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## THE STRUCTURAL BASIS OF MUSCULAR CONTRACTION (\*)

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**Résumé.** — Au cours des vingt-cinq dernières années, des développements importants ont eu lieu dans les techniques de diffraction des rayons X à petits angles et de microscopie électronique appliquées aux matériaux biologiques. Dans le cas du muscle, ces développements ont permis d'étudier un grand nombre de modèles moléculaires se produisant au cours de la contraction. D'après le modèle de structure actuel, la contraction est produite par un mouvement de glissement relatif des rangées de deux types différents de filaments moléculaires. La force de glissement est produite par l'action cyclique de liaisons transverses entre ces filaments de myosine et d'actine. On décrit les résultats expérimentaux qui conduisent à une telle description ainsi que les développements techniques en jeu.

**Abstract.** — During the past 25 years, there have been important developments in the techniques of low-angle X-ray diffraction and electron-microscopy, as applied to biological material. In the case of muscle, these developments have made it possible to study many of the molecular changes which occur during contraction. According to the present structural model, contraction is brought about by a relative sliding motion of arrays of two different types of molecular filaments. The sliding force is generated by the cyclical action of cross-bridges between these myosin and actin filaments. The experimental evidence which led to this picture is described, together with the technical developments involved.

1. **Introduction.** — A previous occasion on which the Croonian lecture was directly concerned with the mechanism of muscular contraction was in 1945, when it was delivered by Professor W. T. Astbury. On that occasion he commented that it was a sign of the times that a physicist should be asked to give the Croonian lecture, and went on to say, and I quote : « We are at the dawn of a new era, the era of « molecular biology », as I like to call it, and there is an urgency about the need for more intensive application of physics and chemistry, and specially structural analysis, to biological problems. » These were very prophetic words, and, as a physicist just entering biology, I was much interested to read them, and to learn about his experiments.

The basic experimental finding which Astbury reported (1947) was that there did not seem to be any important change in the wide angle X-ray diagram from muscle upon contraction ; hence it followed that muscles did not contract by any process which simply involved the large-scale disorientation of originally well-ordered polypeptide chains, nor by an alteration in chain configuration in the well-ordered parts of the structure. Astbury suggested instead that there might be « specifically active foci » which one could perhaps paraphrase as « larger structural units » (i. e. larger than individual polypeptide chains) concerned in contraction, which might be studied in the electron microscope or by low angle X-ray diffraction.

During the past 20 years there have been very important and exciting developments of new techni-

ques for studying biological structures beyond the limit of resolution of the light microscope. As a consequence, structures within the range of dimensions

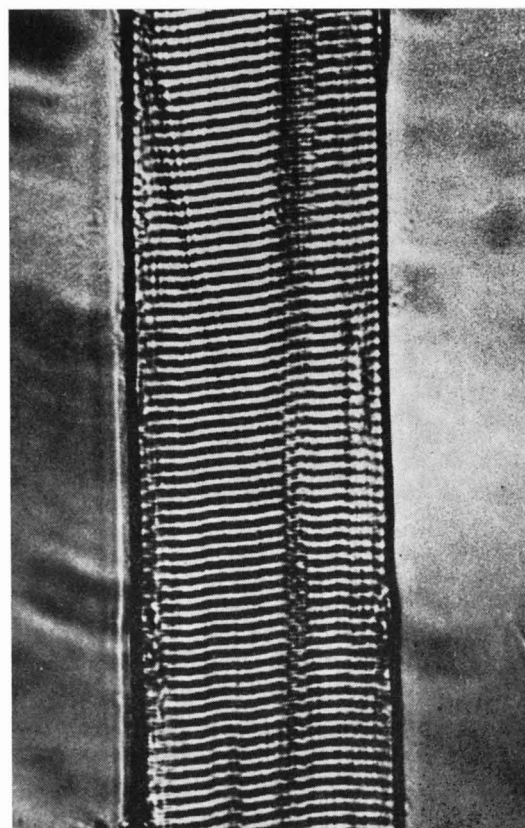


FIG. 1. — A single muscle fibre from a rabbit psoas muscle, photographed in the phasecontrast light microscope. A-bands are dark. I-bands light. The sarcomere repeat is approximately 2.6  $\mu\text{m}$ , and the diameter of this fibre is approximately 50  $\mu\text{m}$ . (Magn.  $\times$  900.)

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between 2 and 200 nm have now become accessible to fairly direct study. Within this size range a great many of the interesting and basic biological processes express themselves in minimal structural terms and amongst these is the phenomenon of muscular contraction. As a result it is now possible to put together a rather detailed hypothesis about muscle structure and the mechanism by which contraction takes place. The hypothesis to which I refer describes muscle contraction in terms of the sliding filament, swinging cross-bridge model, and I will discuss some aspects of its development and of its implications.

The problem is basically the following. Muscles are built up from fibres about 0.1 mm in diameter (Fig. 1). The contractile material within the fibres consists of myofibrils which are long thin structures, usually about a micrometre or so in diameter, many thousands of which act in parallel (Fig. 2). The myofibrils have

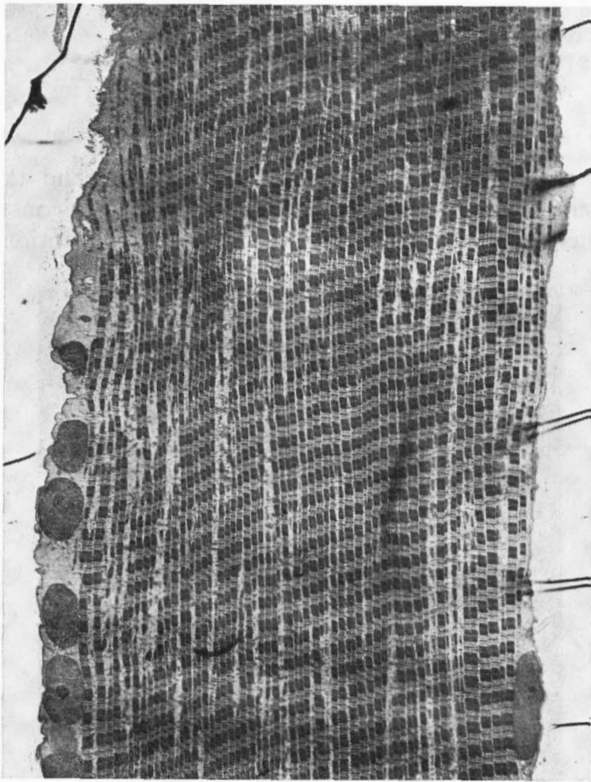


FIG. 2. — A single muscle fibre from a human muscle, fixed, stained, embedded in plastic, sectioned longitudinally, and viewed at relatively low magnification in the electron microscope. It can be seen that the striations visible in figure 1 arise from the characteristic band-pattern on the myofibrils, visible here, with diameters about  $1\ \mu\text{m}$ . The diameter of this fibre in the plane of sectioning is about  $55\ \mu\text{m}$ . At the edge of the fibre, several nuclei can be seen, just below the surface membrane (sarcolemma). (Magn.  $\times 1\ 700$ .)

a very characteristic pattern of bands along their length, repeating at intervals of about  $2.5\ \mu\text{m}$  (Fig. 3) but details of the structures which give rise to these bands cannot be resolved in the light microscope.

In the resting state a muscle is soft and readily exten-

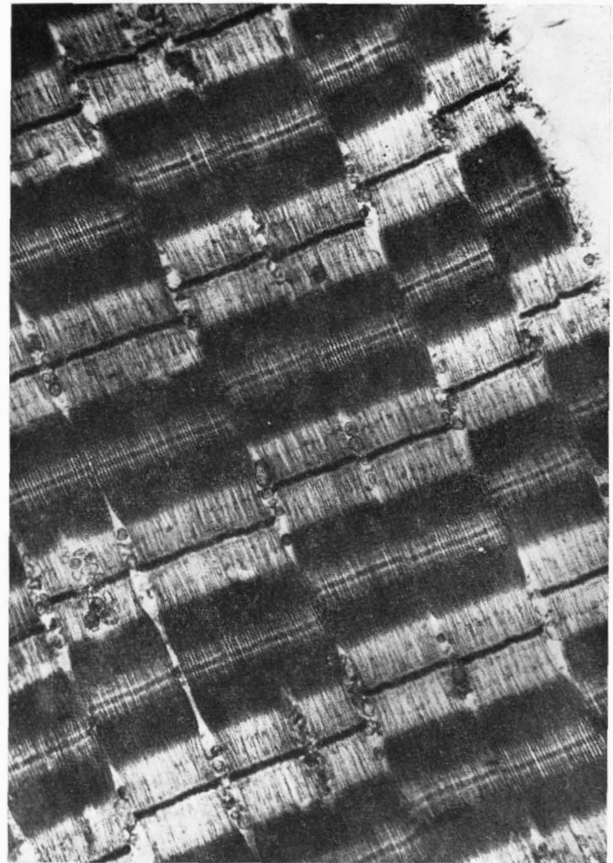


FIG. 3. — Longitudinal section, about 100 nm in thickness, of rabbit psoas muscle. The narrow dense lines, running across the myofibrils (which run from top right to bottom left) are the Z lines, which define the ends of the repeating units of pattern, the sarcomeres. Each sarcomere contains a dense A band and a less dense I band. The central region of the A band, in a rest length muscle, is less dense than the lateral regions, and is known as the H zone. Longitudinal filaments can be seen within the fibrils, but, in sections of this thickness which contain several layers of them, their arrangement cannot be discerned. (Magn.  $\times 15\ 000$ .)

sible. When it is stimulated electrically via its motor nerve, the contractile machinery is switched on by a process in which, it is now believed, calcium ions are rapidly liberated throughout the muscle fibres and activate an enzyme, an ATPase, located in the structural protein complex of muscle, actomyosin. The contractile material then undergoes a very rapid change in its properties: it becomes much more rigid, it develops a large tension — several kilograms per square centimetre — and it can shorten rapidly, often at a rate equivalent to many times its own length per second.

So the question is: what features of the fine structure of the myofibrils confer on them these remarkable properties? More specifically, how are structural changes in individual muscle protein molecules — changes perhaps similar to those now thought to occur during the function of many other enzyme systems — how are these, in a muscle, combined together in an integrated and directed fashion so as



to give the large-scale change of length that is required? I think this is a valid example of « local motion » to which Croonian lecturers are expected to devote their attention.

**2. Early X-ray diffraction and electron microscope evidence.** — In the limited time available, it is not possible to review all the main experiments that have been done in the course of development of the sliding filament model, and instead I will try to consider the nature of the evidence in rather broader terms. The study of striated muscle provides an unusually favourable situation where X-ray diffraction data can be compared and combined with electron microscope observations in such a way that strong inferences from one technique can be confirmed by the other, and in which vital gaps in the picture provided by one technique can often be filled in by findings which are immediately self-evident from the other. This was especially fortunate since some of these developments took place at a time when a whole new world of structure, apparently, was being revealed in biological materials by the electron microscope, but where microscopists, all too aware of the role played by preparation artefacts during the history of light microscopy, were at first reluctant — and quite rightly so — to accept the new structures at their face value without substantiation by other techniques.

In the case of muscle, it is remarkable to what extent all the vital information is contained in the X-ray diffraction diagrams from living muscles, admittedly in somewhat enigmatic form. However, I think it would not have been possible to get the story straight, nor to convince other people that this was the way muscles were constructed, without having at the same time electron micrographs to provide a direct visual picture of the structures in question.

The first step in this work, and perhaps the most important one, was to wonder whether, in a live muscle, where 80 % of the volume is occupied by water, the protein filaments (whose presence was already known from observations on dried material) might, in this aqueous environment, maintain themselves spaced far apart in a very regular arrangement. If this was the case there should be repeating periodicities of the order of a few tens of nanometres which should be detectable by low angle X-ray diffraction techniques, providing that one used cameras which could pick up X-ray reflexions within about  $1/5^\circ$  of the direct beam and providing that those reflexions could be recorded within the limited period for which an isolated muscle would stay alive. The first successful experiments along these lines were carried out in 1951 (Huxley, 1951, 1952, 1953 a) using a specially designed low angle camera and a fine focus X-ray tube designed by Ehrenberg and Spear (1951), and showed from the equatorial X-ray diagram that there is present in a living muscle a hexagonal array of long filaments about 40 nm apart and between 10 and 20 nm in

diameter. The spacing between the filaments was found to vary with muscle length, in such a way that the volume of the array remains approximately constant, like the volume of the muscle as a whole; this would fit in with a model in which the arrays of filaments occupy a large part of the cross-section of each fibre. The live muscle also showed a very nice pattern of axial reflexions, demonstrating that, in the living state, the protein molecules are arranged in the filaments in a very regular way, and are therefore accessible to study, under physiological conditions, by this technique. Most surprisingly, the spacing of these axial reflexions did not change when the muscle was stretched.

The early X-ray results also indicated that there was a second set of longitudinal filaments present lying alongside the primary ones and that these gave rise to especially prominent regions of electron density at the trigonal positions of the hexagonal lattice in muscles in rigor, when actin and myosin were linked together. (Specifically, the X-ray patterns from muscles in rigor showed a very strong reflexion on the equation (the [11] reflexion) whereas in living muscles this reflexion was weaker than the first (the [10] reflexion). Now each X-ray reflexion is associated with a set of lattice planes passing through the structure, and one can see from figure 4a that in the case of the [10] lattice planes

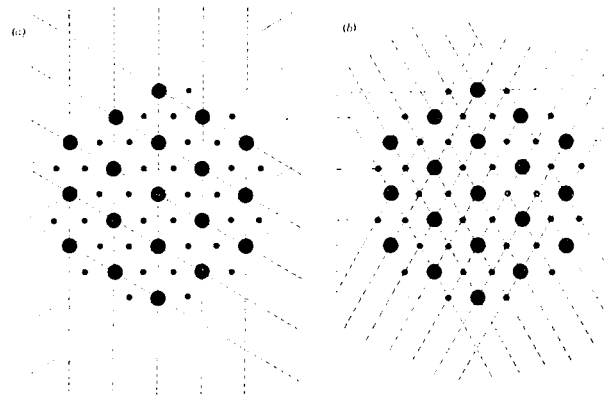


FIG. 4. — *a*) End-on view of double hexagonal lattice of thick and thin filaments, characteristic of overlap region in A bands of vertebrate striated muscle, showing the three sets of lattice planes in the (10) crystallographic directions. The actin filaments at the trigonal points of the lattice will tend to fill in the space between the dense planes of filaments at the hexagonal lattice points, thereby decreasing the intensity of the (10) X-ray reflexions given by the thick filaments on their own. *b*) Similar view, showing lattice planes in (11) crystallographic directions. In this case, both the actin and myosin filaments lie in the same lattice planes and so their contributions to the intensity of the corresponding X-ray reflexions are additive. Hence, as the amount of material at the trigonal points is increased, the intensity of the (10) reflexions decreases and that of the (11) reflexions increases.

filaments at the lattice points contribute strongly to these planes whereas filaments at the trigonal points tend to fill in the spaces between and reduce the amplitude of the density variation; in the case of the

[11] lattice planes, however, which pass through both the lattice points and the trigonal points, filaments in both types of positions will contribute in phase. Thus, as the amount of material at the trigonal points is increased, the [10] reflexion decreases in intensity and the [11] reflexion increases, which was the effect seen when a muscle went into rigor.) It was natural to infer from the X-ray results therefore that there are *two* sets of filaments which could change their relationship to each other, and that myosin might be located in the primary filaments and actin in the secondary ones (Huxley, 1952).

These early results gave no information about the disposition of the filaments within the banded structure of the muscle and the reason I am describing them is to illustrate the way in which the X-ray technique nevertheless gave definite predictions of a structure which should be present in muscle and which was of such a scale as to be visible in the electron microscope. This made it worth while to develop the necessary fixation, staining and ultrathin sectioning techniques so as to try to see it and this in turn led to other features of the structure being recognized. This was at a time when these technical developments were just beginning and it was a great advantage to have a good idea ahead of time of *something* that one should expect to see in a satisfactory preparation.

When the preparative techniques had been brought up to the necessary level, the observations showed not only that the two types of filaments could indeed be seen in electron micrographs lying in the expected double hexagonal arrays with cross-bridges between them but also that the double arrays were not seen everywhere — it depended on which part of the band pattern was examined (Huxley, 1953 b). The double arrays of thick and thin filaments were seen only in the lateral parts of the A bands of muscle. The centre part of the A band (the H zone) contained only thick filaments and the I bands only the thinner filaments (Fig. 5). Now, since we knew with a high degree of confidence from the X-ray results that the double array

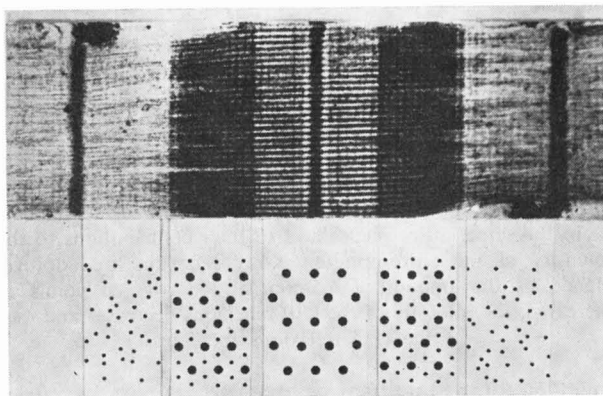


FIG. 5. — Diagram showing the appearance of muscle in cross-sections through the various different parts of the band pattern, indicated by the micrograph of part of a longitudinal section of frog sartorius muscle.

was a real structure, then we were prepared to accept this additional information from the micrographs as being significant. Moreover, at the same time, Jean Hanson and I found from selective extraction experiments that myosin was located in the A bands of muscle, in the thick filaments, and that actin was located in the part of the sarcomere occupied by thin filaments and we realized that this result corresponded perfectly with the implications of the electron microscope images.

### 3. The interdigitating and sliding filament models.

— In the light of this evidence, we were able to put forward the overlapping filament model (Hanson and Huxley, 1953) which I can now summarize in the following terms: the myofibrils are built up of a long succession of partially overlapping, interpenetrating arrays of longitudinal protein filaments. Within the arrays, the filaments are spaced out in a regular hexagonal lattice a few tens of nanometers apart, and separated by an aqueous medium. There are two types of array, in each of which filaments of a particular type and of a fixed length are arranged in register. In vertebrate striated muscle, the arrays are approximately 2 and 1.6  $\mu\text{m}$  in length, and the corresponding filament diameters are approximately 6-8 nm and about 12 nm (Fig. 6).

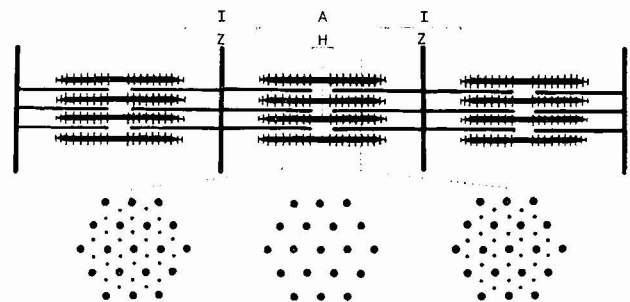


FIG. 6. — Diagrammatic representation of the structure of striated muscle, showing overlapping arrays of actin- and myosin-containing filaments, the latter with projecting cross-bridges on them. For convenience of representation, the structure is drawn with considerable longitudinal foreshortening; with filament diameters and side-spacings as shown, the filament lengths should be about five times the lengths shown.

In terms of the muscle proteins, the model supposes that one of the two principal structural proteins in muscle, myosin, is contained in the thicker filaments, each filament being an assembly of several hundred molecules; there are projections on the filaments which represent (as was realized later, Huxley, 1957) the active ends of these molecules, projecting outwards, presumably so that they can interact with the thin filaments. These contain the other principal protein, actin, again in the form of assemblies of many hundred molecules, and (as we know now) probably in combination with the regulatory proteins, tropomyosin and troponin.

Now, how does this system operate as a contractile mechanism? One of the main clues came from the axial X-ray pattern, which, as I have mentioned, does not change during stretch. This was one of the reasons why Dr. Hanson and I set out to look for constancy of A band and I filament lengths by light microscopy in muscle under various conditions. Such constancy in length was found both by us (Huxley and Hanson, 1954) and by A. F. Huxley and Niedergerke (1954), and this observation enabled both groups simultaneously to put forward the sliding filament hypothesis, in terms of the model which we had already described. This hypothesis was the following:

When the muscle changes its length, either actively or passively, the arrays of filaments slide past each other at almost constant length; the force for contraction is generated by some process which actively translates one type of filament past the neighbouring filaments of the other type.

**4. Other evidence for the model.** — Because of the limited resolution of the light microscope, it was the constancy of X-ray periodicities in passively stretched muscle that first encouraged us to believe we were on the right lines in thinking in terms of filaments of fixed lengths. There are two sets of these reflexions. One set (Fig. 7) was recognized very early as coming

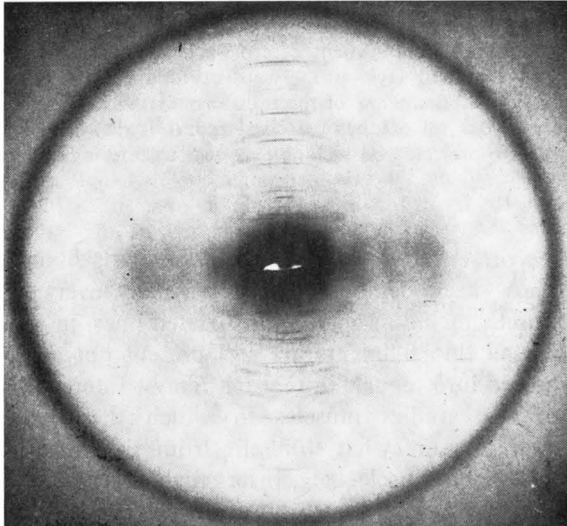


FIG. 7. — X-ray diagram from live frog sartorius muscle (fibre axis vertical) showing the pattern of reflexions in the medium angle region (0.5-5 nm) from the actin-containing filaments.

from the actin filaments, since very similar patterns are given by oriented preparations of purified actin (Astbury and Spark, 1947). A second set of reflexions (Fig. 8a), a set of layer lines corresponding to a 6/2 helix with helical repeat of 42.9 nm and a subunit repeat of 14.3 nm, comes from the myosin filaments (Worthington, 1959; Elliott and Worthington, 1959; Elliott, 1964) and is dominated by the contribution from the projecting cross-bridges (Fig. 8b). Now, the

crucial fact about these sets of reflexions is that they do not change in spacing when a muscle is stretched, even by 20 or 30 % or more. The only straightforward way to interpret this finding is that the filaments do not change in length during stretch, and that accordingly there is an invariant period along each filament at all lengths; only the overlap changes.

More recently, the virtual constancy of both sets of axial X-ray reflexions in actively contracting muscles was demonstrated by Brown and myself (Huxley and Brown, 1967) and by Elliott, Lowy and Millman (1967) and I will return to this topic later.

There are two further pieces of X-ray evidence on living muscle which strongly support the sliding mechanism in an interesting way. Elliott, Lowy and Worthington (1963) noticed that the relative intensities of the equatorial reflexions changed in a characteristic way with muscle length, the second reflexion (11) becoming progressively weaker relative to the first (10) as the muscle was stretched. Given the general arrangements of filaments which had already been established, this result must mean, as the authors realized, that there is a progressive decrease in the amount of material at the trigonal positions of the lattice as the muscle is stretched, and so the observation provided very strong confirmation that the extent of overlap of the thin and thick filaments in resting muscle was dependent on muscle length in the manner which one would expect from the sliding filament model.

More recently Haselgrove (1970), working in our laboratory on contracting muscle, has been able to show in an analogous way, from the equatorial X-ray diagram of muscles allowed to shorten actively to different extents, that changes in overlap do in fact occur during active contraction in just the same way as they do when a resting muscle changes length.

Thus there is quite compelling evidence from X-ray studies alone, on surviving muscles (a technique, I should emphasize, which does not affect the muscle's ability to contract, to give altered X-ray diagrams during contraction, and to give normal «resting» diagrams afterwards) that these muscles contain overlapping arrays of protein filaments spaced apart in an aqueous environment; that these filaments, in turn, are composed of very regular arrangements of protein subunits; that the filaments are mechanically stable structures which are still present in actively contracting muscle, and have approximately the same internal periodicities; and that both active and passive changes in muscle length are accomplished by a process in which the filaments change their extent of overlap. Thus the filaments must slide past each other during contraction.

This picture is strongly supported by electron microscope evidence. In some ways, the most surprising finding was the fidelity with which the main features of the structure were preserved in tissue fixed by what are crude and empirical methods in molecular terms. For example, one might imagine that the

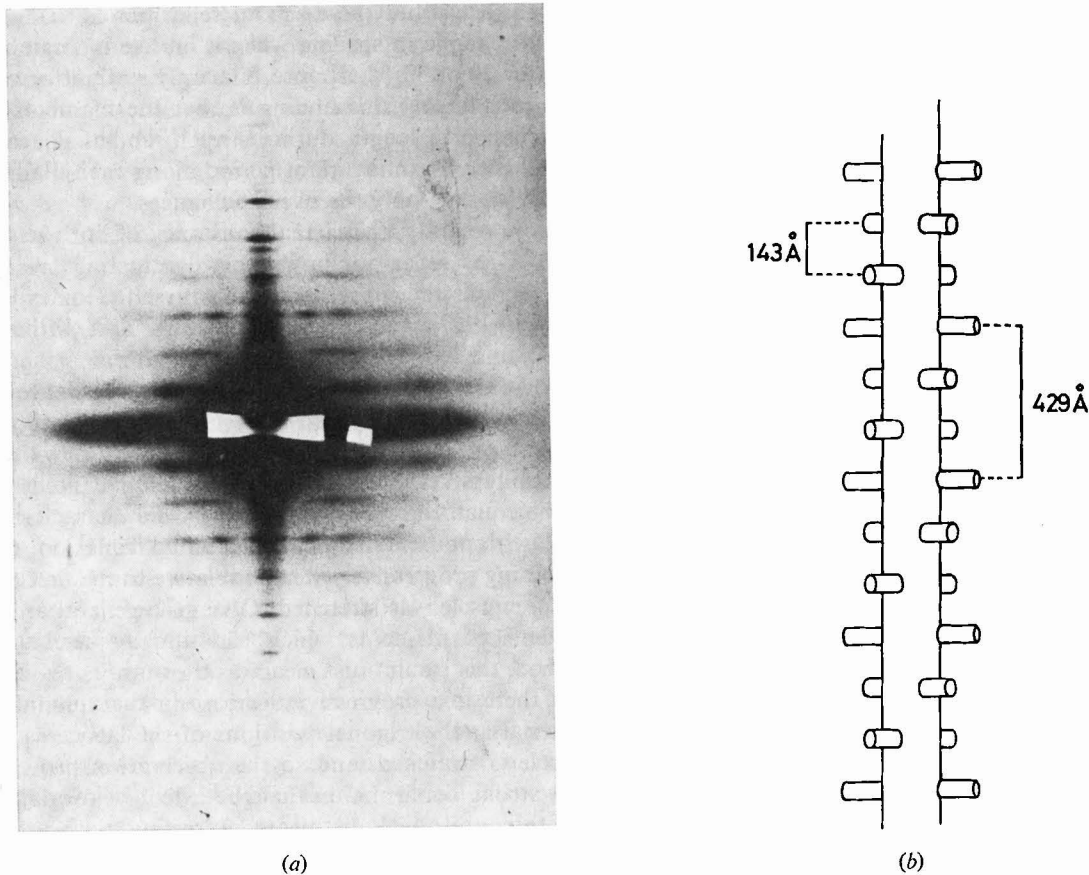


FIG. 8. — *a*) Diffraction pattern from live frog sartorius muscle; fibre axis vertical. The pattern of layer-line reflections corresponds to a repeat of 42.9 nm and is believed to arise from the helical arrangement of cross-bridges on the myosin filaments. Note strong first layer line and strong meridional reflexion on third layer line. The distributions of intensity along the first, second, fourth and fifth layer lines are all similar to each other. Sampling of the transform is evident on the third layer line. *b*) Schematic diagram showing arrangement of cross-bridges on 6/2 helix. Helical repeat is 42.9 nm, but true meridional repeat (i. e. periodicity of the variation in density of structure projected on to long axis of filaments) is 14.3 nm.

double hexagonal array of filaments, maintained in an aqueous medium by some delicate balance of long range forces, would appear only in a very degraded form in fixed, dehydrated and embedded material. On the contrary however, the structural relations are preserved so well that single layers of the theoretically expected lattice, 10 or 20 nm thick, could be cut when a microtome of adequate quality had been developed and when adequate electron staining methods used (Huxley, 1957). Within these single layers of filaments, cross-bridges from the thick filaments to the thin ones could be seen (Fig. 9).

We were therefore encouraged by the good preservation of the lattice geometry to believe that the overall picture of the structure was not likely to be far wrong and that the cross-bridges between thick and thin filaments visible in these micrographs and in the earlier cross-sections were genuine structures. Furthermore, changes in overlap could readily be seen in the micrographs and close constancy of filament lengths has since been demonstrated by Dr. Sally Page (Page, 1964; Page and Huxley, 1963).

The presence of two types of overlapping filaments has now been demonstrated in virtually every type of muscle, including many non-striated ones, in which thick and thin filaments are still present but are not organized into regular in-register arrays. Interestingly, when one studies muscles in which the filament structure is somewhat different from that in vertebrate skeletal muscle, e. g. insect flight muscles, clam adductor muscles, vertebrate smooth muscles, one finds that the difference always lies in the structure of the myosin-containing filaments; the actin-containing filaments seem always to be the same, as pointed out by Hanson and Lowy in their comparative studies (see, for example, Lowy and Hanson, 1962). I will return to this point later on.

Given then that this is the basic structural framework within which the contractile mechanism operates in all muscles, how is the necessary sliding force produced between the filaments?

**5. The mechanism of force generation.** — It is known from a large number of biochemical studies that the ATPase activity of myosin on its own, under



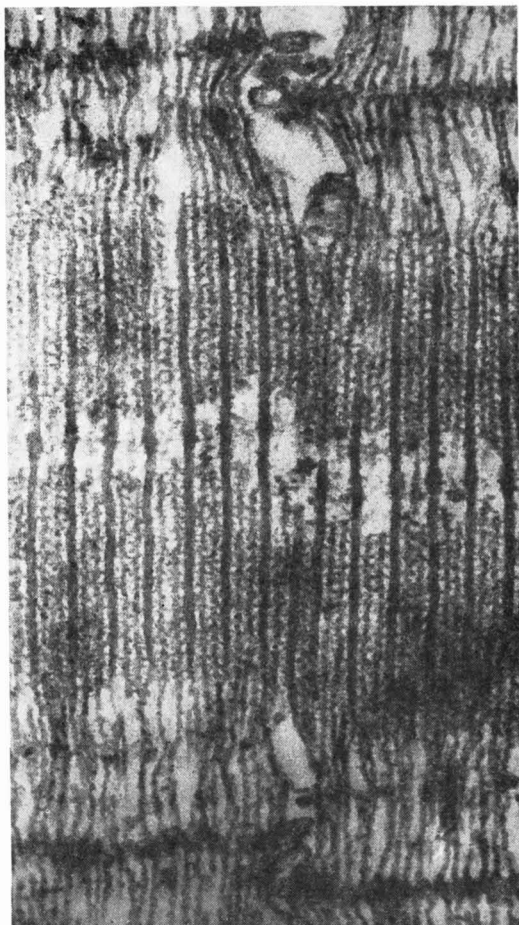


FIG. 9. — An electron micrograph of a longitudinal section through the sarcomeres of two adjacent myofibrils, such as those shown at lower magnification in figure 3. The Z lines bounding the sarcomeres are at the top and bottom of the picture. Two kinds of filaments are visible, thick ones (about 11 nm) in an array confined to the A band, and thin ones (about 5 nm) in two arrays which terminate at the borders of the H-zone in the middle of the picture. The two kinds of filaments interdigitate in the A band (except the H zone): the plane of sectioning through the lattice of interdigitating filaments which will produce a longitudinal section like this one is illustrated in figure 4. Cross links between thick and thin filaments are visible. (Magn.  $\times 160\,000$ .)

the conditions present in muscle, is relatively low, but that it can be greatly activated by the presence of actin (Hasselbach, 1952). Furthermore, the activating influence of actin can be inhibited, in the absence of calcium ions, by the regulatory proteins, tropomyosin and troponin (Ebashi, 1963; Ebashi and Ebashi, 1964; Ebashi and Kodama, 1965, 1966). These have been shown to form part of the structure of the thin filaments in vertebrate striated muscle (Pepe, 1966; Endo, Nonomura, Masaki, Ohtsuki and Ebashi, 1966). I know of no cases where this kind of specific protein-protein interaction has been shown to take place at long range, in the absence of a direct physical combination (if only a transitory one) between the molecules concerned. Moreover, a very specific combination between actin and myosin does in fact take place

very readily in solution to give the so-called actomyosin complex, and cross-bridges between filaments can be seen in muscle. It therefore seems overwhelmingly probable that such a direct physical combination does take place between actin and myosin during the activated splitting of ATP, and that a direct mechanical attachment of the cross-bridges, representing the active parts of the myosin molecule, is made to the actin filaments during the splitting of ATP in muscular contraction. Given that cross-bridge attachment occurs, I believe that this mechanical link provides the most likely site where the mechanical force for contraction is developed, by some structural change accompanying the enzymic splitting of ATP.

More explicitly, the moving cross-bridge model supposes that while a muscle is contracting, these projections go through repetitive cycles of action in which they are physically attached to a site on the actin filaments for part of their cycle, and move in some way, perhaps by a change in tilt, so that the thin filament is pulled along in the appropriate direction, as a consequence of this configurational change; the attachment would then break, enabling the cross-bridge to return to its initial conformation, in preparation for its next cycle of action (Fig. 10); in the

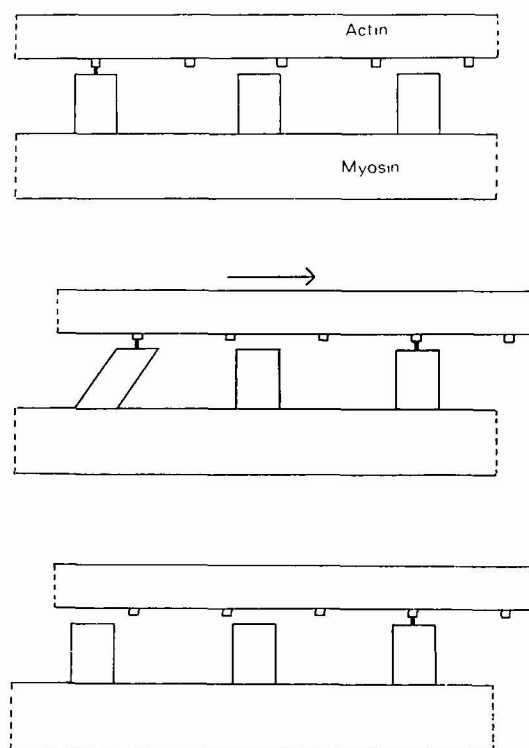


FIG. 10. — Diagram showing, very schematically, possible mode of action of cross-bridges. A cross-bridge attaches to a specific site on the actin filament, then undergoes some configurational change which causes the point of attachment to move closer to the centre of the A band, pulling the actin filament along in the required manner. At the end of its working stroke, the bridge detaches and returns to its starting configuration, in preparation for another cycle. During each cycle, probably one molecule of ATP is dephosphorylated. Asynchronous attachment of other bridges maintains steady force.



course of this cycle, a molecule of ATP is dephosphorylated, releasing energy which is coupled to the configurational change and hence to the performance of external work.

At the same time, it is highly probable that long-range electrical forces, such as have been discussed by Elliott (1968) and by Rome (1967, 1968) are involved in maintaining the transverse separation of the filaments. However, I believe that the study of the structure of the cross-bridges, and their short range interactions with actin, is most likely to yield direct evidence about the contraction mechanism itself.

**6. The internal structure of the filaments.** — The resolution available in tissue sections is limited, and to see more details of structure within the filaments, it was necessary to develop a different technique, in which the organized muscle structure is broken down into its individual components by a combination of biochemical and mechanical procedures (Huxley, 1961, 1963), and then examined by the so-called « negative staining » method. By a comparison of the structure of these separated filaments with those of synthetic filaments made from purified muscle proteins, the composition of the thick and thin filaments was confirmed, and, more interestingly still, it was possible to show that the molecules were built into the filaments with strict structural polarity. The experimental details are described in the papers mentioned and show that all the actin monomers on one side of a Z line are orientated in the same direction and that this direction is reversed on the other side (Fig. 11). The myosin molecules in one half A band

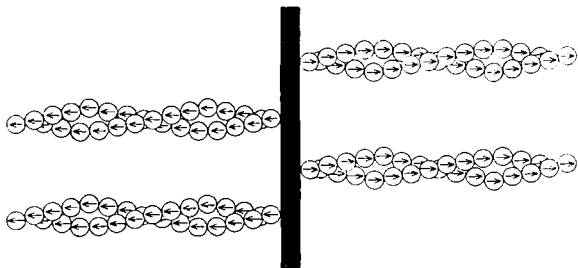


FIG. 11. — Diagram showing structural polarity of the actin molecules in the F-actin filaments, and the reversal of polarity on either side of the Z line, necessary to match up in the correct orientation with myosin cross-bridges in the half A bands on either side.

also all have one particular orientation, and this is reversed in the other half; thus, individual myosin molecules, having a head and tail structure, always have their tails pointing towards the centre of the A band (Fig. 12). It is apparent, therefore, that the interacting sites of the actin and myosin molecules are arranged so that they always have the same relative orientation to each other (Fig. 13) so that if a sliding force is developed at each interacting site, then all the elements of force in the overlap region will add



FIG. 12. — Arrangement of myosin molecules in the thick filaments. The backbone of the filaments is formed from the assembly of the « tails » of the molecules, and the molecules form two antiparallel sets, one in each half of the filament, with a reversal of structural polarity at the centre.

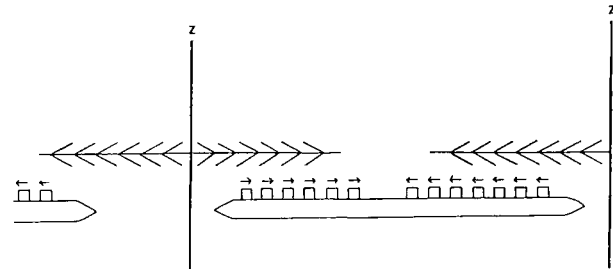


FIG. 13. — Diagrammatic illustration of structural polarity of cross-bridges on myosin filaments, and of molecules of actin in the thin filaments, so that their interaction could produce sliding forces which would move the actin filaments towards each in the centre of the sarcomere.

up in parallel and in the same direction. Furthermore, the sites are arranged so that the absolute direction is reversed in each half sarcomere; thus the two actin filaments interacting with one A filament are always drawn towards each other at the centre of the A band. And finally, the molecules concerned have the ability to assemble themselves *in vitro*, into filaments of this required structural polarity. So we are now beginning to see how, in a muscle, the structure is organized — and indeed organizes itself — so that it can integrate many individual molecular events, in such a way that they all add together with the appropriate orientation to produce a force, and a large scale structural change, in a given direction.

**7. Changes during contraction.** — We must now consider the evidence concerning possible movement of the cross-bridges during contraction, since these bridges are, we believe, participating in continuous cyclical chemical and structural changes during activity. Such cyclic behaviour is not readily accessible to study by present-day electron microscope techniques, which depend on chemical fixation — a relatively slow process. However, since the cross-bridges make a major contribution to the low-angle axial X-ray diagram, one can look for evidence about their behaviour by studying this diagram during contraction. As I mentioned earlier, it was shown both by Elliott and his co-workers (1967) and by Brown and myself (Huxley and Brown, 1967) that the overall repeating periodicities in these diagrams do not change in contraction. However, there was still the question of changes in intensity of the reflections, which were not easy to see in the first diagrams that were obtained.

To study these it was necessary to develop low-angle X-ray diffraction cameras which could record

the meridional pattern with fairly good two-dimensional resolution in about 10 to 20 min ; for this was the maximum period for which an excised muscle could be kept contracting strongly at that time. This total time had to be built up a second or so at a time, at 1 or 2 min intervals to allow the muscle to recover in between. Now, earlier types of camera and X-ray tubes were about 50 to 100 times too slow to give a usable pattern within these time limits, and for a long time the experiment looked extremely difficult. However, Huxley, Brown and Homes (1965) found that a new type of low-angle camera incorporating a bentquartz crystal focusing monochromator working at unusually large aperture and combined with one totally reflecting glass mirror focusing in a direction at right angles to the quartz would, together with the Holmes-Longley fine-focus rotating anode X-ray tube, give us photographs within the time limit. Elliott and his co-workers have used a somewhat different arrangement incorporating two totally reflecting Franks type mirrors arranged at right-angles (Franks, 1955 ; Elliott and Worthingham, 1963) and a fine-focus, stationary anode X-ray tube which also gives a considerable gain, though a smaller one, over conventional cameras.

What such X-ray diffraction photographs showed was perhaps a slight anticlimax, although it was in general what one would expect to see on the basis of the model we have been considering. The « actin » part of the diagram does not change appreciably in spacing nor in the relative intensity of the different lines ; these become, if anything, all slightly more intense. This shows that the main features of the actin structure are in large part unaffected by contraction. On the other hand, the layer lines of the myosin filament diagram, which arise from the regular helical arrangement of the cross-bridges, become very much weaker and would probably disappear almost altogether in a muscle that was maximally active for the full time of the experiment. The meridional 14.3 nm reflexion becomes somewhat weaker but less so than the off-meridional parts of the diagram. At last, then, we can see features in the X-ray diagram which show, unambiguously, structural changes associated with contraction. These experiments show that upon contraction the cross-bridges within any given thick filament move from the highly ordered helical arrangements present in a relaxed muscle to a much more disordered arrangement in contracting muscle. The experiments show that there is some longitudinal displacement or tilting movement of the bridges and that a considerable amount of radial and/or circumferential movement also takes place. This result rules out any contraction mechanism in which the bridges remain stationary throughout contraction. However, what the experiment does *not* prove is that the bridges move only when they are attached to actin ; and there are indications now from very recent experiments by Haselgrove and

myself that activation itself may cause bridge movement, even in the non-overlap zone.

Nevertheless, the result is perfectly compatible with a contraction mechanism in which the cross-bridges move to and fro all the time that a muscle is actively contracting and in which they do so asynchronously so as to develop a steady force. With improved techniques and new methods, we hope that much more detailed structural information about cross-bridge movement will gradually become available.

I should now turn to one apparent difficulty with the cross-bridge mechanism, both to explain how it can be circumvented and to show how it illuminates a very interesting aspect of the action of the cross-bridges. This is the problem of the variable side spacing between the filaments.

**8. Force development between filaments with a variable side spacing.** — The advantage of a sliding filament contractile system working in a fluid medium, so that there is liquid between the filaments (except at the chosen points of contact on the projections) is that this will avoid extraneous short-range interactions between the filaments which would interfere with the sliding process. Essentially it reduces friction. The ability of long rod-shaped structure to maintain themselves some distance apart in an aqueous medium has been known since Bernal and Fankuchen's work on gels on TMV (1941). However, the equilibrium separation between rods in such an array varies with the exact local conditions, as discussed by Elliott (1968) and Rome (1967, 1968) ; in a muscle it varies with the extent of overlap of the thick and thin filaments and in practice a 5 nm or more difference in spacing between the surfaces of the actin and myosin filaments is found over the working range of length of the muscle (Elliott, 1964). Moreover Elliott *et al.* (1967) showed that this variation in spacing is maintained in an actively contracting muscle. Thus one pays a price for a low-friction mechanism, and it is one that raises certain obvious difficulties with a cross-bridge model.

If one protein molecule — myosin — forms a specific complex with another — actin — this will have precisely defined configuration. Then, if some configurational change takes place in this complex as a concomitant of one part of the cycle of enzymic splitting of ATP, it is likely that this will take the form of a transition to another sharply defined structure. We are thus led to the conclusion that the cross-bridges are likely always to need to go through exactly the same structural cycle during the splitting of ATP and the performance of mechanical work. If this is the case, how can they accommodate themselves to a quite substantial variation in spacing between the filaments ? Clearly, they cannot do this by the whole cross-bridge operating over a varying range of tilts.

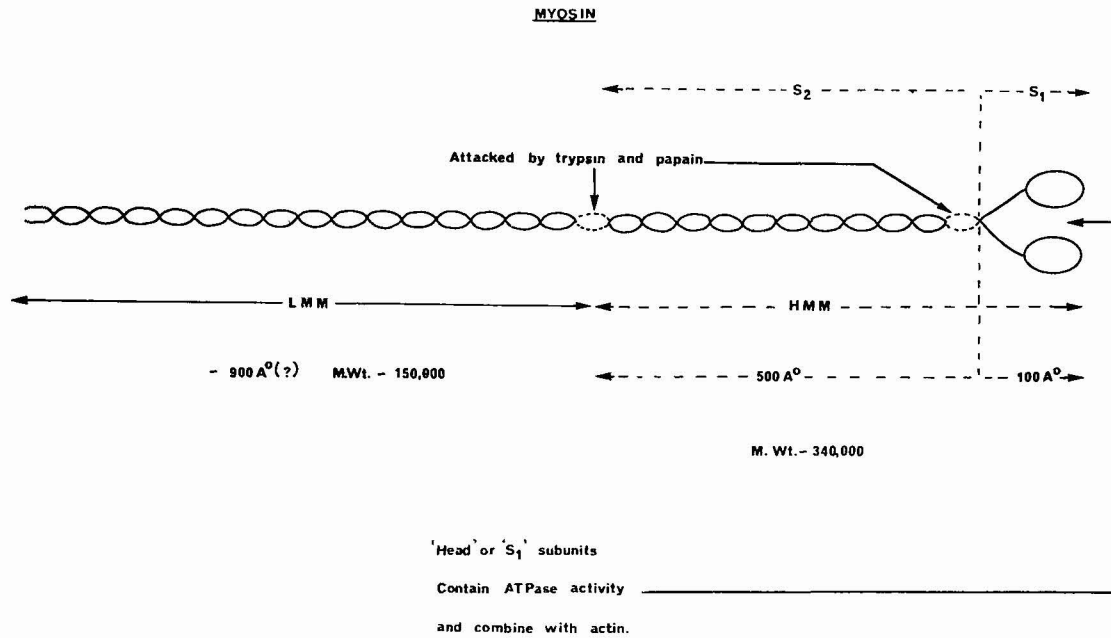


FIG. 14. — Diagrammatic representation of myosin molecules (after Lowey *et al.*, 1969).

Instead, Nature seems to have solved this problem in a rather fascinating way; and, moreover, to have provided very fair experimental clues to tell us how it is done. One clue came from protein chemistry (Lowey, Goldstein and Luck, 1966; Lowey, 1967; Lowey, Goldstein, Cohen and Luck, 1968; Lowey, Slater, Weeds and Baker, 1969). The myosin molecule is a long rod about 150 nm long with the globular regions containing its ATPase and actin binding sites at one end: the rest of the molecule is a two-chain  $\alpha$ -helix (Fig. 14); however it seems to have two points of weakness along its length; these are very susceptible to attack by proteolytic enzymes. The portion of the rod on the left of the first break, away from the heavy meromyosin end, which is known as light meromyosin (LMM) can aggregate at physiological ionic strength into filaments which, though they can grow to large diameters and lengths are probably analogous in structure to the backbone of the thick filaments in muscle. This property of the LMM part of the molecule is presumably utilized when intact myosin molecules assemble themselves into filaments. However, surprisingly enough, the next section of the rod (known as S<sub>2</sub>) between the two susceptible points, does not form aggregates, either with itself or with light meromyosin, and is completely soluble at physiological ionic strength. This feature suggests that in an assembly of myosin molecules this portion of the myosin might not be strongly bound into the backbone of the thick filaments and might enable the whole HMM portion of the molecule to be hinged out in some way (Lowey, 1967).

Another clue came from the equatorial X-ray diagram. This contains two principal reflexions, which are called the [10] and the [11] reflexions,

given by the hexagonal lattice of filaments. When a muscle goes into rigor — that is, when its stores of ATP are used up and the cross-bridges attach to actin — although the extent of overlap of the filaments need not change, the two equatorial reflexions undergo a reversal of intensity so that the second reflexion, the [11], originally weaker than the first in live muscle, becomes, in a rigor muscle much stronger than the first reflexion (Fig. 15). Strangely enough, this was one of the earliest observations made on the muscle diagram (Huxley, 1952, 1953), but it has taken a long time to interpret it properly (Huxley, 1968). The result shows (for reasons explained earlier in this paper) that a large amount of extra material

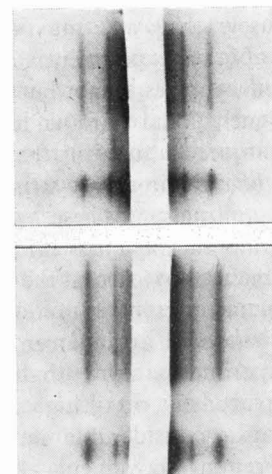


FIG. 15. — Low-angle equatorial X-ray patterns from rabbit psoas muscle: (top) live: (bottom) in rigor. The patterns show the 10 and 11 reflexions from the hexagonal lattice of myosin and actin filaments. The reversal of the relative intensities of reflexions is believed to be caused by the cross-bridges reaching farther out from the myosin filaments in rigor and attaching to the actin filaments at the trigonal positions.

becomes closely associated with the thin filaments in a rigor muscle — enough almost to double their mass per unit length in the overlap region. A straightforward explanation could be that one is seeing the cross-bridges moving outward from the backbone of the thick filaments as they attach to the thin filaments. The extent of the change requires a substantial shift of density within the unit cell, and points rather clearly to a model in which the active part of the myosin molecule, the  $S_1$  subunit, can be hinged out from the backbone, enabling it to link up with the actin filaments over a wide range of inter-filament spacings (Fig. 16). Moreover, given that the links

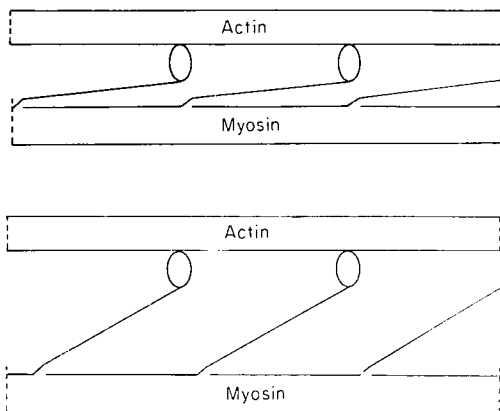


FIG. 16. — Possible way in which « hinging out » of the head subunits of the myosin molecules or the thick filaments could allow attachment of the cross-bridges to the actin filaments to take place over a wide range of interfilament spacings, yet always at exactly the same angle, utilizing assumedly flexible linkages at either end of the linear ( $S_2$ ) portion of the molecule.

on either end of the hinge segment are flexible, then the  $S_1$  sub-unit can always attach to actin in exactly the same orientation.

The system can work as a contractile mechanism on the following assumptions: (1) That some structural change takes place which alters the effective angle of attachment of the  $S_1$  subunit. (2) That there is a system of forces operating in the muscle which, over short changes in overlap, keeps the filament separation approximately constant. Given such constancy, then tilting movements of the cross-bridges, based now, as it were, on the actin filaments, must necessarily lead to relative movement of the thick and thin filaments past each other (Fig. 17). The change in tilt can always be the same, and the tension generated per cross-bridge would be expected to stay fairly constant, even though the filament separation may vary considerably — a type of behaviour that is very difficult to account for by other types of contraction mechanism (especially those involving long range forces) but one which seems to be a characteristic of real muscles, as shown by the studies of A. F. Huxley and his co-workers on the length-tension diagram (Gordon, Huxley and Julian, 1966).

The changes in the equatorial X-ray diagram,

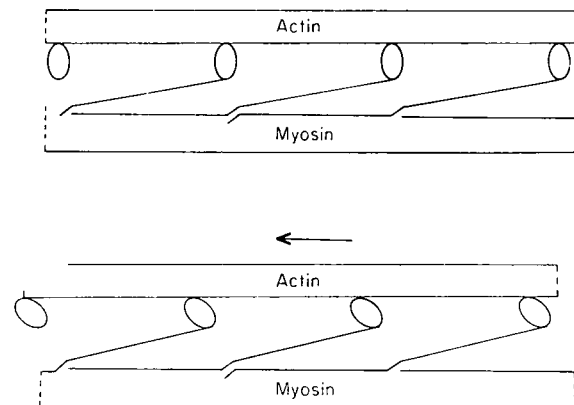


FIG. 17. — Possible way in which a sliding force between actin and myosin filaments could be developed by a change in the effective angle of attachment of the head subunits to the actin filament. The model assumes flexible linkages between the head subunits and the rest of the myosin molecule, and the existence of a force-balance which maintains the filaments at a fixed distance apart for small changes in the extent of overlap.

which we ascribe to outward movement of the active ends of the cross-bridge, have also been studied in our laboratory by Dr. John Haselgrove in actively contracting muscle, where he has confirmed that changes similar to but less extensive than those observed with the rigor muscles — less extensive presumably because all the cross-bridges are not necessarily attached simultaneously during contraction — can be seen in actively contracting muscle, as was indeed noted by Elliott *et al.* (1967); Haselgrove finds that these changes are not artefacts associated with changes in sarcomere length or increasing ordering of the thin filaments in the I-band, but do indeed seem to reflect a genuine outward movement of the cross-bridges when the muscle becomes active.

**9. Other evidence for the swinging tilting bridge mechanism.** — There are a number of other experimental observations which give strong support to this picture, especially to the direction of tilt of the  $S_1$  subunit at the end of its working stroke. First, working with insect flight muscle, Reedy, Homes and Tregear (1965) obtained both X-ray diffraction and electron-microscopic evidence that the cross-bridges are tilted in a rigor muscle and are approximately perpendicular to the thick filaments in a relaxed muscle. Moreover, the direction of tilt was the one required. Secondly, Moore, DeRosier and I have recently completed (Moore, Huxley and DeRosier, 1970) a three-dimensional reconstruction, from electron micrographs, of the structure of actin filaments which have been allowed to combine with  $S_1$  subunits from solution. These  $S_1$  subunits attach in a tilted configuration, and, from previous work on muscle filaments still attached to fragments of Z line, we know that the direction of tilt is again the expected one.

Thirdly, one can see that this mechanism can only



work if the orientation of the  $S_2$  link is such that it will be under tension during contraction. One cannot expect such a structure to be sufficiently rigid to sustain a compressive force without buckling. It is interesting, therefore, that the myosin molecules are indeed packed into the thick filaments with the required orientation.

Finally, I should point out that a mechanism in which the real active unit is an actin filament with the myosin head subunits attached to it places very little restriction on the details of the organization of the « tails » of the myosin molecules into the backbone of the thick filaments. This can vary to suit the particular requirements of various types of muscle, and has in several instances developed along different lines in different species. Indeed, one can imagine motile systems in which actin filaments could move through a relatively unorganized myosin structure thereby providing a basis for cytoplasmic streaming and for cell locomotion (Huxley, 1963). In this connexion it may be noted that Nachmias, myself, and Kessler (1970) have recently found that an actin-like protein from the streaming cytoplasm of a slime mould can bind rabbit myosin  $S_1$  in a manner almost identical to that in which  $S_1$  is bound by rabbit actin; and similar observations on amoeba actin have been made by Pollard and his collaborators (Pollard, Shelton, Weihung and Korn, 1970). Actin-like filaments have also been observed in several other cell types. This has most interesting implications concerning the evolution of different types of muscle from more primitive motile systems.

**10. Correlation with the properties of live muscle.** — It still remains for me to consider the question of how well this model accounts for the detailed physiological behaviour of real muscles. This would be another lecture in itself, or indeed a set of lectures, but I think perhaps there are two crucial features about the behaviour of this type of model which I should emphasize.

First, the model requires that the enzyme sites

function over and over again during one single shortening of a muscle, and not just once; this seems an efficient arrangement. The characteristic shortening velocity of the muscle, which needs to be different in different anatomical situations, then depends on the characteristic ATPase activity of the actomyosin in that particular muscle, and can be regulated by variations in the rate constants of any suitable part of the cycle.

Secondly, if the myosin ATPase can only function when it is attached to actin and goes through the structural cycle and if the physical linkage itself generates the force and is directly coupled, through the muscle structure, to the load, then the splitting of ATP will automatically be linked to the external work done. Thus there is very tight coupling between the external work performed and the molecular events which draw as the energy supplies. This again is clearly a desirable feature from the point of view of efficiency and one that real muscles seem to possess (see, for example, Fenn, 1923).

**11. Conclusion.** — What are the next steps that have to be taken to further explore the details of the contraction process? Many of them are obvious enough in principle, though technically difficult in practice. First, more details about the movement of the cross-bridges must be discovered, by improved X-ray diffraction experiments and by other methods. Next, we have to crystallize the myosin  $S_1$  subunit so that a detailed crystallographic analysis can be performed on it. Thirdly, electron-microscope techniques have to be improved so that we can see meaningful fine detail below the present 2 nm limit. Fourthly, much more intense X-ray sources, perhaps electron storage rings, must be developed so that the time resolution in the X-ray experiments can match that available in mechanical, thermal and chemical measurements. So there is plenty of work to do for the future, and real hope of finding out more and more of the molecular details of this remarkable phenomenon.

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