range of sarcomere lengths from $2 \cdot 0$ to $3 \cdot 5\,\mu$; this is in fair agreement with observation[11]. In arthropod striated muscle, there is a wide range of sarcomere lengths *in situ*, and narrowness of striation appears to be correlated with high speed of contraction[12]. This would be expected if the relative velocity between actin filaments and myosin rods in any one zone of overlap were the same for muscles of different sarcomere lengths, since the number of sarcomeres shortening in series per unit length is inversely proportional to sarcomere length. On this basis it would also be expected that the muscle with longer sarcomeres would be capable of producing a greater tension, but we are not aware of any experimental results on this point.

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Changes in the Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation

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In recent papers[1–3], we have described evidence concerning the location and arrangement of the two principal structural proteins, actin and myosin, in striated muscle at rest length. This evidence indicates that myosin is located in the anisotropic or *A*-bands, in the form of longitudinal filaments about 110 A. in diameter, spaced out in a hexagonal array 440 A. apart; these filaments are continuous from end to end of the *A*-band, and appear to be responsible for its high density and birefringence. Actin, on the other hand, is present in both the *A*-bands and the relatively isotropic or *I*-bands, in the form of filaments about 40 A. in diameter; these extend from the *Z*-lines, through the *I*-bands, and into the *A*-bands, where they lie between the myosin filaments and terminate on either side of the *H*-zone; the myosin filaments seem to have a somewhat greater thickness in this zone. Hasselbach[4] has reached similar conclusions about the location of actin and myosin, though his concept of the details of their arrangement is different from ours. We shall now describe evidence that during stretch, and during contraction down to about 65 per cent of rest length, the length of the *A*-bands remains constant within the limits of accuracy of our measurements (5–10 per cent), the changes in length of the muscle being taken up by changes in the length of the *I*-bands alone; further shortening beyond the point where the *I*-bands vanish (about 65 per cent of the rest length) is accompanied by the formation of contraction bands where the *A*-bands have come into contact with the *Z*-lines. These changes appear to take place by a process in which actin filaments slide out of or into the parallel array of myosin filaments in the *A*-bands; as shortening proceeds, the actin filaments fold up in the *A*-band, and this folding continues after the *I*-bands have been fully retracted. The myosin filaments remain at constant length until forced to shorten by excessive contraction of the sarcomeres.

Previous work on the changes in cross-striation accompanying stretch or contraction (reviewed by Jordan[5] and Buchthal, Knappeis and Lindhard[6]) has given results which in general we consider to be unreliable, for the following reasons. Observations made on intact muscle fibres in conventional light microscopes are liable to be misleading because of optical artefacts due to the thickness of the fibres. (This difficulty has been surmounted by the technique described by A. F. Huxley and R. Niedergerke in the accompanying paper.) Furthermore, normal contraction is so rapid that the changes taking place *during* the process are difficult to see and record. If fixed and sectioned material is used, it is possible to avoid optical artefacts and the necessity for rapid observation, but other kinds of artefacts are introduced. In spite of all these considerations, a number of workers, notably Speidel[7], have given accounts of changes of band pattern during stretch and contraction which we recognize as generally correct; but they do not establish the details of the changes with the precision necessary for satisfactory interpretation.

In order to avoid optical artefacts, we have used isolated myofibrils about 2 μ in diameter prepared by blending glycerol-extracted rabbit psoas muscle[8]. They are admirable objects for high-resolution microscopy in phase-contrast illumination or polarized light, and will contract when treated with adenosine triphosphate[9]. This contraction is a much slower process than contraction *in vivo*, and therefore provides favourable circumstances for detailed observation of the band changes taking place. The evidence that the mechanism of contraction in glycerol-extracted muscle treated with adenosine triphosphate is similar to that of normal contraction in living muscle has already been adequately discussed by Szent-Györgyi[8] and Weber and Portzehl[10]. We have also devised a simple technique for stretching isolated fibrils during observation. A suspension of fibrils, mounted as a very thin layer on a slide under a coverslip, is examined in the microscope until a fibril is found with one end embedded in a fibre fragment adhering to the coverslip, and its other end in a fragment attached to the slide. Movement of the coverslip in the appropriate direction will then produce the desired stretch or will permit the fibril to shorten if adenosine triphosphate is present. Very small movements can be produced with great ease by gentle pressure on the edge of the coverslip, for the thin layer of liquid provides smooth and highly viscous lubrication.

Photographs used for measurement were taken on microfile film at a magnification of × 370 or × 550, and an apochromatic phase-contrast objective of n.a. 1·15 was employed.

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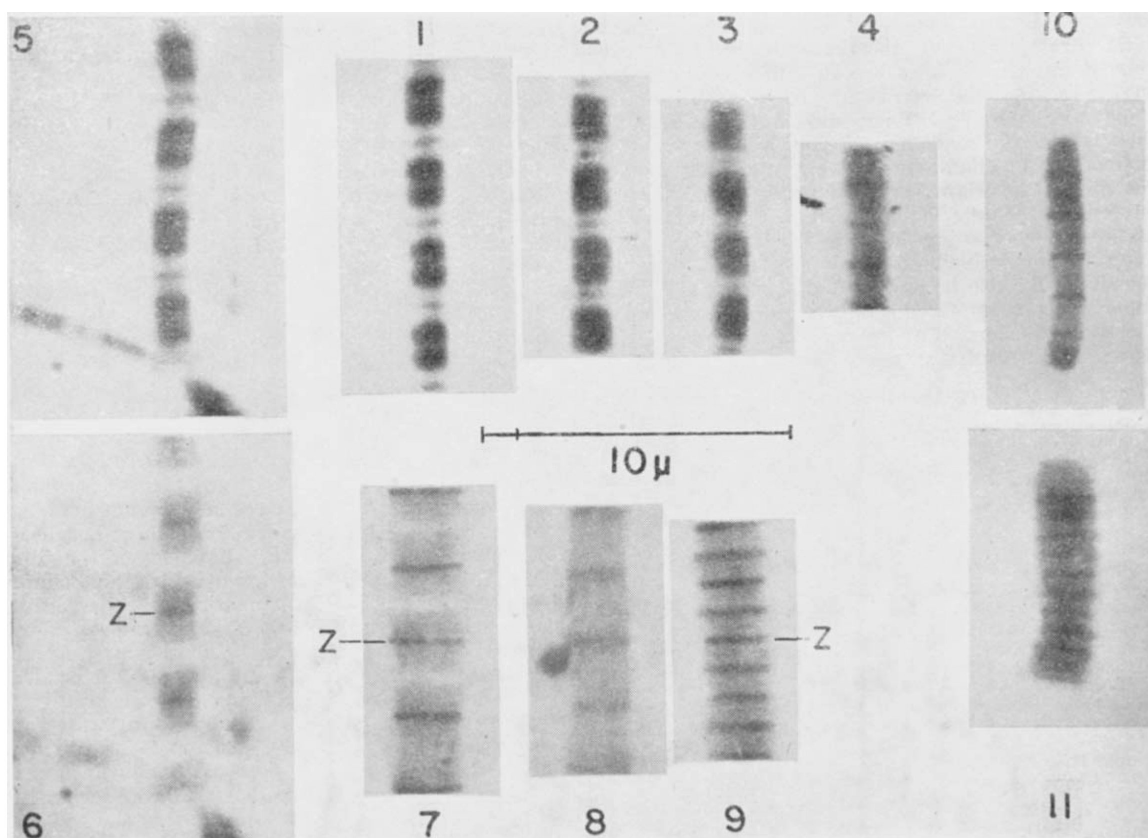

Myofibrils photographed in phase contrast. Magnification, 4,000 ×. Photographs of extracted fibrils are printed so as to give adequate contrast, and the fibrils are in fact much less dense than they appear here

Figs. 1–4. The same four sarcomeres of one fibril photographed during contraction induced by adenosine triphosphate from rest length down to 50 per cent rest length, when contraction bands have formed

Figs. 5 and 6. Stretched fibril (115 per cent rest length) before (Fig. 5) and after (Fig. 6) extraction of myosin

Figs. 7, 8 and 9. Fibrils after extraction of myosin. Fig. 7: rest length. Fig. 8: 90 per cent rest length. Fig. 9: 75 per cent rest length

Figs. 10 and 11. Fibril with contraction bands (50 per cent rest length) before (Fig. 10) and after (Fig. 11) extraction of myosin

Contraction

We have studied and obtained photographic records of the details of contraction in the following systems:

(1) Fibrils contracting freely at room temperature (about 22° C.) in 5×10^{-4} *M* adenosine triphosphate, 0·1 *M* potassium chloride, 10^{-3} *M* magnesium chloride, *p*H 7·0.

(2) Fibrils contracting freely at room temperature in a series of steps achieved by irrigating them with a succession of small amounts of a very dilute solution of adenosine triphosphate (5×10^{-6} *M*), irrigation being stopped as soon as the required degree of shortening had taken place.

(3) Fibrils contracting freely at a low temperature (about 2° C.) in adenosine triphosphate in the almost complete absence of magnesium ions, when shortening takes place very slowly.

(4) Fibrils contracting as in (1) but held at both ends so that shortening is controlled.

Cinephotography was used for system 1; but in systems 2–4 it was possible to take 'still' photographs on fine-grain film. In the first three systems the fibrils were contracting against virtually zero load and showed identical changes of band pattern. System 4 provided information about isometric contraction, and it was found that the changes of band pattern differed in some details from those recorded during contraction against zero load.

During contraction of single fibrils against zero load, we have observed the following changes in band patterns (illustrated in Figs. 1–4). The *I*-bands shorten from a resting length of approximately 0·8 μ until they disappear completely. During this process, the length of the *A*-bands remains constant at approximately 1·5 μ. Changes of density within the *A*-band do, however, occur. The *H*-zone, originally of low density, first becomes indistinguishable from the rest of the *A*-band, and is then replaced (at about 85 per cent rest length) by a narrow zone which is denser than the rest of the *A*-band. At a slightly shorter sarcomere length (about 80 per cent rest length), a very dense line becomes visible at either end of the *A*-band. The overall density of the *A*-bands decreases as the fibril diameter increases during shortening. When the *I*-bands disappear at about 65 per cent rest length, contraction bands form at the lines of contact of adjacent *A*-bands. It is interesting to note that contraction down to 65 per cent of the rest length covers the usual range of physiological shortening. With the further shortening which can usually be produced in isolated fibrils, the contraction bands become denser; during this process (or in some cases just before the *I*-bands disappear) the dense zone in the middle of the sarcomere

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splits into two lines which merge with the incoming contraction bands at approximately 30 per cent rest length.

Fibrils prepared from muscle which was allowed to shorten to equilibrium length ($\sim$ 80 per cent rest length) before glycerol extraction usually lack the *H*-zones characteristic of rest-length fibrils, and have correspondingly shorter sarcomere lengths. Suspensions of untreated fibrils also include some specimens with sarcomere lengths down to about 65 per cent of the rest length. Contraction presumably took place while the muscle was still excitable before glycerol extraction. Such fibrils exhibit the same characteristic band patterns and band lengths as those recorded for each sarcomere length during contraction induced by adenosine triphosphate.

During isometric contraction of isolated fibrils in adenosine triphosphate (achieved by holding the ends of the fibrils) the lengths of the *A*- and *I*-bands do not change. However, a narrow dark zone appears in the centre of the *A*-band (as in free contraction) as though some translation of material within the sarcomere were taking place, presumably accompanied by stretch of a series elastic component. This phenomenon is not observed in fibrils that are attached along their whole length to the coverslip or slide.

Stretch

We have found that isolated fibrils can stretch by two different processes, depending on whether the fibril has been 'plasticized'[10], or whether it is being extended while in rigor or while it is exerting a large contractile force. In each case, however, only the *I*-bands change in length; the *A*-bands remain at constant length.

If fibrils are stretched at 2° C., in the absence of magnesium ions and in the presence of rather high concentrations of adenosine triphosphate (about 10^{-2} *M*)—conditions which favour the plasticizing action of adenosine triphosphate rather than its contracting effect[10]—then the *I*-bands increase in length. The length of the *A*-band remains unchanged, but its central region becomes somewhat less dense, as though the *H*-zone were lengthening; the length of this less dense region increases as stretch proceeds. This process is perfectly reversible: stretched fibrils can be allowed to shorten until contraction bands form, and then re-extended.

If fibrils are stretched without any previous treatment, or in the presence of 10^{-4} *M* adenosine triphosphate and 10^{-3} *M* magnesium chloride at room temperature, when contraction is strong, then it is still the *I*-bands alone which increase in length. However, this increase is now not accompanied by any decrease in density of the central part of the *A*-band; on the other hand, the *Z*-lines now become appreciably fainter in spite of the fact that the diameter of the fibril is decreasing. (In the first type of stretch the *Z*-lines remain normal.) This suggests that some stretch is occurring in the region of the *Z*-line. This type of extension is also reversible.

We believe that the second type of stretch extends the series elastic component (Hill[11]), whereas the first type of stretch produces semi-plastic extension as in relaxed muscle.

The appearance of stretched muscle has also been investigated by examining fibrils prepared from fibres which were stretched immediately after removal from the rabbit. Such preparations contain a very high proportion of fibrils with long *I*-bands and the characteristic long zone of low density in the middle of each *A*-band (Fig. 5). We have made several hundred measurements of the lengths of *A*- and *I*-bands on such fibrils and can detect no significant difference in the lengths of *A*-bands from those found in fibrils with the shorter *I*-bands typical of rest length. These observations provide some evidence that the band pattern changes associated with stretch in whole living muscle fibres are similar to those seen in isolated fibrils which have been plasticized. Much more powerful evidence for this is described in the accompanying paper by A. F. Huxley and R. Niedergerke, to whom we are indebted for early reports of their results.

Myosin Extraction after Stretch and after Contraction

We have extracted myosin from fibrils of different sarcomere lengths by methods similar to those described previously[3]. In these earlier studies a dark zone always remained in the centre of the *A*-band of rest-length fibrils after extraction, although the density of the rest of the *A*-band was reduced to that of the *I*-band. Electron microscope studies showed that the dark band still contained the thicker filaments which elsewhere in the *A*-band had been removed. Hasselbach[4] has shown that this dark band disappears during prolonged myosin extraction, and we have found that this process can be accelerated by using 0·1 *M* pyrophosphate, 10^{-3} *M* magnesium chloride, *p*H 7·0. Accordingly, in the present studies we have employed this method for extracting all the myosin.

The appearance of fibrils extracted at different degrees of stretch and contraction is shown in Figs. 6–9 and 11. The 'ghost' fibril consists of a faint backbone structure (which we believe consists largely of actin) the density of which is about the same as that of the original *I*-bands; the *Z*-lines are also still visible. In fibrils at rest length, an apparent gap is observed in place of the original *H*-zone (Fig. 7). In stretched fibrils, where there was originally a longer zone of low density in the centre of the *A*-band, the length of the gap is correspondingly greater (Fig. 6). The ghost fibrils are, however, still structurally continuous; stretched fibrils shorten spontaneously to a little less than rest length during extraction unless stuck to slide or coverslip; they may be reversibly extended again. This can be done with great ease, and it is apparent that only a very weak force opposes such a stretch; the gap elongates in the process, but the length of the material extending from the *Z*-line to the edge of the gap remains constant; that is, no stretching of an elastic component in the *I*-band region now occurs.

The ghosts obtained from contracted fibrils in which the *H*-zones had just disappeared have no gaps in the centres of the sarcomeres (Fig. 8). Fibrils which had shortened until dark lines appeared in the middle of the *A*-bands retain these lines after thorough extraction of myosin (Fig. 9); these dark lines are also present in ghosts from more strongly contracted fibrils (Fig. 11).

None of these 'ghosts' will contract when treated with adenosine triphosphate.

Electron Microscopy

We have made a preliminary examination in the electron microscope of thin sections of stretched,

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contracted and myosin-extracted muscles prepared by the methods described elsewhere[2,12], and we have obtained results which, so far as they go, are in complete agreement with those obtained by light microscopy. This technique does not readily permit of reliable measurements of the lengths of sarcomeres or bands, for, apart from fixation artefacts, an unknown amount of compression is always present in even the best thin sections. In sections of stretched muscle, the majority of the *A*-bands show the characteristic long central region of low density (also observed by Philpott and Szent-Györgyi[13]); we find that the secondary array of thin (40 A.) filaments[2] is absent from this zone. In stretched muscle which has been subjected to myosin extraction the thick (110 A.) primary filaments are absent, and gaps apparently exist between the groups of thin (40 A.) filaments associated with successive *Z*-lines. In sections of glycerol-extracted muscle contracted in adenosine triphosphate, we have observed the same variety of band patterns seen in the light microscope. Up to the point where the *I*-bands disappear, the primary array of thick filaments remains apparently unchanged; and when contraction bands have been formed, the primary filaments between them are still straight. Our fixation of the secondary filaments has not yet been sufficiently good to allow us to describe adequately any changes, apart from translation into the *A*-bands, that may have taken place in them during contraction. During stretch of living muscle, however, the approximately 400-A. axial period seems to remain unchanged; a similar result was obtained by low-angle X-ray diffraction studies[1].

Conclusions

We believe that most of these changes in the cross-striations of muscle during stretch and contraction may be adequately described in terms of the following fairly simple model: The backbone of the muscle fibril is made up of actin filaments which extend from the *Z*-line up to one side of the *H*-zone, where they are attached to an elastic component (*not* the series elastic component) which for convenience we will call the *S*-filaments. The *S*-filaments provide continuity between the set of actin filaments associated with one *Z*-line and that associated with the next. The series elastic component is provided either by the actin filaments themselves or, more probably, by their mode of attachment to the *Z*-line. Myosin filaments extend from one end of the *A*-band, through the *H*-zone, to the other end of the *A*-band, and their length is unaltered by stretch or by contraction down to the point where the sarcomere length is equal to the length of the *A*-band; when contraction beyond this point takes place, the ends of the myosin filaments fold up and contraction bands form. Thus myosin and actin filaments lie side by side in the *A*-band and, in the absence of adenosine triphosphate, cross-linkages will form between them; the *S*-filaments are attached to the myosin filaments in the centre of the *A*-band by some more permanent cross-linkages.

In this model, plastic stretch takes place when the actin filaments are partly withdrawn from the *A*-band, leaving a long lighter central region and stretching the *S*-filaments in the process. Only the *I*-bands and the *H*-zones increase in length, the length of *A*-band remaining constant. This process would be inhibited by cross-linkages between the actin and myosin filaments; there is good evidence[10] that muscles are only readily extensible when such linkages are absent. When they are present, stretch would take place by extension of the series elastic component; in our model, this would again lead to an increase in the length of the *I*-band, but in this case no change in the length of the *H*-zone would take place. Contraction takes place in this model when the actin filaments are drawn into the *A*-band (until the *H*-zone is filled up) and are then folded up in some way to produce more extensive shortening. Thus, when the model is allowed to shorten, only the *I*-bands decrease in length until adjacent *A*-bands are pulled into contact with the *Z*-lines. When contraction is isometric, translation of actin filaments into the *A*-bands is accompanied by stretch of the series elastic component. It will be seen that in both stretch and contraction the behaviour of this model reproduces the observed behaviour of muscle quite faithfully.

A possible driving force for contraction in this model might be the formation of actin–myosin linkages when adenosine triphosphate, having previously displaced actin from myosin, is enzymatically split by the myosin. In this way, the actin filaments might be drawn into the array of myosin filaments in order to present to them as many active groups for actomyosin formation as possible; furthermore, if the structure of actin is such that a greater number of active groups could be opposed to those on the myosin by, for example, a coiling of the actin filaments, then even greater degrees of shortening could be produced by essentially the same mechanism. The model will remain contracted as long as the splitting of adenosine triphosphate continues; it will relax if the splitting stops and more adenosine triphosphate diffuses in, breaking actomyosin linkages and allowing the muscle to be re-extended. However, our results by no means require that contraction and relaxation be brought about in this way; and indeed, in the light of recent studies on actin[14,15], it would not be surprising if other processes are also involved. Furthermore, our results cannot exclude the possibility that repetitive configurational changes take place within the myosin filaments during contraction, unaccompanied by any overall change in the length of those filaments, and that these changes somehow bring about the observed movement of the actin filaments into the *A*-band.

These results will be described in greater detail elsewhere. We are much indebted to Prof. Francis O. Schmitt for his encouragement of this work, and to the Commonwealth Fund and the Rockefeller Foundation for their support.

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