Electron Microscope Studies on the Structure of Natural and Synthetic Protein Filaments from Striated Muscle

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A technique has been developed for fragmenting striated muscle into its component thick and thin filaments by homogenization in a "relaxing medium". Such preparations are very suitable for examination by the negative-staining technique. The thick filaments so obtained correspond closely in their structure to those seen in the A-bands of sectioned muscles. The thin filaments, often still attached to residual Z-line structures, also resemble closely those seen in sectioned tissue; they show the same characteristic internal structure, first seen and analysed in filaments from smooth muscles by Hanson and Lowy, as filaments of purified actin. Preparations of purified myosin, precipitated at low ionic strength, are found to contain spindle-shaped aggregates similar in appearance and dimensions to the thick filaments, and having a remarkable differentiated appearance along their length. Along part of the length of the filaments numerous projections are visible, probably corresponding to the cross-bridges seen in sectioned material. However, in a central zone, always about 0.15 to 0.2 μ in length, the projections are absent. The same is the case in the naturally occurring thick filaments. This appearance can be accounted for if the myosin molecule has a projection at one end, and if the myosin molecules in either half of the filaments are arrayed with opposite polarities. In confirmation of some recent results of others it is found that isolated myosin molecules, when examined by the shadow-casting technique, do indeed show such a structure. Observations on heavy and light meromyosin confirm this picture of the structure of the thick filaments.

The thin filaments and filaments of purified actin are both found to form the same very characteristic complex structure when allowed to combine with myosin, or with heavy meromyosin. The results obtained show that the filaments are structurally polarized, and in muscle are arranged so that all of them attached on one side of a given Z-line point in one direction, whilst those on the other are oppositely oriented.

A close similarity is found between the lattice structure seen in crystals of tropomyosin B and that formed by the interconnecting system of filaments at the Z-lines.

The functional implications of these results, particularly those concerned with the polarity of the filaments, is discussed.

1. Introduction

Previous studies on muscle structure in the electron microscope (Huxley, H. E., 1957), using the thin sectioning technique, have provided a picture of the general arrangement of the filaments which is in accord with the sliding filament model of muscular contraction (Huxley, H. E. & Hanson, 1954; Huxley, A. F. & Niedergerke, 1954). However, this technique, in its usual form at least, shows very little detail of the internal structure of the filaments, and attempts to improve the technique in this direction by using alternative fixing, staining or embedding methods, have not
been particularly successful (Huxley, H. E., unpublished observations). Moreover, the thin-sectioning technique is not particularly well adapted to the study of purified muscle proteins in the electron microscope. It has therefore been difficult to extend the structural picture of muscle so as to shed more light on the detailed mechanism of contraction, i.e. the details of the process by which a relative force is developed between the cross-bridged filaments of actin and myosin.

In recent years, the negative-staining technique (Hall, 1955; Huxley, 1956; Brenner & Horne, 1959) has proved to be of great value in showing up fine structural detail in biological specimens in the electron microscope. In this technique the specimen is dried down in a thin film of a solution of some dense salt such as sodium phosphotungstate, which comes out of solution as an almost completely amorphous layer in which the specimen is embedded. The relatively low density of the biological specimen (or at least those parts of it which exclude the negative stain) then enables it to be seen by negative contrast. This technique has proved outstandingly successful in the case of viruses, where a great deal of structural detail, which does not show up at all by normal staining techniques, can be resolved (e.g. Horne, Brenner, Waterson & Wildy, 1959).

However, the degree of detail that can be resolved by this technique is limited by the thickness of the specimen. In the case of virus particles a few hundred Ångströms in diameter, partially embedded in a very thin layer of the negative stain, this limitation is not serious. But clearly the technique is not applicable to intact pieces of tissue, and even muscle fibrils 1 μ in diameter are far too thick to be usefully examined. The muscle must first be broken down in some way.

When glycerinated rabbit pectoral muscle is mechanically disrupted in a high-speed blender, it fragments into separated myofibrils, but normally these are very resistant to further breakdown. Presumably the cross-links between the filaments hold them together in a very robust lattice. According to the sliding filament theory, these cross-links represent sites of actin–myosin interaction. A number of systems have been discovered in which the ATP-induced contraction of glycerinated muscle is inhibited, and in which the fibres become much more readily extensible. These are usually known as relaxing systems, and they are believed to operate by inhibiting the enzymic breakdown of ATP and causing the ATP to dissociate actomyosin. In terms of the sliding filament model this would correspond to detaching all the cross-bridges.

Thus we might expect the structure of the fibrils to be greatly weakened by such treatment, and to be much more susceptible to mechanical disruption. This indeed proves to be the case, and fibrils treated with the ATP–EDTA relaxing system are readily broken down into their constituent filaments. These filaments are found to be very satisfactory subjects for examination by the negative-staining technique and some preliminary notes on the technique and some early results have already been published (Huxley, 1961, 1962). Hanson & Lowy (1963) have reported a very elegant study of the structure of actin filaments by the same general technique, and they have also studied the various kinds of filaments in a variety of muscles from invertebrate animals (Lowy & Hanson, 1962; Hanson & Lowy, 1962). In the present paper a detailed study of the filaments from striated muscles of rabbit and chicken will be described, and the structures seen compared with those found in preparations of purified muscle proteins. A study of the interaction between the filaments and specific antibodies against the various muscle proteins is described elsewhere (Pepe & Huxley, 1963).
2. Methods

Preparation of separated filaments

First of all, glycinated muscle was prepared according to the procedure of Szent-Györgyi (1951) as modified slightly by Huxley & Hanson (1957). Strips of rabbit psoas muscle, or of chicken breast or thigh muscle, approximately 4 mm x 2 mm in section and 5 to 10 cm in length, were dissected out from freshly killed animals, tied with wool onto Perspex strips at the required length, and placed in a medium containing 50 vol. glycerol, 40 vol. water, 10 vol. 0.067 M-phosphate buffer, pH 7.0, at 4°C. The glycerol-water mixture had previously been passed over a column of Amberlite IR120H resin (to remove heavy metal contaminants), as had the water used to make up the phosphate buffer; the pH of the whole mixture was adjusted before use. After 24 hr fresh medium at +4°C was substituted, and after a further 24 hr the preparation was transferred to the deep freeze at −20°C and stored there for at least three weeks, but usually much longer, until required for use.

A portion of one strip, weighing approximately 0.3 to 0.4 g (wet), was then transferred to a solution containing 15 vol. glycerol, 85 vol. standard salt solution for 1 hr at 0°C and then50% reduced into very thin bundles of fibres with a needle. (The standard salt solution contained 0.1 M-KCl, 0.001 M-MgCl₂, 0.0067 M-phosphate buffer, pH 7.0). These fibre bundles were then transferred to standard salt solution for a further period of 1 hr to wash out the rest of the glycerol.

The fibre bundles were then cut up into short lengths (about 2 mm) with scissors (by picking up and cutting a lot of them together) and blended in an MSE homogenizer (cooled in ice and running at top speed) in standard salt solution containing in addition 10⁻³ M-EDTA and 10⁻⁴ M-MgCl₂. Blending for three periods of 20 sec, interspersed with periods of 15 sec. low-speed stirring to allow cooling to occur, was usually sufficient to break up nearly all the filaments into separated myofibrils. This was checked in the phase contrast light microscope.

The fibril preparation was then spun down at about 850 g in a bench angle centrifuge for 3 min and the supernatant solution, containing unwanted soluble proteins, was discarded. The fibrils were resuspended in the same volume of ordinary standard salt solution and spun down as before, the supernatant again being discarded. They were then resuspended in a very small volume (<1 ml) of the EDTA medium described above, and carefully cooled to 0°C in melting ice. The relaxing medium, also cooled to 0°C, was then added to make up the normal volume for blending (7 ml). The relaxing medium consisted of standard salt solution containing in addition 10⁻³ M-EDTA, 10⁻⁴ M-MgCl₂, 3 to 5 x 10⁻⁴ M-ATP, pH adjusted (if necessary) to 7.0. The preparation was immediately homogenized again according to the same schedule as before. Any intact fibrils or other debris were spun out as before; the supernatant solution contained the separated filaments.

Such preparations were stable for about 24 hr at 0°C. After longer periods of time, the filaments tended to aggregate again, but could often be satisfactorily dispersed if 1 mg ATP/ml was added and the preparation shaken violently by hand for a few seconds. Satisfactory filament preparations were also made in which the standard salt solution contained 0.15 or 0.20 M-KCl.

Preparations of filaments from fresh insect indirect flight muscle, and from fresh mytilus adductor muscle were made by blending up the tissue directly in the relaxing medium. Such preparations were not as clean as the one described above, but were adequate for the particular purposes for which they were required.

Preparation of muscle proteins

Myosin

Myosin was prepared from rabbit muscle essentially by the method of Szent-Györgyi (1951). Extraction of the tissue, previously passed once through a meat mincer with 5 mm holes, was carried out for 10 min at 0°C in a medium containing 0.3 M-KCl, 0.15 M-phosphate buffer, pH 6.5. All subsequent operations were also carried out at 0°C. Coarse debris was strained off through muslin, finer debris was removed by centrifugation.
at 1000 g for \( \frac{1}{4} \) hr, and lipid by filtration through glass wool.† Myosin was precipitated by
dilution to \( \mu = 0.05 \), separated by centrifugation (finally being packed down into a
small vol. by \( \frac{1}{4} \) hr spin at 40,000 g), and redissolved in a minimum vol. of 1 M-KCl,
0.1 M-phosphate buffer, pH 7.0. Contaminating actomyosin (normally present in only very
small amounts) was removed by dilution to \( \mu = 0.28 \) and centrifugation at 20,000 g
for \( \frac{1}{4} \) hr. The cycle of precipitation and re-solution was then repeated again. Residual lipid
contaminants were filtered out with glass wool again. Such preparations of myosin were
checked in the ultracentrifuge and found to give a single hypersharp boundary.

**Actomyosin**

"Natural" actomyosin was prepared according to the method of Szent-Györgyi (1951).

**Heavy and light meromyosins (HMM and LMM)**

These were prepared by the method of Szent-Györgyi (1953) except that the digestion
medium was buffered with 0.1 M-phosphate at pH 7.0 (instead of 0.01 M-borate at pH 8.8),
and the meromyosins separated by dialysing the preparation against 50 vol. of 0.05 M-KCl,
0.003 M-phosphate buffer, pH 7.0, and centrifuging out the precipitated LMM (modifi-
cations similar to those used by Lowey & Holtzer (1959)). The LMM was then redissolved
in 0.5 M-KCl, 0.1 M-phosphate buffer, pH 7.0. LMM Fraction I was prepared according to
the method of Szent-Györgyi, Cohen & Philpott (1960).

**Tropomyosin**

This was prepared according to the method of Bailey (1948) except that acetone-dried,
butanol-treated muscle powder, prepared according to the method of Tsa & Bailey
(1953), was the material which was treated with 1 M-KCl in the initial extraction.

**Actin**

Actin was extracted according to the method of Tsa & Bailey (1953) from butanol-
acetone dried powder except that water (instead of 30% acetone), containing \( 10^{-4} M \)
neutralized ATP but no ascorbic acid, was used as the extracting medium. After the
initial isoelectric precipitation, redispersion and dialysis against water containing
\( 10^{-4} M \) ATP, the actin was further purified by ultracentrifugation (2 hr at ~100,000 g),
first in the absence of salt, when the supernatant fraction was preserved, then in
the presence of 0.1 M-KCl, \( 10^{-2} M-MgCl_2 \), 0.005 M-phosphate buffer, pH 7.0, when the
resultant pellet was redispersed in the same medium.

**Negative staining**

The basic method used was that in which a drop of the suspension under examination
is applied to a carbon-filmed specimen grid, the grid then rinsed with pure solvent so
that only particles adhering to the grid remain (still immersed in solvent), and the solvent
replaced (without drying) by the negative-staining solution, which is then allowed to dry.
A number of variations of this technique were used; some of them have already been
mentioned (Huxley & Zubay, 1960). The more important ones were as follows.

1. The substance most usually used as a negative stain was uranyl acetate. The
advantageous properties of this as a negative stain were noticed during the work just
referred to, and became even more apparent when working on muscle. Muscle filaments
are largely destroyed if attempts are made to stain them negatively in sodium phospho-

tungstate without prior fixation, whereas they are well preserved in uranyl acetate.
Moreover, better contrast was obtained using uranyl acetate, particularly over holes
(see below).

The media in which the preparations were suspended often contained phosphate
buffer, and, if allowed to mix with the uranyl acetate solution, this tended to produce
a precipitate which adversely affected the negative staining. Such preparations were
therefore rinsed with 0.1 M-KCl alone before applying the negative stain, which was
generally used in unbuffered 1% aqueous solution, pH around 4.25 to 4.5. There was no
sign in general that the preparations were adversely affected by this pH.

† Caution: glass wool should be washed free of possible heavy-metal contaminants.
(3) When maximum resolutions and contrast were required, together with absence of background "noise" due to surface structure in the supporting film, preparations were made on perforated carbon film, as described by Huxley & Zubay (1960), so that the specimens were embedded in thin films of the negative stain extending over the holes.

(3) Preparations destined for negative staining with sodium phosphotungstate were always fixed beforehand, either in formalin as below, or by floating the grid on which they had been placed face downwards on 0.1% osmium tetroxide solution buffered in the usual way with M/15 veronal acetate, pH 7.0, or by brief (30 sec) exposure of the grid, still wet with a thin film of solvent, to the confined vapour of a 2% aqueous solution of osmium tetroxide.

(4) Even when uranyl acetate was used as a negative stain, some details of the structure were best preserved by prior fixation. This included, in particular, the cross-bridges on the natural thick filaments. Fixation was effected by mixing the preparation with an equal volume of 10% formalin (4% formaldehyde) in M/15 veronal acetate buffer, pH 7.0, leaving it 1 hr at 0°C and then proceeding in the normal way.

(5) In some cases, the whole process was carried out in a cold room at 0°C and the drying down of the negative stain took place over a water bath so that it was very slow. Although it was not always a decisive effect, one often had the impression that preparations made in this way were superior to those made at room temperature, particularly in the case of the LMM lattices. It appeared, however, that it was the stage when the drop of suspension was applied to the grid, rather than the final drying, which was more sensitive.

**Shadow casting**

The procedure originated by Hall (1956) and applied to myosin preparations by Rice (1961a,b) was followed closely, except that an extremely low shadowing angle (giving a shadow length/height ratio of about 15 : 1) was used.

**Electron microscopy**

Preparations were examined in a Siemens Elmiskop I using an accelerating voltage of 80 kV, beam current 13 μA, double condenser illumination (15 clicks of KI), a 200 μ aperture in condenser II, and a 60 μ molybdenum objective aperture.

3. Results

(i) General characteristics of the muscle preparations

After the first stage of homogenization described above, the glycerinated muscle is broken down into isolated myofibrils, which can be examined in the phase-contrast light microscope. These are still visible, slightly swollen, immediately after they have been resuspended in the relaxing medium containing ATP and EDTA. At first they are uncontracted but, after standing for a few minutes on a slide under a coverslip, they begin to shorten and eventually contract very strongly into tightly packed and recognizable masses. The same process takes several hours in the bulk suspension and probably occurs as a result of the gradual lowering of the ATP concentration by slow hydrolysis to a level which is insufficient to cause relaxation.

After the second stage of homogenization, i.e., in the relaxing medium, a considerable proportion (which varies somewhat from preparation to preparation) of the fibrils are broken down into fragments invisible in the light microscope, and examination in the electron microscope shows that they have dispersed into separated filaments. A typical preparation of these is shown in Plate I.

A number of different components is present. These comprise thick filaments, thin filaments, I-segments and vesicles.
(a) **Thick filaments**

These are present in great profusion and show up very clearly in negatively stained preparations. The filaments are 100 to 120 Å in diameter and are predominantly about 1.5 to 1.6 μ in length. The ends of the filaments are tapered, and often fragments of thin filaments are still attached to them, so that it is not easy to give a precise estimate of length; but filaments are not found, for example, with lengths of 1.8 μ or 2.0 μ or longer. The filaments have large numbers of irregular-looking projections on their surfaces (see Plate II). These are present right out to the tips of the filaments, but they appear to be absent from a central zone about 0.15 to 0.2 μ in length. In the middle of this bare central zone, a slight thickening of the filament can often be seen. Some thick filaments are found having lengths less than 1.5 μ, e.g. as short as 0.6 to 0.8 μ, but these show signs of having been formed by mechanical fracture of the longer filaments; thus they have the same diameter as the longer filaments, but the bare zone is no longer centrally disposed, and the tapering is either uneven or absent.

Thus the appearance of these separated thick filaments corresponds in all respects with the appearance of the thick A-band filaments seen in muscles which have been fixed, positively stained and sectioned for examination in the electron microscope; some examples of the latter preparations showing the tapering, the projections or cross-bridges present on the filaments right out to their tips, and the bare central zone with the central thickening are reproduced in Plate III. It is clear that the homogenizing procedure has released these A-band filaments virtually intact.

(b) **Thin filaments and I-segments**

A second characteristic type of filament is present, having a diameter of 60 to 70 Å. These occur predominantly as separated filaments of somewhat variable length. They are often 0.5 to 1.0 μ long, but substantially longer ones can also be found. We will see later that these are probably formed by the polymerization of the shorter fragments. However, a certain proportion of them, which varies from one preparation to another, are present as bundles of filaments, of the kinds shown in Plates IV and V. In these, the filaments seem to be attached together at a central plate and to extend about 1 μ on either side of it. Both the attached and the separated thin filaments have the same characteristic beaded appearance, first noticed by Hanson & Lowy (1962, 1963) in preparations of filaments from smooth muscles.

It is clear that the bundles of thin filaments with this central zone of adhesion correspond to the arrays of thin filaments seen in sectioned material attached to a Z-line and extending on either side of it to terminate at the edges of the H-zones. The lengths of these separated bundles, or "I-segments", correspond to the value we should expect from the model of muscle proposed earlier and their general appearance is unmistakable. Thus we can conclude that the separated thin filaments are to be identified with the thin filaments seen in thin sections of muscle. The diameter of the filaments measured by the latter technique (about 50 Å) is somewhat smaller than that seen here, but it is not clear at present whether the difference is a significant one; certainly the treatment of the material has been very different.

(c) **Vesicles**

In addition to the filamentous components, membrane-bound vesicles of a wide variety of sizes (300 to 5000 Å) are also present. Many of these no doubt derive
from the sarcoplasmic reticulum, but the method of preparation—including the use of glycerinated material—is ill-suited to preserve its original structure. We will not be concerned any further with this component here.

(ii) Other properties of the separated filaments

(1) If the ionic strength of the medium in which the filaments are suspended is increased (by adding KCl) to \( \mu = 0.6 \), the thick filaments dissolve, but the thin ones remain. If they are suspended in a medium containing 0.6 \( \text{m-KCl} \), however, both types of filament dissolve. Thus the separated filaments have solubility properties which are the same as those deduced earlier (Hanson & Huxley, 1953, 1955) from observations in the phase-contrast light microscope of the behaviour of intact muscle fibrils when treated with salt solutions of various kinds. These observations led to the conclusion that the protein myosin was located in the thick filaments, and actin in the thin ones.

(2) If the original fibril preparation is treated with solutions known to extract myosin selectively (e.g. Hasselbach & Schneider’s solution (1961)), and the residue then resuspended in the standard salt medium and a filament preparation made either in the normal way, or simply by homogenization in the absence of a relaxing medium, then large numbers of the thin filaments, showing their characteristic structure, are released, but no thick filaments are found.

(3) The length of the thick filaments, and of the I-segments, is the same whether they are prepared from muscles at rest length, or stretched by up to 25%; and their length is still constant when the preparation has stood for a length of time sufficient to lower the ATP concentration, so that fibrils to which this preparation is added will undergo strong contraction. The filaments and I-segments also remain unchanged when the grids bearing them are, before negative staining, rinsed with solutions containing \( 10^{-2} \text{m-ATP} \), \( 10^{-1} \text{m-KCl} \), \( 10^{-3} \text{m-MgCl}_2 \) (which cause fibrils to contract violently), even when the filaments are lying in holes in the carbon film and are therefore unrestrained.

(4) In many instances thick filaments are to be seen with thin filaments lying alongside them (Plate IV(b) and (c)). The thick and thin filaments do not lie with their surfaces in close contact, however; they are usually 100 to 200 Å apart and the projections on the thick filaments extend across this gap and touch the thin filaments. Again, this corresponds closely to the structure of muscle structure derived earlier by other techniques.

To proceed further with our observations and deductions, we must now turn to the examination of purified muscle proteins, the structures which can be formed from them in vitro, and the comparison of these with the naturally occurring thick filaments.

4. Examination of Preparations of Purified Myosin

(i) Myosin aggregates

If a solution of purified myosin in 0.6 \( \text{m-KCl} \) is examined by the normal negative-staining technique, no particles are usually visible. The reason for this remains obscure, and we shall not be concerned with it further at this point; the molecules can be rendered visible by use of the shadow-casting technique, as has been demonstrated by Rice (1961a), and we shall illustrate and discuss their appearance later.
However, if the ionic strength is lowered to 0·2 or 0·1 μ, then rod-shaped particles are readily visible in negatively stained preparations. These particles may be up to 2 μ in length and 150 Å in diameter, and clearly represent aggregates of myosin molecules. For a single myosin molecule has dimensions of the order of 1500 Å × 20 to 40 Å. More commonly the aggregates are 0·3 to 1·0 μ in length and 60 to 100 Å in diameter, and typical preparations are shown in Plates VI and VII. Short aggregates are formed by rapid dilution of the stock myosin solution in 0·6 m-KCl with standard salt solution. Longer aggregates by dialysis of the stock myosin solution against standard salt solution. The preparation shown in Plate VI gave a rapidly spreading peak in the ultracentrifuge with a sedimentation coefficient of the general order of magnitude of 50 s. The aggregates are characteristically somewhat spindle-shaped and, like the naturally occurring thick filaments, have very rough surfaces due to the large number of projections on them (Plate VIII). Similar aggregates of myosin to these were observed by Jaksis & Hall (1947) using the shadow-casting technique.

If 10\(^{-3}\) or 10\(^{-4}\) m-ATP is added to such a suspension of synthetic filaments in 0·1 or 0·2 m-KCl, no change is observed in their appearance as seen by negative staining. If the myosin is precipitated at lower ionic strength, say at 0·05 μ—as is done during the usual myosin preparation—the denser aggregates then formed consist of large clumps of the same kind of needle-shaped filaments as are found at higher ionic strength, rather than of larger, thicker filaments.

Closer examination of these synthetic filaments of myosin reveals some rather remarkable and significant features. The shortest filaments seen are about 0·25 to 0·3 μ in length, and in many cases consist of a relatively bare central shaft, about 0·15 to 0·2 μ in length, with irregular projections at either end, as shown in Plate IX (top). Larger filaments, say 0·5 μ in length, again frequently have a bare central shaft of about the same length as before, but now there is a longer section on either side of the centre where the irregular projections are visible all the way out to either end of the particle (see Plates IX and X). The character of these projections is somewhat variable. Often there are many fewer of them, but they are much larger, than on the naturally occurring thick filaments; it may be that they arise from clumps of myosin molecules nearly in register rather than a succession of exactly spaced ones as is believed to be the case in vitro. The same characteristic appearance may be found in even larger filaments still, even in ones as long as 1·5 μ (see Plate X). Throughout the whole range of lengths the bare shaft has always about the same length, i.e. 0·15 to 0·2 μ, and is situated near to the centre of the filament, never at one end.

That a structure differentiated in this way along its length can form spontaneously by the aggregation of an assumedly pure protein seems at first remarkable. But it can be accounted for in a very simple way, which it will be convenient to describe here rather than in the Discussion: the significance of some of the other results will then be easier to see.

The myosin molecule is generally supposed to be of the order of 1500 Å in length. It can be cleaved by brief tryptic digestion into two well-defined types of fragment, light meromyosin (LMM) and heavy meromyosin (HMM); and the actin-combining ability and ATPase activity reside in the HMM moiety (Szent-Györgyi, 1953). It appears probable that each myosin molecule contains only one subunit having the properties of HMM, at least if we accept the straightforward view that the purified HMM molecules do not consist of dimers or higher polymers of units which were
Protein Filaments from Muscle

Originally separated in the intact myosin. In the intact muscle, cross-bridges can be seen in the electron microscope extending from the thick (myosin) filaments and attaching to the thin (actin) filaments. It was suggested (Huxley, H. E., 1957, 1960) that these corresponded to the HMM part of myosin (or a part of it) and that the LMM part was contained in the backbone of the thick filaments.

If the projections seen on the synthetic myosin filaments are formed of HMM too, then the appearance of the shortest aggregates seen can be explained in two possible ways. Either:

1. The myosin molecules could have an HMM unit at both ends, and be somewhat longer than current estimates, and could be laid down side by side with no restrictions on their polarity; or

2. The myosin molecules could have an HMM at one end only, could be 1500 Å long, and could form a short filament with the molecules at one end all pointing in one direction (with the HMM end outwards) with those at the other end pointing in the reverse direction, and with the anti-parallel molecules partially overlapped in the centre. In this way the projections due to the HMM could be separated by a bare central shaft whose length could be up to twice the length of the straight part (as opposed to the "HMM projection") of the myosin molecule. All the LMM would be in the core of the filaments. This arrangement is illustrated in Fig. 1(a).

Fig. 1(a). Possible arrangement of myosin molecules, with globular region at one end only, to produce short filaments of type observed, with globular region at either end and straight shaft in centre. The polarity of the myosin molecules is simply reversed on either side of the centre. (b) Possible arrangement of same myosin molecules to produce longer filaments in which the straight shaft in the centre is still present but in which a longer region on either side now has globular projections on it. The polarity of the myosin molecules is reversed on either side of the centre, but all the molecules on the same side have the same polarity.

The first of these possibilities conflicts with the model of myosin having only one HMM subunit per molecule and seems to be ruled out completely by the appearance of the somewhat longer filaments (i.e., those with lengths 0.5 to 0.7 μ), which still show the bare central shaft. If there were projections at either end of the myosin molecule then the only way in which a central bare shaft could be preserved as more and more myosin molecules were added to the original group of molecules which formed the shortest filament would be to displace these additional molecules along the filament, relative to the original group, by a distance only slightly less than their length, i.e., to place them so that the projections at their ends fell just at the edges of the original bare shaft and so did not fill it up. However, if this were done, then filaments whose overall length was around 0.6 to 0.75 μ (i.e., three times the length of the shortest filaments) should show a second bare shaft on either side of the central bare shaft, separated from it by a cluster of projections, with another cluster of projections.
at the ends of the filament. These other bare shafts could not be filled up with projections until the length of the filament reached about 1 \( \mu \). Such effects are not observed. Outside the central zone, projections are observed all the way along to the ends of the filaments, whatever the length of the filament may be. Thus this type of electron microscope observation provides independent evidence that a myosin molecule does not have an HMM subunit projecting out at either end of it.

Let us now consider the alternative way of accounting for the visible structure of the filaments, i.e. to suppose that the myosin molecules, with a projection at one end only, are assembled together, each one staggered longitudinally somewhat relative to its neighbours, but all pointing in the same direction on one side of the central zone of the filament, and having their polarities all reversed on the other side. The polarity is such that the projections are at the ends of the molecules nearer the ends of the filament, and the straight part of the molecules point towards the centre of the filament. The molecules in the central zone which overlap in antiparallel are placed so that the projections at their ends are 0.15 to 0.20 \( \mu \) apart (i.e. if the straight part of the myosin molecule is, say, 1200 \( \text{Å} \) long, then the overlap would be 400 to 900 \( \text{Å} \)). Filaments of any desired length can then be constructed simply by adding more and more myosin molecules by the same rule. This model, illustrated in Fig. 1(b), seems entirely satisfactory.

It will also be noticed that it accounts for the tapered appearance of both the synthetic and natural filaments, for there must always be progressively fewer and fewer molecules in parallel over the last length \( l_{ms} \) of the filament, where \( l_{ms} \) is the length of the non-projecting part of the myosin molecule. Furthermore, as the cross-bridges can be seen in both cases (but especially clearly in the case of the naturally occurring thick filaments (see Plate III)) to occur right out to the tips of the filaments, it requires that the projection on the myosin molecule be very near to the end of the molecule.

There are of course important functional implications of this particular model. These will be dealt with in the Discussion.

It became clear during this work that it was important to try to confirm the apparently rather compelling conclusion that a myosin molecule has a projection at one end of it only, in case some artifact, or some impurity, was being overlooked. Attempts to do this by examination of single myosin molecules by the negative-staining technique were largely unsuccessful. In some experiments, apparently because of unknown and unrepeatable combinations of factors in the preparation of the carbon-coated supporting grid and of the myosin itself, filamentous structures having the appropriate dimensions to be myosin molecules could be seen in certain areas; but the evidence of degradation was so obvious as to make such occasional observations of limited value. A better technique is required.

\[(ii) \text{Examination of individual myosin molecules by shadow casting}\]

We have already mentioned that myosin molecules—or, more accurately, structures which may be single, intact myosin molecules—can be seen using the shadow-casting technique. Although this technique gives substantially lower resolution than negative staining when used to examine the internal structure of particles and filaments and is a good deal more difficult and complicated to use, it does have the advantage that particles of approximately the expected dimensions can be seen, quite consistently, in preparations of isolated myosin molecules, whereas
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apparently because preparation of the us structures having en in certain areas; ch occasional obser-
oo casting
more accurately, be seen using the substantially lower internal structure of implicated to use, it expected dimensions molecules, whereas
nes can be seen by normal negative-staining methods; and I am indebted to Dr. Rice for kindly showing me details of how it is carried out. Rice (1961a, b; 1963) has ob-
served a number of characteristic structures in such preparations, consisting of rod-
shaped molecules of somewhat variable length with one or more globular regions
attached, and has lately favoured the view that the myosin molecule, about 1000 to
1500 Å long, has only one globular region on it. He has supported this view with the
observations that HMM molecules, visualized by the same technique, often appear
as globules of the same size as those on the intact myosin, with short tails (up to
800 Å) attached, whereas LMM molecules appear principally as simple rods up to
900 Å in length. It therefore seemed worthwhile to find out how consistently such
structures could be found by a different observer and, if practicable, to photograph
a sufficient number of them to do a statistical study at least of the lengths of the
different particles seen.
The patterns seen are at first disappointing, and it is clear that most of the material
is not present as distinct, separate and identical particles. Large aggregates of
material are deposited at the centre of the droplets as they dry up. However, there is
a region around many of the droplets in which a considerable number of particles of
similar appearance can be seen. Presumably these represent protein salted out from
the droplet as the salt concentration rises during drying (they do not appear to be
deposited during the initial impact of the droplets, for when such a heavy spraying is
used that the droplets merge and then separate into a different droplet pattern as
the preparation dries, the particles are found deposited mainly around the final droplets).
Many of these particles, as others have described, consist of a rod-shaped structure
with a globular region at one end (Plates XI and XII). In many other cases, however,
particles lacking the globular region are present in considerable numbers. Under such
conditions, a bias on the part of the investigator as to which structures most closely
resemble the normal ones can influence the collection of data. In the present study
fields were photographed in which at least a few of the particles with a globular end
were present (it was very easy to find such areas) but when the photographs were
analysed, all the particles in the field photographed (perhaps 10 times as many as
were used to select the field) were included in the measurements. Thus there was some
bias, but a limited one.
Altogether, 43 fields containing about 1700 particles were analysed. The predomi-

nating species of particle was rod-shaped with a globular region at one end. For every
100 such particles, there were about 20 particles with no globular region, and about
20 either with more than one globular region, or with a globular region not at the
end. This seems to be quite strong confirmation that a rod with a single globular
region at one end is the most likely approximation to the intact myosin molecule.
Only about one in five of the rod-shaped particles without globules was longer than
the average length of the rods with globules, so it seems unlikely that the latter par-
ticles are formed from the former by the folding up of one end; it is much more
probable that the simple rods are formed by breakage of the longer compound
particles during preparation for electron microscopy.
The lengths of the 1200 or so "typical" particles are plotted in the histogram shown
in Fig. 2. The lengths of the particles showed a wide scatter. The average length was
1520 Å and 50% of the particles had lengths within ±280 Å of this value (i.e. within
the range 1240 to 1780 Å). Particles in the better-looking fields (i.e. those with least
debris and aggregation) often tended to be slightly longer than the over-all average.
Thus the average length of the particles shown in Plate XII, which were obtained from the best fields, is 1680 Å. No doubt many of the shorter particles were produced by breakage during the drying of the sprayed droplets, and the longer particles may equally well represent aggregates of broken particles and intact ones. Alternatively, they may represent particles which have been stretched by surface-tension forces during drying. They may also represent intact particles at their true length, for these longer particles are not anomalous in any other respects and all the shorter particles,

![Diagram](image)

**Fig. 2.** Histogram showing the distribution in lengths of about 1200 myosin molecules. Only molecules consisting of a straight rod with a globular region at one end only are included in the distribution.

including those near the average length, may be breakdown products. In these circumstances, it is not possible to say what is the relation between the initial length of the particles—assuming their initial lengths were all identical—and the average length measured here. But as there appears to be an excess of rods without globules left over at the end of these events, the average length of the observed rods with globules must represent an underestimate of the initial length of these structures; thus it is probably safe to say that the length of the molecule is very likely to lie between 1400 and 2000 Å.

The diameter of the rod-shaped region was about 15 to 20 Å, that of the globular region about 40 Å. These are of course the dimensions of the *dried* particle. The length of the globular region was about 150 to 250 Å.

The general conclusion from these observations agrees with that described by Rice (1961a,b; 1963) and lately by Zobel & Carlson (1963), namely that myosin, as seen in the electron microscope, is a rod-shaped molecule of the general order of 1500 Å long, with a globular region at one end. This is in good accord with hydrodynamic
studies (e.g. Lowey & Cohen, 1962), which we will mention later, and of course it
fits in very well with the conclusions we have already reached on the basis of the
negative-staining studies of myosin aggregates, if we equate the projections seen
there with the globular regions seen here. This picture is further confirmed by obser-
vation on HMM and LMM.

Appearance of LMM and HMM when studied by the shadow-casting technique

When LMM in 0.6 M-ammonium acetate is sprayed onto mica and examined in the
usual way, rod-shaped particles 15 to 20 Å in diameter are seen, as well as larger
aggregates (see Plate XIII). These particles always lack the thicker globular region
seen in the intact myosin molecules. Their length shows a very wide scatter indeed
(possibly the structure has been weakened by the trypsin) and one is even more
uncertain than in the case of myosin what significance to attach to the average
value (for 1112 particles), which was found to be 610 Å ± 100 Å. We shall see later
that other electron microscopy evidence suggests a molecular length of about 530 Å,
960 Å, or 1380 Å, etc. (i.e. 430m Å + 100 Å).

The significant observation is the absence of the globular region seen in the intact
myosin and the fact that the molecule is very much shorter. The fact that the
diameter of the molecules is about the same as the measured diameter of the intact
myosin may be significant, but it is possible in principle that the intact myosin could
contain two LMM side by side so that the height of the particle, and hence the
shadow-length—which is what we are measuring here—was the same.

When HMM is examined in the same way, globular particles, often with a short
rod-shaped tail attached to them (Plate XIV), are seen. The globular regions are
identical in size with those seen in the intact myosin. The over-all length of the particles
is again extremely variable, too much so to allow average values to have very much
significance, but the length often lies in the general range 600 to 900 Å. The globular
region most commonly occurs at the end of the rod, not near the middle of it. These
observations on LMM and HMM, made on a large number of preparations, are in
general agreement with those of Rice (1963).

The observations naturally suggest, as Rice (1963) has pointed out, a very simple
model for the myosin molecule, consisting of HMM and LMM joined end to end,
LMM being a linear molecule, and HMM being linear for part of its length but with
a globular region at one end, which also lies at the end of the intact myosin molecule.
This model is in excellent accord with the model which was deduced from the obser-
vation on aggregates of myosin, and it also fits in reasonably well with the hydro-
dynamic data. However, the amount of degradation and aggregation that has clearly
occurred in the preparations is disturbing, and had the results turned out to be in
conflict with other types of observations, one would have hesitated to place complete
reliance on them.

Observations on light meromyosin (LMM) aggregates by negative staining

As with myosin, LMM, when dissolved in 0.6 M KCl and so present as single
molecules, gives very disappointing results when attempts are made to visualize it
by the negative-staining technique. Sometimes tangled and degraded-looking filaments
of 10 to 20 Å in diameter can be seen, but no useful conclusions can be drawn.
However, when the ionic strength is reduced to 0.1 or 0.2 M, then spindle-shaped
aggregates of LMM are found, and these can grow to microscopic dimensions, as was
shown by Szent-Györgyi (1953) and by Philpott & Szent-Györgyi (1954), who drew attention to the fine axial periodicity of 425 Å often seen in them. These aggregates show up very well when negatively stained, and a typical preparation is shown in Plate XV(a). They seem to be able to grow indefinitely in length and diameter, in contrast to the myosin filaments which rarely exceed 100 to 200 Å in diameter and 2 μ in length. Their surfaces are completely smooth—as can be seen in Plate XV(b)—and one forms the impression that the aggregates are formed from straight and uniformly rod-shaped molecules lacking in any projections which would interfere with their orderly stacking into arrays of any size. Very fine filaments 10 to 20 Å in diameter and 20 to 30 Å apart can often be discerned within the spindle-shaped aggregates. According to the concepts developed earlier, the rod-shaped part of the myosin molecule, which includes the LMM moiety, packs together neatly to form the bare central shaft of both the synthetic and the natural thick filaments, and also form the backbone of the rest of the thick filaments. The present findings are clearly in very good agreement with this picture.

When the aggregates are formed by rapid lowering of the ionic strength (by dilution), they rarely show any sign of an axial periodicity, and a typical such preparation is shown in Plate XVI. However, when the ionic strength is reduced more slowly (by dialysis), many of the filaments show one very pronounced axial period (of about 430 Å) by the negative-staining technique, as already shown in Plate XV(a). The main feature of this repeat is the light band, about 100 Å in width. The band shows up most strongly in filaments which are deeply immersed in negative stain, indicating that it does not arise from positive staining of the rest of the repeating pattern. This could correspond to a region of greater protein density than the average. It may reflect a difference in mass per unit length along the length of each molecule. Alternatively, it could arise from an assembly of molecules, which were relatively uniform along this length, but which were arrayed in register with successive sets of molecules overlapping by 100 Å. This model would imply a molecular length of 430n Å + 100 Å where n = 1, 2, 3 . . . depending on whether there is approximately a whole, half, one-third . . . molecular length displacement between successive sets of molecules.

Again, the staining pattern may arise because a certain region of the molecule has a specific charge distribution which tends to exclude the negative stain. In that case, it would be more difficult to draw conclusions about molecular length from the band pattern.

Light meromyosin can also aggregate in a very different manner, into two- or three-dimensional open lattices, which we will describe later, they are not immediately relevant here.

Attempts to examine HMM molecules by negative staining have been unsuccessful so far, and it appears that their structure is either destroyed or completely infiltrated by the negative stain.

Observations on natural and synthetic actin filaments

When preparations of purified F-actin are examined by the negative-staining technique, they are found to consist of filaments all having the same diameter (60 to 70 Å) and of indefinite length (see Plate XVII). These filaments show the same characteristic beaded structure as the thin filaments in the homogenized muscle preparations and have the same diameter. This structure has been analysed in detail
PROTEIN FILAMENTS FROM MUSCLE

by Hanson & Lowy (1963) and seems to consist of two chains of units (probably G-actin monomers) wound around each other in a double helix. The pitch of the helical path pursued by each chain is approximately 700 Å and there are 13 monomers per turn, with a spacing of about 55 Å. The two helices are displaced relative to each other by half a turn, and the monomers in the two helices are displaced relative to each by half the separation of the monomer along either helix. Thus the resultant structure repeats after 700/2 Å = 350 Å. The clarity with which this double helix can be seen is somewhat variable. Some filaments which show it rather well are shown in Plate XVIII. The fact that the thin filaments from striated muscles show exactly the same structure confirms conclusively that they contain actin, in the form of a uniform filament of F-actin, about 1 micron or so in length. The appearance in the muscle homogenate of some thin filaments longer than 1 μ is probably due to the ease with which F-actin filaments can join end to end to form extremely long polymers. Some experiments were done to find out whether the filaments changed in length at all during the negative-staining procedure. First of all, the lengths of a number of 1 segments from rabbit psoas muscle, which were sufficiently thin for the ends of the filaments in them to be accurately defined, were measured. The average value for 35 such measurements was 1.06 μ ± 0.02 μ, for the distance from the Z-line to the end of the filaments, giving a length of 2.12 μ for the total length of the 1 segment.

A rather accurate value for the length of the 1-segments in intact, wet fibrils was found in the following way. When the sarcomere length of a fibril is such that a gap exists between the ends of the thin filaments in the centre of the A-band, then a narrow lighter band, the H-zone, is seen there. When the sarcomere length is such that the ends of the thin filaments overlap slightly in the centre of the A-band, then a darker line appears there. Experience showed (1) that in many fibrils the sarcomere length was apparently very uniform over 10 or 20 sarcomeres, which all exhibited identical band patterns and (2) that the point at which the light H-zone disappeared, and was replaced by a dark line, could be judged quite accurately with a little practice. Thus it was found that in fibrils where the average sarcomere length was 2.27 μ more, the H-zone could be seen in all the sarcomeres. In ones with a sarcomere length between 2.26 and 2.23 μ, neither an H-zone nor a dark line could be seen. In fibrils with an average sarcomere length of 2.22 μ or less, a dark line could be seen in all sarcomeres. This then gives a length of about 2.24 μ for the length of the intact hydrated 1-segment of rabbit psoas muscle, to be compared with the value 2.12 μ of segments after negative staining. The amount of shrinkage that can have taken place is thus about 5%. It may in fact be less, for the I-filaments measured in the electron microscope were never perfectly straight and it was not practicable to try to correct for slight kinks in them. Thus if the periodicity of the I-filaments is 350 Å measured in negatively stained preparations, that in the intact muscle, assuming that all that can have happened is that the filaments may have changed in length slightly, can at a maximum be only 5% higher, i.e. about 372 Å.

Actin filaments treated with HMM and with myosin

It was shown by Szent-Györgyi (1953) that HMM retains the ability of the parent myosin to form a complex with actin, and it was observed subsequently in the phase-contrast microscope (Hanson & Huxley, 1955) that the I-segments of muscle fibrils from which the myosin had been extracted would take up considerable amounts
of myosin and of HMM from solution, undergoing a large increase in density all along their length in the process. This experiment provided another line of evidence that actin was located in the I-filaments and was uniformly distributed along their length. It therefore seemed worth while to examine these actin–myosin or actin–HMM complexes by the negative-staining technique. Composite filaments of actin and HMM are readily formed by placing a drop of a solution of HMM in 0.1 M KCl onto a carbon-filmed specimen grid previously treated with either an F-actin solution or a muscle-filament preparation, and rinsed but not dried, so that many filaments remain attached to the carbon film and still evidently able to combine with HMM. This method was less successful in the case of myosin, but composite filaments are readily obtained here by mixing the two proteins in solution, or from preparations of natural actomyosin.

The appearance of the actin–HMM filaments as seen by negative staining is shown in Plates XIX and XX. This remarkable composite structure, whose diameter (200 to 300 Å) is considerably greater than that of the actin filaments, has a very strongly defined long axial period. Measurements of a total of 870 such periods in 118 different filaments negatively stained with uranyl acetate gave an average value of 366 Å ± 15 Å, very close to the period of the double helix in the F-actin structure. Measurements in the electron microscope of the lengths of intact I-segments treated with HMM show that they are the same (within 2%) as those of untreated I-segments. Measurements in the light microscope, of the kind already described, of the lengths of the I-segments in extracted muscle fibrils treated with HMM show that here too the addition of the HMM alters the length of the I-filaments by less than 5%. The composite filaments also show a well-marked shorter axial period of 50 to 60 Å, similar to the periodicity of the monomers along the F-actin helix. The filaments have a very definite structural polarity, perhaps best seen in terms of the tendency of their rather complicated internal structure to give an appearance of arrowheads which all point in the same direction along the whole length of any given filament.

Exactly the same appearance is seen whether the complex is formed with purified actin or with the naturally occurring thin filaments from striated muscle or from insect indirect flight muscle, or from mytilus adductor muscle (a smooth muscle), filaments found by Hanson & Lowy (1963) to show the actin structure. No significant difference in period is observed in any instance, including experiments in which the complex was fixed in either formalin or osmium tetroxide before negative staining, or in which the filaments were suspended over holes in the carbon film so that they were not in contact with a carbon substrate.

If unixed preparations of the actin–HMM complexes are treated with standard salt solution containing 10−4 M-neutralized ATP and then examined by the negative-staining technique, the normal actin structure is seen again; the complex has apparently been dissociated by the ATP, as we should expect.

The appearance of actin–myosin complexes when deposited onto the specimen support from 0.6 M KCl (see Plates XX(c) and XXI) is rather similar to that of actin–HMM. The composite filaments show the same polarized "arrowhead" structure, and have approximately the same periodicities. Unlike the actin–HMM filaments, however, they tend to associate together in rafts, in which the side-to-side distance is about 220 Å, the filaments all point in the same direction, and the long axial periodicities of adjacent filaments are displaced relative to each other by about half a period. When such actomyosin complexes are derived from natural actomyosin,
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contoured onto the specimen similar to that of actin–d “arrowhead” structure, he actin–HMM filaments, a side-to-side distance cition, and the long axial to each other by about from natural actomyosin, a considerable proportion of the filaments are around 1 μ in length, as though they were individual I–filaments to which the myosin had become attached. These filaments probably correspond to those seen by Hall, Jakus & Schmitt (1946) in positively stained preparations of (acto)myosin.

In the presence of 10−5 M-ATP (in 0.6 M-KCl), only ordinary actin filaments could be obtained from actin–myosin solutions.

At lower ionic strengths (e.g. in 0.15 M-KCl), the same type of actin–myosin complex was observed as in 0.6 M-KCl, provided, of course, that the complex was initially formed at high ionic strengths, where myosin is in the form of single molecules. If the myosin was in the form of aggregates at the lower ionic strength, before actin was added, then the compound filaments were not formed, but merely associations between the myosin and actin filaments. The existence of these two quite distinct forms of actomyosin should be borne in mind. When 10−5 M-ATP was added to the compound filament type of preparations (in very dilute solution), the actin–myosin complexes all apparently dissociated, and only actin filaments and some myosin aggregates could be found by the negative-staining technique.

The natural interpretation of these observations is that an HMM (or myosin) molecule has combined with each of the G–actin units in the F–actin structure, giving rise to a double helix of HMM molecules wound around the outside of the original actin filament. The looser packing of the molecules at the greater radius would enable the periodicities in the structure to be seen more easily.

The exact details of the structure of the molecules of HMM and the manner in which they are attached are not evident from the electron micrographs obtained so far. Isolated molecules of HMM are not visible by the negative-staining technique, which perhaps degrades them in some way. Thus the structure seen in the composite filaments may contain only the degraded remnants of the HMM molecules, remnants large enough, however, still to be visible. Filaments of actomyosin (deposited on the grid from 0.6 M-KCl) have a very similar appearance to those of actin–HMM, and, except in a few places, show little sign of 1500 Å long myosin molecules attached to the actin monomer; possibly a good deal of the myosin molecule has been degraded too.

There are two conclusions, however, which do emerge unambiguously from the general appearance of these composite filaments. One is a relatively trivial one, that one HMM molecule attaches per G–actin subunit; if this were not the case if, for instance, only one HMM molecule was present per two G–actin units and was shared between two G–actins on opposite chains, or on the same chain, then the long period would have twice the observed value. The second is more important, and follows from the structural polarity of the complex filaments. This polarity is not a consequence of a unidirectional growth of the complex. Actin filaments treated very briefly with low concentration of HMM will show the composite structures along short stretches of their length, separated by long stretches of normal actin. These stretches of composite structure are all polarized in the same direction. Thus the structural polarity must be imposed by the underlying actin structure, and so the actin filament itself must have a structural polarity. This means that the monomeric units in the F–actin structure in both chains must all be oriented in the same direction (of course, if every n-th monomer, where n > 2, along either or both chains was reversed, then a polarized structure would still be produced, but such a structure should then show other periodicities in electron micrographs, and these are in fact not observed).
If the actin filaments have a polarized structure, the question immediately arises as to how this is arranged in situ in the muscle. It is possible to use the same technique to answer this question. As already mentioned, preparations of separated filaments often contain bundles of thin filaments still attached to a Z-line. When such a preparation is treated with HMM, the filaments take up the HMM and form the complex structure as before, and it can be seen (Plates XXIII and XXIV) that all the filaments on one side of the Z-line are structurally polarized in the same direction, and all the ones on the other side in the reverse direction. The sense of these polarizations (the "arrowheads" pointing away from the Z-line) is the same for all I-segments examined (several hundred). As in the case of the myosin filaments, the functional implications of this will be considered in the Discussion. There are some further structural implications, however, which it will be convenient to mention before describing observations concerning the nature of the Z-line.

It is quite clear that the actin filaments do not pass uninterrupted and unchanged through the Z-line. This is apparent from many electron micrographs and the nature of the interconnections at the Z-line has recently been admirably described by Knappes & Carlsson (1962). The basic observation is that actin filaments on either side of the Z-line do not lie in the same straight line. They are all displaced relative to each other and interconnected by very thin diagonally oriented filaments. What the present observations show is that these connections cannot be, in whole or in part, simply a continuation of the actin filaments in, say, an untwisted form. Such a continuity is ruled out by the reversal of polarity on either side of the Z-line. The actin structure has to be reversed in some way within the width of the Z-line, and one might think that the best way of achieving this was to connect the actin filaments to some other structure which possessed a twofold rotation axis perpendicular to the long axis of the muscle.

We shall describe in the next sections some observations which bear on the possible nature of this substance. The observations made on troponyosin B were stimulated by the appearance of certain structures in LMM preparations, and so we shall describe these first, though they may not have any direct relevance to the structure of muscle.

**Lattice structures in LMM aggregates**

The most usual type of aggregate formed when the ionic strength of a solution of LMM was reduced to below 0.2 M is the spindle-shaped filament already described. However, occasional preparations—and the exact conditions have not been successfully defined so far—contained, in addition to the usual filaments, remarkable open lattice structures, as shown in Plate XXV. These hexagonal lattices seem to be formed by three groups of molecules or filaments, meeting or crossing each other at 120° angles at each lattice point. Within each group, a small amount of scatter in direction and position seems to be present, after negative staining anyway. Sometimes isolated groups or "stars" are seen (Plate XXVI) and in these the scatter is considerable. The separation of the lattice points is slightly variable between 550 and 600 Å, and the width of the structures which cross at the lattice points is 100 to 150 Å. This is of course much greater than the width of a single LMM molecule. The width of the isolated stars is 800 to 900 Å. Sometimes normal-type LMM filaments grow out of the lattices along one of the principal axes (see Plate XXVIII). Preparations of LMM which show both types of structure in their aggregates give only a single peak in the
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ultracentrifuge. Preparations of LMM Fraction 1 can also give both types of aggre-
gate. It is not apparent at present whether there is any sort of a lattice structure
present in the third dimension, or whether one is simply looking at a simple two-
dimensional lattice or a number of such lattices in register and in contact.

These observations demonstrate a rather novel way in which a fibrous protein can
aggregate, but they probably have no direct relevance to the way the LMM units
of the myosin molecules are arranged in muscle. However, they did serve to remind
the author of the open framework lattices seen by Hodge (1959) in positively stained
and unstained crystals of tropomyosin B, and to suggest that these might with
advantage be examined for them by the negative-staining technique.

Observation on aggregates of tropomyosin B

Tropomyosin B was precipitated by dialysis against ammonium sulphate as
described by Bailey (1948) so as to give crystals of the protein. These were dispersed
in a mechanical blender into smaller fragments suitable for negative staining. When
examined in this way in the electron microscope, the preparations were found to
contain lattices of the type shown in Plates XXVII and XXVIII. The lattices are
approximately square and the lattice spacing in all cases examined was about 200 Å.
Two filaments cross at each lattice point, as opposed to three in LMM. The structure
is clearly the same as that seen by Hodge in sectioned crystals, but some additional
features can now be discerned. The structures which cross at the lattice points are
very narrow and measure 20 to 30 Å across. In many places they can be seen to
consist of two fine filaments about 25 Å apart, and measuring about 10 to 15 Å in
diameter. It is tempting to suggest that these filaments represent single α-helices, or
coiled-coils of two α-helices. In some instances, filamentous aggregates may be seen
growing out of the lattices (Plate XXVIII(a)).

On close examination, some curious features of the lattice itself become apparent.
First of all, no cases have been observed where the lattice is accurately square;
the relevant angle is always 5 to 15° different from 90°. Secondly, the structure often
presents a somewhat "woven" appearance. This occurs because successive lattice
points along each of the two principal directions do not lie precisely on a straight
line. They lie, or have a tendency to lie, alternately on either side of such a straight
line. Thus the lattice points fall into two groups and define two new lattices. These
"superlattices" are again of course approximately square, have a spacing 1/2 times
that of the original lattice, are rotated relative to it by ~45°, and are displaced
relative to each other as shown in Fig. 3.

The simplest way of accounting for this effect is to suppose that the two sets of
lattice points lie in two different planes—or lay in two different planes before the
preparation was dried in the negative stain—and that this has given rise to a fairly
systematic displacement of the lattice points during the preparation, depending on
which of the two planes the lattice point belongs to.

This structure seen in the fragmented tropomyosin crystals therefore bears a
startling resemblance to the arrangement at the Z-line (Plate XXVIII(c)). The
general organization of filaments there into an approximately square lattice was
noted by the author some time ago, and the exact structure has recently been worked
out in detail by Knappes & Carlson (1962) and by Reedy (1962 in preparation). We
will go into the matter in a little more detail in the Discussion, but mention at this
point that the structure found by them is defined by two sets of lattice points in two
planes, with the same spacings and arrangement of connecting filaments between them as seen in the present negatively stained preparations of tropomyosin. It seems too much of a coincidence for this not to suggest that some at least of the tropomyosin B is located in the Z-line filaments.

Fig. 3. Lattice structure seen in tropomyosin crystals and resembling closely that seen in cross-sections through the Z-line. The two types of lattice point in the crystal may lie in two separate planes, in each of which the lattice spacing is \( \sqrt{2} \) times the spacing in the lattice formed by their superposition. The two lattices would correspond to the attachment points of the two sets of actin filaments on either side of the Z-line.

5. Discussion

(i) General structure of muscle

The finding that discrete filaments of well-defined length, equal to that of the A-bands, can be recovered from homogenized preparations of striated myofibrils provides a new kind of evidence in support of the view that an array of such filaments is present in the A-bands of striated muscle and is responsible for the characteristic high density and birefringence of that zone (Hanson & Huxley, 1953). Admittedly such discrete filaments have already been seen clearly in osmium tetroxide-fixed PTA-stained (positive-staining) sections of muscle (Huxley, H. E., 1957), but no harm is done by confirming such observations by a very different technique. The further finding that such homogenized preparations also contain structures, again of a clearly defined length, which are obviously sets of I-filaments still connected to a Z-line and ending, in register, about 1 \( \mu \) on either side of it, confirms the other part of the picture of muscle structure put forward earlier.

The results described above are also in full agreement with the earlier conclusions concerning the location of actin and myosin in muscle, the general nature of the filaments they form, and the invariance in length of those filaments under different physiological conditions. These various issues have already been fairly well covered during the description of the results, and the points made will not be labourd further here. The results provide further evidence that the cross-bridges are to be associated with the HMM component of myosin, with the LMM component present in the backbone of the thick filaments, and they indicate that it may be worth while exploring further the possibility that part of the tropomyosin B in muscle is located in the Z-lines.

On the question of whether additional protein components besides actin and myosin are present in the thin and thick filaments, the evidence is still incomplete. Thus the remarks in the following two sections are necessarily of a somewhat
PROTEIN FILAMENTS FROM MUSCLE

(ii) Other possible components in thin filaments

Hanson & Huxley (1955) showed that when muscle fibrils, from which myosin had already been extracted (so that they consisted simply of a succession of I-segments), were further extracted with 0.6 M-KI (known to depolymerize and extract actin), much of the material of the thin filaments was removed. However, a very tenuous backbone structure still remained when the extraction was observed, in phase contrast, taking place in fibrils attached to the coverslip or slide. This indicated that some other filamentous substance as well as actin might be present in the thin filaments, but the evidence could not be said to be completely conclusive because of the possible presence of denatured actin filaments in these glycercinated fibrils.

Later work (Huxley & Hanson, 1957) showed that the I-substance in such fibrils accounted for some 24% of the total muscle protein (36% of fibril protein) and the "Z-substance" (i.e. the material responsible for the additional density of the Z-line) about 4% (6% of fibril protein). Estimates by Hasselbach & Schneider (1951) had shown that the quantity of actin which could be extracted from whole muscle amounted to some 14% of the total protein. The difference between these two values could be ascribed to the presence of some material besides actin in the thin filaments; but it might also be at least partly due to incomplete extraction of actin.

It has been shown that actin and tropomyosin tend to be extracted together from muscle (Corsi & Perry, 1958) and also that they tend to remain associated together in some way once extracted (Martonosi, 1962). Again this suggests the possibility of actin and tropomyosin being located together in the thin filaments, but it makes quantitative arguments based on earlier estimates of actin content (which may have included an appreciable amount of tropomyosin as well) less precise than ever. Another type of argument is that advanced earlier by the present author (1960) where it is calculated that if the thin filaments were built out of the helical arrangement of nodes described by Selby & Bear (1956) and if a node represented one actin monomer, then the quantity of actin present should be either 16.5% or 20.25% of the total muscle protein, depending on whether a molecular weight of 57,000 or 70,000 was taken for actin. This figure represented the maximum quantity of actin which could be present, if the entire cross-section of the muscle were occupied by fibrils. There is thus a distinct discrepancy between this value and the value of 24% for the amount of I-substance deduced from interference microscope measurements and, as suggested earlier, this difference might be accounted for by the presence of tropomyosin.

In that case, tropomyosin would have to be present side by side with actin all the way along the thin filaments, for the interference microscope observations include measurements of density within the I-heads but outside the region of the Z-line (that is, they were not all measurements of density integrated over the whole I-region), and the density throughout the I-region is too high. The possibility that tropomyosin is also present in the form of a lattice in the Z-line has already been mentioned, and there is a certain attraction in the idea that just as lattices and filaments of both tropomyosin and of L-meromyosin may be observed growing out of each other in vitro, so filaments of tropomyosin extend out from either side of a double layer...
of tropomyosin lattice at a Z-line. It is perfectly possible for such a lattice to have the appropriate symmetry requirements mentioned earlier (2-fold rotation axis in plane of lattice) such that the projecting tropomyosin filaments will have opposite polarity. Along these filaments actin is laid down again with opposite polarity on either side of the Z-line. The dimensions required for such a lattice may be easily calculated from the known spacing of the hexagonal array in the A-bands of muscle. Taking a value of 440 Å for this, and remembering that there are two thin filaments for every thick filament in the array, then, if the Z-line lattice is 10° out from being a true square, the I-filament separation in the region on either side of the Z-line will be about 292 Å and the separation of the lattice points in the Z-line, seen face on, so that the two layers of the lattice are superimposed, will be \( \sqrt{2 \times 292} \approx 206 \) Å. This value agrees very well with the observed lattice spacing in the tropomyosin crystals.

It is shown elsewhere (Page & Huxley, in press) that the periodicity visible in the thin filaments in sectioned muscle, when corrected for shrinkage effects, lies very close to 406 Å, a value decisively different from the helical period found by Hanson & Lowy (1963) in filaments of actin (\( \sim 350 \) Å), and that found here in the actin-HMM filaments (\( \sim 366 \) Å). As we have already seen, this difference is not a consequence of any length change in the thin filaments when they are negatively stained, or when they combine with HMM and are negatively stained. It is very interesting and a little disappointing that no obvious changes in the actin structure take place when it combines with myosin or HMM; but such changes may lie below the resolution of the present techniques. It is of course by no means established that the pitch of the actin helix in intact muscle is of the order of 406 Å rather than 350 Å (although recent very accurate X-ray studies by Worthington (1959) do support a value of 411 Å), only that some structure in the I-bands, probably superimposed on the actin structure, has this period. This possibility is discussed by Hanson & Lowy (1963). All these considerations point to the desirability of studying the interactions of tropomyosin B and actin, particularly by the negative-staining technique. Of course, it may simply turn out that the difference in period arises merely from a change in helical parameters unaccompanied by significant length changes of the filaments during negative staining.

If the four very fine filaments seen by Knappes & Carlson (1962) to continue on from the ends of the thin filaments near the Z-line represent continuations of tropomyosin filaments extending throughout the I-filaments, we can place limits on the amount of tropomyosin present. If tropomyosin consists of two \( \alpha \)-helical polypeptide chains side by side (Tsao, Bailey & Adair, 1951) then a filament containing four such molecules in parallel, and also containing 13 actin monomers of molecular weight 62,000 per 350 Å, would have an actin:tropomyosin ratio of approximately 3:6:1. Perry (1960) has estimated that tropomyosin B may represent 6 to 10% of the total muscle protein (cf. about 14% of actin) so there is certainly enough tropomyosin present to build such a structure; on the basis of these figures, there would be almost enough to have at least 8 molecules in parallel in the thin filaments, and still have enough left over to build a lattice at the Z-lines.†

Another question that presents itself, and which we can do little to answer at present, is how in a muscle all the thin filaments come to be laid down with such a

† It should be appreciated that only a part of the Z-line density is accounted for by the very fine filaments passing through it; a good deal of other material appears to be present between the fine filaments.
such a lattice to have the fold rotation axis in plane will have opposite polarity, site polarity on either side if the Z-line will be about seen face on, so that the ~ 206 Å. This value agrees osin crystals.

(iii) Other possible components in the thick filaments

In the case of the A-band filaments in muscle, the question of whether myosin is the sole protein in them can again not be decided satisfactorily at present.

There is no evidence from the electron micrographs that the naturally occurring thick filaments and those reconstituted from purified myosin differ in their appearance in such a way as to suggest that the former contained a substantial amount of some other protein, although such differences would not necessarily be apparent. There is one small difference in that the thick filament seen in sectioned material, and often the isolated ones, have a slight thickening half-way along their length (in the middle of the central bare zone) but the amount of material involved would at most be 2 to 3% of their total mass. There is no sign of any residual core when the thick filaments are dissolved at higher salt concentrations. There are nevertheless some rather stubborn discrepancies, not large but perhaps significant just the same, which arise when one tries to describe the situation on a strict quantitative basis.

If one takes values of 110 Å for the diameter of the thick filaments, 40 Å for the diameter of the cross-bridges and 180 Å for the length of the cross-bridges, then, knowing the number of filaments per ml. of muscle (about 2.4 × 10^6), the contribution made to the total wet weight of the muscle by the thick filaments may be calculated to be 6.3 mg/ml. Taking a value for the myosin content of 34% of the total protein, and a protein content of 20% of the wet weight, then the myosin content may be calculated to be 6.8 mg/g or 7.1 mg/ml. On this basis then the agreement is reasonably good.

On the other hand, using the same dimensions for the filaments, one may calculate that the “molecular weight” of each of them is about 1.8 Å. Now the number of cross-bridges associated with one thick filament appears to be about 200 to 220 (Huxley, H. E., 1957). If there is one cross-bridge per myosin molecule, one can calculate the contribution made by myosin to the molecular weight of the filament, if one knows the molecular weight of myosin. Current values for myosin lie around either 470,000 (Lowey & Cohen, 1962) or 620,000 (Kieley & Harrington, 1960). This leads to contributions of either about 100 × 10^6 or 130 × 10^6, leaving a substantial contribution to be made by some other substance to bring the value up to 160 × 10^6. And the difficulty cannot be avoided satisfactorily merely by accepting that one has assessed the dimensions of the filaments and cross-bridges incorrectly, for then the agreement with the total “myosin” content will no longer hold. If a contribution from some other substance is admitted, then it also has to be accepted that this substance is erroneously estimated with the myosin. Alternatively, one might suppose that there
were two myosin molecules per cross-bridge, that the size of the filaments was being underestimated, and their number overestimated.

Perhaps it is pushing the data too far at present to expect perfect numerical agreement, but it does appear that at least one of the values being used must be wrong.

As in the case of the thin filaments, there exists the question of what determines the lengths of these filaments, in muscle, within such close limits; again, it is difficult to see how this could reliably be achieved by the controlled polymerization of myosin on its own, although preparations of synthetic myosin filaments can be made in which most of the filaments have lengths of the right order of magnitude. Again, one wonders about the possibility of a copolymer with some other structure of a slightly different period taking place so that stable structures were formed only at lengths equal to the lowest common multiple of the two periods. There exists the following curious numerical coincidence. The periodicity of the myosin filaments seems to be 435 Å whilst the other axial periodicity in muscle which can be determined very accurately by X-ray diffraction is 411 Å (Warthington, 1959). (This second period is probably equivalent to the approximately 406 Å period in the I-filaments mentioned earlier. The lowest common multiple (to a good approximation) of these two lengths is 7400 Å (435 × 17 = 7395, 411 × 18 = 7398) which lies close to one-half the length of the A-band.

(iv) The structural polarity of the actin and myosin filaments

When a structure consists of two helical chains of molecules wound round each other, then it is not necessarily so that the resultant structure has to be directionally polarized; it would not be, for instance, if alternate molecules in each of the two chains were reversed, or if all the molecules in each chain pointed in the same direction but the two chains pointed in opposite directions. It is all the more interesting then to find that when the G-actin molecules polymerize to give F-actin, it is a polarized structure that they form, in which all the molecules in both chains are oriented in the same equivalent direction.

Furthermore, the actin filaments at the Z-line do not simply run straight through it, but are interrupted and connected to a fairly elaborate structure there in such a way that all of them on one side point in one direction and all on the opposite side of the Z-line point in the opposite direction. This all suggests very strongly that there is a functional requirement that all the actin monomers on one side of a Z-line should point in one direction, and all those on the other in the reverse direction.

According to the sliding filament model, the relative force that is developed between the actin and myosin filaments on either side of a given Z-line has to pull all the myosin filaments towards that Z-line. If the force is generated as a consequence of short-range interactions between the actin monomers and the cross-bridges, and if spatial activation effects are not invoked to provide directionality—and the fact that myofibrils seem to contract quite normally when merely irrigated with solutions of ATP strongly indicates that such effects are not involved—then the direction of the force developed at any given site must be locally determined. It could be determined by the orientation of the actin monomer, or by the orientation of the myosin molecule of which the cross-bridge is a part, or by the orientation of both. Furthermore, the presumably very specific interaction of two protein molecules,
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Thus the findings concerning the strict polarity of the actin monomers are not 

ely consistent with the sliding filament model, but seem to be an essential 

uirement of it.

In the same way, the arrangement of myosin molecules in the thick filaments, 

their polarity reversed in either half of the A-band, seems again to be an essential 

uirement of the sliding filament model. It is particularly interesting that myosin 

olecules aggregate to form this type of structure spontaneously in vitro, and it 
suggests that the built-in ability of myosin molecules to form filaments with the 

ropriate pattern of polarity may be a requirement of the processes by which muscle 

ure is built up in vitro. It also suggests the beginnings of possible ways out of the 

ceptual difficulties which arise when one tries to apply the sliding filament theory 

to the contraction of a synthetic actomyosin fibre. It has generally been assumed 

such a fibre contains none of the precisely ordered structures present in a striated 

ule, and so it has been a little difficult to see how the sliding mechanism could 

ill work; and yet it would be surprising if a different mechanism was also available. 

ever, it is now becoming apparent that such synthetic fibres will contain myosin 

olecules not only organized into ordered filaments but even into filaments whose 
polarity is reversed at either end, and so even if the difficulties are not fully over-

ome, they are at least reduced.

Thus while the negative-staining results have so far not added a great deal to our 

nowledge of the detailed molecular architecture of the cross-bridges, they have 

vealed a number of new items of information about the structure of the thick and 

in filaments, all of which either confirm or are in agreement with various aspects of 

e sliding filament theory, and which may help us to approach the problem of con-

ction a little more closely.

(v) Speculations on contraction mechanisms 

Whilst questions of structural polarity have always been an inherent feature of 

iding filament models which depend on short-range interaction between protein 

olecules in the two types of filament, the present results, demonstrating that such 
polarities actually exist and can be seen in the electron microscope, are perhaps 

elpful in that they decrease the size of the speculative element in discussions about 

ossible mechanisms involved in contraction. The problem can now be stated 
as follows.

Two types of filaments, built up of different proteins, lie side by side in an aqueous 

edium of ionic strength 0.1 to 0.2 M. The filaments have diameters of about 65 Å 

nd 110 Å and their surfaces are about 170 Å apart. The thick filaments contain 

osin, the thin ones actin. There are projections, 30 to 40 Å in diameter, on the 

ick filaments which extend out sideways and touch the thin filaments. These 

jections probably contain the enzymic site of the myosin molecule, and also 

ie site responsible for combination with actin. These sites are not necessarily the 

me, but have at least to interact with each other. The sites on all the projections 

riented in the same sense. It is not known whether the projections are rigid or 

lexible, nor whether they move during activity. There is a projection between a
given pair of myosin and actin filaments at regular intervals, and the interval is probably 435 Å. The actin filaments contain two helical chains of actin monomers, and the pitch of the helix is probably 410 Å, though there is a possibility it may be only about 350 Å. The actin monomers in each chain are about 55 Å apart; they are all oriented in the same direction and each of them can interact with the cross-bridges from the myosin filaments. Each actin filament has three myosin filaments around it, with which it interacts, and probably the cross-bridges from these filaments touch the actin filament at intervals of 435°/3 = 145 Å (rather than all in register at 435 Å intervals). The cross-bridges provide the only apparent mechanical connection between the two types of filament, and so they are likely to be responsible in some way for the relative force which can be developed between the filaments in an axial direction.

A relative force is developed between the actin and myosin filaments when ATP is split by the system; the filaments can slide past each other by distances which are very large compared to the separation of the active sites on the actin and on the myosin and continue to generate tension. The problem is to try to define the different types of mechanism which could exist to produce the relative force, and to think of means of differentiating between them. This is not the place to embark on such a general discussion, but there are a few specific points which could be made now that we can think of the system in concrete terms.

The difference in period between the actin and myosin filaments is probably about 25 Å, and there are 3 bridges directed at the actin filament in each 435 Å period. Thus, on average, successive bridges will be progressively further out of register with the active sites on the actin monomers by 8 Å per bridge. Thus if the interaction at one bridge can result in the actin filament moving along by as little as 8 Å, the next site can start to interact. Provided that there are at least seven bridges in the initial distance of overlap (≥ 1000 Å), the actin can therefore be pulled along by a distance equal to the separation of monomers in each chain; another cycle of events can then start at the first cross-bridge involved and a continuous sliding could occur. The system could probably work with even a shorter average initial overlap than this, as a result of longitudinal Brownian motion of entire thick filaments, which would not necessarily always stay exactly in register with each other. All this is not to say that the amount of movement produced by one cross-bridge is only 8 Å, but merely that it could in principle be as little as this.

There are a number of ways in which the interaction of actin and myosin at the cross-bridges could produce movement and these have been dealt with in various degrees of detail by several authors (Hanson & Huxley, 1955; Huxley, A. F., 1957; Huxley, H. E., 1960; Spencer & Worthington, 1960). Clearly, the relatively small amount of movement required, and the precise orientation of the active sites, make it perfectly possible to think of the two molecules having a certain area of mutual contact and moving relative to each other during a reaction in which ATP is split. Alternatively, the actual observation of two different periodicities in muscle, apparently arising from the actin and myosin filaments (Worthington, 1959), provides a continuing stimulus to think of models of the type (amongst others) discussed by Hanson & Huxley (1955) which specifically depend on such a difference in period to stretch the actin filaments and interrupt the actin helix when actin–myosin combination takes place. This type of mechanism has also recently been favoured by Oosawa, Asakura & Ooi (1961) on the basis of their work on actin.
A final point may be made on the subject of the interaction between actin and myosin filaments. If an actin filament can only interact with myosin filaments which are appropriately oriented, and if the result of this interaction is a relative force directed along the actin filament, then such a filament would tend to be maintained in motion in a constant direction when placed in a suspension of myosin filaments, in the presence of ATP. This might have interesting and measurable consequences on the diffusion of actin under these conditions. Furthermore, an oriented gel of actin in which a preponderance of filaments was polarized in one direction might tend to propel itself along under similar circumstances. Possibly some such mechanism is involved in the streaming of cytoplasm.

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**PLATE I.** Typical preparation of separated filaments from glycerinated rabbit psoas muscle, ac-
etively stained with uranyl acetate without previous fixation. A number of intact thick
ilaments, about 1-5 to 1-6 μ in length, may be seen, together with some broken ones. The surfaces
of the filaments seem to be covered with small projections and the ends of the filaments are tapered.
Also visible are separated thin filaments, some thin filaments still attached together in large
ortion or small (upper left) bundles, and a few vesicles. Magnification: 45,000 x.

**PLATE II (a) to (e).** Thick filaments from muscle, showing projections all the way along their
length except for a short central region about 0-2 μ long, which is comparatively bare but has
a slight thickening in the middle. These specimens were fixed in formalin before negative staining,
since it was found that the projections were better preserved under these conditions. (f) Synthetic
"thick filament" made from purified myosin. Note close similarity to appearance of natural
thick filaments, including the bare central shaft. Magnification: 150,000 x.

**PLATE III (a) to (e).** Arrays of thick filaments as seen in the A-bands of fixed, embedded and
sectioned tissue. The tapered appearance of the filaments can be seen (e.g. in (a)), together with
cross-bridges all the way along the filaments, except for a central region, which can be seen well
in (c), which is relatively bare but which has a zone of greater thickness half-way along it. The
filaments are foreshortened by compression during sectioning. Magnification: 150,000 x.

**PLATE IV (a).** Isolated I-segments, from muscle filament preparation, consisting of bundle of
thin filaments held together at the Z-line and extending a distance of about 1 μ on either side of
it. Magnification: 50,000 x. (b), (c). Thick and thin filaments in homogenized muscle prepara-
tion still attached together by cross-bridges. Magnification: 150,000 x.

**PLATE V.** Group of thin filaments, still held together in register at Z-line showing characteristic
beaded structure along their length. Magnification: 150,000 x.

**PLATE VI.** Preparation of purified myosin, at an ionic strength μ = 0-15, showing spindle-
shaped aggregates of the protein, predominantly around 0-5 μ in length. These were formed by
rapid lowering of the ionic strength from μ = 0-8. Magnification: 50,000 x.

**PLATE VII.** Another preparation of purified myosin at ionic strength μ = 0-15, showing the
longer aggregates formed when the ionic strength is reduced slowly from μ = 0-8 by dialysis. Fil-
aments up to 1-5 μ and longer may be found in such preparations. Magnification: 50,000 x.

**PLATE VIII.** General view of typical field showing a number of filaments prepared by lowering
the ionic strength of purified myosin solution to μ = 0-15. The filaments show the characteristic
bare central zone, and the irregular projections at either end. Magnification: 145,000 x.

**PLATE IX.** Synthetic myosin filaments of different lengths ranging from 2500 to 4500 Å,
showing characteristic bare central shaft of approximately constant length (1500 to 2000 Å),
together with irregular projections along the rest of the filament. Magnification: 145,000 x.

**PLATE X.** Synthetic myosin filaments with lengths ranging from 5000 to 9000 Å, together
with one of length greater than 14,000 Å (1-4 μ). All of them show the same characteristic pattern
of a bare central shaft and projections all the way along the rest of the length of the filament.
The appearance of the projections is rather variable, perhaps due to clumping together in some
cases. Magnification: 145,000 x.

**PLATE XI.** A number of fields showing appearance of individual myosin molecules examined
by the shadow-casting technique. A high proportion of the rod-shaped particles seem have a
characteristic thickened region at one end. Particles are varied in length, often around 1500 to
1700 Å, and sometimes stick together, but the separated ones have a very characteristic appear-
ance, being rod-shaped with a thickened region at one end. The diameter of the long shaft of the
molecule measures 10 to 20 Å, that of the head about 40 Å. TMV rods are for location and
calibration purposes. Magnification: 38,600 x.

**PLATE XII.** A number of selected myosin molecules from shadowed preparations, dem-
strating the existence of the "head and tail" structure in a large number of instances. The average
length of the particles in this particular group is 1670 Å. Magnification: 100,000 x.

**PLATE XIII.** Preparations of light meromyosin (LMM) as seen by the shadow-casting technique.
The molecules generally appear as simple rods, without globular regions, often 800 to 700 Å in
length. Magnification: 30,880 x.
LEGS TO PLATES

PLATE XIV. Preparations of heavy meromyosin (HMM) as seen by shadowing technique. The particles very frequently resemble those visible in myosin preparations, in being rod-shaped with a globular region at one end, but are much shorter. The actual lengths are very variable, ranging from 100 to 200 Å (when only the thickened region, or the "head" is seen) to about 800 Å. Possibly the HMM is particularly sensitive to breakage during the drying of these sprayed preparations. Magnification: 38,000 x.

PLATE XV (a). Light meromyosin "crystal", negatively stained (with uranyl acetate). Widened structures like this are readily formed when the ionic strength of LMM solutions is reduced below 0.06 to 0.15. There are indications of longitudinal filaments slowly (by dialysis) from $\mu = 0.6$ to $\mu = 0.15$. Within the fibre, the axial periodicity is about 430 Å. Magnification: 155,000 x. (b). Light meromyosin filament formed when ionic strength is reduced rapidly to $\mu = 0.15$, by dilution. The filament appears to be composed of well-oriented, rod-shaped molecules, and the absence of an axial periodicity suggests that in this case they are not aligned in a transverse register. The sharp outline of the filament, which is free of projections, indicates that the width of the molecules is very uniform, and that they are straight. Magnification: 143,000 x.

PLATE XVI. Normal appearance of LMM preparation when ionic strength reduced quickly to $\mu = 0.15$. The protein aggregates into needle-shaped structures, frequently up to 500 Å in length, which may be many microns in length. The surfaces of these filaments are completely smooth. Occasionally, a lattice structure is observed (bottom left) (see also Plates XXVII and XXVIII). Magnification: 38,000 x.

PLATE XVII. Purified F-actin, prepared from acetone-dried muscle powder. The actin forms filaments of indefinite length but constant width 60 to 70 Å, with the characteristic banded structure. Magnification: 155,000 x.

PLATE XVIII. Purified F-actin preparation, prepared by homogenizing muscle from which all myosin had been previously extracted. The field shown here is one in which the actin is embedded in a film of uranyl acetate over a hole in the carbon supporting film, a procedure which gives very good contrast and detail. In many of the filament, the double-helical structure can be seen (e.g. bottom right indicated by arrows). Magnification: 155,000 x.

PLATE XIX. Filaments of F-actin, treated with solution of heavy meromyosin. A very characteristic compound structure is formed, showing a strong axial periodicity of approximately the same value (330 to 370 Å) as that seen in F-actin. The filaments also have a well-marked structural polarity ("arrowheads") which has the same sense all the way along any given filament. Magnification: 155,000 x.

PLATE XX (a), (b). Thin filaments from muscle, treated with solution of heavy meromyosin. The filaments show the characteristic compound structure (see Plate XXI), and in the upper picture (a) two filaments pointing in opposite directions may be seen. Magnification: 155,000 x. (c). Preparation of "natural" actomyosin, deposited from solution at an ionic strength of $\mu = 0.6$. The same type of "arrowhead" complex filament structures are seen as in the actomyosin (HMM). Filaments. This preparation was made using a normal supporting film, whereas (a) and (b) are filaments photographed over holes, and the greater degree of flattening, etc., in the present case produces a somewhat different appearance. All preparations negatively stained with uranyl acetate. Magnification: x 150,000 Å.

PLATE XXI. Another preparation of "natural" actomyosin at $\mu = 0.6$, fixed in formalin and negatively stained with sodium phosphotungstic acid showing characteristic polarized compound structure. Many of the compound filaments are about 1 µ long. They frequently line up in small parallel arrays in which all the filaments have the same polarity and in which the pattern seen in adjacent filaments is displaced by about half a period, so that an approximate lattice is formed. Magnification: 143,000 x.

PLATE XXII. Untreated I-segment, showing bundle of thin filaments extending out on either side of their Z-line attachment. This micrograph is a control for Plates XXV and XXXI. Magnification: 108,000 x.

PLATE XXIII. I-segment treated with solution of heavy meromyosin. The thin filaments now show the characteristic polarized structure, and all filaments on the same side of a X-line point in the same direction. Those on the other side of the X-line point in the opposite direction. Magnification: 108,000 x.

PLATE XXIV. Another small I-segment, treated with heavy meromyosin solution, again showing all filaments with same polarity on given side of Z-line region, and reverse polarity on other side. All I-segments examined show identical arrangement. Magnification: 108,000 x.
seen by shadowing technique. The preparations, in being rod-shaped, the actual lengths are very variable, the "head", is seen) to about 800 Å, during the drying of these sprayed particles (with uranyl acetate). Wide magnification: 150,000 ×. (b) Light meromyosin just beginning to aggregate, showing isolated "stars" (with diameters up to about 800 Å) and some of the more normal filamentous aggregates, together with indications of individual LMM molecules in background. Magnification: 150,000 ×.

PLATE XXVII. Lattice structure, obtained by homogenization of tropomyosin B crystals, negatively stained with uranyl acetate. Lattice spacing ~200 Å. The "woven" appearance arises because alternate lattice points along either of the two principal lattice directions are displaced alternately slightly to either side of the mean position. Possibly the lattice points do not all originally lie in one plane, but in two, and the displacement seen is a consequence of the drying down of this structure. The lines joining the lattice points frequently appear double, consisting of two fine filaments measuring about 10 to 15 Å in diameter and being about 25 Å apart. Magnification: 150,000 ×.

PLATE XXVIII (a). Tropomyosin B preparation, showing some of the typical lattices, with fibrous aggregates growing out of them along the principal lattice directions. Magnification: 150,000 ×. (b). Another example of tropomyosin B lattice. The lattice is always rhombo, but the angle by which it differs from an accurately square lattice is somewhat variable in the range 3' to 13'. Magnification: 150,000 ×. (c). Cross-section through a number of fibrils of rabbit psoas muscle in 1-band and Z-line region; the denser fibril in the lower part of the picture has been sectioned through the Z-line, and the approximately square lattice in that region can be seen. On the left-hand side of this fibril one is seeing the lattice on one side of the Z-line, but at the top right of the fibril one is seeing the lattice on both sides simultaneously, and a lattice with 1/2 the spacing of the first one and rotated 45° to it is seen (see text). Magnification: 50,000 ×.