

OBLIQUELY STRIATED MUSCLE

III. Contraction Mechanism of *Ascaris* Body Muscle

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ABSTRACT

Segments of the obliquely striated body muscle of *Ascaris* were fixed at minimum body length after treatment with acetylcholine and at maximum body length after treatment with piperazine citrate and then studied by light and electron microscopy. Evidence was found for two mechanisms of length change: *sliding* of thin filaments with respect to thick filaments such as occurs in cross-striated muscle, and *shearing* of thick filaments with respect to each other such that the degree of their stagger increases with extension and decreases with shortening. The shearing mechanism could account for great extensibility in this muscle and in nonstriated muscles in general and could underlie other manifestations of "plasticity" as well. In addition, it is suggested that the contractile apparatus is attached to the endomysium in such a way that the sarcomeres can act either in series, as in cross-striated muscle, or individually. Since the sarcomeres are virtually longitudinal in orientation and are almost coextensive with the muscle fiber, it would, therefore, be possible for a single sarcomere contracting independently to develop tension effectively between widely separated points on the fiber surface, thus permitting very efficient maintenance of isometric tension.

INTRODUCTION

Recent studies of the body musculature of *Ascaris* have shown that this muscle, which was formerly thought to be "smooth," has properties in common with both classical cross-striated muscle and classical smooth muscle (22, 24, 25). On the one hand, it has a regular double array of myofilaments, thick and thin, which interdigitate with each other and which produce the appearance of oblique banding at low magnification. On the other, it lacks Z lines, and possesses dense bodies instead; its fibers are spindle shaped and relatively short; contraction and relaxation are slow, and the muscle is spontaneously active and has a dual innervation (4). Moreover, it has been shown that, despite the similarity of the contractile

apparatus to that of cross-striated muscle, the myosin ATPase of *Ascaris* muscle has the biochemical characteristics of that in a "smooth" muscle (chicken gizzard) (2; M. Bárány, unpublished data). This muscle thus appears to occupy an intermediate position, and it is of interest, therefore, partly because of insights which it may bring to an understanding of the contractile process in smooth muscle as well as striated. It has a distinct practical advantage over typical smooth muscle in that it exhibits enough order so that its three-dimensional structure can be analyzed readily, particularly from examination of transverse sections.

Accordingly, the previous investigation of this

muscle has been extended by comparing samples of *Ascaris* muscle fixed in extension with specimens fixed in contraction in an effort to reconstruct the events that occur during the contraction-relaxation cycle. The results indicate that a sliding filament mechanism occurs here as it does in cross-striated muscles (12); but that, in addition, there is a second potential mechanism for length change, referred to as "shearing," which does not occur in cross-striated muscle, but which may be

responsible for the great length range over which smooth muscle contraction is possible (26). Shearing may also underlie the slow time course of length changes in *Ascaris* muscle. In addition, it is suggested that individual sarcomeres may be able to act independently in this muscle, resulting in very efficient maintenance of tone under isometric conditions.

Ultrastructural studies of other invertebrate muscles, particularly those by Hanson and Lowy

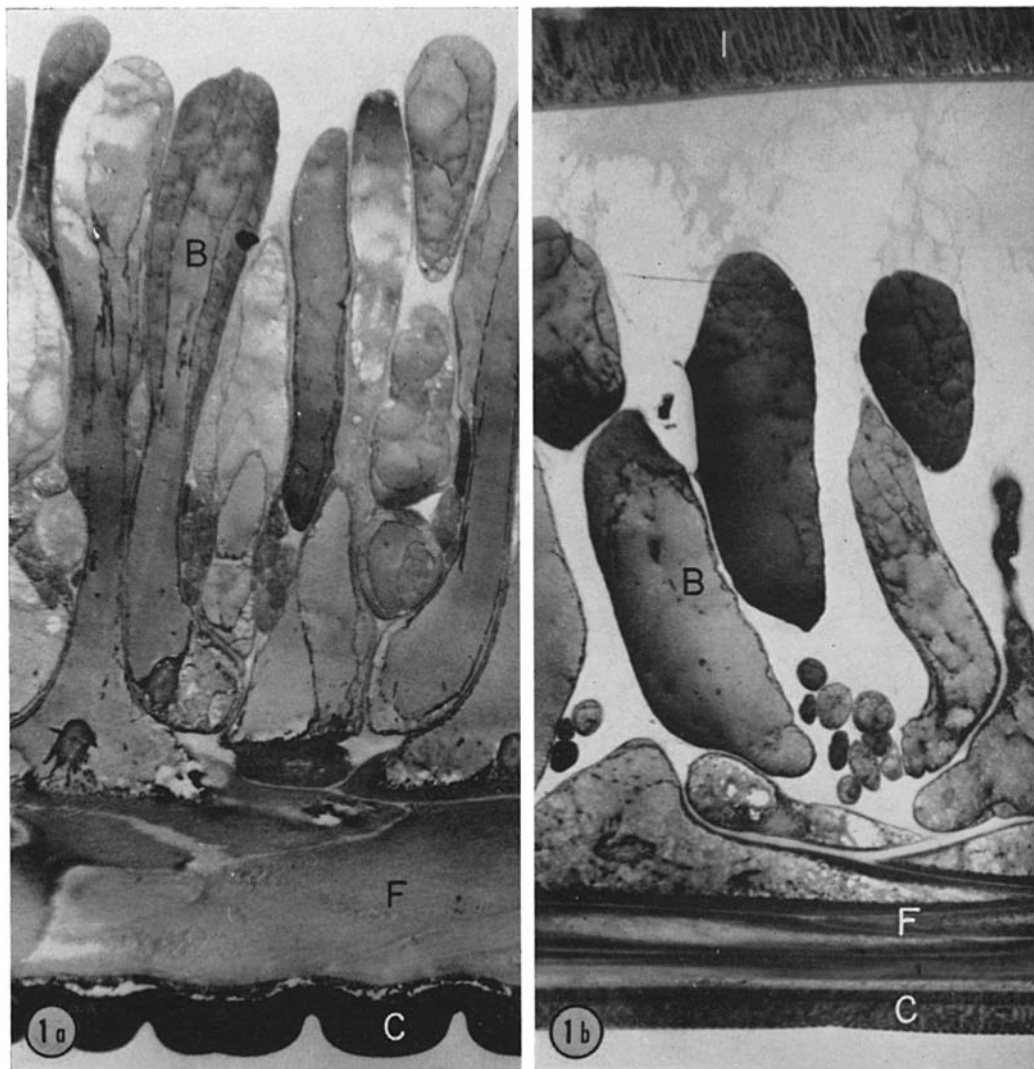


FIGURE 1 Photomicrographs of two longitudinally sectioned segments of the same worm. *a*, Contracted. The cuticle (*C*) is corrugated; the layer of fibers (*F*) is approximately 80% taller than in *b*; the muscle bellies (*B*) are compressed together. *b*, Extended. The cuticle is smooth and the muscle bellies are spread apart. *I*, intestine. *a*, $\times 100$. *b*, $\times 110$.

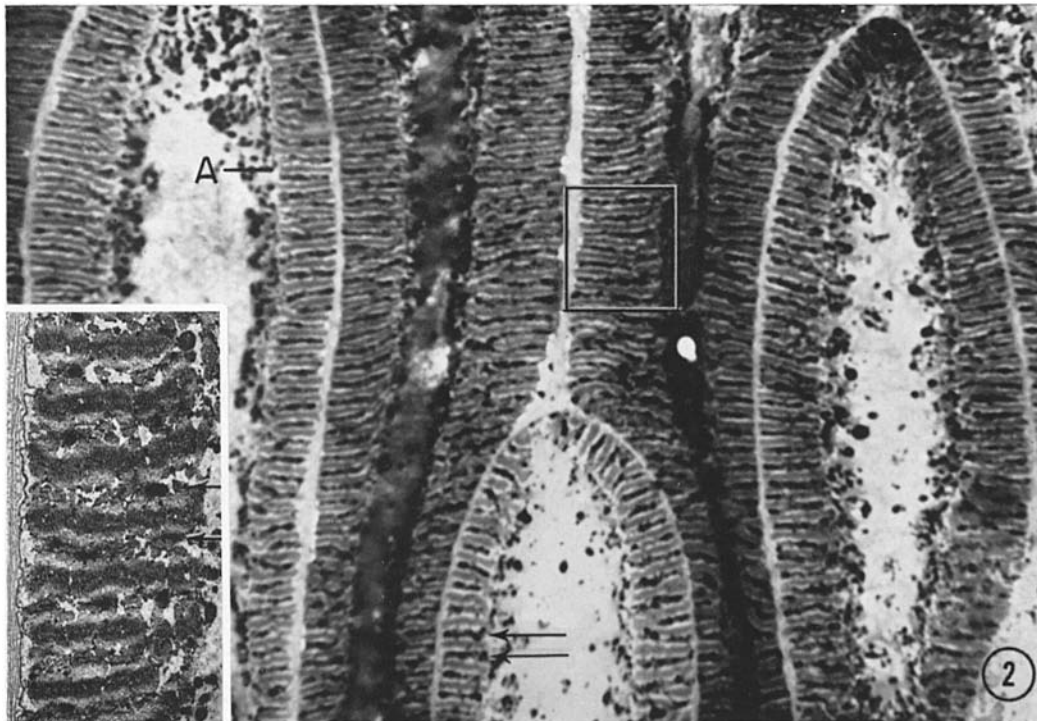


FIGURE 2 Photomicrograph of extended fibers sectioned transversely. The beaded dense bands (arrows) are prominent. Between each pair of dense bands are two light bands and a central denser band *A*. Inset. Low power electron micrograph of an area like that shown in the box in Fig. 2. $\times 1400$. Inset, $\times 5000$.

(9), Ikemoto (13), and Kawaguti and Ikemoto (17), have shown that an oblique array of myofilaments, far from being unique, occurs in a variety of invertebrate phyla. There are, however, differences of opinion about the mechanism of contraction in this type of muscle (10, 19).

MATERIALS AND METHODS

Live *Ascaris lumbricoides* were obtained from a hog slaughterhouse and were kept in warm mammalian Ringer's solution during transport to the laboratory. On arrival, the worms exhibited moderate muscle tone and were partially contracted. Their spontaneous motions consisted of longitudinal waves of contraction, resulting in a slow lashing from side to side in a plane perpendicular to the plane through the two lateral lines.

Muscle extended to "maximum body length" was obtained as follows: the anterior tip of a worm was snipped off and about 5 ml of a 10^{-3} g/ml solution of piperazine citrate (Burroughs-Wellcome & Co., Tuckahoe, N. Y.) in mammalian Ringer's solution was perfused through the anterior third of the animal. Piperazine has the effect of an inhibitory neurohumor

(6). The solution was introduced through a syringe needle penetrating into the body cavity at approximately the junction between the anterior and middle thirds of the animal; efflux was from the cut anterior tip. Almost as soon as the perfusion was begun, the anterior segment of the worm lost tone. Very quickly it became completely flaccid and also lengthened noticeably under its own weight. The limp perfused segment was then extended maximally and pinned at that length onto a wax plate. Further extension was apparently limited by the cuticle. The extended segment was incised along one lateral line, opened out, and pinned flat; the intestine was stripped out and the interior of the worm then flooded with a 2.8% solution of glutaraldehyde (biological grade) in 0.1 M phosphate buffer (pH 7.4) for several minutes. The tissue was then immersed in the fixative for about 3 hr still pinned to the wax plate. In some instances, the fixative was perfused through the worm immediately after the piperazine citrate and dissection carried out subsequently. After glutaraldehyde fixation, the tissue was no longer contractile. It was then rinsed briefly in 0.9% NaCl and immersed for 1-4 hr in a 1% solution of OsO_4 in acetate-Veronal buffer (pH 7.4) with salts added to yield

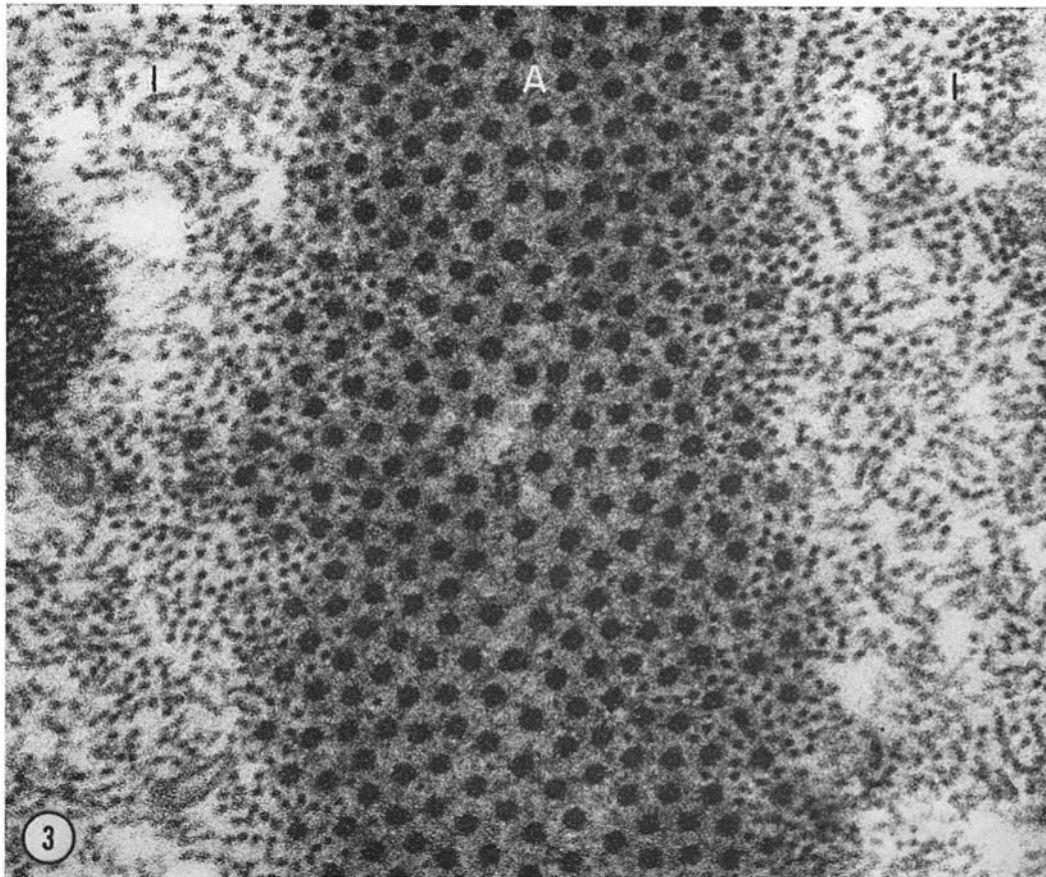


FIGURE 3 Electron micrograph of extended fiber sectioned transversely. The central moderately dense A band is composed of 10-15 rows of thick filament profiles. The light I bands on either side consist of thin filament profiles. No thin filaments are present in the center of the A band, but the thick filaments are surrounded by a finely fibrillar matrix of moderate density. Part of a dense body appears at the extreme left. $\times 162,000$.

an ionic composition approximately equal to that of mammalian Ringer's solution.

Muscle specimens at "minimum body length" were obtained by the same procedure except that in place of the piperazine citrate, a 5×10^{-3} g/ml solution of acetylcholine (5) was perfused through the anterior portion of the animal, causing marked contraction with shortening of the perfused segment. In order to eliminate asymmetry, the worms were kept straight during fixation. Presumably, bending would be accompanied by extension of the muscle on the convex side of the animal.

In some instances, in order to eliminate individual differences between worms, an animal was perfused with acetylcholine to maximal contraction and the anterior third of the animal marked off and measured. The worm was next perfused with piperazine citrate,

extended maximally, pinned, and opened along one lateral line, and the intestine removed as described above. The other lateral line was then incised, dividing the worm into two symmetrical halves. One-half was fixed at the extended length and the other removed, flooded with acetylcholine, and allowed to shorten down to the contracted length measured originally (approximately 60% of the extended length¹). It was then pinned and fixed, and the two halves, one extended and one contracted, processed and embedded together.

¹ Strips of muscle from which the cuticle, the hypodermis, and the lateral lines have been removed are capable of much greater shortening. Evidently these structures, which are in parallel with the muscle, restrict its range of motion.

All specimens were dehydrated in a graded series of methanol solutions and embedded flat in Araldite (18). Sections were cut with a Porter-Blum MT-2 microtome, mounted on bare grids, stained with uranyl acetate (23) or lead hydroxide (15) or both, and examined with a Philips EM 200 electron microscope at 60 kv. 1-2- μ sections were stained with toluidine blue (21) and examined by light microscopy.

OBSERVATIONS

Light Microscopy

Ascaris body muscle cells consist of three parts: a longitudinally oriented contractile *fiber*, a *belly* containing the nucleus, and an *arm*, or innervation process. The ultrastructure of these components has been described previously (24, 25).

The most obvious distinction between extended and contracted worm segments is to be seen in longitudinal sections, in which the cuticle investing a shortened muscle strip appears prominently corrugated (Fig. 1 *a*), in contrast to that in an extended specimen, in which it is smooth (Fig. 1 *b*). Evidently, the cuticle is relatively inelastic and

buckles as the subjacent muscle shortens, the degree of corrugation providing some measure of the degree of shortening. At its midportion, the fiber of the shortened muscle cell is about $\frac{1}{3}$ mm high. This is approximately 80% greater than in an extended cell. Contraction also results in a compression of the muscle bellies together such that they, too, become taller in the radial direction. The bellies of extended muscle cells, in contrast, are separated and somewhat flattened in appearance.

In transverse sections viewed at high magnification, it is clear that the band pattern in the U-shaped contractile cortex of each fiber is very different in contracted and extended specimens. In the extended fibers (Fig. 2), "dense bands," which contain the "dense bodies," are thin, prominent, and beaded. They repeat approximately every 1.5 μ . Between each two dense bands, three other bands can be distinguished—two light ones immediately adjacent to the dense bands and a central darker band. This pattern was noted previously in fibers fixed at random lengths, but did not occur consistently. In the

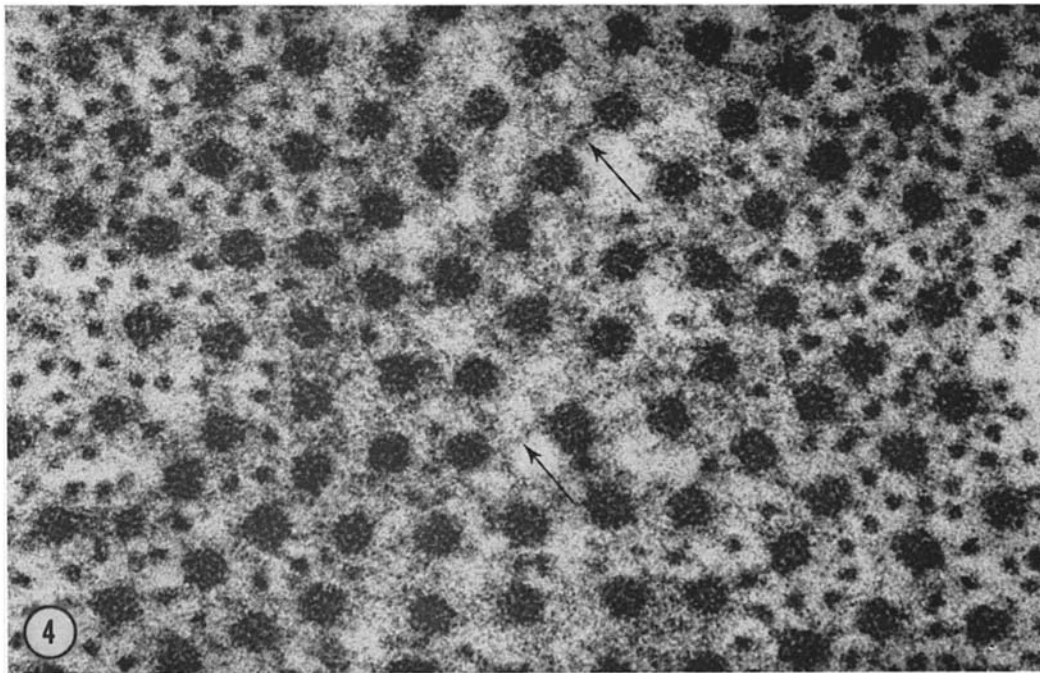


FIGURE 4 Extended fiber. The thick filaments appear to be composed of subunits. In the center of the figure thin filaments are absent, but strands (arrows) of a finely fibrillar matrix seem to bridge between neighboring thick filaments. $\times 296,000$.

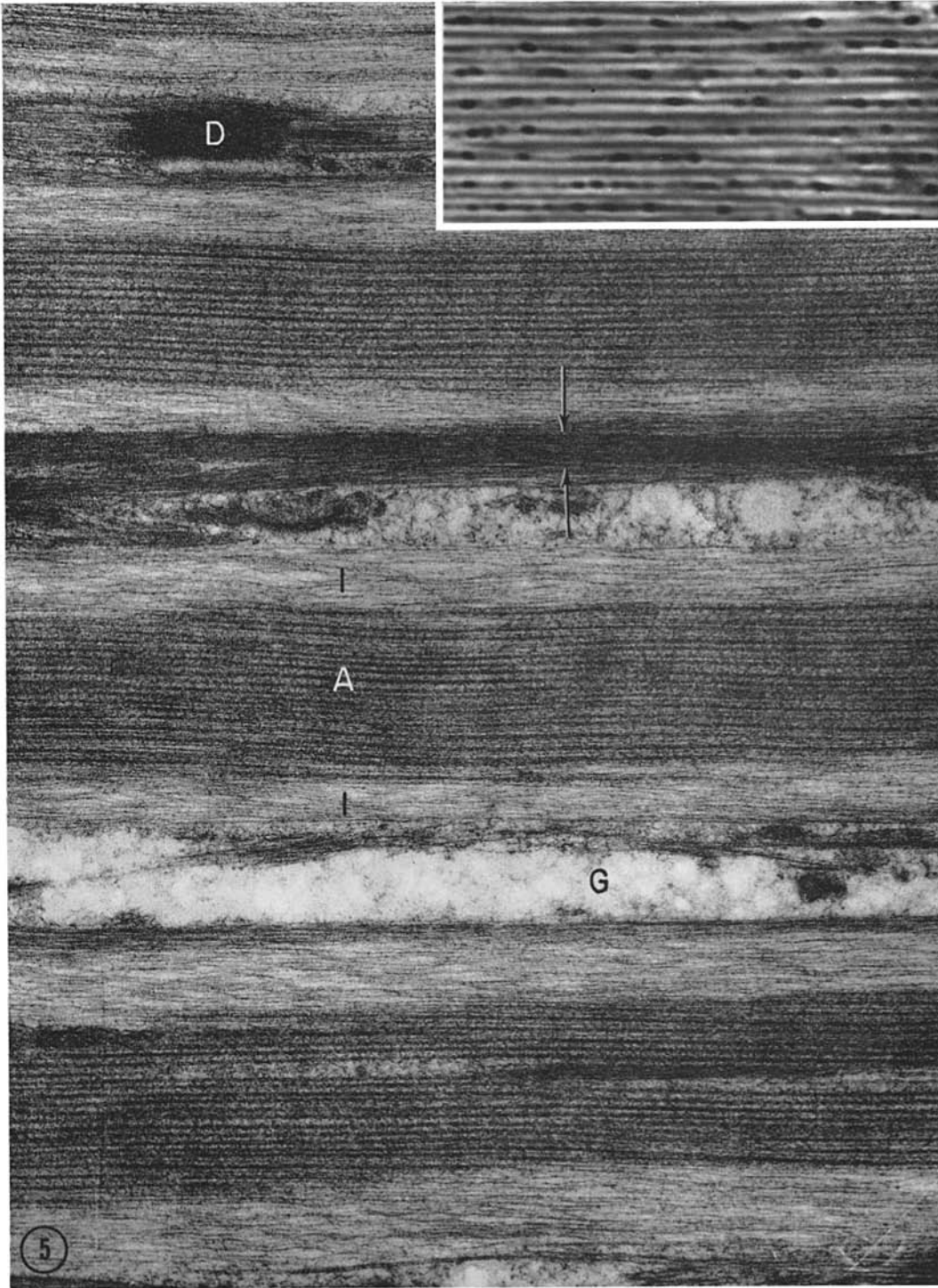


FIGURE 5 Extended fiber sectioned longitudinally. I bands composed of thin filaments appear to lie at the sides of the A bands containing both thick and thin filaments. The dense bands between I bands contain dense bodies (*D*), fibers of the intracellular skeleton (between arrows) and glycogen (*G*), which in this preparation is unstained. Inset: Photomicrograph of extended muscle. The dense bodies appear to be linked together by the strands of the intracellular skeleton. $\times 40,000$. Inset, $\times 2,200$.

extended fibers described here, however, the pattern occurs regularly in all fibers. In longitudinal sections (Fig. 5) the same pattern appears, and here the "beads," or dense bodies, which were described previously as disconnected structures (24) appear to be linked together by strands of the "supporting fibrils" (24) or "intracellular skeleton" forming continuous chains.

In transverse sections of contracted specimens (Fig. 7), the dense bands are less distinct and further apart from one another. They repeat approximately every 2.3μ . Between each pair of dense bands, three bands are again distinguishable, but only very faintly. The central band is now very broad and slightly paler than the two narrow, darker bands flanking it. The same pattern appears in longitudinal section (Fig. 11). However in this plane, the dense bodies now appear to have a somewhat oblique orientation and to be dis-

continuous, presumably due to folding and coiling of the intracellular skeleton so that it passes in and out of the plane of section.

Electron Microscopy

In transverse sections of extended muscle (Figs. 2 inset, and 3), it is clear that the pale zones adjacent to the dense bands are composed of thin filament profiles and, therefore, constitute I zones. Between each pair of I zones is a darker A zone consisting of approximately 10-15 rows of thick filaments which are $\sim 230 \text{ \AA}$ in maximum diameter. The width of the A zone is about 30% of the repeating period, and the H zone occupies about half of the A zone.

In the H zone, in which thick filaments occur without associated thin filaments, there is, nevertheless, a finely fibrillar matrix surrounding the thick filaments. Whether this matrix is composed

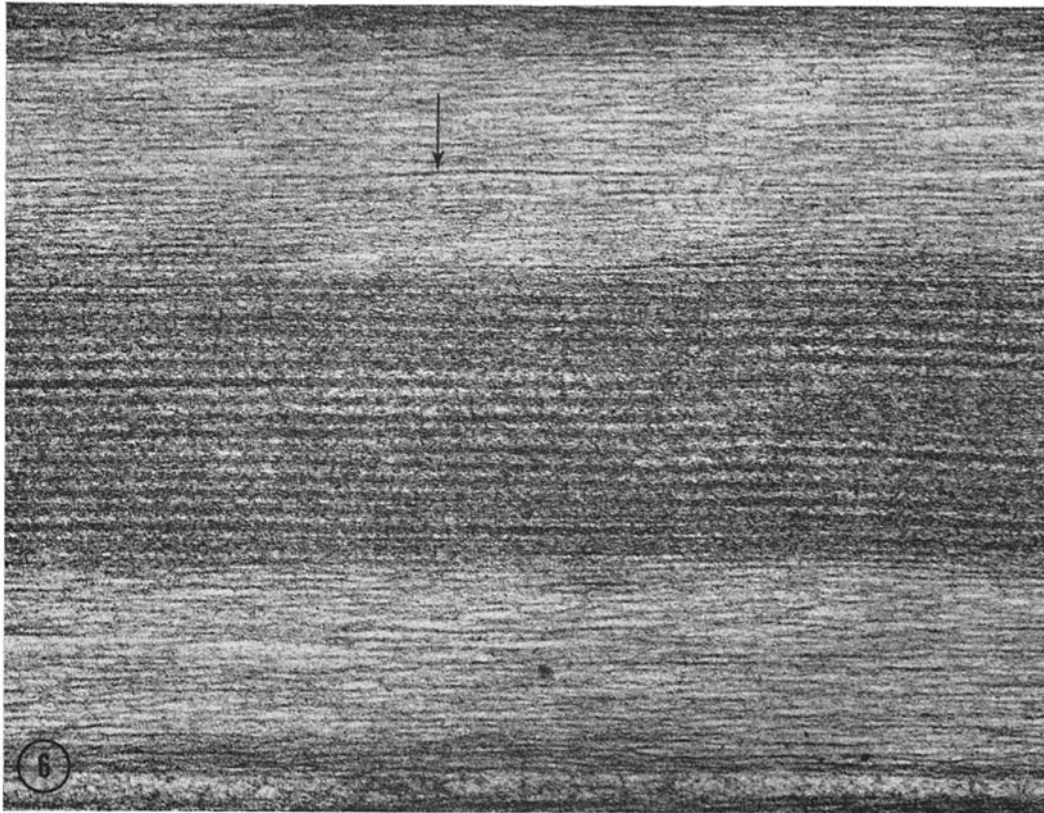


FIGURE 6 Extended muscle in longitudinal section. Some of the thin filaments appear to be denser than others (arrow) and some display a $\sim 380\text{-\AA}$ periodicity (just below arrow). The filament-to-band angle is barely detectable. $\times 82,000$.

of projections from the thick filaments or is independent of them is not clear. The matrix is present throughout the A zone, but is most conspicuous in the middle. In some preparations (Fig. 4), fine bridges appear to link the midportions of neighboring thick filaments in much the same manner as M band bridges interconnect adjacent thick filaments in cross-striated muscle (8, 20). In the *Ascaris* muscle, however, discrete bridges are seen only infrequently and without the high degree of regularity exhibited by those of cross-striated muscle. This difference could reflect merely greater randomness in the three-dimensional orientation of the bridges such that fewer appear in any one plane. In addition, the "amorphous" component of this matrix appears denser in the *Ascaris* muscle than in cross-striated muscle. To what extent this represents a real structural difference and to what extent it can be attributed to differences in section thickness, staining method, or other preparative procedures is not clear. It can at least be said, however, that in the A zone of the *Ascaris* muscle, especially at its midportion, a matrix of moderate density, but

unknown composition surrounds the thick filaments. This matrix is not present in the I zone.

The three-dimensional structure of the muscle is such that the widths of the various zones that appear in transverse sections are directly proportional to the lengths of the corresponding bands in longitudinal section. It can be concluded, therefore, that the length of the thin filaments in each half sarcomere approximates that of the thick filaments in the whole sarcomere. Similarly the H band occupies approximately half the length of the A band or, in other words, approximately the terminal fourths of each thick filament are overlapped by thin filaments in the extended muscle.

Longitudinal sections confirm the inferences made from examination of the transverse plane. A and I bands are distinguishable; however, because in the extended muscle the bands form only a very small angle with the filament axis, instead of a right angle as in cross-striated muscle, the I bands appear to be shifted from the ends of the A band to its sides (Figs. 5 and 6). Indeed, from examination of longitudinal sections alone, one would probably conclude that the bands and

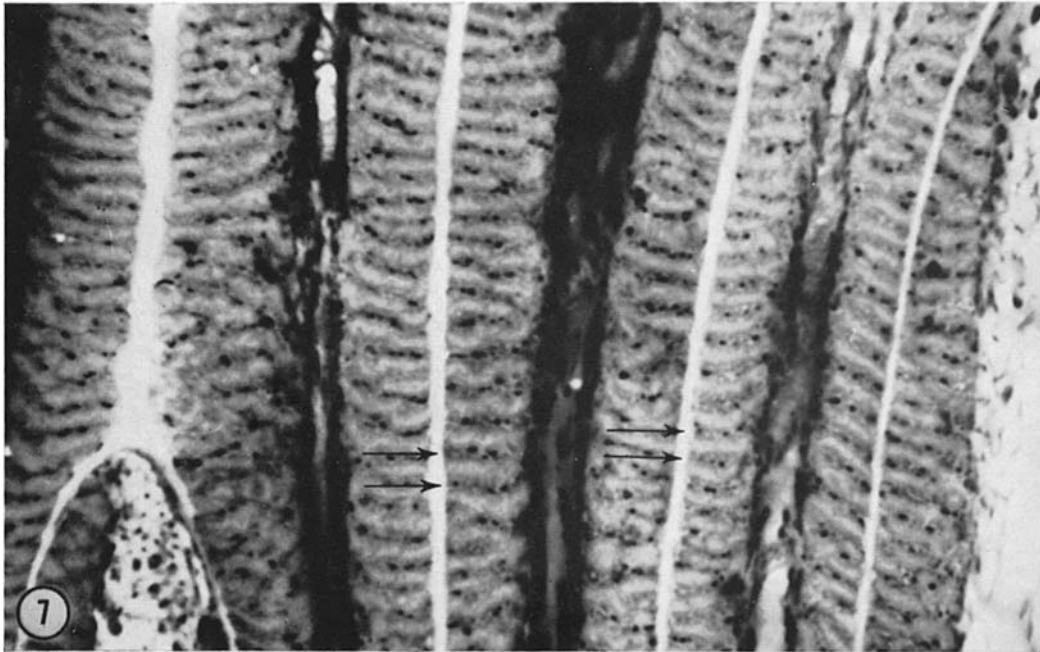


FIGURE 7 Photomicrograph of contracted muscle in transverse section. The band pattern is less regular than in extended muscle. Between each pair of beaded dense bands (arrows) there appear to be three bands of which the central one is lighter than those on either side. $\times 1,500$.

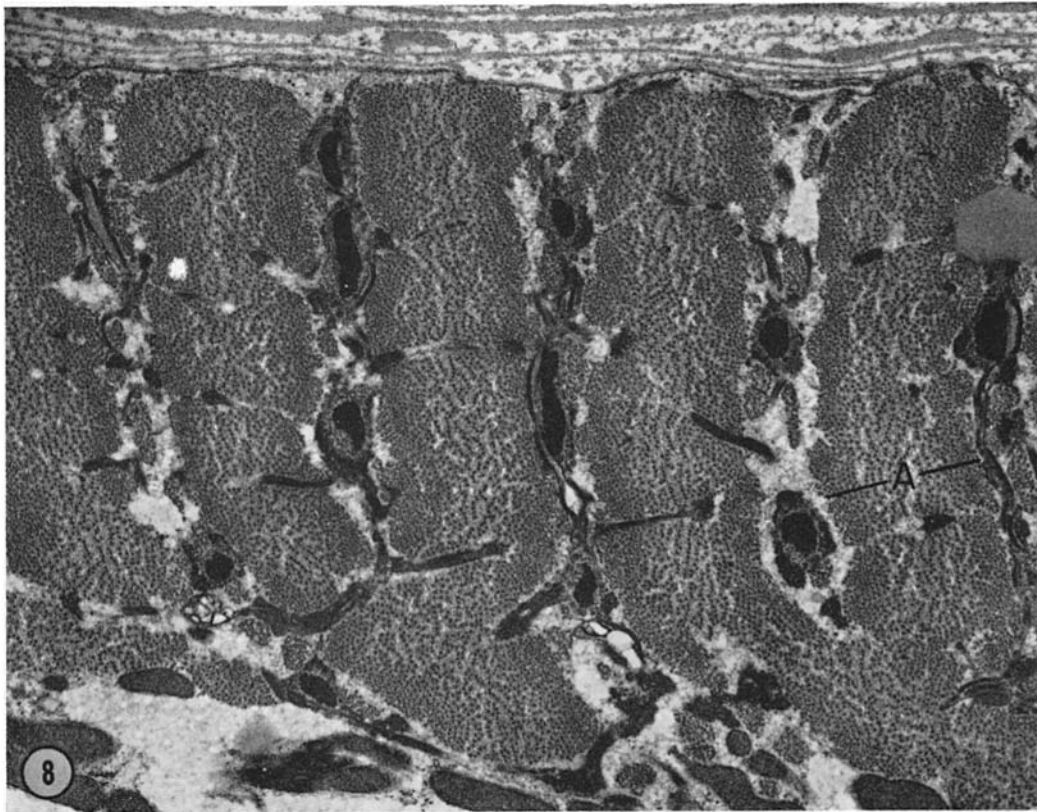


FIGURE 8 Electron micrograph of contracted muscle in transverse section. No I bands are present. In the middle of the A zones, rows of thick filaments are widely spaced. $\times 15,000$.

filaments are parallel to each other; it is only on close examination that the slight filament to band angle can be detected. The filaments of the intracellular skeleton, which are denser than the I band filaments, run in the dense bands in parallel with the I bands. Thus, filaments of three different kinds appear to course almost in parallel in extended specimens of this muscle.

Contracted muscle in transverse section (Fig. 8) differs in several respects. I zones consisting of thin filaments alone are virtually absent, and the H zone containing only thick filaments is replaced by a new zone of "double overlap" in which the rows of thick filaments are widely separated from each other by a double complement of thin filaments (Fig. 9). The width of the new central zone is approximately one-half the width of the A zone which now extends virtually all the way from one dense band to the next. The over-all width of the A zone is increased about threefold in the contracted

specimens, partly because the average lateral spacing of the thick filaments is increased and partly because the number of rows of thick filaments in the A zone is increased approximately twofold; that is, the A band in the contracted muscle consists of 20–30 rows of thick filament profiles instead of 10–15 as in the extended muscle. The implication of this difference is that the degree to which the thick filaments overlap each other is greater in contracted than in extended muscle. It was pointed out previously (24) that the number of rows of thick filaments per band in transverse section is inversely proportional to the degree to which neighboring thick filaments are displaced. If 10 rows of thick filaments appear per band, then the adjacent rows are displaced by $\sim 10\%$ of their length; if 20 rows, then the displacement is only $\sim 5\%$.

The angle of striation with respect to filament axis, as calculated from A band width and thick

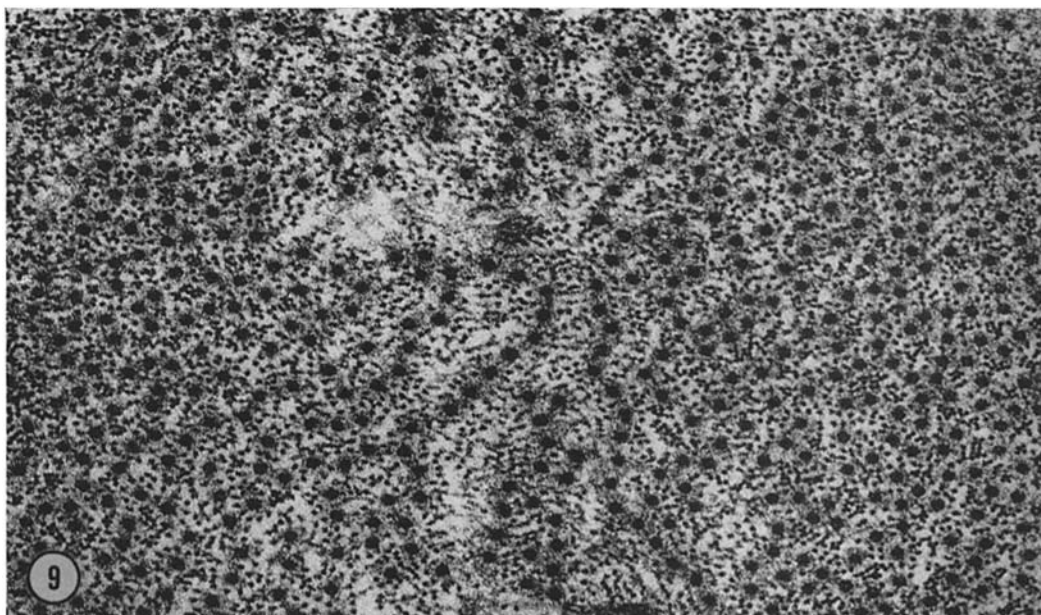


FIGURE 9 Contracted muscle, A zone. The space between the rows of thick filaments in the middle of the A zone is occupied by an excess of thin filaments. The members of each row and, in some cases, the adjacent rows appear to be glued together by an amorphous matrix in which no thin filaments are to be found. $\times 89,000$.

filament length (24), also increases about threefold, i.e., from $\sim 4^\circ$ in maximally extended muscle to $\sim 12^\circ$ in muscle fixed at minimum body length.

The matrix surrounding the thick filaments, which is so prominent in the H zone of the extended muscle, is visible in the contracted muscle as well. Although the *rows* of thick filaments are separated from each other by a double complement of thin filaments, the *members* of each row usually remain "glued" together by the matrix with no thin filaments interposed between them (Figs. 9 and 10). This close association between thick filaments is prominent in the midportion of the A zone of the *Ascaris* muscle; throughout the remainder of the A zone, however, even though a matrix of moderate density surrounds each thick filament, thin filaments commonly intervene not only between the rows of thick filaments but also between the members of each row.

In longitudinal sections of contracted muscle as in transverse sections, I and H bands are absent and multiple thin filaments can be seen separating the thick filaments (Figs. 12 and 13). In both contracted (Fig. 13) and extended muscle (Fig. 6),

some of the thin filaments exhibit an axial periodicity of ~ 380 A.

The thick filaments must bend during muscle shortening in order to become more widely spaced at their midportions than at their ends, as the traverse sections show them to be; and, indeed, in

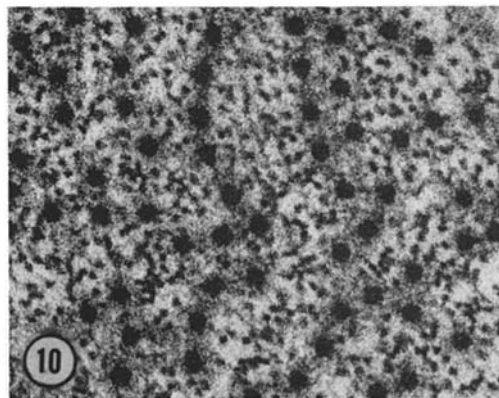


FIGURE 10 Contracted muscle, mid A zone. Some of the thick filaments appear to be interconnected. $\times 130,000$.

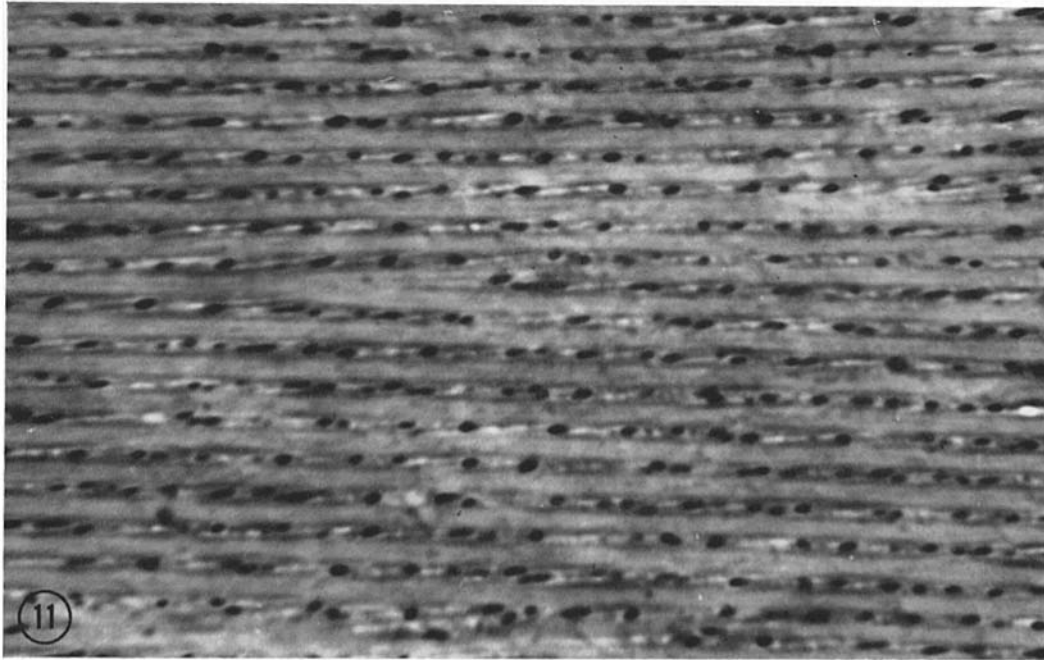


FIGURE 11 Photomicrograph of contracted muscle sectioned longitudinally. The dense bodies appear to be discontinuous and somewhat oblique in contrast to those in extended muscle (See Fig. 5, inset). $\times 1,600$.

longitudinal sections the thick filaments sometimes do appear to have a slightly sigmoid shape as Kawaguti et al. noticed previously (16). Whereas the obliquity of the bands to the filament axis is very inconspicuous in the extended specimens, it is clear in the contracted muscle. Because of the bending of the thick filaments, however, there is some ambiguity about the direction of the thick filament axis.

Thin filaments extending into opposite ends of dense bodies appear taut in the contracted muscle, but the filaments of the intracellular skeleton are markedly coiled and folded (Fig. 12).

Relationship of the Contractile Apparatus to Connective Tissue

The muscle fibers are surrounded individually by strands of connective tissue which are generally thicker than collagen fibrils and which display no periodic structure. In both longitudinal (Fig. 15) and cross-sections (Fig. 8), strands cut lengthwise can be found next to strands cut transversely or

obliquely. The connective tissue thus appears to form a stocking-like, two-dimensional network around each muscle fiber with components oriented longitudinally, circumferentially, and obliquely with respect to the muscle fiber axis. The connective tissue strands come into direct contact with the sarcolemma periodically all along its length and also follow the sarcolemma into the clefts that invaginate the fiber cortex (Fig. 16). In contracted fibers, some of the strands fold on themselves (Fig. 14 *a*) and in some cases, the redundant folds appear to fuse along their sides with neighboring connective tissue strands.

Adjacent muscle fibers are separated from each other by several layers of this connective tissue. In some instances, longitudinal and circumferential strands reside in alternate layers (Fig. 8, top left). Except at the distal extremities of the muscle arms, no instances have been seen in which muscle cells are directly joined together with no intervening connective tissue. The muscle fibers are, therefore, attached together only indirectly by way of the

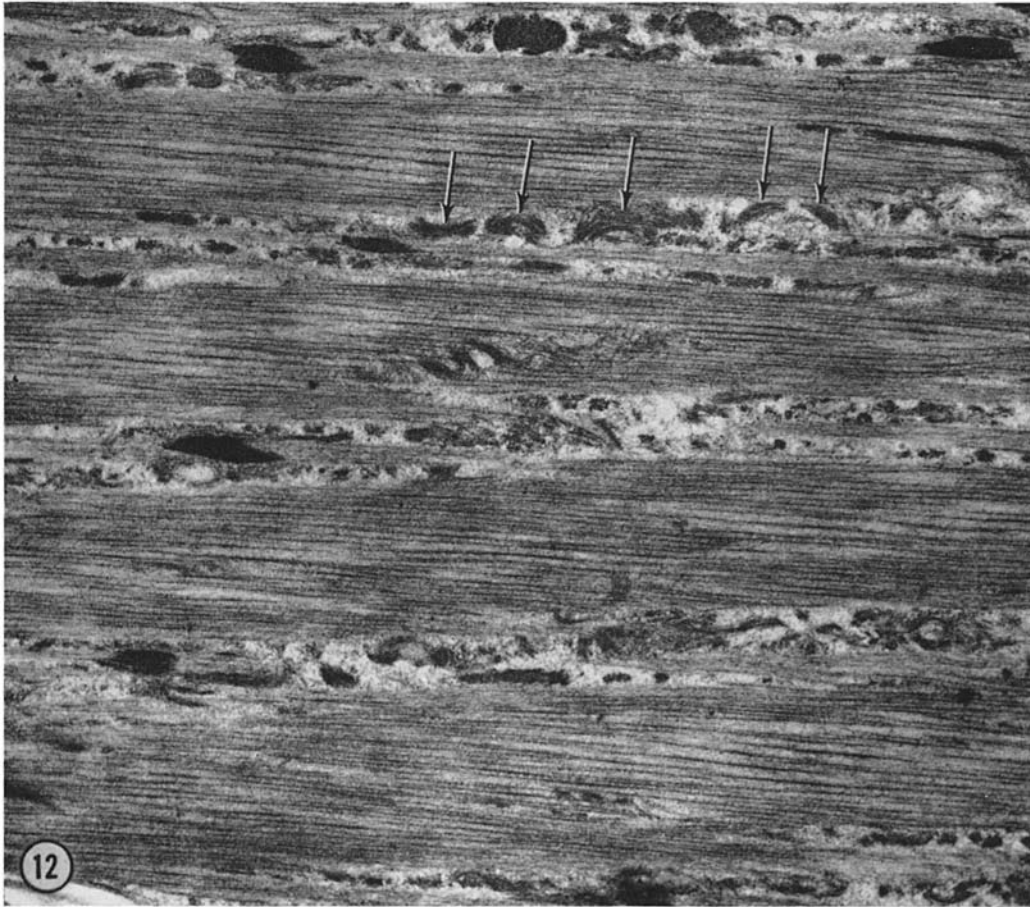


FIGURE 12 Electron micrograph of contracted muscle in longitudinal section. Thin filaments insert into opposite ends of the dense bodies. Profiles of coiled strands of the intracellular skeleton (arrows) occur in the dense bands between dense bodies, and occasionally among the myofilaments. Myofilaments are distinctly oblique to the axis of the dense bands. $\times 15,000$.

connective tissue network in which they are all embedded.

The contractile apparatus of the muscle cells is attached to the surrounding connective tissue in two ways. In some instances, bundles of intracellular skeletal filaments approach the plasma membrane obliquely and attach to its inner surface at a dense region resembling a hemidesmosome (Fig. 15). The outer surface of this specialized region of plasma membrane is, in turn, attached to a connective tissue strand. The second type of attachment (Fig. 14 *b*) consists of a dense body fused to the inner surface of the plasma membrane also immediately opposite a

connective tissue strand attached to the outer surface of the plasma membrane. Neither type of attachment is confined to the ends of the muscle cell. They occur at frequent intervals along its length both at its outermost surface and along the sides of plasma membrane invaginations (Fig. 16). Since the I band filaments attach to the dense bodies and since the dense bodies appear to be connected to each other by the intracellular skeleton, it is probably through these latter two structures, both of which are attached to the sarcolemma, that the contractile apparatus transmits its force to the surrounding connective tissue. The connective tissue network, in turn, is attached

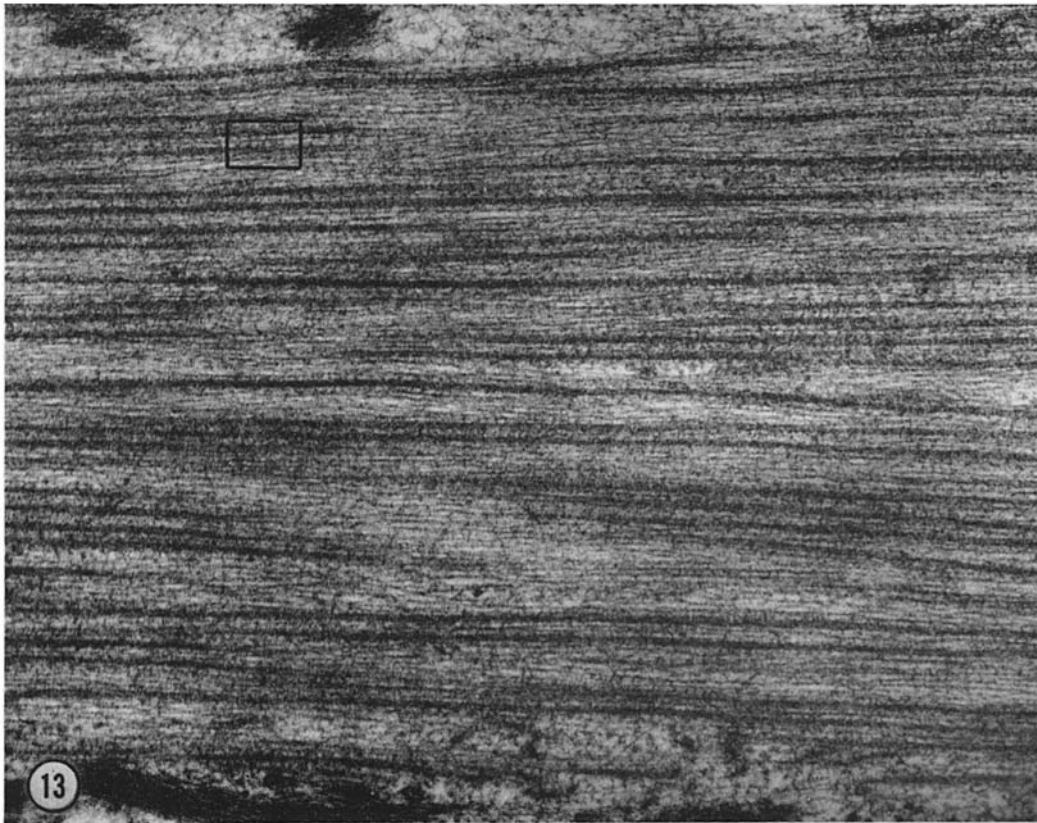


FIGURE 13 A band, contracted muscle in longitudinal section. An abundance of thin filaments is present throughout the band. In some regions, cross-links between thick and thin filaments are visible (box). In others, thin filaments exhibit a $\sim 380\text{-\AA}$ periodicity (cf. Fig. 6). $\times 56,000$.

to the hypodermis and cuticle all along its length. Presumably, this network is able to transmit the force developed by individual fibers or by short chains of fibers obliquely to the immediately adjacent cuticle, resulting in the shortening of localized segments of the worm. Contraction of successive small groups of fibers should, therefore, produce a wave of shortening traveling lengthwise along the animal.

DISCUSSION

In the original study of this muscle, it was surmised, on the basis of the presence of a double array of filaments with partial overlap between them, that a sliding filament mechanism occurs here as in cross-striated muscle (24). The present study supports this inference by showing that the degree to which thin filaments overlap thick ones

increases on contraction and decreases on extension. Moreover, it is shown that, at full contraction, a "double overlap" of thin filaments occurs in the middle of the sarcomere (cf. 9). The latter phenomenon has been described in cross-striated muscles and is believed to account, in part, for the diminution in maximum tension as they contract below minimum body length (11). Thus, despite its biochemical relationship to smooth muscle (2; M. Bárány, unpublished data), the interaction between thick and thin filaments in this obliquely striated muscle appears to be substantially like that in cross-striated muscle.

However, in order to account for the increased number of thick filament profiles per band in cross-sections of contracted muscle, it becomes necessary to postulate a second kind of movement consisting of a shear of the thick filaments with

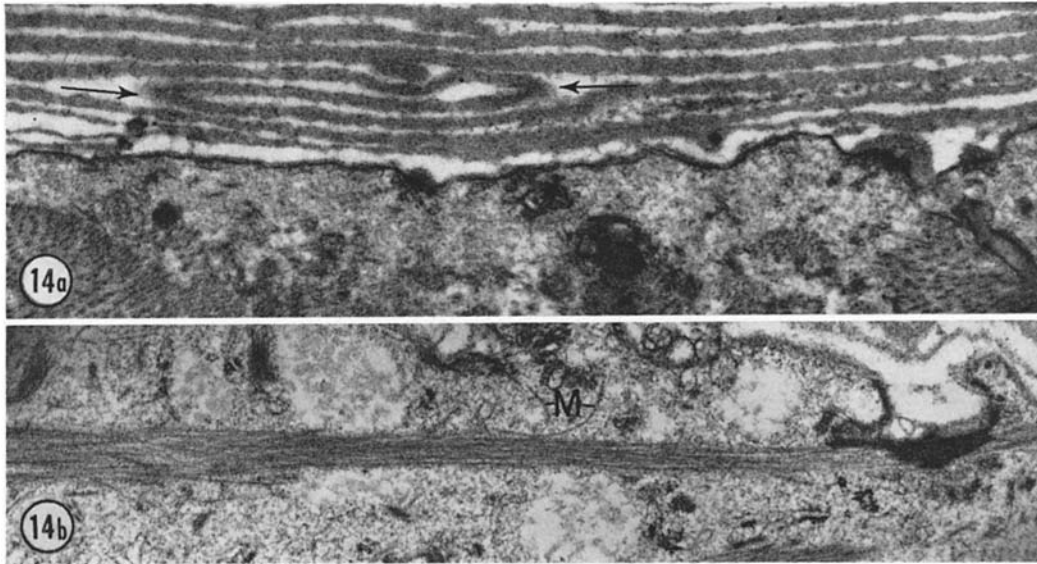


FIGURE 14 *a*, Contracted muscle sectioned obliquely. One of the connective tissue strands (arrows) folds on itself. *b*, Intracellular skeletal filaments insert obliquely into a dense body located at the cell surface. Microtubules (*M*) occur near the cell surface. *a*, $\times 20,000$. *b*, $\times 20,000$.

respect to one another (cf. 13). On contraction of the fiber, the thick filaments shear together; that is, the degree to which they overlap increases and the extent of stagger decreases. During extension the thick filaments shear apart. Because of the shear, the separation of the dense bodies increases on extension, and eventually the strands of the intracellular skeleton which appear to interconnect the dense bodies are pulled taut and presumably prevent further shearing. Shear in the opposite direction during shortening of the muscle results in a diminution in the distance between dense bodies and, correspondingly, the strands of the intracellular skeleton fold and coil. If no shearing occurred, then the reverse would be true; that is, assuming that the volume of the sarcomere remains approximately constant during the contraction-relaxation cycle (7), contraction of the muscle would result in spreading of the myofilaments in the direction perpendicular to their axis with the result that the dense bodies would tend to separate, whereas during muscle extension the dense bodies would approach each other.

Shearing probably accounts for the marked changes in band angle known to accompany length changes in muscles of this type (9, 19). If

the bands on opposite sides of an obliquely striated fiber are optically superimposed, they criss-cross (27), the angle between them increasing as the muscle shortens and decreasing again as the muscle is extended. This shift in angle can be explained very simply on the basis of changes in the degree of stagger of the myofilaments during contraction and extension.

The forces that might produce shear in either direction and the time at which shear occurs are unknown. During the active state, actin-myosin cross-links might be expected to impede shear. On the other hand, if, during shortening, one end of a thick filament reaches a dense body, further sliding of that thick filament in the same direction may be impeded. Because of the oblique array, however, the adjacent thick filament should still be able to slide freely over some distance before reaching the same dense body. As a result, the two thick filaments would tend to shear together and the angle of striation to increase accordingly.

External forces arising, for example, from the elasticity of the longitudinally oriented connective tissue strands around the fibers or from the contraction of antagonist fibers in the contralateral half of the worm might also operate to produce shear. In order to be effective, however,

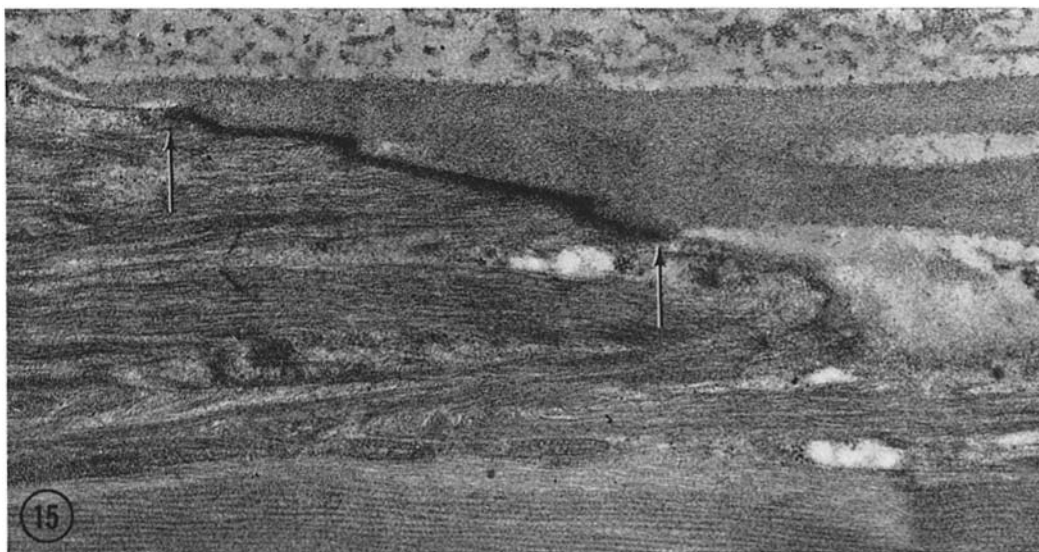


FIGURE 15 Extended muscle in longitudinal section. Intracellular skeletal filaments insert obliquely into a hemidesmosome (between arrows) at the cell surface. A thick connective tissue strand appears to be attached to the outer surface of the sarcolemma over the hemidesmosome. $\times 28,000$.

external forces would have to be applied non-colinearly to the sarcomeres. It is probably significant, in this regard, that connections between the contractile apparatus and the connective tissue occur all along the *sides* of the *Ascaris* fibers and are not confined to opposite tips. Smooth muscle cells also exhibit attachments to connective tissue all along their sides (23).

If the interaction between thick and thin filaments is comparable in this obliquely striated muscle to that in cross-striated muscle, what is the importance of the obliquity? Presumably, an arrangement which occurs so widely in invertebrate phyla has functional significance. On the basis of the ultrastructural organization of the *Ascaris* muscle, several possible functional consequences of the oblique arrangement have been inferred:

1. *Extensibility*

Some soft-bodied animals are capable of great variations in length and accordingly, must have muscles which are correspondingly extensible. The range of extension of cross-striated muscle is, however, limited to a maximum of approximately two- to threefold. Furthermore any change in the length of a cross-striated muscle is accompanied

by a change in the degree of overlap between thick and thin myofilaments and, correspondingly, by a change in the force which the muscle is capable of developing. In obliquely striated muscle these problems are circumvented, for here, length change by a different mechanism—shearing of the myofilaments—is possible (Fig. 17).

In cross-striated muscle, the Z lines are at minimum length; that is, any change in the 90° angle between the Z lines and the myofilament axis would require an increase in the length of the Z lines. Thus the Z lines, assuming they are inelastic, will tend to resist shearing and maintain the rectilinear array of the sarcomeres. In obliquely striated muscle, however, shearing of the myofilaments is not restricted in this way. Although the intracellular skeleton may impose a limit to the separation of dense bodies during muscle extension, shearing together of the myofilaments and dense bodies on shortening would have no such limitation and presumably could continue until the oblique striations become transverse (cf. 10). The muscle is, therefore, capable of a markedly increased range of length change. Furthermore, there need be no relation between the degree of overlap between thick and thin filaments and the degree of stagger of adjacent thick filaments.



FIGURE 16 Contracted muscle in transverse section. Intracellular skeletal filaments (arrow) insert into a hemidesmosome located deep in a plasma membrane invagination. A connective tissue strand extends into the invagination. $\times 17,000$.

Accordingly, length and tension could be dissociated in muscles of this kind.

2. Velocity of Length Change and Plasticity

Because of the stagger of adjacent thick filaments, a single sarcomere extends obliquely along a considerable length of the fiber (cf. 13). In a fiber at rest length, if the myofilaments were assumed to be approximately parallel to the fiber axis and the angle of striation to be 6° , then a sarcomere beginning on one side of a fiber 100μ up from the hypodermis would extend obliquely down the side of the fiber along a length of 1 mm and then obliquely back up the other side along another millimeter to a point again 100μ up from the hypodermis. The sarcomere would describe a half-turn of a helicoid somewhat flattened from side-to-side, and the length of this half-turn would approximate that of the fiber itself. If the fiber were O-shaped as in cephalopod muscles (1) rather than U-shaped—that is, if the contractile cortex continued around the whole

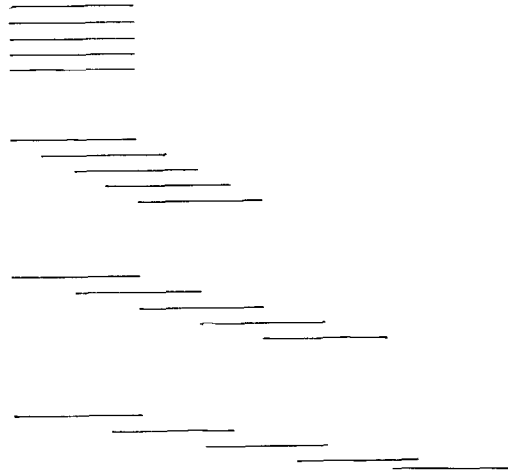


FIGURE 17 Length change by shearing. The band-to-filament angle decreases from 90° at the top to about 10° at the bottom. Approximately every 15th thick filament is shown.

periphery of the fiber—the helicoid would cycle along the entire fiber regardless of its length.

As is pointed out in the preceding section, such a bundle of filaments could undergo marked changes in length by shearing. However, evidence is presented here that the midportions of the thick filaments are adherent. Depending on the interaction of the thick filaments with one another and with the matrix surrounding them, shearing might, therefore, be a very slow process. The matrix might be highly viscous; or under certain conditions, related perhaps to pH or ionic milieu, the thick filaments might become rigidly bound together so that length change by shearing would virtually cease. If the thick filaments interacted with one another all along the length of their overlap, then the impedance to shearing would be approximately proportional to sarcomere length; if the thick filaments' interaction occurred only where there were no intervening thin filaments, then the impedance to shearing would be proportional to the length of the H band. In the latter case, during muscle contraction thin filaments might extend past the midline and prevent interaction between the thick filaments so that *shortening* by shearing might occur readily. An externally applied stress to the shortened muscle might then remove the thin filaments from the center of the sarcomere, thereby permitting interconnections between the thick

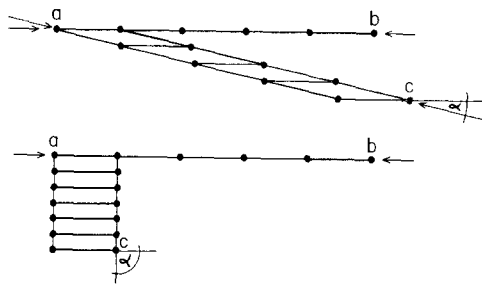


FIGURE 18 Contraction of a single sarcomere in obliquely striated vs. cross-striated muscle. In the obliquely striated muscle (upper diagram) if one sarcomere contracts, tension develops between *a* and *c*. If angle α is small, the longitudinal component of this vector is large. Contraction of the series of sarcomeres results in tension development between *a* and *b*. In the cross-striated muscle (lower diagram), contraction of one sarcomere results in negligible tension between *a* and *c*. If α is 90° , the longitudinal vector is zero. Contraction of the series of sarcomeres still results in tension development between *a* and *b*.

filaments to form, with the result that the rows of thick filaments might not be readily able to shear apart. Thus, the muscle fiber could become "reset" at the new shorter length. If the thick-thick interconnections were plastic and permitted slow shear over an extended period, then a continuous external stress would gradually restore the original length of the fiber. On the same basis, tension produced by an external stress would be expected to decay gradually (cf. "stress relaxation" described in reference 3); and, similarly, tension produced by active contraction when the length of the fiber was fixed would also be expected to decay gradually, the sliding of thin filaments with respect to thick providing the force tending to shear the thick filaments apart. In short, the oblique—almost longitudinal—bundles of thick myofilaments may constitute a viscous resistance whose magnitude depends on the nature and extent of interaction between thick filaments, and which may be responsible for the slow time course of length changes in this muscle and for other manifestations of plasticity.

The same considerations may apply to invertebrate smooth muscles, which also possess thick filaments, but lack Z lines. In the limiting situation, rigid binding of thick filaments to each other at their midportions could also account for the phenomenon of "catch," provided the over-

lapping bundles of thick filaments extended the whole length of the fiber (cf. 14). Vertebrate smooth muscle is not known to possess thick myosin filaments; however, if there were lateral interaction between actin filaments, this, too, could result in impedance to shear and other plastic properties.

3. Independent Linkage of Sarcomeres to Connective Tissue

A cross-striated myofibril consists of a large number of sarcomeres linked together in series and attached at the ends to the fiber containing it. In order for contraction of such a fibril to be most effective, the members of the chain should contract synchronously. If only one sarcomere were to contract and the rest remain relaxed, the primary effect of the contracting sarcomere would be merely to stretch the relaxed sarcomeres in series with it; little of the force developed by the contracting sarcomere would be transmitted by way of the relaxed sarcomeres to the connective tissue at the ends of the fiber. It might be possible for some force to be transmitted to the endomysium along the sides of the fiber by way of the Z lines which extend to the sarcolemma; however, because the Z lines are oriented perpendicularly both to the endomysium and to the direction of the force developed by the sarcomere, they would be in a poor position to transmit the force of contraction effectively (Fig. 18, bottom). Thus, contraction of a single sarcomere in such a fibril would be expected to result primarily in internal shifts in sarcomere length within the fibril, but not in significant tension development in the tendinous structures between which the fibril is attached.

This may not be true of the obliquely striated muscle of *Ascaris*. If it is assumed that, on extension, the dense bodies are interconnected by taut strands of the intracellular skeleton and that the thin filaments insert into the dense bodies, then the intracellular skeleton would be well adapted to transmit the force of contraction of a single sarcomere, or even only part of a sarcomere, to the surrounding connective tissue even though the other sarcomeres in series with it were slack. Since the intracellular skeleton inserts obliquely into the sarcolemma and is nearly parallel both to the myofilaments and to the surrounding connective tissue,

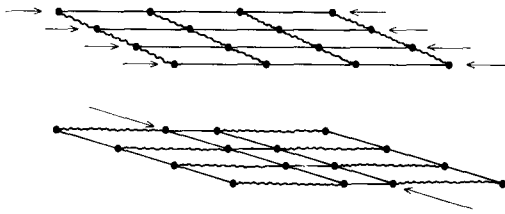


FIGURE 19 Synchronous vs. asynchronous contraction of sarcomeres in obliquely striated muscle. In the upper diagram, the sarcomeres contract synchronously and the intracellular skeleton is taut. Tension is transmitted longitudinally between the arrows through the series of sarcomeres. In the lower diagram, only the middle sarcomere contracts and the intracellular skeleton is taut. Even though the two lateral sarcomeres are slack, tension can still be transmitted along the intracellular skeleton between the arrows in a direction oblique to the myofibril axis. Wavy lines represent slack links; straight lines are taut links.

the force developed by the one sarcomere would be transmitted with little loss, and would virtually equal the force developed by contraction of the entire fiber² (Fig. 18, top). Furthermore, since the sarcomere extends along a considerable length of the fiber, the force would be developed between widely separated points on the fiber surface. Thus, because of the oblique arrangement, such a fiber under stretch could generate and maintain tension isometrically even if only a small portion of the contractile apparatus were active. The fiber could, therefore, subserve a tonic function very efficiently, if, for example, successive sarcomeres were to contract independently but in sequence. Even in a shortened fiber, in which the intracellular

² The tension developed by contraction of a number of sarcomeres in series is no greater than that developed by any one member of the series (see reference 23 for discussion).

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skeleton was slack, tension developed by a single sarcomere could be transmitted through that sarcomere by way of the actin-myosin links. In this case, however, the tension would be much smaller, equaling only that developed by the terminal members of the bundle. Regardless of whether the tension developed by contraction of a single sarcomere was carried by the intracellular skeleton or by the cross-linked actin and myosin filaments, the tension would be transmitted in a direction parallel to the sarcomere and slightly oblique to the filament axis. If all the sarcomeres contracted simultaneously, then the fiber would be equivalent to a cross-striated myofibril and the force of contraction would be transmitted along the chain of sarcomeres in a direction parallel to the filament axis and slightly oblique to the sarcomere axis. In short, the oblique arrangement may be adapted for either synchronous or asynchronous contraction of the sarcomeres (Fig. 19).

These various possible consequences of the oblique pattern are of interest partly because all of them—the additional mechanism for length change, the slow time course of length changes and plasticity, and the independent linkage of sarcomeres to connective tissue with the consequent potential for great efficiency in maintaining tension under stretch—are all relevant to properties of smooth muscle. Even though the basic process of contraction seems to be the same in the *Ascaris* muscle as in vertebrate cross-striated muscles, the oblique organization itself may provide a structural basis for properties like those of smooth muscle.

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