Recent X-ray Diffraction and Electron Microscope Studies of Striated Muscle

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ABSTRACT The sliding filament model for muscular contraction supposes that an appropriately directed force is developed between the actin and myosin filaments by some process in which the cross-bridges are involved. The cross-bridges between the filaments are believed to represent the parts of the myosin molecules which possess the active sites for ATPase activity and actin-binding ability, and project out sidewise from the backbone of the thick filaments. The arrangement of the cross-bridges is now being studied by improved low-angle X-ray diffraction techniques, which show that in a resting muscle, they are arranged approximately but not exactly in a helical pattern, and that there are other structural features of the thick filaments which give rise to additional long periodicities shown up by the X-ray diagram. The actin filaments also contain helically arranged subunits, and both the subunit repeat and the helical repeat are different from those in the myosin filaments. Diffraction diagrams can be obtained from muscles in rigor (when permanent attachment of the cross-bridges to the actin subunits takes place) and now, taking advantage of the great increase in the speed of recording, from actively contracting muscles. These show that changes in the arrangement of the cross-bridges are produced under both these conditions and are no doubt associated in contraction with the development of force. Thus configurational changes of the myosin component in muscle have been demonstrated: these take place without any significant over-all change in the length of the filaments.

The general picture of the fine structure of striated muscle that has been built up in the last 10 or 15 years is now very familiar, and I will not repeat it here in detail. In summary, we believe that the myofibrils contain overlapping arrays of actin- and myosin-containing filaments, and that cross-bridges between these thick and thin filaments generate a relative sliding movement of one set of filaments past the other; the filaments themselves remain virtually constant in length. The tension generated by the cross-bridges along a given thick filament adds up in parallel, so that the tension generated by the system as a whole varies according to the extent of overlap of the filaments, and also according to the number of cross-bridges which

have time to attach to the active sites on the actin molecules alongside them. When the muscle is allowed to shorten rapidly, the time available for links to form is reduced, and, if we assume appropriate reaction constants, as has been done, for example, in the systems suggested by A. F. Huxley (1) and Podolsky (2), we can account for the observed variation of force with velocity of shortening. It is also important to note that the force generated by the cross-bridges must act in the appropriate direction. That is, all the elements of force in one half of the A band must act in one direction and those in the other half of the A band must act in the reverse direction, so that the actin filaments are moved toward the center of the A band from either side. When the muscle is no longer activated, we suppose that the cross-bridges detach from the thin filaments, which are then free to be drawn out of the A bands again, and the muscle thereby is reextended to its resting length. Studies on the assembly of the myosin and actin molecules into synthetic filaments (3) have indicated that the myosin molecules are arranged so that they all point in the same direction in one half of the A band, and that the direction of all the molecules in the other half of the A band is reversed. In this way the orientations of the active sites in the two halves of the A band would be reversed relative to each other, thereby achieving the necessary directional relationships of the forces that they can generate. Similarly, it has been shown that actin filaments have a structural polarity and that this polarity is reversed on either side of the Z line. These electron microscope studies, though valuable in showing up some aspects of the arrangement of the molecules of the contractile proteins in the muscle filaments, were not able to show the details in the very regular repeats of the molecules that we should expect to find. This is probably because some disorder of the structure takes place during the preparation of the specimen in electron microscopy, especially in the thick filaments.

We are now using improved low-angle X-ray diffraction techniques to study the detailed internal structure of the thin and thick filaments in muscle, the detailed arrangement of the actin and myosin molecules in these filaments, and the changes that occur in the arrangement when the two proteins interact with each other when the muscles contract, and also when they pass into rigor. In the latter condition, all the ATP in the muscle has been used up, and it is believed that permanent cross-connections are established between the two types of filaments. In the former case, in contraction, we believe that there is repetitive interaction between the myosin cross-bridges and the active sites on the actin filaments, and that this leads to the relative sliding motion of the filaments past each other. Dr. K. C. Holmes and Dr. W. Brown have contributed to various aspects of the work.

The low-angle X-ray reflections that we are now studying in detail are the ones which I first described in 1951–1953 (4–6) as being given by living muscles; recently, we have greatly improved the technology associated with the

recording of these reflections and are now able to obtain much better pictures in much shorter times. This is not the place to describe the techniques in detail, but, in summary, we are employing a high-powered, fine-focus, rotating-anode X-ray tube (designed by Holmes and Longley) and a new type of camera consisting of a combination of a bent quartz focusing monochromator, which gives very high resolution in one direction, combined with a total reflecting bent glass plate, which gives focusing in another direction at right angles to the first and therefore essentially leads to point collimation. The

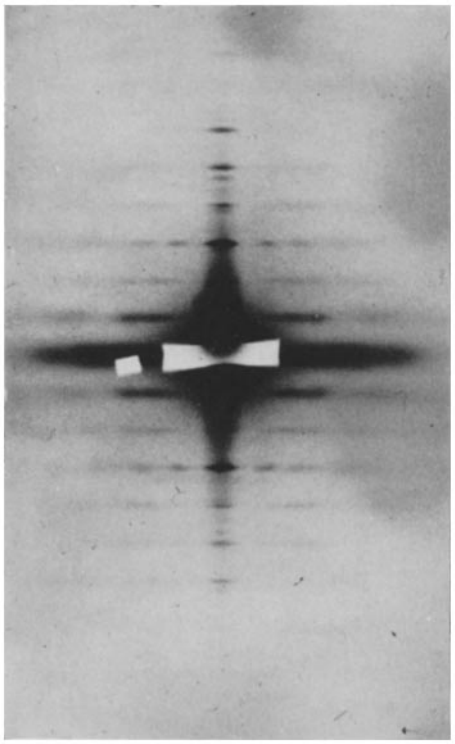

FIGURE 1. Low-angle X-ray diffraction diagram from surviving frog sartorius muscle, showing characteristic system of layer line reflections, arising from helical arrangement of cross-bridges on myosin filaments. Arrangement approximates to 6/2 helix (three pair of cross-bridges at 143.0 A intervals in full repeat of 429.0 A), but occurrence of "forbidden" reflections on meridian on layer lines other than 3rd, 6th, etc., indicates departure from simple helix. Other reflections are observed between layer lines.

fastest of our cameras can record the pattern from collagen fibers in less than 30 sec, and the main features of the muscle diagram in about 10 min.

The X-ray diagram given by surviving frog sartorius muscle contains a wealth of interesting, informative, and intriguing detail (Fig. 1). The main feature of this diagram is a system of equally spaced layer lines, which were in fact the reflections first seen in 1952. These were later identified by Elliott (7) and Worthington (8) as arising from the myosin filaments, and indeed there is good reason to suppose that the cross-bridges (which should show up, as it were, with higher contrast against the surrounding sarcoplasm) will contribute strongly to this part of the pattern.

The pattern we see is the characteristic one to be expected from a helical

distribution of scattering matter. That is, we see a characteristic variation of intensity along the different layer lines, which themselves are separated by a distance corresponding to a helical repeat of 429.0 A. We observe that there is a strong meridional reflection lying on the third order of this repeat, which would correspond to the true axial repeat, i.e. the axial repeat of the subunits which are arranged in the helical pattern. In this case the subunits would correspond to the cross-bridges, and the pattern shows that these must occur at intervals at 143.0 A. The distribution of intensity along the layer lines can be identified with that expected from a so-called 6/2 helix, i.e. one in which we have pairs of cross-bridges, one on either side of the filament, repeating at the 143.0 A spacing, with successive repeats rotated relative to each other by 60°, so that the whole structure repeats at 3 × 143.0 A, i.e. the helical repeat. The distribution of scattering intensity along the layer lines corresponds to that to be expected with a center of mass of the scattering centers at a radius of about 110 A, which corresponds to the position of the cross-bridges as seen by electron microscopy.

If the arrangement of the cross-bridges were perfectly helical, then the only meridional reflections which could occur would be the ones on the third, sixth, ninth, etc., layer lines. However, although the reflections on these layer lines are strong ones, there are meridional reflections on all the other layer lines except the first, showing us that there is some degree of distortion from an ideal helix.

At wider angles we can see the well-known system of reflections originally reported by Astbury (9) as being given by the actin structure and interpreted in a number of possible ways by Selby and Bear (10) in 1956. It was later shown by Hanson and Lowy (11) that the actin filament did indeed consist of a double helix of apparently globular subunits, in agreement with one of the models put forward by Selby and Bear. The actin reflections from living frog sartorius muscle can be indexed on a helical net in which the pitch of the helix is 355 A × 2–370 A × 2, with 13–13½ subunits per turn in each of the chains. There are, however, some lower-angle reflections which have characteristics similar to those of the other actin lines and which have a spacing of about 400 A, and it is not clear at present what exactly is the origin of these latter reflections.

When living muscles are stretched, the spacings and relative intensities of both these systems of reflections are unchanged, as was originally shown in 1952. This provides a very clear demonstration that changes in filament length do not occur during passive changes in length of the muscle.

So far we have been concerned with the most well-known features of these diagrams. However, if we examine them in greater detail with cameras of improved resolving power, we can see that they contain a number of other well-defined reflections of a rather more complicated and intriguing nature

(Fig. 2). In the first place, there are strong reflections on the meridian of these diagrams from living muscles at spacings of 388 A and 444 A units. These do not correspond to the periodicity of either the actin or the myosin helices, and apparently arise from some other system in the muscle. As the system which gives rise to these reflections clearly has exactly the same orientation as that which gives rise to the reflections we have already described, and as the reflections are quite strong ones, it seems very likely that they arise from some other component directly associated with the myofibrils rather than, say, with the mitochondria or with the reticulum. In the electron microscope (Fig. 3), especially in relatively thick sections, a

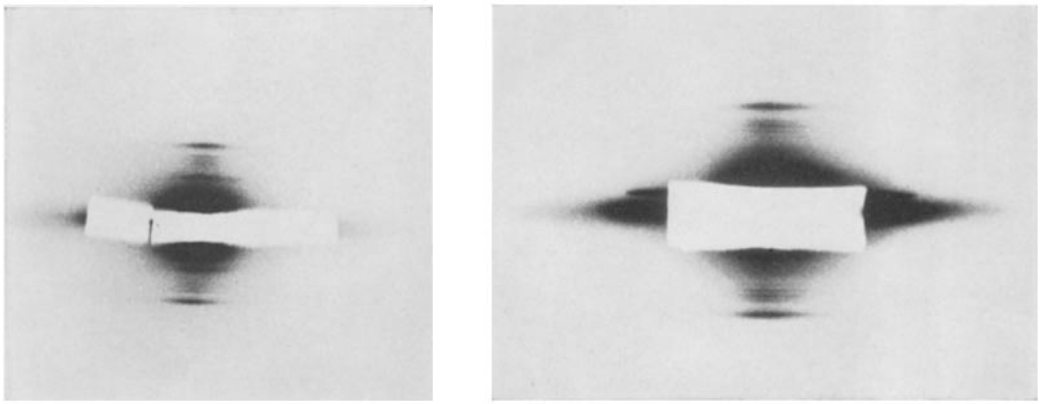

FIGURE 2. Very low-angle meridional reflections, as seen in fixed, stained, and embedded frog sartorius muscle. Outer reflection (which corresponds to the one having a spacing of approximately 385–390 A in surviving muscle) is believed to arise from material associated with thin filaments. Inner reflection, associated with thick filaments (444 A in surviving muscle), shows here additional lines which are near submultiples of 7500 A (see text).

well-defined periodicity is visible in both the I and A bands, which has long been recognized as having a value in the region of 400 A. More accurate measurements recently have shown that the periodicity in the I bands corresponds to the shorter of these two new spacings, i.e. to the value of 388 A in living muscle, and that in the A band corresponds to the longer spacing, i.e. 444 A. Furthermore, it was subsequently shown by Cohen and Longley (12) that a periodicity of about 380–390 A could be detected in crystals of tropomyosin. There has been a good deal of evidence recently indicating that tropomyosin and actin are associated together in some way in thin filaments, and it therefore seems that it is this associated tropomyosin that we are picking up in the low-angle X-ray diagram. There have also, of course, been indications that the tropomyosin may play some role in the regulation of the activity of the actomyosin, and its repeating periodicity

may therefore turn out to have some important significance in relation to that of actin and myosin.

The periodic structure that gives a repeat of 444 A in the A band is rather more mysterious, for as yet no additional component has been identified in the thick filaments, apart from myosin. There is, on the other hand, a certain amount of evidence (13, 3) that all the protein of the thick filaments cannot readily be accounted for by those myosin molecules having cross-bridges which are visible in the electron microscope, and that there might therefore be either additional myosin molecules buried deeper in the filaments

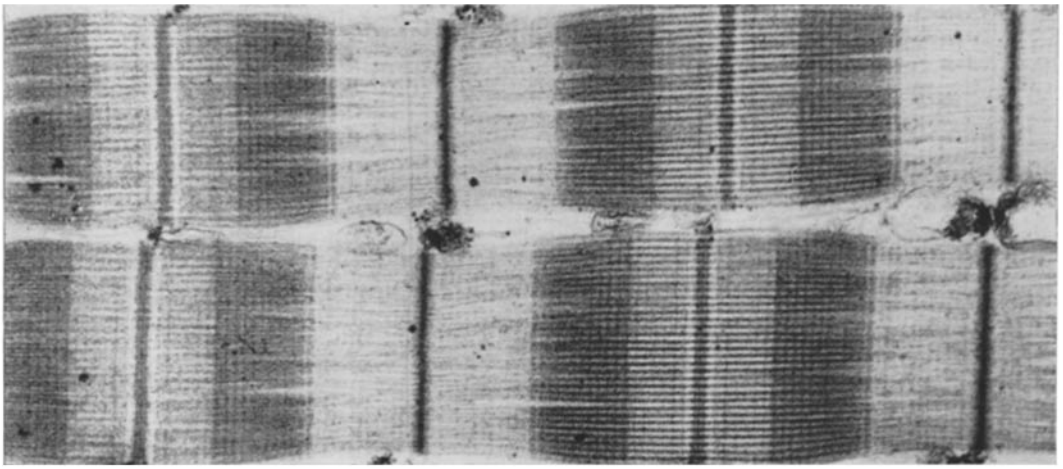

FIGURE 3. Section of muscle, similar to that giving X-ray diagrams in Fig. 2, but stained with lead instead of phosphotungstic acid. Note fine transverse periodicity in I and A bands, including the H zone. Period is about 12% shorter in the I band than in the H zone, and hence system would give rise to reflections at two different spacings on meridian because of large lateral extension of the narrow zones of higher density. × 28,800.

or some extra protein component. This should be investigated by protein chemistry.

A problem we have been aware of for some time is the question of how the length of the filaments in muscle is determined. The thick filaments appear to have a very constant length of about 1.55–1.6 μ in the muscles from a large variety of animals. The filaments are built up of many hundred myosin molecules, and it does not seem to me plausible that such a precise and constant length could be achieved by a kinetic mechanism. It appears more likely that there is some structural mechanism involved which assures that the filaments always assemble themselves so as to have this precisely predetermined length.

When one is building up long filaments by polymerizing small molecules,

a possible way in which the length can be determined would be for two or more components to be present, each of them building structures with slightly different repeating periodicities so that a match would be obtained after n repeats of one type of molecule and $n + 1$ repeats of the other. Very recently, using a camera of exceptionally high resolution, capable of separating reflections equivalent to the successive orders of a long repeat of more than 10,000 A, and using muscles stained with phosphotungstic acid to enhance the intensities of these low-angle meridional reflections, we have found that they have a more complicated nature than was at first apparent. The reflection which comes from the A filaments can be resolved into a group of very closely spaced reflections, all of which are submultiples of approximately 7500 A, that is, half the length of the A band. Since the spacing of these reflections is not affected by changes in sarcomere length, they do not apparently arise as a high order of a repeat, say, of 3 μ. The entire group of reflections must arise from each A band independently, and within such a band there is clearly not room for a number of repeats of 7500 A. It would appear, then, that we are seeing a rather unusual diffraction phenomenon, which may be equivalent or identical to the diffraction from three or four structures of slightly different periodicity which all share the same lowest common multiple, i.e. 7500 A. It is tempting to suppose that this may be related to the filament length-determining mechanism in muscle—indeed, that there may be some relationship between these reflections and certain features of the scheme proposed by F. Pépé (personal communication)—and the subject requires further work, which is now in progress. At higher angles we can again see a very rich pattern of reflections which do not index on either the actin or myosin lattice repeats. So we see, then, that the low-angle X-ray diagram from living muscle still contains plenty of new information in it!

Let us now turn to the diagram of muscles in rigor, i.e. muscles in which combination between the cross-bridges and actin filaments alongside has taken place, and let us first of all consider the diagrams of muscles near to rest length or shorter, in which there is a large region of overlap between the actin and myosin filaments. If we look at the very wide angle actin pattern, i.e. that at spacings of less than 50 A, we find that there is virtually no detectable change in either the spacing or the intensity of these reflections, except for the first true meridional reflection at 27.3 A, which is somewhat more intense in the rigor specimens. This indicates that the local packing of the actin monomers is virtually unaffected when the cross-bridges attach to them. The actin reflection near to 59 A moves nearer to the meridian as though it were becoming associated with scattering matter at a larger radius than that of the actin filaments alone.

In contrast to the behavior of the actin reflections, the diagram of myosin filaments is greatly altered when the muscle passes into rigor. We still observe

a strong meridional reflection near 143 A, showing that the average axial repeat of the cross-bridges is still the same. But the characteristic pattern of regularly spaced layer lines at intervals of 429.0 A is no longer present, showing us that a considerable change has taken place in the helical arrangement of the cross-bridges. In the part of the diagram where the cross-bridge reflections originally showed so prominently (i.e. between 60 and 430 A), we now see a series of somewhat diffuse reflections, which can be indexed on an actin-like lattice, indicating that the position of the cross-bridges now conforms to the repeat of the active sites along the actin filaments. Such a change can only be achieved if the cross-bridges move, so that the result shows first of all that the cross-bridges move when the muscle passes into rigor and that they do not represent a constant pattern for all states of the muscle. Furthermore, there is quite a strong reflection now on a first layer line at 370 A, and another reflection at 239 A; this, together with the subunit repeat of 143 A, can be indexed on a nonintegral helix with 2.5 residues/turn, i.e. 5 residues in two turns of the helix at a true repeat of about 720 A. [I should say that another nonintegral helix was first detected by electron microscopy in insect flight muscle by M. K. Reedy (manuscript in preparation).] There are thus indications that a change in the myosin filament helix may occur when the muscle passes into rigor, but from these observations we cannot see whether this is due directly to the absence of ATP, to the combination with actin, or to the presence of calcium.

We have also examined very stretched muscle in rigor, i.e. muscles which have been extended to a sarcomere length of approximately 3–3.2 μ, so that there is now only a 0.2–0.3 μ region of overlap between the thin and thick filaments. The diagrams from these muscles still show the pattern of actin reflections, but the movement of the 59 A actin reflection toward the meridian is now much reduced, as we would expect if a smaller proportion of actin monomers were becoming labeled by the heavy meromyosin cross-bridges. Moreover, these highly stretched muscles no longer show a pattern of reflections at spacings longer than 59 A, which, as we mentioned, can be indexed on an actin-like lattice in muscles in rigor at rest length. Nevertheless, the original regular layer line pattern given by the living muscles is no longer present in these stretched rigor muscles, indicating that a disorganization of the cross-bridge pattern may take place when the muscle passes into rigor, and that the presence of overlapping actin filaments may serve to stabilize the cross-bridges in a new regular pattern.

Now let us turn to the study of the X-ray diagrams given by actively contracting muscles. It is not possible to keep an isolated muscle contracting continuously in an oxygenated Ringer bath. The muscle must be left for a time between successive stimuli to enable it to recover. A schedule often employed is one in which the muscle in stimulated for about 1 sec at intervals of

1 min, and carefully dissected muscles can continue to respond satisfactorily in this way for 10 or 20 hr or more. By arranging to have the shutter on the X-ray camera open only when the muscle is generating tension, we can build up the required total exposure time of 10 or 20 min by adding together 600–1200 individual 1 sec contractions, so that the total time of the experiment will be 10 or 20 hr; as mentioned previously, the fastest cameras that we now use will record the main features of the low-angle X-ray diagram within this time. When the diagrams from actively contracting muscles are examined

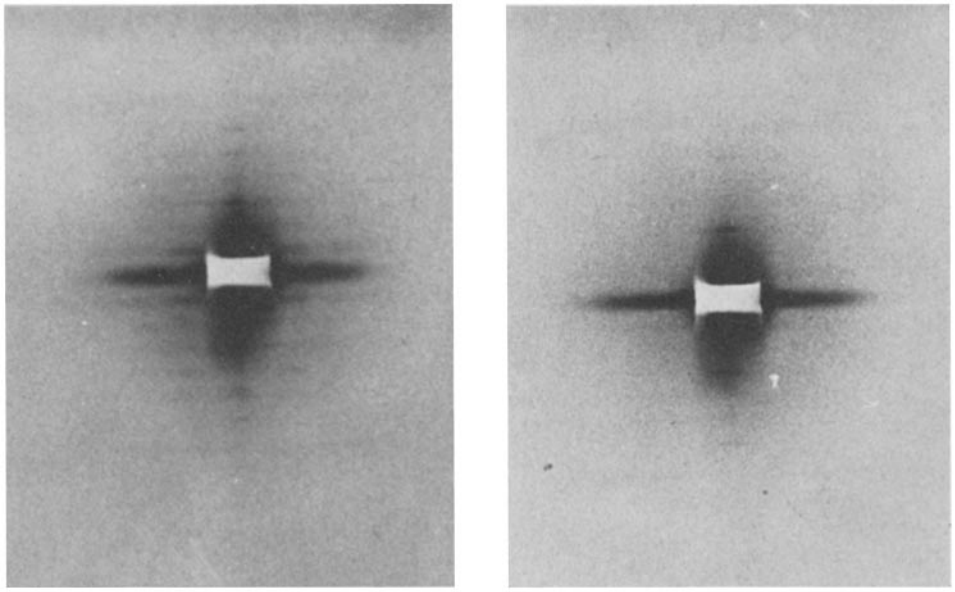

FIGURE 4. X-ray diagram from resting (left) and isometrically contracting (right) frog sartorius muscle. Total exposure time was the same in both cases. Third-order meridional reflection from myosin system, and 59 A actin reflection, remain approximately unchanged in spacing. A very large decrease in intensity of off-meridional part of myosin layer lines is observed (see text).

(isometric, and also with shortening by 10% of rest length), we find that the strongest characteristic reflections of the axial pattern, namely, the 143.0 A meridional reflection (showing the average repeat of the cross-bridges) and the 59 A off-meridional actin reflection, are approximately unchanged in spacing. There may be a small decrease in the intensity of the 143.0 A reflection, but that of the actin reflection appears unchanged, and we have not so far detected any movement of this reflection nearer to the meridian. These results (14), which were also independently obtained by Elliott, Lowy, and Millman (15), show that the repeating periodicities of the two types of filaments and, hence, the over-all lengths of those filaments are almost un-

changed during contraction. On the other hand, we find that a large change is visible in the intensity of the off-meridional part of myosin layer lines, which all become very much weaker (Fig. 4).

This indicates that a substantial change has occurred in the helical pattern of cross-bridges when the muscle becomes active, and that the arrangement of cross-bridges does not merely change to a new but equally highly ordered form. Surprisingly, a large component of this movement must be either radial or circumferential rather than axial, for a random axial displacement of cross-bridges would be expected to diminish greatly the intensity of the 143 A meridional reflection. One can say that an unsynchronized movement of the cross-bridges takes place, so that a consistent helical repeat is not present over a substantial length of the filament at any given time. The fact that the 59 A actin reflection is relatively constant in position, and that there is little sign in the diagrams we have obtained so far (with presently available exposure time) of any additional reflections characteristic of muscles in rigor, indicates that all the cross-bridges are not attached to the actin filaments simultaneously during isometric contraction. If all the cross-bridges *were* attached simultaneously, this would indicate that the range of movement of the cross-bridges (supposedly oscillating to and fro in contraction) lay in the region of 50–100 A. If, say, only one third of the cross-bridges are attached at any given time, this would indicate a movement of approximately 15–30 A, and therefore it is important to obtain data from much longer exposures than have been possible so far, to investigate this matter further by setting limits on the proportions of bridges attached at any given time.

To summarize, then, the actin and myosin molecules are arranged in their respective filaments in a very regular helical manner. The pitch of the two helices in resting muscle is not the same, nor are their subunit repeats equal. The presence of additional components is indicated by the X-ray diagram. When the muscle passes into rigor the position of the cross-bridges on the myosin filaments changes, so that a substantial number of them fall into a pattern compatible with the helical arrangement of active sites on the actin filaments. The actin filaments themselves do not undergo any substantial change in their local packing, but there are indications that a small change in their long helical period from 355 to 380 A may take place. When the muscles become active, the regular arrangement of the cross-bridges is disturbed, so that they no longer give rise to a readily detectable X-ray pattern, and we can therefore say that muscular contraction is associated with movement of the cross-bridges, though much further work is needed to define the exact nature of this movement more precisely.

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Discussion

Dr. Gordon W. Whaley: I would like to ask Dr. Huxley about the first two pictures he showed, in which there appeared to be some cross-bridges between the actin filaments. Why are they there?

Dr. Huxley: The reason I think that one apparently sees cross-bridges between adjacent actin filaments in the A band is that these sections, which are about 150 A thick, have another layer of thick filaments lying just above the plane of sectioning, and then a second layer of thick filaments lying below the plane of sectioning; you more or less exclude these when you cut the section, but each of these filaments outside the section will be sending cross-bridges upward toward the actin filaments which are included in the section, and if any of these cross-bridges are included in the section, these can give the appearance of cross-bridges between actin filaments.

When you look at cross-sections of the structure, where this kind of artifact doesn't arise, you see that in the overlap zone, the only bridges which are apparent are those between actin and myosin.

Dr. Francis D. Carlson: My question relates to possible causes for the loss of diffraction spots in the contracted muscle. Recently we have made a very preliminary study of the loss of coherence of a laser beam on transmission through resting and contracting muscle, Without going into the experimental details, the results show that an isometric tetanus produces a loss of coherence, indicating that there is a component of the muscle system that is in a state of violent motion during contraction. We do not yet know whether this incoherence is the result of some kind of movement of the cross-bridges, or of a rapid fluctuation in sarcomere length or myofibril position about a mean position, or some combination of these possibilities. The question is, if sarcomere length or myofibril position were undergoing rapid fluctuations of small amplitude (a kind of "microjitter") during contraction, could this have produced the X-ray changes that you observed?

Dr. Huxley: The X-ray diagrams which we get are those we would expect to get

from individual A filaments diffracting on their own. The only feature which arises from the arrangement of the filaments in a lattice, in register with one another, is the spots on the lines which you sometimes see, but individual, isolated thick filaments, if they are all pointing in the same direction, will give rise to this pattern of layer lines without their having to have any particular regular relationship, either to thick filaments alongside them, or to thick filaments in neighboring sarcomeres, so that the only thing that can destroy the regular pattern given by the cross-bridges is a change in the position of the cross-bridges relative to each other on the same thick filament.

Now, it may well be that you have successive sarcomeres jittering in and out. My only point is that there is a change in the position of the cross-bridges relative to each other, indicated by the diagram.

Dr. A. F. Metherell: I would like to refer to the opening remarks on contraction. Accepting that the filaments slide during contraction, and accepting that the ATP is broken down at the sites where the cross-bridges contact the actin filaments, I don't see that it necessarily follows that the force is transferred through the cross-bridges.

I wonder if Dr. Huxley has considered the second alternative, that the load is transferred through viscous shearing in the myoplasmic fluid that surrounds the filaments. The viscous shearing can be set up by gradients in interface tension caused by the release of energy at the cross-bridge contact sites. Do you have any comments on that, please?

Dr. Huxley: No. I think the only thing one can say is that the cross-bridges are the site where the events which are responsible for the release of contractile energy take place, and that the number of sites in operation is proportional to the amount of energy that would be released, and that one can, from a number of experiments, produce evidence for a change in orientation of the cross-bridges.

The simplest way of thinking about this, then, seems to be to suppose that the cross-bridges in fact physically move the thin filaments along. But I don't think one can rule out other mechanisms of the type you have described on the present evidence.

Dr. Elliott: This is perhaps a corollary on the last one. I don't want to take too long because I'm coming back to this tomorrow, but it does seem to me that the evidence which Professor Huxley has from the disappearance of the layer lines, and which we were able to get as well, doesn't really tell you that the projections ("cross-bridges") move in a coherent way. All it really tells you is that there is an increase in the (crystallographic) temperature factor and disorientation of the projections when the muscle is contracting, and it seems to me that you would expect this in almost any model of muscular contraction which you have.

Dr. Perry: I wonder if I may ask a question. How close do you believe, Dr. Huxley, that the heads of the myosin molecules get to the actin subunits?

Dr. Huxley: I don't have any evidence about it, and all we can say is that when you look at them in the electron microscope they look very close, so there's not a gap between them, but this is very poor evidence indeed. I would have thought that the good evidence that there was likely to be a direct attachment of the cross-bridges would come from evidence more in Professor Perry's field; namely, from the rather specific influence that actin has on the ATPase activity of myosin, and I would find

it difficult to think that such a specific influence could be transmitted over long distances. I think it would be much more reasonable to suppose that the two proteins are brought into fairly intimate contact.

Dr. Donald A. Fischman: Regarding the 380 A repeat in the I band, Professor Huxley, we have recently been examining tropomyosin crystals, prepared by the method of Cohen and Longley, which give this repeat. If one examines shadowed replicas of these crystals, there is a spiral or helical pattern on the surface of the crystals, suggesting that the linear molecules may be able to aggregate in a helical fashion. We are currently making measurements of this helical form.

Dr. Huxley: That is extremely interesting because it's a little odd in a way, because the 380 A reflection lies very, very close to the meridian, and therefore this could really only be the subunit repeat along the helix.

Is there a much longer helical repeat of which the 380 is the subunit repeat?

Dr. Fischman: Well, this spiral repeat is of fairly large magnitude; it is not of the order of the helix within the tropomyosin molecule itself. The repeat of this spiral is approximately 7 times the 400 A length of tropomyosin molecule, or about 2800 A.

Dr. Hayashi: I seem to see an increase in intensity of reflection between the relaxed and contracted muscle, which Elliott has suggested might be due to an increased ordering of the actin filaments in contraction.

Now, assuming that the difference between decreased ordering and increased ordering is a certain amount of slack in the actin filaments in the relaxed state, about what sort of change, what order of magnitude of change in length of the total length of the actin filament, would be required to bring about this sort of change?

Dr. Huxley: Maybe Dr. Elliott should answer this question, but could I answer it for him? My recollection was that the reflection which increased in intensity is not one of the meridional reflections. It was one of the equatorial reflections, and this is a question of the ordering of the actin filaments in between the thick filaments, and it's a question of how accurately they're placed at the trigonal points in the lattice. Small deparatures from accuracy in this represent extremely slight changes in the filaments and therefore wouldn't represent appreciable changes in their over-all resolved length, along the lengths of the muscle.