CONTRACTION IN GLYCERINATED MYOFIBRILS OF AN INSECT (ORTHOPTERA, ACRIDIDAE)

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ABSTRACT

The A substance of glycerol-treated myofibrils of the femoral muscles of the locust *Gastrimargus musicus* (Fabr.), removed by a salt solution of high ionic strength, has the properties of actomyosin. A phase contrast study of these fibrils, contracted by the addition of ATP, has revealed that the A bands of most myofibrils shorten during contraction. Changes in density within the A band lead to the formation of C_m and C_z bands while I bands are still present. The A band region between the contraction bands is of much lower density than it is in the uncontracted fibril. During contraction in some fibrils the I bands disappeared and the A bands remained unchanged in length until contraction bands appeared. These results have been interpreted in terms of coiling and stretching of the thick filaments of the sarcomere.

INTRODUCTION

The existence of an ordered double array of filaments in the striated myofibrils of vertebrates and arthropods has been amply demonstrated by studies employing the techniques of electron microscopy and x-ray diffraction (1). Observation of changes in band pattern during contraction (made by phase contrast and interference microscopy) have suggested that the two sets of filaments slide relative to one another, without themselves changing in length. It has also been established, for vertebrate muscle, that the thick filaments are composed mostly of myosin and the thin filaments mostly of actin, a spatial separation of the two major muscle proteins which adds logical weight to the sliding filament model. This model now enjoys wide support and forms the basis of recent attempts to explain contraction in molecular terms (2–5).

Hanson (6) has concluded from a phase contrast study of the myofibrils of the flight muscles of *Calliphora* that contraction in these muscles is also consistent with the sliding filament mechanism, but, since I bands are usually not detectable in the flight muscle sarcomeres at rest length, it is difficult to establish whether their shortening is the result of a sliding process or of coiling of elements within the A band. De Villafranca et al. (7) have shown that the A substance of the myofibrils of the arthropod *Limulus* is a protein complex of the myosin B or actomyosin type, and, moreover, the A bands of these fibrils are reported (8) to shorten during contraction. But De Villafranca's photographs of fibrils before and after contraction show little detail of band pattern, and his preliminary communication has had little impact on the status of the sliding filament theory.

Our interest in the band pattern changes in the myofibrils of locust femoral muscle was stimu-

lated by the finding that the A substance of these muscles is also a complex of the myosin B type. Examination of isolated glycerol-extracted myofibrils under phase contrast illumination has revealed changes in the length and internal structure of the A band which are clearly inconsistent with the sliding filament mechanism. As a result of this study we propose a new model for contraction in arthropod muscle.

MATERIALS AND METHODS

Preparation of Glycerinated Myofibrils

Adults of the locust *Gastrimargus musicus* (Fabr.) were caught in the field at Canberra during the summers of 1961–62 and 1962–63. Their hind legs were removed under CO_2 anesthesia: then the ventral keels of the femora were cut off by scissors, and the legs were dropped into an ice-cold mixture of equal parts of glycerol and 0.01 M Tris buffer at pH 7.0. This procedure removed most of the depressor tibialis muscles, but left the large levator tibialis muscles intact. The femora were left overnight in the refrigerator to allow penetration of the glycerol. The next day, the femoral muscles were dissected out and suspended in fresh glycerol mixture, ready for storage in a deep-freeze cabinet. In some cases precautions were taken to glycerinate the muscles at rest length. This was done by holding the tibiae appressed to the undersides of the femora by means of small rubber bands during the initial overnight treatment in glycerol. It was found, however, that this procedure had no noticeable effect on the distribution of band patterns in the fibrils as ultimately prepared, and was later abandoned. Presumably the majority of the fibrils relaxed spontaneously during glycerol extraction.

The muscles of a single femur were used for each preparation. After storage in glycerol for a period of from 2 to 8 weeks, they were washed in a mixture of 0.1 M KCl, 0.01 M Tris, pH 7.0, and then homogenised by hand with 4 ml of the same solution in a glass-teflon homogeniser. The myofibrils were collected by centrifugation and then resuspended by light homogenisation in another 4 ml of solution. This procedure was repeated, and the washed myofibrils finally suspended in 2 ml of the buffered KCl solution.

Optical Equipment

A Zeiss Opton microscope was used, with phase contrast objectives 40/0.63 or 100/1.25. Some early photographic records were made with a Praktica 35 mm reflex camera body mounted directly on the microscope tube without supplementary lens, but the majority of the photographs were taken with a Leitz camera attachment and Leica camera body using a 10 × Leitz eyepiece. Kodak Micro-File film was used.

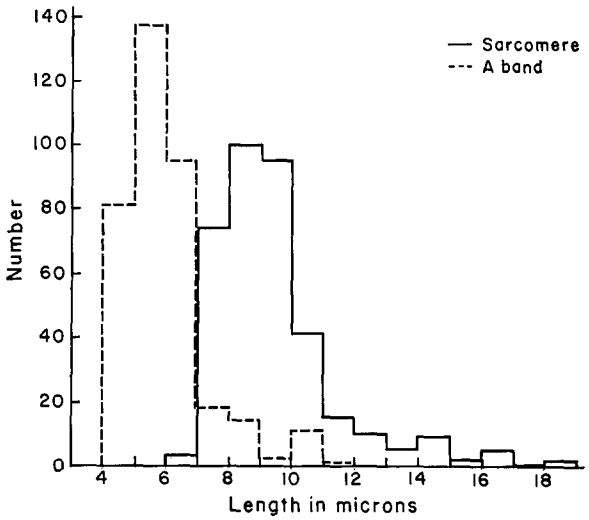


FIGURE 1. Distribution curves for lengths of sarcomeres and A band in uncontracted fibrils

RESULTS

Appearance of the Myofibrils

Distribution curves of the lengths of sarcomeres and A bands of the isolated relaxed myofibrils are illustrated in Fig. 1. Variation in size was quite extensive, but in general the ratio of A band length to sarcomere length was reasonably constant. This is illustrated by the sharp peak of the distribution curve for this ratio, which is shown in Fig. 2, and suggests that most of the myofibrils

examined were in an equally relaxed state. Fibril diameters varied from 1.5 to 4.0 μ. Most preparations contained a number of strongly contracted fibrils, and a few were seen at an intermediate stage of contraction (Fig. 11 *a*). In relaxed fibrils (Figs. 4 *a*, 5 *a*, 8 *a*) A and I bands and Z lines were clearly distinguishable, and an H zone was present in the mid region of the A bands. M lines were not visible.

Removal of the A Substance

We have confirmed Hanson's (6) finding that the A substance of insect muscle is more resistant to solution than is that of vertebrate muscle, and found, in fact, that ionic strengths even higher than those she recommends were necessary for *Gastrimargus* femoral muscle. A solution containing 1.0 M KCl, 0.01 M sodium pyrophosphate, 0.001 M $MgCl_2$, and 0.01 M Tris, pH 7.0, was found to be routinely successful. The appearance of a myofibril before and after washing with this solution is seen in Fig. 3. Fig. 3 *c* shows the same myofibril after further treatment with 0.6 M KI. The KCl/pyrophosphate solution removes the A substance (presumably the thick filaments) more or less completely, while the KI solution removes the lighter background material (thin filaments) between the Z lines. These photographs cannot establish that *only* thick filament material is removed by the KCl/pyrophosphate, and if some thin filament material is dissolved, then the resultant solution would contain actomyosin. On the other hand, the photographs show changes similar to those obtained with rabbit muscle treated to remove the thick and thin filaments, and the proportion of the total protein removed by two washings was rather less (45 to 50 per cent, as compared with 60 per cent) than that ascribed to the A band of rabbit muscle (9). Of the protein removed by the KCl/pyrophosphate solution from the insect myofibrils, about 90 per cent precipitated at an ionic strength of 0.04, and had the properties of an actomyosin with an ATP sensitivity (10) of about 100. This indicates a high actin content, and suggests that the thick filament material is, in fact, actomyosin.

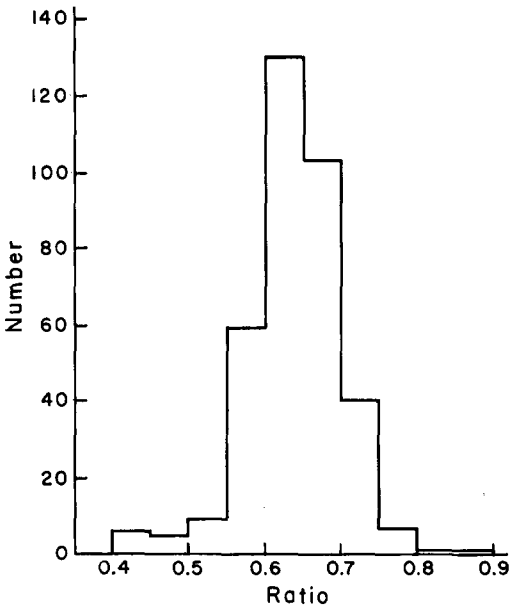


FIGURE 2. Distribution curve for the ratio of A band length to sarcomere length in uncontracted myofibrils.

Contraction Induced by ATP

Myofibrils prepared as described responded to ATP for a period of 2 to 3 hours after preparation. Contraction was elicited by ATP alone (Sigma disodium ATP dissolved in 0.1 M KCl, 0.01 M Tris, pH 7.0); addition of either Mg^{++} or Ca^{++} appeared to have no effect. The threshold ATP concentration varied among fibrils and among preparations. A concentration of 1×10^{-5}M never elicited a response, whereas 1×10^{-4}M caused contraction in some fibrils but not in others. Most studies were made at a concentration of either 5×10^{-4}M or 1×10^{-3}M. Relaxation did not occur after the ATP was washed out, but, in some fibrils which contracted isometrically in the presence of higher ATP concentrations (above 1×10^{-3}M), what appeared to be a rapid contraction-relaxation cycle was observed.

Changes in Band Pattern during Contraction

ISOMETRIC CONTRACTION

Contraction of the isometric type was observed most frequently and was most easily photographed in the myofibril preparations. This was because the glycerinated fibrils had a strong tendency to stick to either slide or coverslip. On the addition of ATP they developed tension, but did not shorten appreciably. An example of this sort of contraction is seen in Fig. 4. Measurements of sarcomere and A band lengths from photographs of this fibril (Table I, fibril I/17) establish that, although there

is very little change in sarcomere length, the A bands have shortened by about 10 per cent, while the I bands have lengthened by a corresponding amount. Measurements of other fibrils in which contraction approached the isometric condition (shortening of no more than 2 per cent) indicate that shortening of the A band is a constant feature, whereas the I band may lengthen, remain unchanged, or also shorten slightly (Table I). Perhaps more significant than these measurements, which are of limited accuracy in phase contrast micrography, are the changes in density which occur within the A band. The H zone disappears and is replaced by a darkened region, the C_m band, which produces a bulge in the outline of the fibril. Darkening also occurs, with varying intensity, at the outer edges of the A zone. Between these regions the A band decreases in density.

Shortening

Two distinct types of band pattern changes were seen in fibrils which were free to shorten under the influence of ATP. In the first of these, both A and I bands shortened, the I band sometimes being reduced to very small dimensions, but remaining distinct in even the most strongly contracted fibrils. This contraction pattern was encountered in the great majority of the fibrils. Measurements of the lengths of A and I bands before and after contraction in a series of fibrils are collected in Table I. Reduction in length of the A band is a constant feature in this series of measurements, which range from what we have called isometric contraction down to a fibril which shortened to 55 per cent of rest length. I bands were also reduced in all fibrils which shortened appreciably, often by greater amounts than were the A bands. Changes in density within the A band were similar to those already described for isometric contraction. A heavy C_m band usually developed in the mid region, along with thickenings of varying intensity at the outer edges of the A band (C_z bands). Between the C_z and C_m regions the A band decreased in density to varying degrees, becoming in some instances almost as light as the I bands.

Photographs of a number of fibrils from which the measurements in Table I are derived are collected in Figs. 5, 7, and 8. In Fig. 5 (fibril I/27) the sarcomeres photographed have undergone varying degrees of shortening. Those on the left are practically isometric, whereas farther to the right appreciable shortening has taken place. Sarcomere 4, for instance, has shortened by 13 per cent, while its A band has shortened by 9 per cent. I bands remain clearly visible. The fibrils in Fig. 7 (fibril Q/6) are those in which the greatest degree of shortening was recorded. These photographs are less clear since the fibrils were not lying entirely within the plane of the objective, but it is possible to distinguish I bands in the contracted sarcomeres, although the contracted sarcomere length is less than the relaxed A band length. Fibril I/8 (Fig. 8) also shows greatly reduced, but distinguishable, I bands in the contracted sarcomeres. When the I bands were very much reduced, it was sometimes difficult to distinguish the structures in the Z region. The oil objective was usually needed to resolve into separate Z and C_z bands the structure which

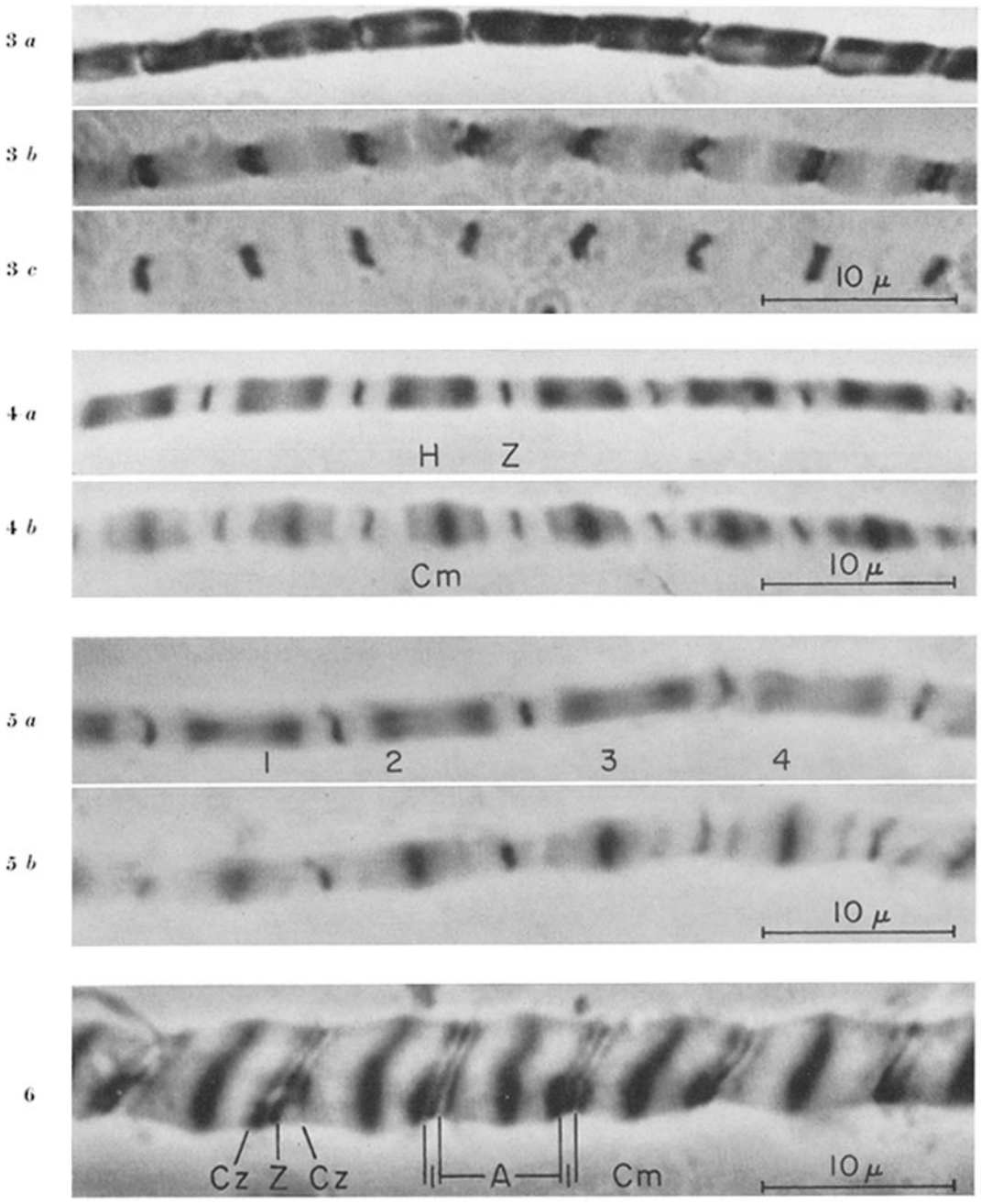
Figs. 3 to 12 are glycerol-treated myofibrils from the femoral muscles of *Gastrimargus musicus*. All figures except Fig. 10 were made with a Leica camera body and Leitz camera attachment. Fig. 10 was made with a Praktica camera body mounted directly on the microscope. The Zeiss objective 100/1.25 was used for all photographs except Fig. 7 *a* which was made with the 40/0.63 objective.

FIGURE 3 *a*. Uncontracted fibril.
b. Same fibril after treatment for 10 minutes with a solution containing 1.0 M KCl, 0.01 M sodium pyrophosphate, 0.001 M $MgCl_2$ and 0.01 M Tris, pH 7.0.
c. Same fibril after further 10-minute treatment with 0.6 M KI.

FIGURE 4 Fibril I/17 before (*a*) and after (*b*) treatment with ATP. "Isometric" contraction.

FIGURE 5 Fibril I/27 before (*a*) and after (*b*) treatment with ATP.

FIGURE 6 Fibril P/6 after treatment with ATP.


3 a
3 b
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under the 40/0.63 (air) objective appeared as a single dark C_z region. The triple-banded structure in the Z region, so revealed, was, in fact, the most characteristic feature of strongly contracted fibrils which had undergone contraction of this type. It was seen not only in experimentally contracted fibrils, but also in some untreated fibrils which presumably had retained the natural contraction pattern. The fine structure of the Z region in a contracted fibril is well illustrated in Fig. 6. Although most contracting fibrils developed strong C_m bands with lighter C_z bands, in some the reverse occurred, and occasionally C_z bands

It proved difficult to obtain photographs of this second type of contraction, since it happened less frequently than the first type described earlier, and also because the sequence of events was usually more rapid. In Fig. 10 are seen two rather blurred images of a fibril contracting in this way. The I bands originally present have all disappeared in the contracted fibril, with the exception of one half I band, while heavy C_z and lighter C_m bands have appeared. Fig. 11 also illustrates part of this kind of contraction sequence. These photographs are of a fibril which was already partly contracted before the application of ATP.

TABLE I

Measurements of Sarcomere and A Band Lengths Before and After Contraction

Fibril	No. of sarcomeres measured	Mean sarcomere length uncontracted (S_r)	Mean A band length uncontracted (A_r)	$S_r - A_r$ (I_r)*	Mean sarcomere length contracted (S_c)	Mean A band length contracted (A_c)	$S_c - A_c$ (I_c)*	$\frac{S_c}{S_r} \times 100$
		μ	μ	μ	μ	μ	μ	
I/17	6	7.8	4.5	3.3	7.6	4.1	3.5	98
D/1	5	11.7	5.3	6.4	11.4	5.1	6.3	98
G/3	2	10.6	6.6	4.0	10.4	6.4	4.0	98
I/23	4	7.6	5.1	2.5	7.4	5.0	2.4	97
I/27	4	10.1	6.5	3.6	9.6	6.1	3.5	96
I/3	5	9.5	6.3	3.2	8.8	6.0	2.8	93
I/1	2	7.7	4.5	3.2	6.9	4.0	2.9	90
Q/7	10	9.0	6.6	2.4	7.9	6.1	1.8	88
I/25	4	7.2	4.3	2.9	6.0	4.1	1.9	85
Q/10	4	10.9	7.4	3.5	8.3	6.8	1.5	76
I/8	2	16.7	11.0	5.7	11.5	9.7	1.8	69
Q/6	4	10.5	7.5	3.0	5.7	4.9	0.8	55

* I_r and I_c, which are taken as measures of I band length, actually include two half I bands and two half Z discs.

only were formed. A contracted fibril of this kind is shown in Fig. 9. This photograph is also notable for its clear demonstration of I bands in a strongly contracted fibril.

In the second type of contraction pattern, which, as stated above, was observed in only a few fibrils, the sequence of events was very similar to that described by Hanson and Huxley (11) for rabbit fibrils. The A bands of these fibrils apparently remained unchanged in length until the Z lines impinged on their outer edges. At this stage the I bands had disappeared and the H zones had darkened. With further shortening, dark C_z bands developed in the Z region, and were usually accompanied by narrower C_m bands.

Shortening in this case is accompanied by an increase in the width and density of the C_z bands, by the appearance and disappearance of small C_m bands, and by a decrease in the density of the A band between these regions. Finally, Fig. 12 shows a group of strongly contracted fibrils which have shortened in this way. These fibrils are distinguished by the complete absence of I bands, the presence of very dense, broad C_z bands within which no fine structure is visible, and the occasional presence of light C_m bands. The A band region between the C_z bands is of low density.

DISCUSSION

We are not the first to observe that contraction in insect muscle is accompanied by a shortening of

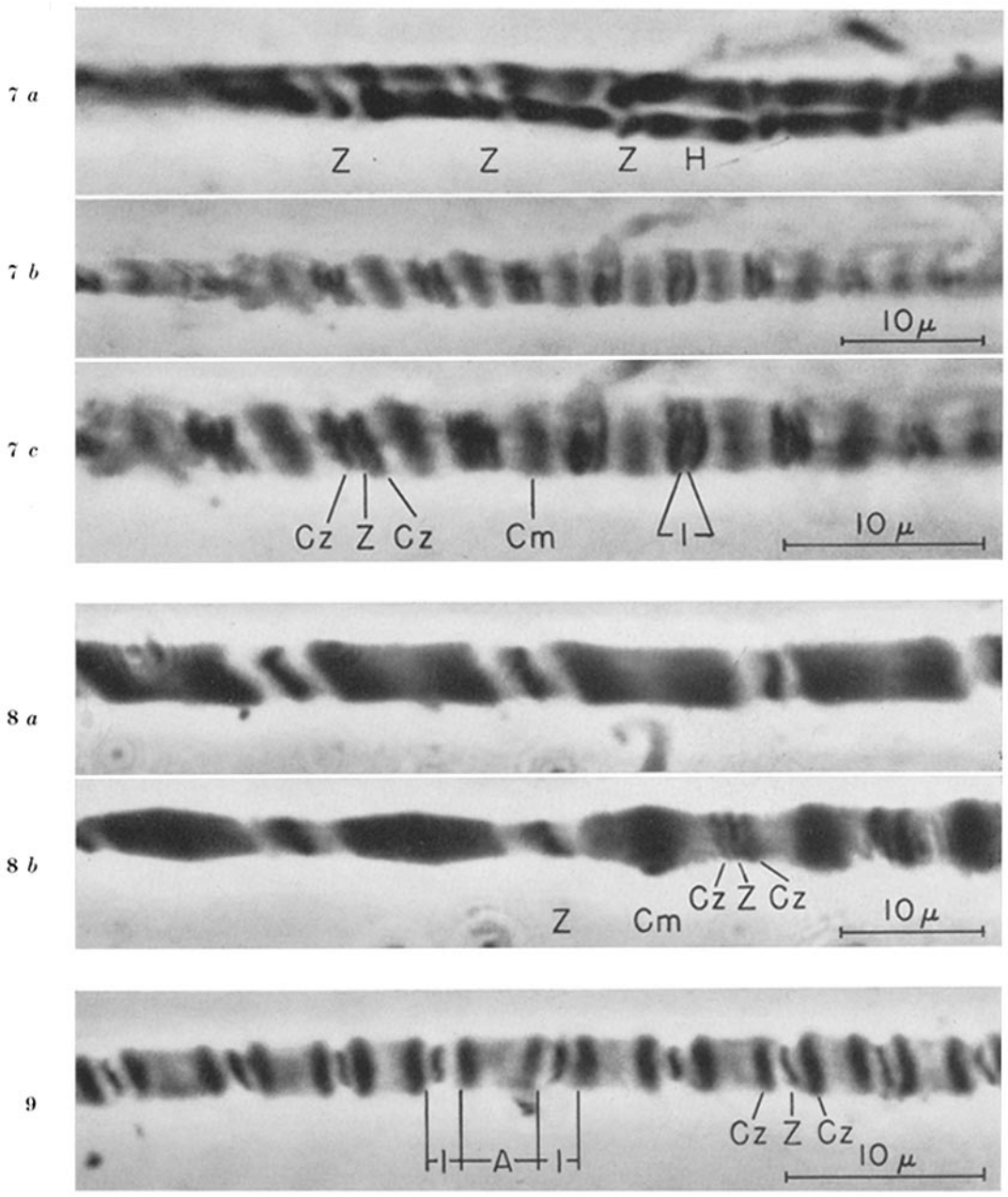

FIGURE 7 Fibril Q/6 before (*a*) and after (*b*) treatment with ATP; 7 *c* is a further enlargement of the same negative used for 7 *b*.

FIGURE 8 Fibril I/8 before (*a*) and after (*b*) treatment with ATP.

FIGURE 9 Fibril M/4 after treatment with ATP.

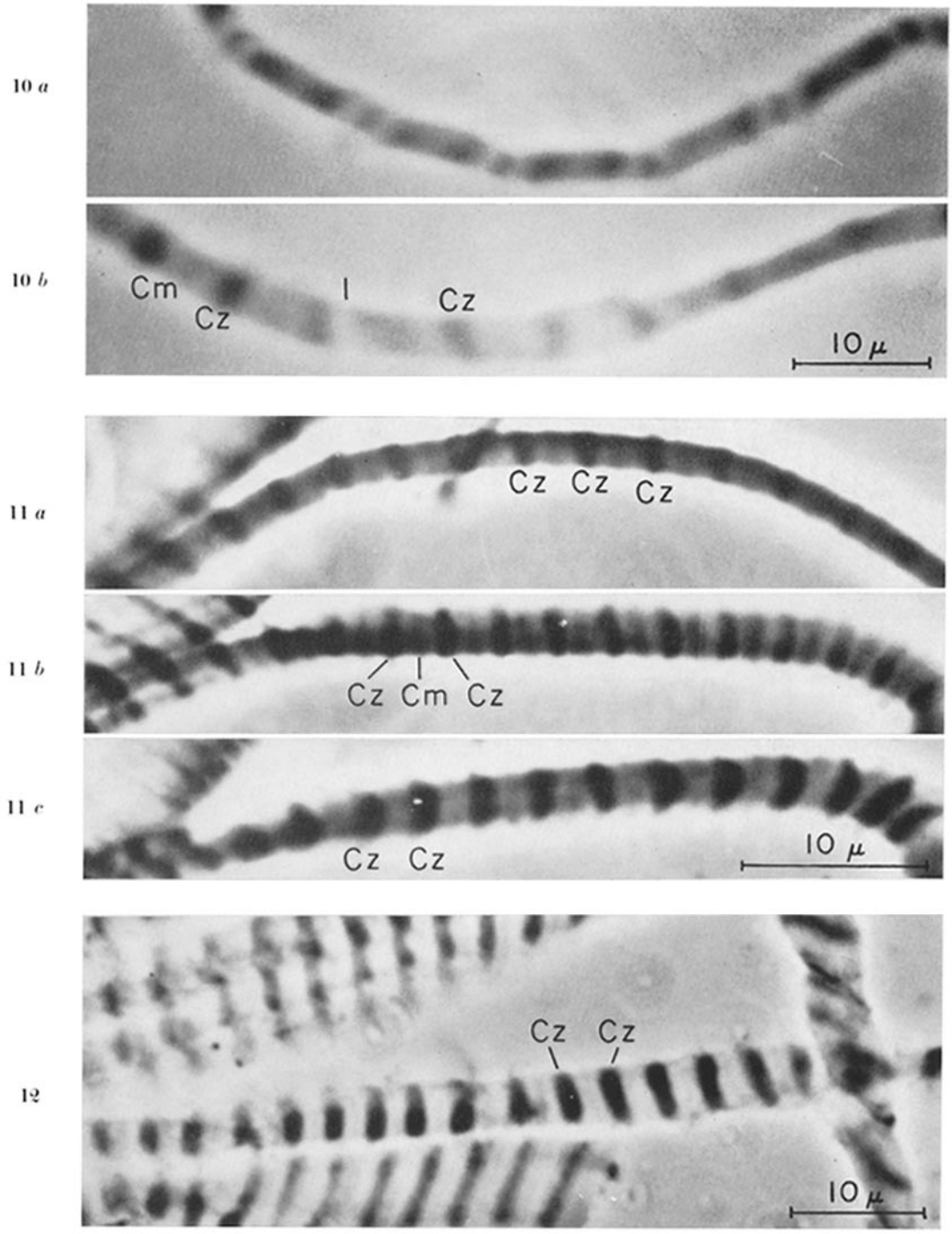

FIGURE 10 Fibril B/1 before (*a*) and after (*b*) treatment with ATP. All but one half I band disappeared.

FIGURE 11 Fibril K/4 before (*a*) and at successive stages of contraction after (*b* and *c*) treatment with ATP. Fig. 11 *a* has been printed more lightly than Figs. 11 *b* and *c* for the sake of showing detail, so the decrease in density between the contraction bands is underemphasised.

FIGURE 12 Contracted fibrils after treatment with ATP.

the A bands. The histologists of fifty and more years ago reported the same change, with a weight of evidence which is difficult to assess nowadays but is not to be ignored. Hürthle's (12) measurements on living and fixed muscle of *Hydrophilus* led to the conclusion that the A band shortened, while the I band lengthened, during contraction, whereas Engelmann's (13) observations on several insects agree with ours in demonstrating shortening of both A and I bands. These results reinforce our belief that the glycerinated fibrils we have studied can be considered to be a valid model of living muscle. The fibrils studied by Hürthle had quite short I bands at rest length, and it is possible that what he called A bands in contracted fibrils were, in fact, C_m bands, the C_z bands being unresolved in the general Z region. This would account for the gross changes in length of the A and I bands that he reported. In defending the sliding filament theory, A. F. Huxley (14) has discounted the findings of the older histologists, and it is true that these studies became the basis for erroneous concepts about muscle *in general*, but the error may have been not in the observations themselves, but in the argument from them, in particular, to a general theory to cover all types of muscle. We submit, however, that a general theory based on the events in vertebrate muscle may be equally erroneous.

The evidence we present is, we believe, sufficient to establish that in the glycerinated fibrils of *Gastrimargus* both A and I bands may shorten during contraction. Moreover, the changes in density within the A band, considered in relation to the known double filament structure of the sarcomere as revealed by the electron microscope (15), suggests that contraction is produced by the coiling or folding of the thick filaments in certain preferred areas. We are faced, however, with the dilemma of explaining two apparently different contraction sequences within the one set of muscles. Whereas in most fibrils both A and I bands shortened, with accompanying changes in density in the A band which were consistent with coiling of the constituent filaments, some fibrils followed a sequence in which I bands shortened and disappeared, in a manner which suggested relative sliding of interdigitating filaments. We have rejected the idea that two fundamentally different types of contraction mechanism exist in the same muscles, and have sought an explanation based on a concept of contractile thick filaments.

The theory we propose is based on the following assumptions:—

1. In the relaxed state the thick and thin filaments are free to slide relative to one another.
2. The activation process involves the formation of bonds between neighbouring sets of thick and thin filaments, followed by coiling of the thick filaments either in their mid regions, or at their ends, or at both sites. This results in either the movement of the Z discs toward one another or the development of tension by the stretching of other regions of the thick filaments.
3. Activation occurs progressively from the outside to the inside of the fibril. In our experiments this is a consequence of the inward diffusion of ATP. In living muscle other processes are presumably involved. Thus some sets of thick and thin filaments may start to contract while others nearer the interior of the fibril are still at rest length and free to slide relative to one another.

Under conditions of zero load, such as in isolated fibrils lying freely in solution, the activation and contraction of only a very small proportion of the full set of thick and thin filaments might be needed to start the movement of the Z discs towards one another. Meanwhile the majority of the thick filaments would remain unaffected, and the appearance of the A band under phase contrast would be unaltered. If shortening were rapid in relation to the movement inward of the activating process, the Z discs might impinge on the ends of uncontracted thick filaments before activation had spread through the whole fibril. Observed in the light microscope, the I bands of such a fibril would first disappear, after which further shortening would be accompanied by the appearance of C_z and C_m bands.

Under conditions of positive load, a larger proportion of filament sets would need to be activated before the Z discs would start to move towards one another, and under these circumstances there would be a much stronger possibility of the whole fibril being activated before the Z discs impinged on the ends of uncontracted thick filaments. Thus contraction bands would develop while I bands were still visible. Such conditions of positive load clearly apply to many of the isolated fibrils observed on a microscope slide. They stick to the slide at one or more points; developing tension is made manifest by the straightening of

the fibril between points of attachment; often attachments are seen to give way and further shortening ensues. Isometric contraction would represent an extreme case of this condition, where sarcomere shortening would be negligible, while contraction band patterns would develop within the A band.

Obviously the proposed mechanism depends on the conduction time of the activation process across the fibril being appreciable in relation to the shortening time. Calculation of the probable conduction time on present knowledge must rely heavily on assumptions. In our experiments, for instance, the mode of application of the ATP solution (drawing it between slide and coverslip with the aid of filter paper so that it flowed through and around a heavy suspension of fibrils, of which only one was being observed) introduces uncertainty as to what concentration of ATP actually bathes the fibril at the start of contraction. Moreover, the threshold for activity differs among fibrils and possibly also among sets of filaments within the fibril. Finally, no figure exists for the rate of diffusion of ATP into insect myofibrils. However, if one extrapolates from Edman's (16) data on the rate of diffusion of ATP into glycerinated rabbit *fibres*, and assumes that the initial concentration on the outside of the fibrils we examined was double the threshold concentration, then one arrives at a time of 0.1 to 0.2 seconds for the interval between activation of the outside and the activation of the inside of a fibril 3 μ in diameter. This would be sufficient to allow for the differential effects we propose, and the time may well be an understimate, since Edman's data are for diffusion into fibres, and includes diffusion between as well as into fibrils. Since we do not know what the conduction process is in living muscle, we can make no prediction about the conduction time for activation across a fibril in such conditions, although it is clear that all the events of contraction are much faster in living muscle than those observed in glycerinated models.

The elements of the theory we propose are presented diagrammatically in Fig. 13. On the left side (*A*) are shown the supposed series of changes in the thick and thin filaments of a fibril shortening under zero load. Movement of the Z discs is shown as being effected by the successive activation of sets of thick and thin filaments. We have shown the sets which were activated first as no longer participating in the contraction process when a later set becomes activated (indicated by a wave in the thin filament between the end of the thick filament and the Z disc), but this is obviously not necessary to the theory. These sets of filaments could continue to play an active role in shortening by an extension of the coiling process. Nor do we exclude the possibility that bonds between thick and thin filaments, once formed, may later relax, especially in filament sets which are no longer exerting tension because of the more rapid movement of other sets. *A* (*3*) of Fig. 13 may be considered to represent the condition seen in Fig. 10 *b*, whereas Fig. 11 *b* might correspond with *A* (*4*) and Fig. 11 *c* with *A* (*5*). On the right side (*B*) of Fig. 13 are seen successive stages of a sarcomere shortening under positive load. Tension within the sarcomere is suggested by the reduction in diameter of the thick filaments between the zones of coiling. In *B* (*4*) and *B* (*5*) the zones of coiling are all shown in register for the sake of convenience, although they need not be strictly aligned. Clearly the fibrils in Figs. 5 *b*, 6, 7 *c* and 8 *b* correspond in varying degrees with the condition shown in B (*4*), while Fig. 9 shows a fibril in the state of *B* (*5*).

The explanation of the observed sequence of events in contraction that we offer is based on a concept of coiling of the thick filaments. This concept is supported biochemically by the observation that the thick filaments apparently consist largely of actomyosin, which could be expected to "contract" under the influence of ATP. But we have extended the concept to a number of fibrils in which the observed sequence of events was superficially similar to that seen by Huxley and Hanson in rabbit muscle, and explained by them on the basis of sliding filaments. It is instructive to enquire whether any evidence can be adduced from insect material which would allow discrimination between these two conflicting models. Three observations favour the explanation we offer over that of Huxley and Hanson. They are: (*a*) The wide C_z bands shown in Fig. 10 *b* have developed at a sarcomere length which is roughly equal to the length of the A band of the relaxed fibril. This suggests that the C_z band may have been the result of coiling of the thick filaments, rather than crumpling against the advancing Z discs. On a sliding filament model, such heavy C_z bands would have been expected at much shorter sarcomere lengths. (*b*) In Fig. 11 it can be seen that in some sarcomeres the C_m

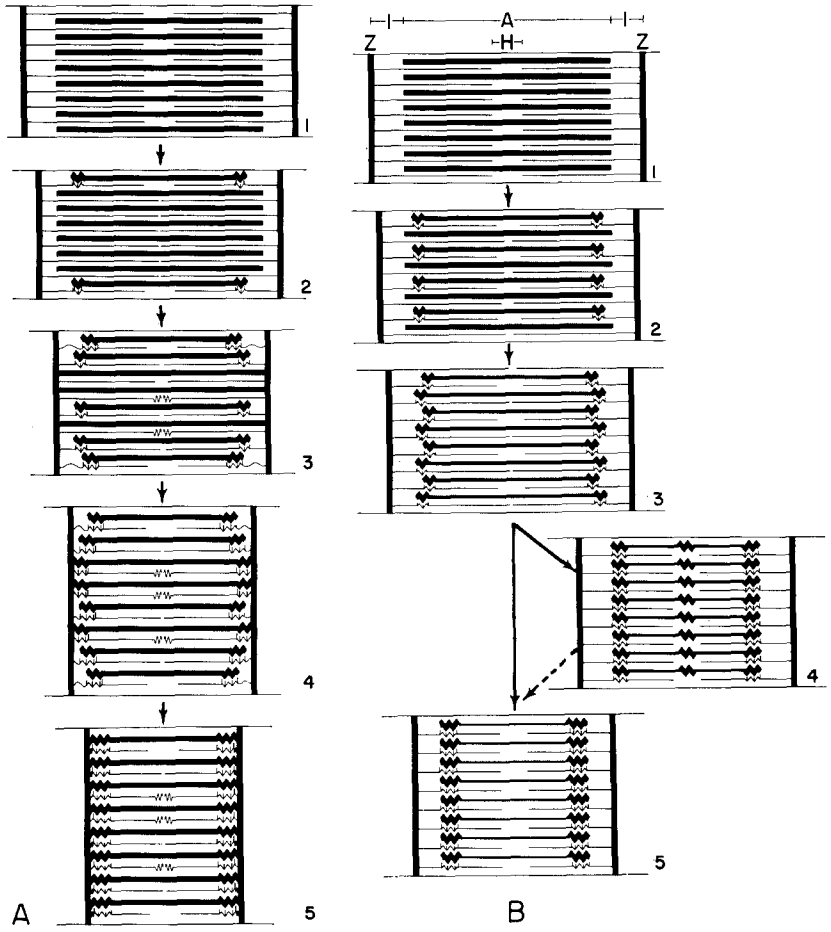

FIGURE 13 Diagrams of the proposed sequence of changes in the filaments of a sarcomere contracting under zero load (*A*) or under positive load (*B*).

bands formed at an intermediate stage of contraction have later disappeared. Huxley and Hanson have explained the C_m band (as distinct from the M line, which appears in the centre of the H zone of some uncontracted fibrils) as being the result of the folding up of the ends of the thin filaments when they meet in the centre of the sarcomere. It is difficult to see, on this model, how C_m bands, once formed, could later disappear. On the other hand, if C_m bands are due to coiling of the thick filaments, as we suggest, then it is conceivable that such coiling could later be pulled out by the stronger contraction in the C_z region. (*c*) A striking feature of strongly contracted fibrils is the very low density of the A band region between the C_z bands. Lightening of the A band has also been observed by Hodge (17) and Hanson (6). Hodge explained it in terms of migration of the A substance, but Hanson pointed out that some reduction in density is to be expected even without movement of the A substance, because of the increase in diameter of the contracting fibril. This controversy can only be resolved by precise measurements of changes in protein density and fibril diameter, but our subjective impression is that the decrease in density, seen under phase contrast, is much greater than that to be expected from the increase in fibril diameter (cf. Fig. 12). We therefore favour Hodge's explanation, although we state it in terms of coiling and stretching of the A band filaments.

Contraction of the thick filaments occurs preferentially either at the ends or in the middle. This may suggest some longitudinal differentiation

of the thick filaments, but an alternative explanation may be that elements of the sarcoplasmic reticulum, which are known to persist in glycerinated fibrils, may aid or be necessary for the inward movement of the activation process at these points.

The model of contraction discussed above is advanced solely as a rational explanation, on the basis of present knowledge, of the events we have observed in glycerinated insect fibrils. We are not in a position to express an opinion about the possibility of extending it to other groups. It may be that striated muscle has evolved separately in arthropods and vertebrates, and that, in spite of the similarity in appearance, their contraction mechanisms are different. On the other hand, contraction patterns apparently similar to the ones we have described have been encountered in living frog muscle (18), and, although they have been regarded as atypical, for a variety of reasons, they encourage the belief that a unifying theory valid for both groups may be within reach. Further work with the light microscope on living muscle and with the electron microscope on fixed specimens will be needed to resolve these differences.

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