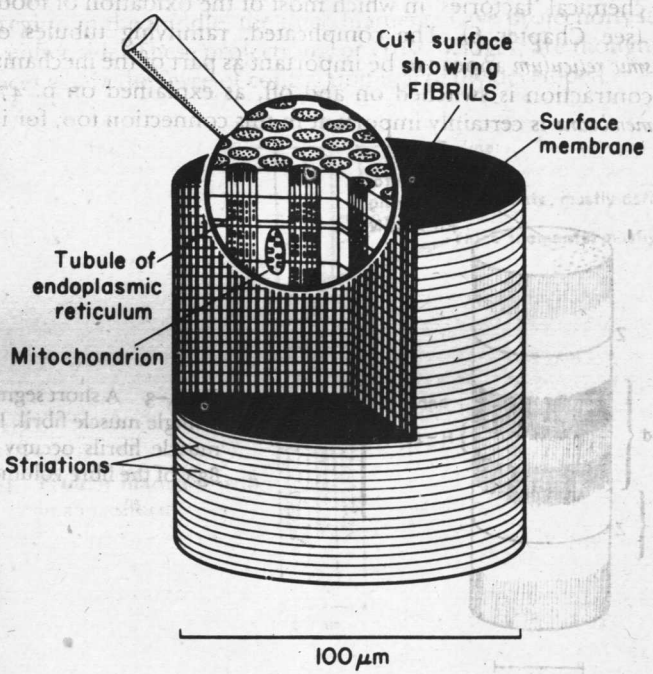


Fig. 1–2 A short segment of a single muscle fibre.

It also needs a nerve supply so that its contraction can be regulated according to the instructions sent down along motor nerve fibres from the central nervous system, that is, the brain and spinal cord. Finally, in order to co-ordinate movement, the central nervous system needs to be informed about the actual length of the muscle and about the tension in its tendons. This information is provided by special sense organs and signalled along sensory nerve fibres back to the central nervous system.

The muscle has a grainy appearance because it is made up of smaller subunits, the fibres. In between them is the extracellular space containing blood vessels, connective tissue etc; which constitutes about 20% of the total volume.

The muscle fibre (Fig. 1–2). This is a cylindrical structure which may be many centimetres long. After staining, or when suitably illuminated, it is seen to have regular striations which extend right across inside the fibre, dividing it up into *sarcomeres* which are stacked one on top of the other like coins in a pile. The details of this striation will be dealt with under Fig. 1–3.

Within the fibre can be seen many cylindrical subunits, the *fibrils*. These are the structures that actually contract, and, in between them, in the *sarcoplasm*, are other structures of functional importance. The *mitochondria* are the chemical 'factories' in which most of the oxidation of foodstuffs occurs (see Chapter 6). The complicated, ramifying tubules of the *endoplasmic reticulum* appear to be important as part of the mechanism by which contraction is switched on and off, as explained on p. 47. The *surface membrane* is certainly important in this connection too, for it only

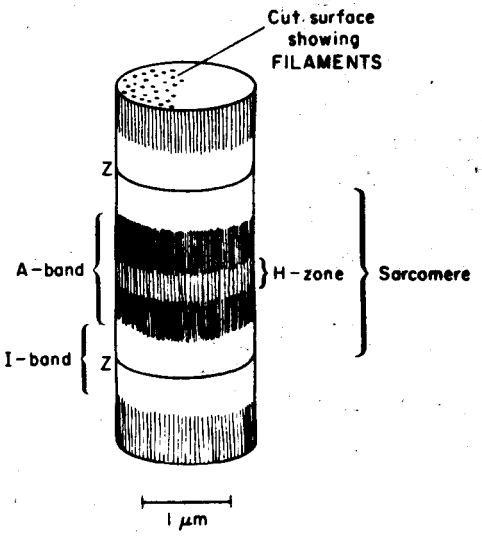


Fig. 1–3 A short segment of a single muscle fibril. In frog muscle fibrils occupy about 83% of the fibre volume.

allows certain ions to pass through; this leads to the generation of the resting potential (p. 45) and of the action potential (p. 45).

In the living body the process of excitation and contraction is only set going when an action potential arrives down the motor nerve fibre from the central nervous system. The nerve fibre is connected to the muscle fibre at a special junctional region called an *end-plate*, whose properties are described on p. 47.

The fibril or myofibril (Fig. 1–3) is a rod of contractile protein which runs

from one end of the fibre to the other. In life it is perfectly transparent, but if it is observed through a special microscope that detects differences of refractive index or of polarization, a pattern of cross-striations can be seen. The correct understanding of this pattern has led to great advances in our knowledge about contraction, as explained on p. 20.

The fibril is divided up into segments by thin partitions called Z-lines or Z-discs. These also run from fibril to fibril right across the fibre, thus dividing it into sarcomeres. In the middle of the sarcomere is the A-band of high refractive index, with a less refractile central H-zone. The rest of the sarcomere is occupied by I-band. The fibril itself is composed of longitudinal fine protein *filaments*.

The protein filaments (Fig. 1–4) are of two types, thick and thin, and they interdigitate with each other as shown in the diagram. The H-zone is that part of the A-band from which thin filaments are absent. Except for a short region in the middle, the thick filaments have projections sticking out to either side. These projections, or cross-bridges, are thought to be the places where the force of contraction is actually developed.

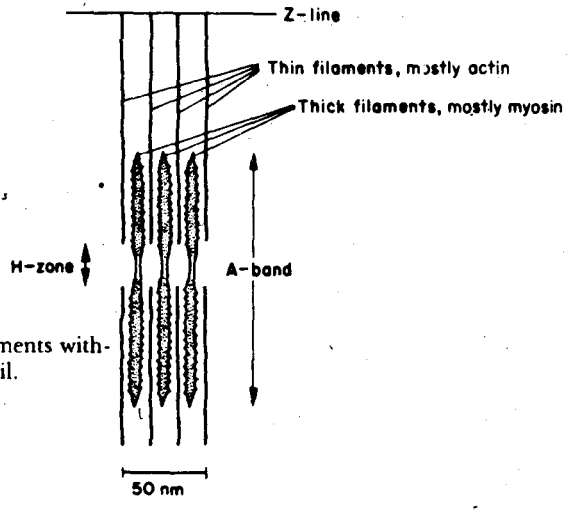
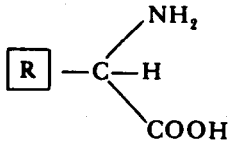


Fig. 1–4 Protein filaments within a myofibril.

The structure of both types of filament will be described later (pages 11, and 13). We will next jump down to interatomic dimensions to see how proteins are built up.

The chemical structure of a protein (Fig. 1–5). It is formed by the polymerization of amino acids. Each amino acid has the formula shown on page 6, where R — is one of twenty or so different chemical groups that characterize the different amino acids. The —CO OH group of one amino acid readily reacts with the —NH_2 group of another, with the elimination of H_2O, and this leads to the structure shown in Fig. 1–5. This has a

Amino acid formula (see p. 5)



central spine of —C—C—N—C—C—N— atoms, with the R — groups sticking out sideways.

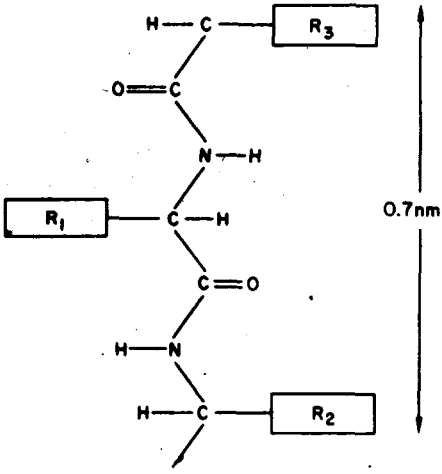


Fig. 1–5 The chemical structure of a protein.

Secondary structure; the α-helix (Fig. 1–6). The central spine readily curls up into a helical (corkscrew-shaped) coil, as shown. The R — groups then stick out radially like the bristles on a test-tube brush, so that the whole forms a cylindrical structure.

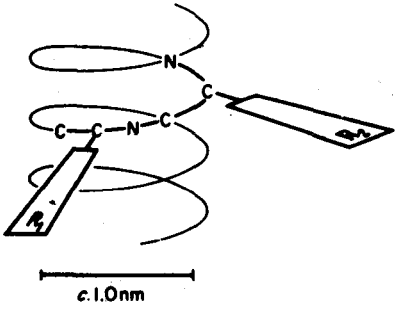


Fig. 1–6 Secondary structure of a protein; the α-helix.

Tertiary structure (Fig. 1–7). The particular sequence of amino acids along the spine gives rise to forces that bend the α helix into a characteristic and often very complicated *tertiary structure* as indicated in highly simplified and diagrammatic form in Fig. 1–7. The forces originate in several different ways. The water-repelling hydrophobic amino acids will tend to be pushed to the centre of the structure while the water-

attracting hydrophilic ones will be pulled to the outside. This brings into proximity groups that were formerly distant, which enables other forces to develop, such as hydrogen bonds and electrostatic attractions. In this subtle process the unique properties of water play an essential role.

The tertiary structure of many proteins, including enzymes, has now

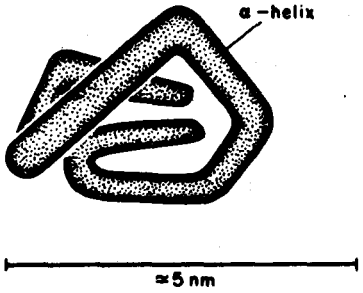


Fig. 1–7 The hypothetical tertiary structure of a globular protein unit.

been determined, using X-ray diffraction (see p. 23). It is necessary first to crystallize the protein, which unfortunately nobody has yet achieved, with the most interesting proteins of muscle. Their detailed structure is thus not yet known.

1.1 Experimental methods

Anatomical dissection and observation suffices for Fig. 1–1. However, the functional significance of what is seen is not so obvious as you might imagine, as explained on p. 61. Light microscopy would reveal most of Figs. 1–2 and 1–3, but remember that since a living muscle fibre is perfectly transparent, you must use a phase contrast or interference microscope (sensitive to differences of refractive index) or a polarizing microscope (sensitive to optical anisotropy: quartz crystals show anisotropy and so does the A-band, hence its name). With an ordinary microscope, slightly out of focus, a sort of phase-contrast effect makes it possible to visualize striations, but you have to be very careful over identifying them. If it is below proper focus, the A-disc will appear darker; above focus, the converse. This effect was well understood by early microscopists, who could not regard their instruments merely as something bought 'off the shelf'. Later workers were not so careful. The result, as related by A. F. HUXLEY (1957), was a half-century of confusion.

A light microscope cannot resolve objects closer than the wavelength of light—say 0·5 μm—so for Figs. 1–3 and 1–4 an electron microscope would be used. X-ray diffraction (see p. 23) detects structures that repeat regularly with a period from less than 0.1 nm up to about 100 nm, so this method has been used to confirm Fig. 1–4 and to establish Figs. 1–6 and 1–7. Finally, the amino acid sequences in Fig. 1–5 must be determined by purely chemical means.

2 The composition of muscles

In this chapter we are not concerned simply with the composition of muscle as shown by chemical analysis. In muscle, composition structure and function are so closely interwoven that all three must often be discussed together. Even chemical analysis, which may seem a rather prosaic approach to the question of how muscles actually work, presents us with some major unanswered questions; certain substances are present in quite high concentration without having any known function. The most challenging substances are the iminazole base carnosine (in some species replaced by anserine) which has not so far been shown to play an intelligible part in contraction or recovery though it does act as a buffer; and zinc, which is present in almost the same concentration as calcium but has not yet been assigned a functional role in normal contraction.

2.1 Water

Muscle contains about 80 per cent water and it is not there simply to fill up the space: it plays a vital role in contraction because of its unique physico-chemical properties. It cannot be successfully replaced even by the closely related compound deuterium oxide, D_2O. Part of the uniqueness of water arises from the fact that even at room temperature the water molecules are not randomly arranged like the molecules of a perfect gas. On the contrary, because of the polar nature of the individual molecules, water has a marked tendency to form liquid crystals, especially if given an electrically charged ion or a membrane (such as abound in muscle) to act as a nucleus around which water molecules can arrange themselves. The formation and dissolution of such hydration shells are accompanied by very large changes of energy. For example, to strip off the hydration shell from K^+ ions and Na^+ ions requires 322 and 405 kJ mol^{-1} respectively.

Under normal conditions about 20 to 25% of the water is present in the interspaces between the fibres. Movement of water in and out of the fibre is regulated by osmotic forces; the water tends to move into regions where the concentration of solute is highest. Thus in weak solutions the fibres tend to swell and in strong ones they shrink. Consequently, one of the important considerations in making up a physiological solution (see p. 26) in which the muscle will function normally, is that its osmolarity should be the same as that of the interior of the muscle fibre. There is still a good deal of discussion over the question whether the water in muscle is 'free' to take part in physico-chemical processes or whether part of it is 'bound' to protein so tightly as effectively to separate it into a separate

compartment. In support of the idea that part of the water is bound is the fact that not all the water freezes at moderately low temperature, and that an exceedingly dry atmosphere, i.e. a very low water vapour pressure, is needed to dry a muscle completely: moreover, although a muscle fibre behaves as a perfect osmometer when it is placed in solutions of various concentrations, it does so as though part of its water was not participating in these changes. On the other hand, all the water seems able to dissolve salts and urea with the normal depression of vapour pressure, so the situation is still not entirely clear.

2.2 Proteins

These make up most of the solid matter of muscle, roughly 20% of its wet weight; this is why meat is a valuable food. The contractile machinery itself is made of protein, as mentioned in the Introduction. Much of the protein is firmly attached within the muscle fibre as shown by the fact that it is far more difficult to extract the protein from the muscle in the first place than to keep it in solution later. Tests of extractability and solubility are an important way of distinguishing one type of protein from another, since some proteins can be extracted by salt solutions that are too weak to extract others. The key property of the solution that decides how effective it will be in extracting protein is not really the concentration as such, but the *ionic strength*, which is compounded of the concentration and the valency, as follows: multiply the concentration of each ion, in mol per kilogram solvent, by the square of the valency; add all these terms together and divide by 2.

Thus for 0.01 mol kg^{-1} NA_2SO_4,

$$\text{ionic strength} = (0.02 \times 1^2 + 0.01 \times 2^2)/2 = 0.03 \qquad (2.2.1)$$

For monovalent salts such as KCl, the ionic strength is numerically equal to the molality (mol kg^{-1}).

Other tests to separate and characterize proteins depend on the mobility of the protein in a strong gravitational field (ultracentrifugation) or an electric field (electrophoresis) or through a column of a suitable absorbant (chromatography). Nowadays some proteins can even be identified by the appearance of their individual molecules in the electron microscope. Tests for enzyme activity or for reaction with specific antibodies can also be extremely useful in special cases.

From the functional point of view, the many proteins in a muscle fibre can be classified into three main groups:

1. Stroma protein. About one fifth of the protein is very insoluble and it seems to function solely as an inert structural element, or skeleton, to hold the rest of the structures in place. Part of this protein is extracellular and can be identified with the collagen and elastin filaments that bind the fibres together and transmit their tension to the tendons. The rest of the stroma protein plays an analogous role within the fibres.

2. *Ordinary cellular proteins.* These are the proteins that are not specifically characteristic of muscle but are also found in other metabolically active cells, and they comprise about another fifth of the total protein. The most interesting of these proteins are the enzymes, more than fifty of which are responsible for guiding the chemical reactions within the cell and thus for keeping the contractile system supplied with ATP. Some of the enzymes can be extracted easily into solution but others, notably those concerned in the oxidation of foodstuffs, are bound to the mitochondria.

3. *Special contractile proteins.* Two types of protein, *myosin* and *actin*, are known to be absolutely essential for contraction: they constitute roughly 33 and 15% of the total protein respectively. They were formerly thought to be characteristic of muscle but more recent research has detected them in a bewildering variety of other tissues. Their presence and function in blood platelets has already been briefly described (p. 2). Their presence in other mobile cells—spermatozoa, amoebae and green algae which exhibit protoplasmic streaming—is at least intelligible. More puzzling, and even more fascinating, has been the detection of actin and myosin in appreciable quantities in organs such as vertebrate brain which we do not normally associate with motility. There is some doubt whether the myosin from all these sources is identical. This is no great surprise since, as we shall soon see, myosin has a complicated molecule whose architecture could well vary slightly without much functional consequence. On the other hand, actin from all sources appears identical in every test at present available.

Myosin can be extracted from fresh minced muscle by salt solutions whose ionic strength is about 0.4 to 0.5. However, a crude extract made in this way contains other proteins, notably some actin, which very much affect its properties so special solutions have been evolved which do extract myosin in relatively uncontaminated form. In solution the myosin molecules have a very characteristic shape, as shown in Fig. 2–1 (a); in electronmicrographs they are seen to have a compact 'head' region and a long 'tail'. Further detail has been revealed by the methods of biochemistry and physical chemistry, and is shown diagrammatically in Fig. 2–1b. Digestion with the proteolytic enzyme papain splits off the 'tail' (MW 210 000) from the head, which itself falls apart into two very similar subunits, each called S1 and with a MW of 120 000. The tail can be broken by trypsin into two unequal fragments, as shown, called light meromyosin (LMM) and S2. These names arose by historical accident and do not express a functional difference since the whole tail region consists simply of two long α helices round each other like a two-stranded rope. Its function is purely mechanical: as shown in Fig. 2–2 the tails pack together to form the thick filament, leaving part, perhaps S2, sticking out (see Fig. 4–9) so as to tether the head to the thick filament while still permitting appreciable movement at right angles to the axis.

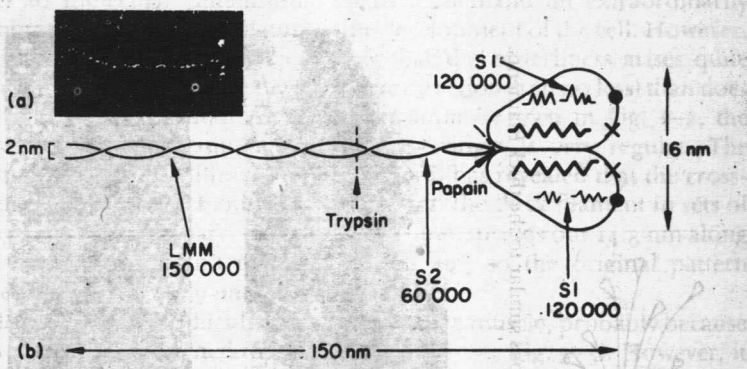


Fig. 2–1 (a) Electron micrograph of a single myosin molecule in solution. (b) Diagram to illustrate the structure of a rabbit myosin molecule as revealed by biochemical and other methods. The numbers indicate the molecular weight of the various subunits, making up a total of 450 000. The LMM 'tail' extends far off to the left. The complex, composed of two S1 units and one S2, is called heavy meromyosin or HMM. The zig-zag lines indicate, completely diagrammatically, the heavy and light chains that have been identified within S1 and • and × indicate the existence of separate binding sites for ATP and for actin on each S1. (Electron-micrograph from ELLIOT, OFFER and BURRIDGE, 1976, *Proc. R. Soc. B.*, 193, 45–53.)

The really interesting structures involved in the transformation of chemical energy into mechanical energy reside in the head. Each S1 has an enzymatic group which can bind ATP and subsequently hydrolyse it: each also has a binding site for actin through which mechanical force can be transmitted. The central problem in muscle physiology is to discover how the hydrolysis of ATP is constrained in such a way that part of the free energy change of the reaction is conserved as mechanical work.

By various chemical treatments, the S1 unit can be further fragmented into a heavy chain, which seems to extend the full length of the unit, and two or more light chains. The latter differ in different types of muscle. The functional significance of the various fragments is not clear at present. It is not certain whether the two S1 units in the head are identical, nor has it been established whether they act independently or co-operatively.

In solutions of high ionic strength, say 0.4, the individual myosin molecules are not attached to each other, but if the ionic strength is lowered by adding water, the myosin molecules aggregate together to form rods that are big enough to be seen in the ordinary light microscope. The way in which this aggregation occurs is illustrated in Fig. 2 (a) and (b). In the middle of the rod is a region about 0.2 μm long which is composed solely of tails. Elsewhere the heads stick out from the surface of the rods, which thus bear a very strong resemblance to the thick filaments of myosin found in living muscles (see also Fig. 3.4). At first sight the orderliness of the organization of a striated muscle, extending down as it

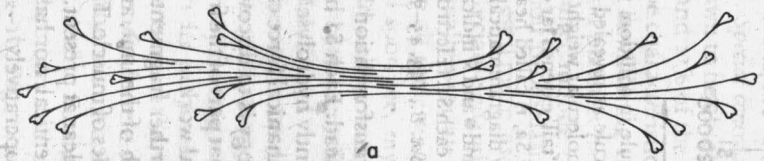


Fig. 2–2 (a) Diagram to show how the myosin molecules aggregate in solution to form rods similar to the thick filaments.

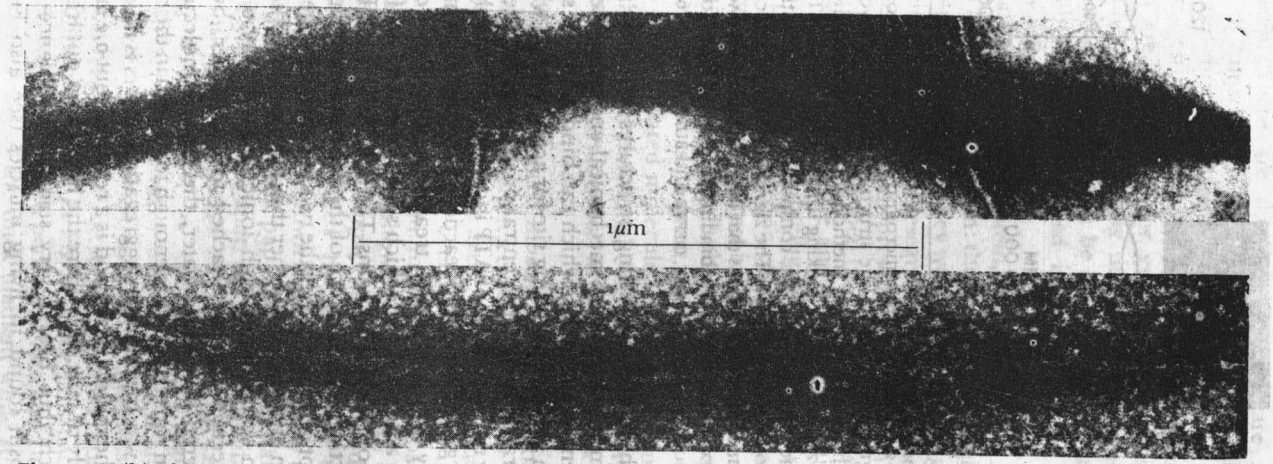


Fig. 2–2 (b) Electronmicrographs of myosin filaments. *Above:* from a muscle: *below:* formed in solution. Note how similar they are. (From H. E. HUXLEY, 1963. *J. Mol. Biol.*, **7**, 281–308.)