

OBSERVATIONS BY ELECTRON MICROSCOPY ON CONTRACTION
OF SKELETAL MYOFIBRILS INDUCED WITH
ADENOSINETRIPHOSPHATE*

BY CHARLES A. ASHLEY,† M.D., KEITH R. PORTER, PH.D.,
DELBERT E. PHILPOTT, AND GEORGE M. HASS, M.D.

*(From the Rush Department of Pathology, Presbyterian Hospital, Chicago; The Electron
Microscope Laboratory, University of Illinois College of Medicine, Chicago; and the
Laboratories of The Rockefeller Institute for Medical Research, New York)*

PLATES 6 TO 11

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The literature contains several reports on the fine structure of isolated myofibrils (1-5). From these it is clear that the extended fibril is composed, in part, of longitudinally arranged filaments which in the dry state are about 200 Å. thick and which show nodosities and densities arranged at regular intervals of about 400 Å. In the intact fibril these densities are likely to be in perfect phase or register and thus give to the fibril a fine cross-banding quite distinct from the relatively macroscopic striations. There is some evidence (3, 4) that these filaments are arranged along the periphery of the fibril, thus making of it a tubular structure. From electron microscopy the major striations, the A and Z bands, seem to result from concentrations of a non-fibrous, amorphous material. The association, if any, between this and the filaments has not been clarified. In contraction, the major part of the amorphous material within the A band moves into the I and Z band regions. That more attention has not been given the phenomenon is doubtless due to difficulty in controlling contraction of small muscle units and obtaining intermediate stages in the process.

Schick and Hass (6) have described the isolation of fibrils from frozen sections of muscle with the aid of tryptic digestion at 0° C. When the isolated fibrils were treated with adenosinetriphosphate, the fibrils progressively shortened to approximately 25 per cent of their original lengths. During this shortening, the cross-striations became indistinct and the fibrils broadened. After a time no further contraction was observed. The action was not reversible.

More recent studies (7) have indicated that the contraction could be slowed

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† Fellow, Graduate School, University of Illinois, Chicago.

by increasing the H ion concentration, lowering the temperature, or decreasing the concentration of ATP. The use of a buffer solution of potassium phosphate and citric acid in 80 per cent water and 20 per cent glycerin as a suspending medium allowed the addition of ATP at -5°C . At this temperature, contraction did not occur for periods up to 30 minutes. However, when the temperature was raised, contraction began and could be halted by the addition of formalin. Thus, almost any degree of contraction could be obtained.

It has been the interest of this study to observe with the electron microscope the effects of trypsin on the myofibril and the changes in fine structure during contraction induced by the addition of ATP. Incidental observations on the effects of hydrogen ion concentration and the ionic strength of the medium have been included.

Methods

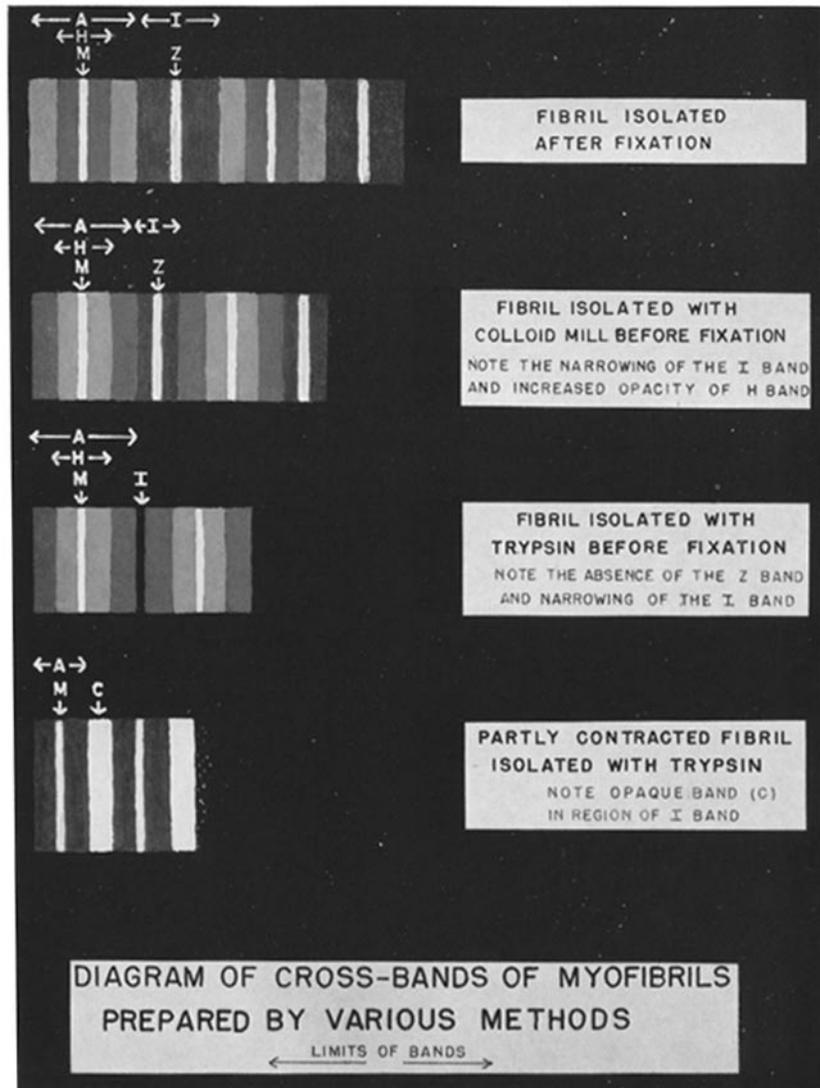
The anterior thigh muscles from young rabbits killed by air embolism were used. A 5.0 gm. block of muscle was frozen and then cut into sections with a freezing microtome in a cold room at -15°C . The sections were then washed with potassium phosphate, citric acid buffer solution (pH 7.0, ionic strength (μ) 0.25) in a second room at 0°C . To 100 ml. of this suspension, 0.005 gm. of crystalline trypsin was added. After 20 to 40 minutes, the fibers were broken up into individual fibrils by agitation in a Waring blender for 3 seconds. Myofibrils were also prepared in an Eppenbach colloid mill (type QV-7-1), using the same buffer solutions. This machine eliminated the necessity of cutting frozen sections, and trypsin was not required for adequate separation. After both procedures the fibrils were purified by repeated washing and centrifugation in a potassium phosphate, citric acid buffer solution (pH 7.0, μ 0.154).

The control or uncontracted fibrils, prepared both by tryptic digestion and with the colloid mill, were suspended in aqueous and in glycerinated (80 per cent water and 20 per cent glycerine) buffer solutions (pH 7.0, μ 0.154) to which ATP was not added. They were then fixed with 10 per cent formalin buffer solution (pH 7.0, μ 0.154) directly on collodion-coated screens.

Contracted myofibrils were prepared by two methods. The temperature of those suspended in glycerinated buffer solution was lowered to -5°C . and the sodium salt of adenosinetriphosphoric acid added to give a final concentration of 0.0025 M. The total contraction time, determined by direct observation with the light microscope, for 0.5 ml. of this suspension was approximately 1 minute when placed directly on a collodion-covered 160 mesh wire screen at 8°C . Myofibrils in various stages of contraction were obtained by formalin fixation of a series of preparations at intervals of 15 seconds. Screens were also prepared using fibrils suspended in aqueous buffer solution. 0.1 ml. of this suspension at 8°C . was placed on each collodion-coated screen. An equal amount of 0.005 M adenosinetriphosphate was added after which the fibrils were fixed with formalin at intervals of 15 seconds.

The effect of changes in ionic strength and hydrogen ion concentration on the structure of the uncontracted fibril was studied by soaking the isolated fibrils in appropriate buffer solutions for 12 hours followed by fixation with formalin buffer solutions of the same pH and ionic strength.

After fixation the screens were washed, dried, and shadowed with 0.003 gm. of chromium at angles of either 10° or 12° from a distance of 15 cm. An RCA electron microscope, type EMU, was used. Most micrographs were taken at magnifications between $\times 4,000$ and $\times 6,000$ and enlarged photographically.



TEXT-FIG. 1

OBSERVATIONS

The Uncontracted Myofibril

Myofibrils separated by treatment in the colloid mill in neutral buffer solutions (pH 7.0, μ 0.154) *without use of trypsin* show structures now familiar from electron micrographs of similar material. Major cross-striations can be identified (Fig. 1 and Text-fig. 1). The fibril shown in Fig. 1 has an unusually narrow I

band.¹ The A band is composed of longitudinally oriented fibrous components about 400 Å. in diameter with some evidence of nodosities, but the spacing of these is not clearly regular. However, fibrils suspended and fixed in slightly acid buffer solutions (pH 6.0, μ 0.154) are narrower, have longer I bands, and more evident nodosities along the filaments.

The I band in the dry fibril appears as a marked depression bisected by a ridge which is the Z band. In drying, the I band has flattened more than the A band, the difference in the middle of this fibril being of the order of 300 Å. Other investigators (1, 3) have indicated that the filaments of the A band traverse the A-I junction and are continuous with those of the I band. This relationship is not apparent in our preparations.

The preparations of *trypsin-treated* muscle show many clean myofibrils of various lengths and widths (Figs. 2, 3, Text-fig. 1). No pronounced differences are noted between those fibrils in glycerinated buffer solutions and those in aqueous buffer solutions. Measurements fail to reveal any characteristic width, and the larger fibrils are not multiples of the smaller.

The most striking feature of these preparations when compared with those not treated with trypsin is the relative absence of the Z band substance and the greater part of the I band (Figs. 2, 3, Text-fig. 1). It seems that the digestion more or less destroys this region of the fibril and leads in some cases to a complete separation of the sarcomeres (Fig. 4). Prolonged tryptic digestion of fibrils isolated with the colloid mill results in a thorough removal of the Z and I bands with no sign of residue in these regions.

The sarcomere length in trypsin-treated fibrils varies from 1.37 to 1.65 μ . This is shorter than in the intact fibril and apparently results from destruction of the I band. It follows that the range of A band widths (1.34 μ to 1.6 μ) essentially coincides with total sarcomere length. This range is similar to that found for the A band by other investigators.

The M band, which appears as a slight ridge precisely bisecting the A band, is 640 Å. wide. It is bounded on either side by a narrow depression which coincides in position with the H band. Within the A band and adjacent to this depression there is generally a band of greater opacity which varies in width from 2,600 Å. to 7,700 Å. (Figs. 3, 4, and Text-fig. 1). It is more than simply a region of greater density. It has as well a greater bulk in the dry state and seems to represent a piling-up of the A substance. The location of this band in fibrils prepared with the colloid mill and in those treated with trypsin coincides with a band-like depression, the H band, described by other workers, using fibrils which were first fixed with formalin and then separated mechanically.

The thickness of the dry myofibrils varies considerably, depending somewhat on how the fibril has dried down. In Fig. 3 the fibril is fairly uniform in thick-

¹ The various bands are transverse to the long axis of the fibril. The portion of the sarcomere occupied by each band is measured in the long axis of the fibril and is termed the width of the band.

ness across its entire width and the lateral margin measures 120 Å. On the other hand, in myofibrils where the center of the fibril is obviously thicker (Fig. 2) the margins are usually extremely thin, not more than the thickness of a single filament, *i.e.*, about 30 or 40 Å. Along the middle of such sarcomeres, however, the thickness may be 300 to 600 Å., depending on the size or diameter of the fibril.

Previous electron microscopy of myofibrils has provided some evidence that the non-volatile matter of the fibril (the filaments and A substance) is organized around its periphery, as if in a tubular arrangement (3, 4). This is of course not readily observed in fibrils which in drying flatten out with their longitudinal axes parallel to the supporting membrane. However, on at least one occasion in these preparations a sarcomere was observed which had dried while oriented in a semivertical position relative to the screen (Fig. 5). It is evident in this micrograph, although image contrast is greatly reduced by specimen thickness, that there is a definite ring of density around the outside.

The filamentous organization of the A bands is not greatly altered by the trypsin treatment. The longitudinal component with a width of 400 Å. which is prominent in the untreated fibril (Fig. 1) is present but less conspicuous in some of the treated fibrils (Figs. 5, 7). The surface of the fibril appears to have been smoothed out, probably through the dissolution of an amorphous, non-fibrillar surface component. In other trypsin-treated myofibrils, the larger strand organization is replaced by a greater number of closely packed filaments, each 125 to 175 Å. in width (Figs. 3, 6). In places (Fig. 7), the impression is gained that two of these fine ones are associated to make one of the large strands which are 400 Å. in width. More precise observation on this structure is prevented by the presence of an amorphous matrix, the A substance.

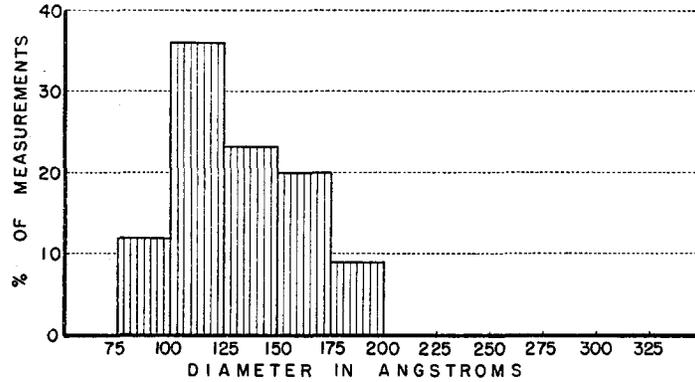
In some places, there is a breakdown at the margin of the fibril and a consequent dissociation of filaments and A substance (Fig. 8). Under these conditions the filaments are more readily measured (Text-fig. 2), but the value of such measurements is in doubt since the filaments may differ considerably from the form and size they had in the intact fibril.

Fibrils suspended and washed in more concentrated buffer solution (pH 7.0, μ 0.4) and then fixed in formalin, spread out enormously, leaving only the filaments and a few remnants of the M band. The Z band cannot be identified in these preparations, and the amorphous material in the A band seems to have been completely removed, suggesting that it is the A substance which binds the individual filaments together to give the longitudinal components with a diameter of 400 Å. seen in the intact fibril.

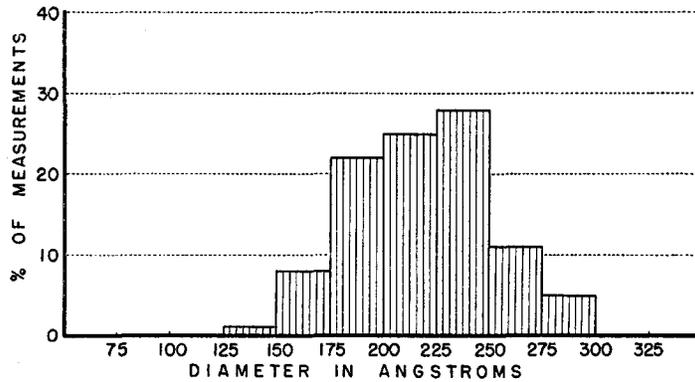
The Adenosinetriphosphate-Contracted Fibril

Treatment of the fibrils with ATP after trypsin brings about changes shown in Figs. 9 to 16. The most striking response is a shortening which is usually uniform throughout the length of the fibril. Along with this decrease in length,

there is an increase in girth. Occasionally, fibrils are encountered which exhibit a greater degree of contraction at one end than at the other (Fig. 13). Micrographs of these are especially useful for observing sequences in contraction.



FILAMENTS IN UNCONTRACTED PREPARATIONS



FILAMENTS IN CONTRACTED PREPARATIONS

TEXT-FIG. 2

All degrees of shortening have been found (compare Figs. 9 and 10), even down to as little as 25 per cent of the original length. If the fibrils remain un-fixed for 30 seconds after contraction appears completed, disorganization of the fibril ensues and the resultant mass loses all semblance of a myofibril (Fig. 11).

The contraction in total length of the fibril is accompanied by a proportional contraction in each sarcomere. Simultaneously there is a shift in the density

pattern of the sarcomere. The material of the A band appears to concentrate in the I band region. Fig. 12 represents an early stage in this process. It shows that the increase in density in the I band region is not merely an accumulation of electron-scattering ions, but represents an increase in non-volatile matter which is sufficient to produce an elevation in the contour of the desiccated fibril. Along with this development, the central portion of the A band loses some of its density (Fig. 13, Text-fig. 1). Hence some shift in the location of the A substance seems to be involved in this piling-up of material at the A band-I band margin.

At the completion of this shortening phenomenon (Figs. 9 and 10), the conventional organization of the sarcomere is lost. What was M band now becomes the most transparent part of the fibril, and no evidence of the H band margins or A-I band separation remains. The filaments, that were formerly organized in parallel array, may now criss-cross the fibril in every direction (Fig. 14). The fibril is further disorganized in the disruption of its surface, which is probably facilitated by the previous treatment with trypsin.

This fraying-out of the fibril margins as in the case of the uncontracted fibril provides an opportunity to observe isolated filaments and their relation to other elements of the fibril. In many cases, a fairly dense, non-fibrous material is associated with the filaments and apparently adherent to them (Fig. 15). This may be the substance of the A band which concentrates on contraction.

Where the filaments are cleaner at margins of contracted fibrils, measurements of their diameters are feasible (Fig. 16). From 1000 such measurements, it developed, as shown in Text-fig. 2, that the filaments from contracted fibrils have larger diameters than the filaments from uncontracted material. Whether any of this increase is due to adsorption of A substance is not apparent.

No branching filaments are found, as might be expected if filaments were double.

Filament length varies enormously. In uncontracted preparations, no filaments longer than the width of a single A band are found. In contracted material, however, some filaments are longer than the sarcomeres of neighboring fibrils, but all are less in length than the width of the A band of uncontracted fibrils.

Fibrils isolated with the colloid mill and treated with ATP show essentially the same general structural rearrangement (Fig. 17). In the early stages of contraction, the dense material localizes in the Z band, in contrast to the trypsin-isolated fibrils in which the initial localization is at the A-I junction. In later stages, the appearance is similar to that of the trypsin-isolated fibrils, although there is a more marked increase in the elevation of this material which has concentrated in the I band. In Fig. 17 the contraction band is 760 Å. high. The same criss-crossing arrangement of filaments can be seen.

DISCUSSION

The isolation of myofibrils by either tryptic digestion or the colloid mill permits the study of a relatively pure intracellular structure. Since these myofibrils retain the ability to contract on the addition of ATP, not only the uncontracted structure can be studied by electron microscopy, but also the fine structural changes associated with contraction. It can be assumed that the observed structural changes represent the response of the myofibril to chemical reactions in which only ATP and certain components of the myofibril are involved. A better understanding of the significance of these changes during contraction will depend largely upon chemical studies.

Tryptic digestion employed in isolation brings about a partial destruction of the myofibrils. The Z band and the greater part of the entire I band are removed. This differential action of the trypsin on the Z band material probably explains its effectiveness as an aid in freeing the fibrils from each other and the sarcolemma, for Draper and Hodge (3) have shown that the myofibrils are joined to the sarcolemma and to each other at the Z bands.

The lytic action of the trypsin goes beyond the Z band and includes the filaments of the entire I band region. Despite the belief of others that filaments are continuous throughout the sarcomere, the action of trypsin on filaments stops at the A-I junction. It is suggested, therefore, that the A substance may provide a protective cover for the filaments of the A band, either because it resists digestion or because it occurs in greater bulk. It is also conceivable that the filaments in the two regions are of different composition. Proteolytic enzymes of bacterial origin produce a similar lysis (3).

If permitted to continue long enough, the trypsin treatment brings about a complete separation of the sarcomeres along the A-I junction; but in the preparations studied here, enough connection generally remained to keep the fibril intact lengthwise even during contraction. The residual I band components (some shown as filaments in Fig. 4) appear to draw the opposing A bands closer together, thus reducing the I band width to a small fraction of its former size.

In most preparations (Figs. 2 to 7 and Text-fig. 1), there is a region of increased density where the H band should be. This conflicts with observations of others (1, 3, 5) in which this region is described as an area of decreased density. The difference can be explained by assuming that the fixation of intact fibers with formalin at temperatures *above* 0° C., used by others, induces an early stage of contraction which would be represented according to classical histology (8) by slight migration of the material of the A band toward the A-I junction. At 0° C. it is assumed that this does not happen before the formalin accomplishes fixation. Hence in the resting or relaxed state the A substance is probably concentrated next to the M band.

The enzyme treatment, by bringing about various degrees of fibril disintegration, is useful in revealing structural features not otherwise available for

study. For example, isolated sarcomeres (Figs. 4 to 6), being short and cylindrical, are occasionally found standing on end. Arranged thus, they are useful for studying the cross-sectional morphology of the fibril. Our preparations might have been improved if they had been dried by Anderson's method (9), which eliminates the compressing effects of surface tension. In Figs. 5 and 5a, the ring of density around the margin of the fibril conforms to the concept developed by Pease and Baker (4) and by Draper and Hodge (3) that the fibril is a thin-walled tube. It is reasonable to draw the same conclusion from the form assumed by the dry fibril shown in Figs. 2, 3, and 4. Solid cylinders (rods) of filaments and A substance, after being hardened by formalin fixation, would not be expected to reorganize in drying to form a thin layer of nearly uniform thickness (as in Fig. 3). Instead, the structure required for this would need to be pipe-like. This implies, then, that the fibril which has been washed with buffer solution (pH 7.0, μ 0.154) is a tube with filaments and A substance located around the outside and most of the water in the inside. Since some of the proteins isolated from muscle are soluble at this ionic strength (10), it is conceivable that they are also located within this tubular structure in the intact muscle.

It was mentioned above that the filaments in the uncontracted fibril appear to be associated in pairs to produce a longitudinal component with a width of 400 Å. This double structure is accentuated by treatment with trypsin, which apparently removes some of the amorphous A substance from the surface of the fibril. It is not clear from these preparations whether the paired filaments are coiled about each other to form a double spiral. Actually, the appearance of fibrils further disintegrated by trypsin as well as by buffers of higher ionic strength suggests neither a coiled structure nor even the paired arrangement.

It seems worthy of note that the A substance is removed from myofibrils by salt solutions similar in ionic strength to those used for the extraction of myosin from bulk preparations. This suggests that the A substance may be identical with myosin and the filaments actin. Rozsa *et al.* have reported a similar opinion (5).

The observations of classical histology (7) indicate that the first change in contraction is a migration of dense material within the A band toward its margins. This is followed by a gradual increase in density and width of the Z band, resulting in the formation of a contraction band and a reversal of the cross-striations. These observations were confirmed by Hall, Jakus, and Schmitt (1) and later by Draper and Hodge (3) using the electron microscope. It is evident, then, that the addition of ATP to the isolated fibril initiates a series of changes at least similar to those seen in living muscle which has been stimulated through the intact nerve.

This shift in density, pictured both optically and by electron scattering, is not simply a shift of some ion such as K or PO_4 , but involves most of the ma-

terial of the sarcomere. It is as though the A substance were concentrated in the Z-I band and formed there a new pattern of organization.

In this respect, the sequences in the density shift seem of some significance. In the fibrils with intact Z bands, the initial change associated with contraction is a migration of A substance from the part of the A band nearest the A-I junction and a concentration of this substance at the Z band. As contraction proceeds, it seems that material progressively nearer the M band migrates to and condenses at the Z band. Fibrils isolated by tryptic digestion do not have Z bands and only remnants of I bands, yet in these the structural changes are similar except that the concentration of A substance begins at and continues at the A-I junction. The actual site of action of the ATP has not been determined, but these observations indicate that neither the Z band nor the organized I band is an essential structure for the contraction mechanism. The ATP-induced contraction, however, has not been reversed, so that the relationship of these structures to the process of relaxation is not known.

The contraction of the myofibril down to as little as 25 per cent of its original length is associated with a disorientation of the individual filaments from the parallel arrangement found in the uncontracted fibril. This degree of shortening is greater than that which occurs in the normal intact fiber. Since disorientation of filaments is not seen in the early stages of contraction, it is probably an artefact introduced by an excessive amount of ATP.

It would seem that a decrease in length and an increase in width of filaments should accompany shortening of the myofibril during contraction. It has not been possible to measure changes in length, but there is a definite increase in the average width of filaments during contraction. However, it is possible that the increase in width is due to an adsorption of A substance, rather than any intrinsic change in the structure of the filaments.

The periodicity of 400 Å. observed by other workers in the uncontracted fibril is not seen in these preparations, so that a shift in period size as suggested by Draper and Hodge (3) cannot be measured. Possibly this shift could be studied at slightly higher hydrogen ion concentrations in which the periodicity is more evident.

SUMMARY

Skeletal myofibrils isolated either by tryptic digestion at 0° C. or by a colloid mill and suspended in buffer solution (pH 7.0, μ 0.154) containing 20 per cent glycerin and 0.0025 M adenosinetriphosphate at -5° C. contracted slowly and progressively when the temperature was raised above 0° C. Formalin fixation halted this contraction. With the aid of these procedures myofibrils in progressive stages of contraction were then studied with the electron microscope.

Electron micrographs showed that uncontracted fibrils isolated by the colloid mill were structurally similar to those described by other workers. Treat-

ment of fibrils with trypsin removed the Z bands and disorganized the I bands. This enzymatic modification of structure did not impair the contractile response. The principal structural changes during contraction consisted of a migration of dense material from the A band into the A-I junction or the Z band, a gradual increase in width of the fibril, a gradual decrease in length of sarcomeres, an apparent increase in the mean diameter of filaments, and a disorientation of these latter from their parallel arrangement.

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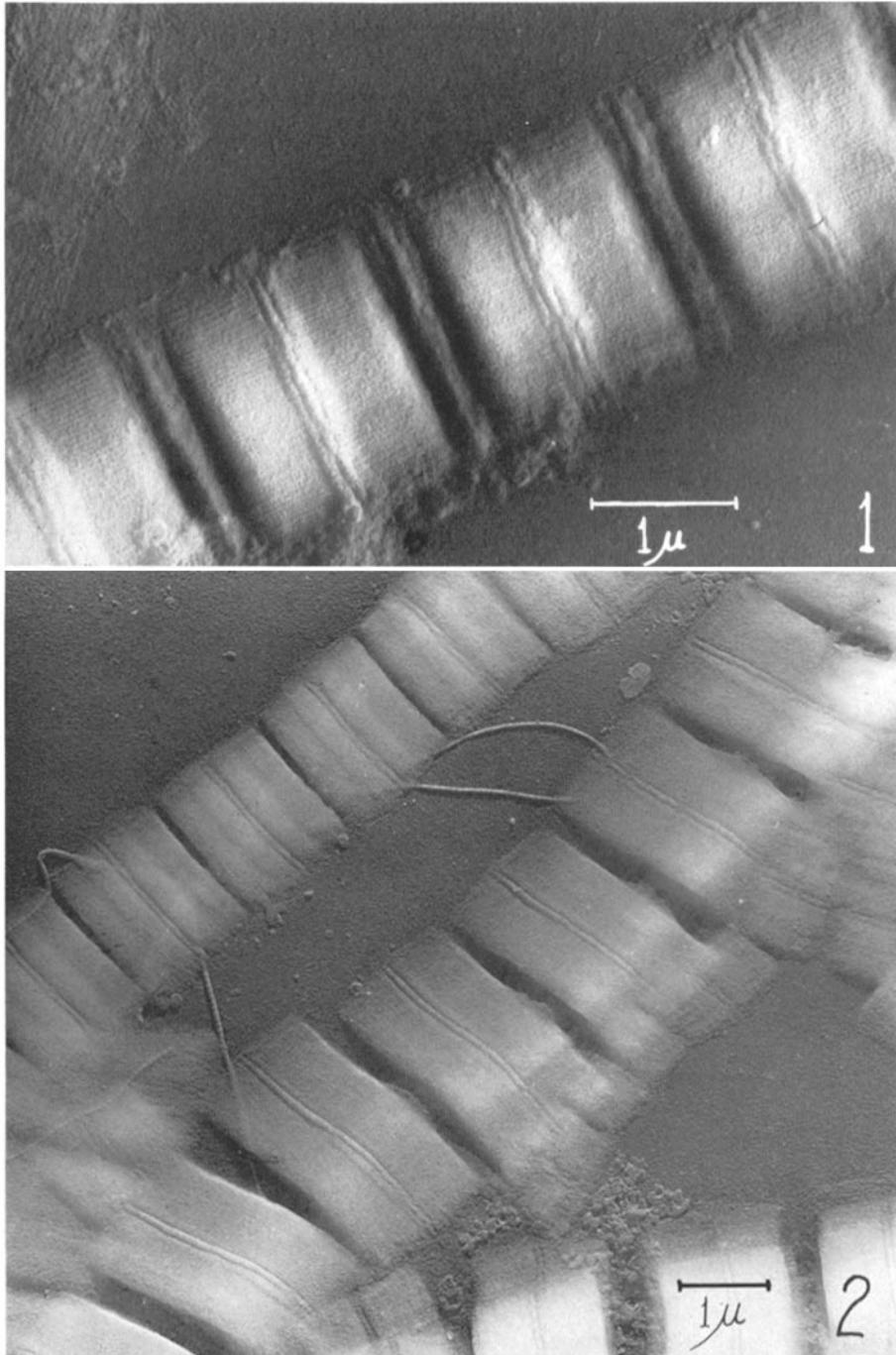
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EXPLANATION OF PLATES

PLATE 6

FIG. 1. Electron micrograph of skeletal myofibril isolated with a colloid mill, fixed with 10 per cent formalin buffer solution (pH 7.0, μ 0.154), and shadowed with chromium. The major cross-bands are shown, as are the longitudinal filaments. The latter show some nodosities, but not clearly enough to demonstrate that they occur at regular intervals. \times 20,100.

FIG. 2. Electron micrograph of myofibril isolated by tryptic digestion, fixed with formalin in buffer solution, and shadowed with chromium. The Z band is absent, and the I band is no more than residual. In several places, the A bands are almost torn apart. A considerable variation in the width of the fibrils may be noted. The central portion of these fibrils is moderately thick, whereas the margins are extremely thin (30 to 40 Å). The M band is distinct, as are the depressions on either side of it. The raised area of increased opacity on either side of the M band is seen to vary markedly in width in the two nearly adjacent fibrils. Several collagen fibers are present. \times 12,000.

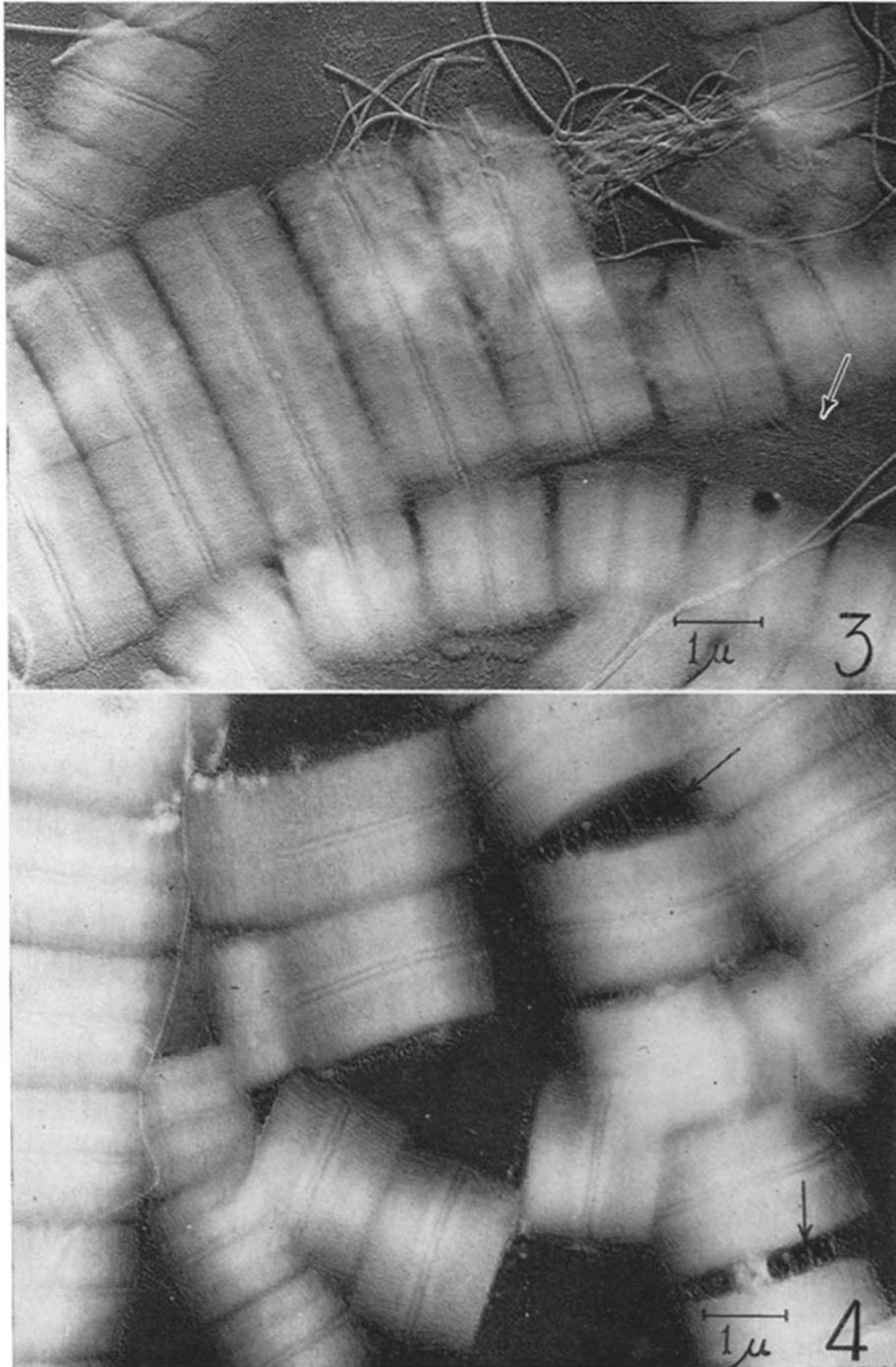


(Ashley *et al.*: Adenosinetriphosphate contraction of skeletal myofibrils)

PLATE 7

FIG. 3. Micrograph of myofibrils isolated by tryptic digestion, fixed with formalin in buffer solution, and shadowed with chromium. The Z bands are absent, and the I bands are very narrow. The filamentous composition of the A band is shown, and numerous single filaments detached from sarcomeres are present in the background (arrow). The central fibril is fairly uniform in thickness across its entire width, and the lateral margin is approximately 120 Å. thick. Collagen fibers are present. × 11,900

FIG. 4. Micrograph of myofibrils isolated with trypsin, suspended in 20 per cent glycerinated buffer solution, fixed with formalin in buffer solution, and shadowed with chromium. The Z bands are absent, and the I bands are narrow if not missing. Separation of sarcomeres at the A-I junction is shown. A few filaments can be seen connecting several of the A bands, which are almost torn apart (arrows). × 11,400.



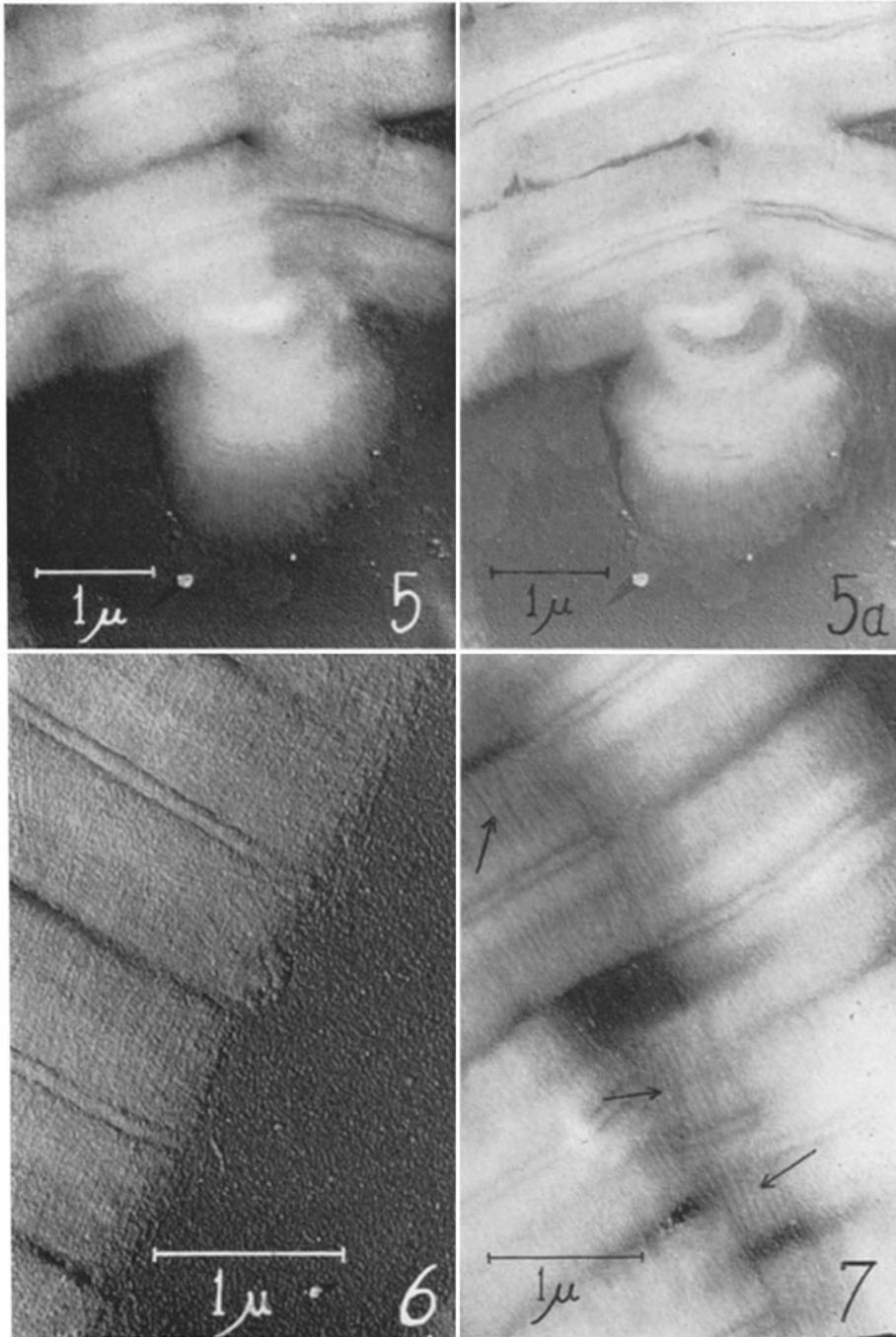
(Ashley *et al.*: Adenosinetriphosphate contraction of skeletal myofibrils)

PLATE 8

FIG. 5. Micrograph of myofibrils isolated with trypsin, suspended in 20 per cent glycerinated buffer solution, fixed with formalin, and shadowed with chromium. An isolated A band which is essentially standing on end is shown. The peripheral ring of increased density, as emphasized by retouching in Fig. 5*a*, indicates a tubular arrangement. $\times 16,000$.

FIG. 6. Micrograph of portion of myofibril prepared as for Fig. 5. Presented to show filamentous character of sarcomere surface after trypsin treatment. $\times 25,900$.

FIG. 7. Micrograph of myofibrils prepared as for Fig. 5. The double form of the individual filaments is shown (arrows). $\times 21,000$.



(Ashley *et al.*: Adenosinetriphosphate contraction of skeletal myofibrils)

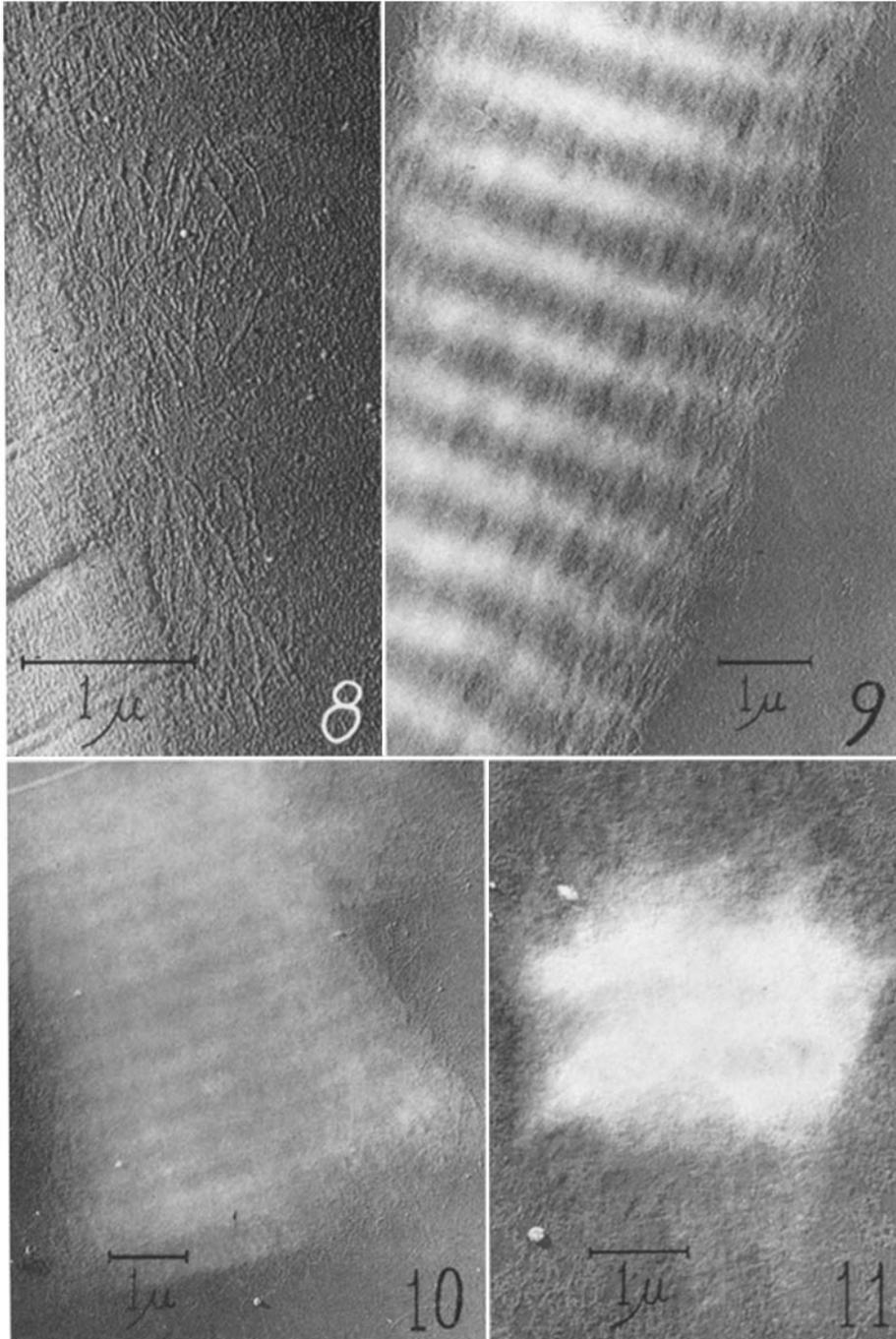
PLATE 9

FIG. 8. Micrograph of margin of myofibril isolated with trypsin, suspended in aqueous buffer solution, fixed with formalin, and shadowed with chromium. The fraying of the margin into individual filaments is shown. The filaments within the fibril do not appear to be double as in Fig. 7. $\times 23,700$.

FIG. 9. Micrograph of trypsin-isolated myofibril, suspended in glycerinated buffer solution, contracted with ATP, fixed with formalin, and shadowed with chromium. The sarcomere length is 50 per cent of that of uncontracted fibrils. There is a complete structural rearrangement, with the formation of a dense contraction band which has covered the A-I junctions of adjacent A bands (compare with Fig. 2). Structural features other than the filaments cannot be seen in the A band, which, at this stage of contraction, is the least dense portion of the sarcomere. $\times 12,900$.

FIG. 10. Micrograph at a lower magnification of an ATP-contracted myofibril prepared as in Fig. 9 except for the use of aqueous, instead of glycerinated, buffer solution. The sarcomere length is 30 per cent of that of uncontracted fibrils. Numerous filaments are shown around the margin of the fibril. $\times 10,400$.

FIG. 11. Micrograph of myofibril prepared as for Fig. 10 (aqueous buffer solution). Formalin was not added until 30 seconds after completion of contraction. The fibril is completely disorganized. $\times 13,500$.

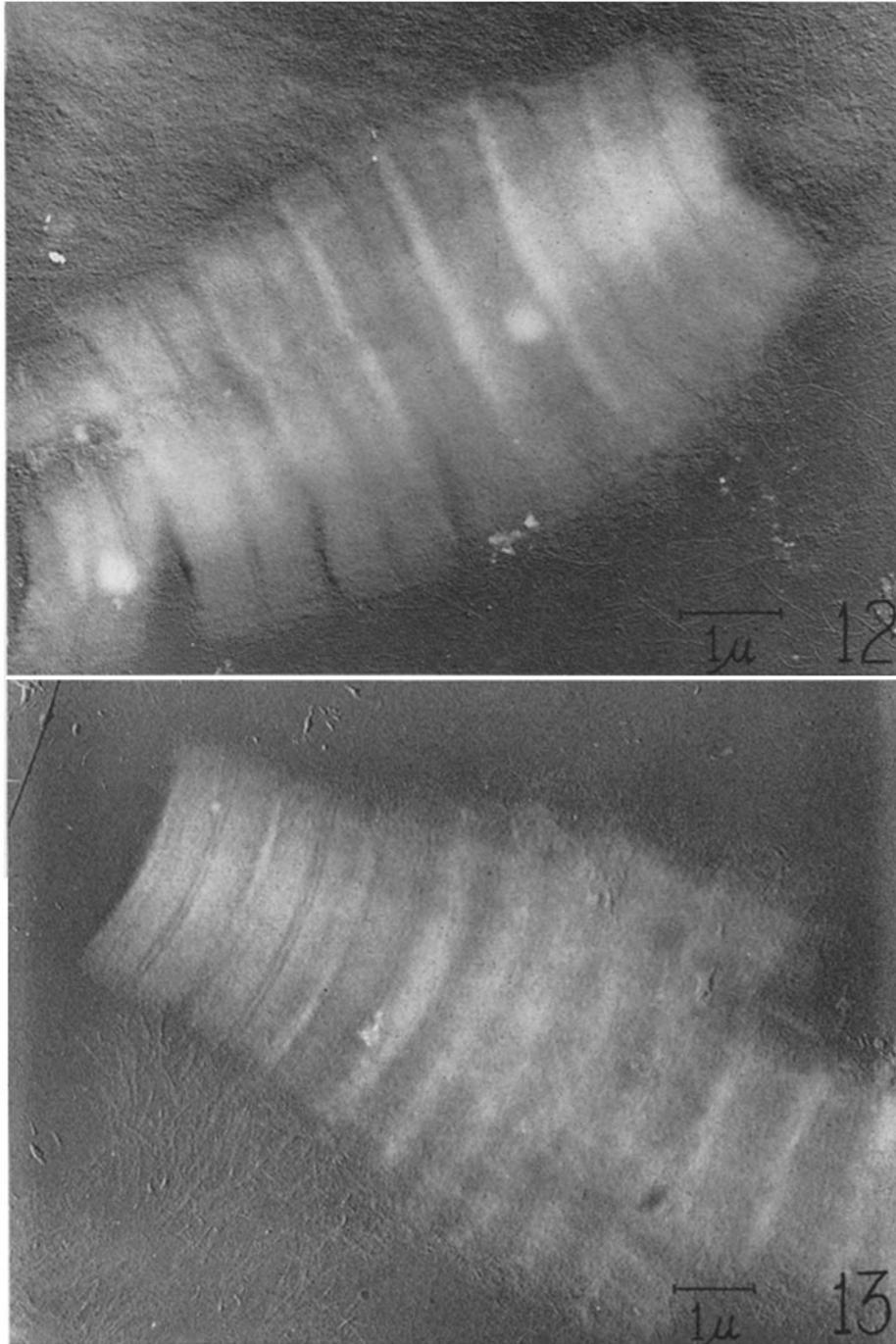


(Ashley *et al.*: Adenosinetriphosphate contraction of skeletal myofibrils)

PLATE 10

FIG. 12. Micrograph of myofibril isolated with trypsin, suspended in glycerinated buffer solution, contracted with ATP, fixed with formalin, and shadowed with chromium. This is presumably an early stage of contraction. The M band can be identified, as can the dense, elevated contraction band overlapping the A-I junctions of adjacent A bands. Contraction is most advanced in the sarcomeres in the central portion of the fibril, and it can be noted that the A bands of the more contracted sarcomeres are less dense than those of the sarcomeres at the ends of the fibril, which show almost no contraction. Fibrils of the type shown here and in Fig. 13 which show variation in the degree of contraction along the length of the fibril are found only rarely in the preparations. $\times 13,500$.

FIG. 13. Micrograph of myofibril prepared as for Fig. 12, showing no evidence of contraction in the sarcomeres at one end but with progressive shortening and broadening toward the other. The shift in the density pattern is shown; *i.e.* the wider and denser the contraction band, the less dense the A band. This suggests a shift of material from the A band into the A-I band junction. $\times 10,900$.



(Ashley *et al.*: Adenosinetriphosphate contraction of skeletal myofibrils)

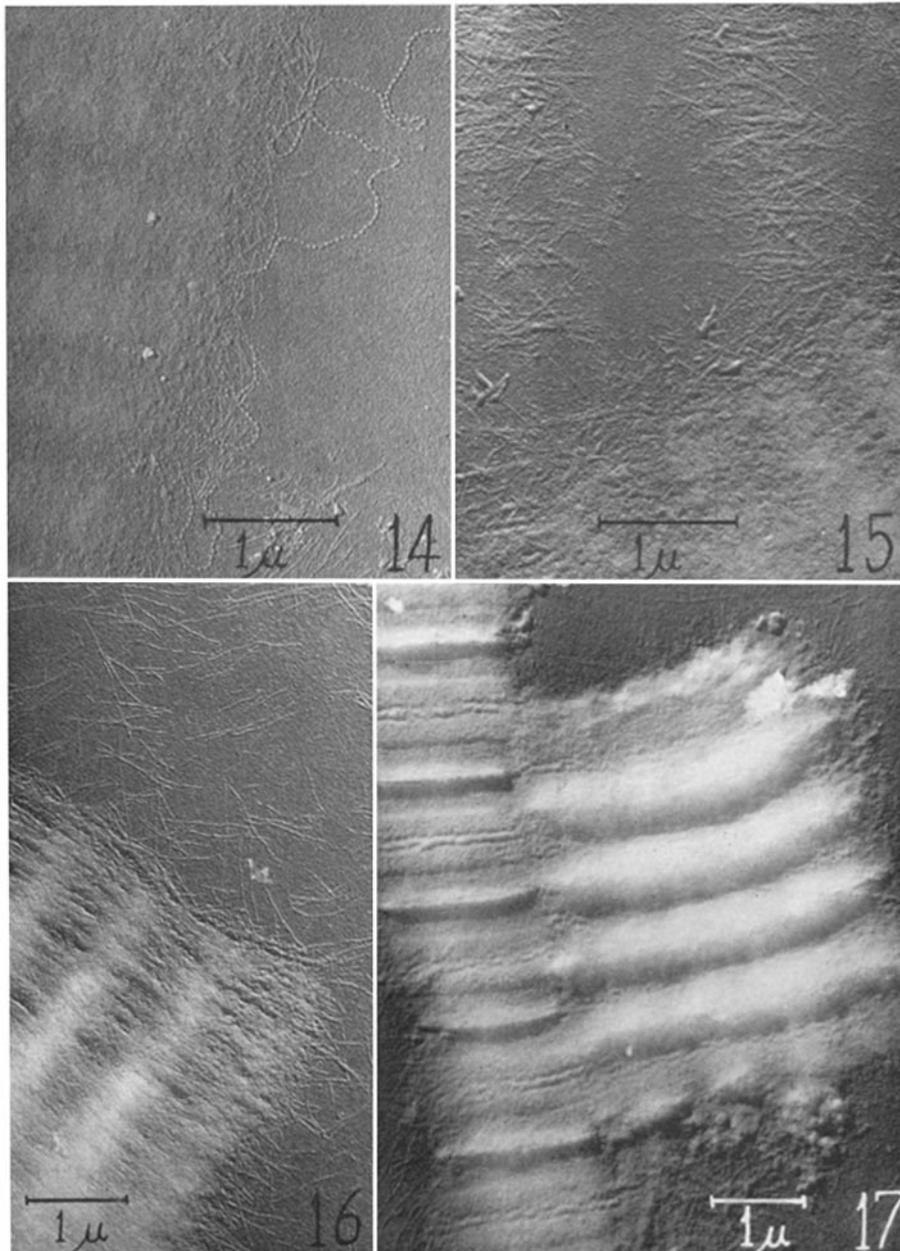
PLATE 11

FIG. 14. Micrograph of the margin of a myofibril isolated with trypsin, suspended in aqueous buffer solution, contracted with ATP, fixed with formalin, and shadowed with chromium. The criss-crossing arrangement of the filaments in advanced contraction is shown both at the margin and in the body of the fibril. $\times 17,800$.

FIG. 15. Micrograph of myofibril prepared as for Fig. 14, except for the use of glycerinated buffer solution as a suspending medium. Many filaments are shown near the margin of the fibril. There is an extremely dense, amorphous material, probably A substance, which is apparently adherent to many of the filaments. $\times 18,700$.

FIG. 16. Micrograph of a portion of a myofibril prepared as for Fig. 15. The sarcomere length is 55 per cent of that of uncontracted fibrils. Many clean filaments are seen adjacent to the myofibril. $\times 13,700$.

FIG. 17. Micrograph of two myofibrils isolated in a colloid mill without trypsin, suspended in glycerinated buffer solution, contracted with ATP, fixed with formalin, and shadowed with chromium. In the more contracted fibril the sarcomere length is 50 per cent of that of the average uncontracted, mill-isolated fibril. The sarcomere length of the other fibril is at the lower limit of the range of sarcomere lengths found for fibrils to which ATP has not been added. The contraction band in the shorter fibril is denser and more elevated than in the trypsin-treated fibrils (compare with Fig. 9). The M band can be identified between the contraction bands. $\times 12,800$.



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