OBLIQUELY STRIATED MUSCLE

IV. Sarcoplasmic Reticulum, Contractile Apparatus, and Endomysium of the Body Muscle of a Polychaete, *Glycera*, in Relation to Its Speed

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ABSTRACT

Body muscle cells of the bloodworm *Glycera*, a polychaete annelid, were studied by electron microscopy and compared with muscle cells of the more slowly acting nematode *Ascaris*, which have been described previously. Both muscles are obliquely striated. The predominant type of bloodworm fiber is characterized by a prominent transversely oriented sarcoplasmic reticulum with numerous dyads at the surface of each cell. Thick myofilaments are ~3 μ long and overlap along ~60% of their length in extended fibers and ~80% in shortened fibers. There is virtually no endomysium and very little intracellular skeleton, and the cells are attached by desmosomes to one another rather than to connective tissue. Dense bodies are absent from the fibers and in their place are Z lines, which are truly linear rather than planar. Scattered among the predominant fibers are others, less orderly in arrangement, in which the SR is much less prominent and in which the thick filaments are thicker and longer and overlap to an even smaller degree. It is suggested that physiological differences between bloodworm and *Ascaris* muscles derive from differences in the proportion of series to parallel linkages between the contractile elements, differences in the amount and disposition of the SR, and differences in the impedance to shear within the myofibrils.

INTRODUCTION

In the previous papers in this series, it was shown that *Ascaris* body muscle, which has many of the properties of a smooth muscle and, indeed, was long considered to be one, in fact has a very regular "obliquely striated" organization (24, 25). Analysis of this muscle at shortened and extended lengths indicated that the sliding filament hypothesis (13, 14) is applicable to it as well as to cross-striated muscle, but that the peculiar structure of *Ascaris* muscle has other potential consequences not relevant to cross-striated muscle (27). Specifically, it was suggested that the oblique arrangement could confer great extensibility and other plastic properties onto the fibers and, because of the prominence of the intracellular skeleton and connective tissue investment of the fibers, could also permit them to maintain tone effectively with only partial or asynchronous contraction.

The polychaete *Glycera* is another example of a class of animals whose body muscle fibers appear, by light microscopy, to be composed of unstriated, longitudinally oriented myofibrils (5). Like *Ascaris* fibers, these, too, prove to have a

typical obliquely striated organization; i.e., myofilaments are staggered in a regular manner so that a line drawn through corresponding points on adjacent filaments forms an angle of only a few degrees with the filament axis instead of a right angle as in cross-striated muscle. *Glycera* differs from *Ascaris*, however, in that its movements are visibly much more rapid, and a question arises as to whether this difference in speed has any structural basis in the muscle cells of the two animals.

Fast and slow cross-striated muscles have been shown to differ with respect to their innervation (11), myosin ATPase activity (3), amount and disposition of sarcoplasmic reticulum, and details of myofibrillar structure (11, 18). In the present study, differences in the sarcoplasmic reticulum, contractile apparatus, and endomysium of *Glycera* and *Ascaris* obliquely striated muscles are demonstrated and their functional implications discussed.

MATERIALS AND METHODS

Live bloodworms, *Glycera dibranchiata*, are readily obtainable along the Atlantic coast; specimens used in this study were purchased at local fishbait shops and stored in the refrigerator. Only animals exhibiting active motility on being returned to room temperature were used. Fixation was accomplished by pinning the worms onto a wax plate, slitting the animals dorsally and opening them out, and then flooding the interior with a 3% solution of glutaraldehyde (biological grade) in 0.1 M phosphate buffer (pH 7.4). The fixative was used at room temperature. Specimens were fixed at shortened, extended, and intermediate lengths. Shortened specimens were obtained either by pinching the worm or by soaking in a 10% solution of ethyl carbamate before pinning it. Extended specimens were obtained by soaking in 7.5% $MgCl_2$ until completely flaccid or by stretching. After approximately 1–3 hr in glutaraldehyde, the pinned segments were separated from the rest of the worm, rinsed in sea water, or 0.9% NaCl, and transferred to a solution of 1 or 2% osmium tetroxide in acetate-Veronal buffer (pH 7.4) for an additional 1–3 hr. The fixed segments were then dehydrated in a graded series of methanol solutions and embedded flat in Araldite (17). Sections were cut with a Porter-Blum MT-2 microtome, mounted on bare or carbon-coated grids, stained with uranyl acetate or lead hydroxide or both, and examined with a Philips EM 200, a Zeiss EM 9, or a Siemens 1 electron microscope at 60, 60, and 80 kv, respectively.

OBSERVATIONS

In cross-sections through the body wall, longitudinally oriented muscle fibers are cut transversely and appear as striated elliptical profiles. The striations are perpendicular to the long diameter of the ellipse (Fig. 2) and run all the way across each fiber uninterrupted by a sarcoplasmic core such as occurs in *Ascaris* fibers (25). The maximum cross-sectional dimensions of extended fibers are approximately 4 × 20 μ and of shortened fibers 4 × 30–40 μ; i.e., the volume redistribution that occurs during muscle fiber shortening is accompanied by an increase in only one diameter. Among these profiles are others of smaller size (Fig. 2, upper left), which presumably represent sections across the tapered ends of the fibers.

In longitudinal sections parallel to the short diameter of the ellipse the fibers have broad, virtually transverse striations (Fig. 1), and in longitudinal sections parallel to the long diameter of the ellipse the fibers exhibit fine striations which are almost longitudinal in orientation (Fig. 3). This apparent change in striation angle with plane of section is characteristic of obliquely striated muscle and can be calculated for any plane of section (25).

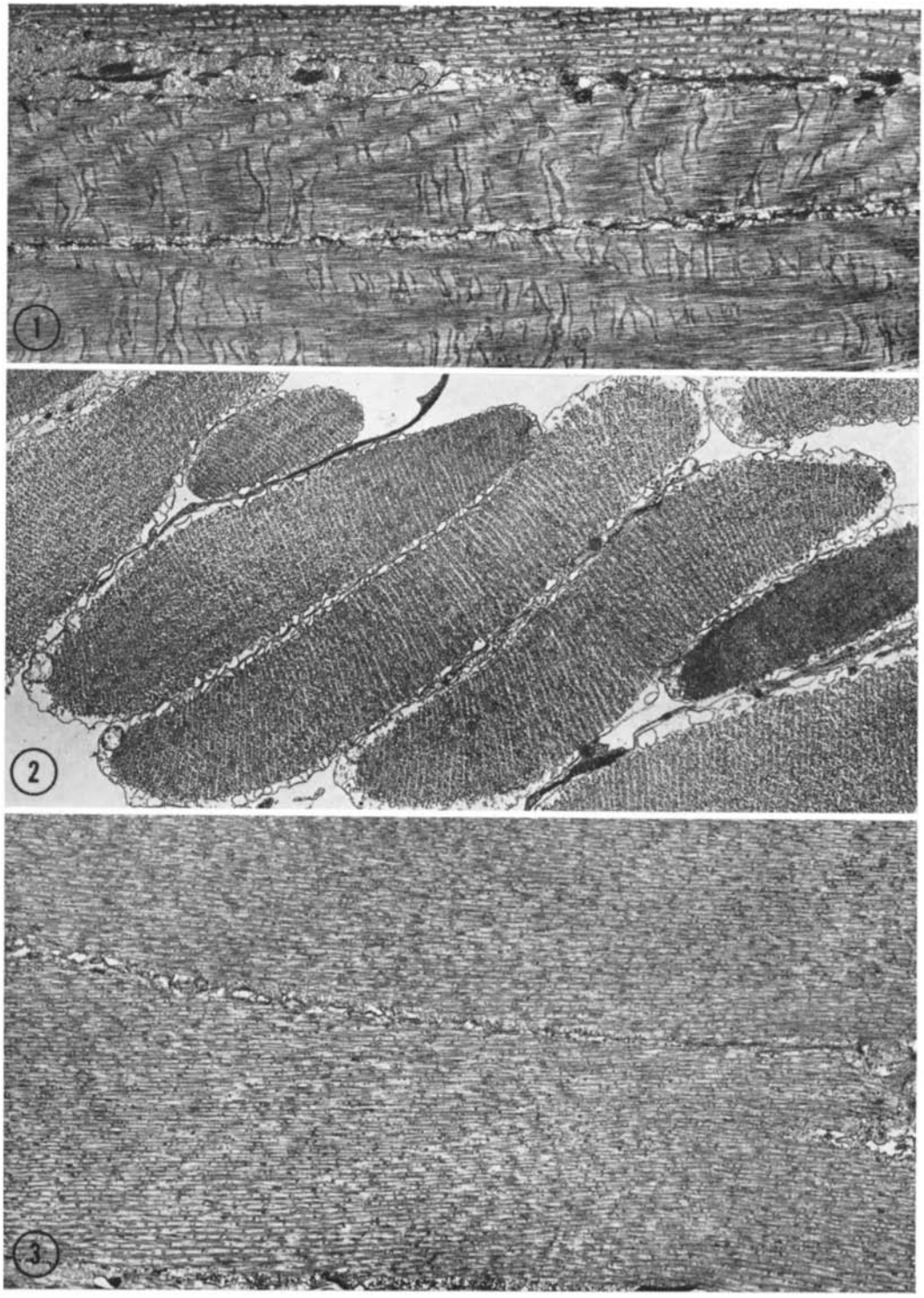
Nonmyofibrillar components—the nucleus, mi-

FIGURES 1–3 Muscle fibers fixed in extension and sectioned in three different planes.

FIGURE 1 Longitudinal section. The middle fiber is cut through its short diameter. In this plane, striations are broad and almost transverse. × 5000.

FIGURE 2 Cross-section. Each fiber has a long and a short diameter. Fine striations run parallel to the short diameter. The variation in dimensions of the fiber profiles suggests that the fibers taper longitudinally. In contracted fibers the short diameter is approximately the same size as here, but the long diameter is increased about twofold. × 5000.

FIGURE 3 Longitudinal section. The fibers shown are cut through their long diameters. In this plane, striations are fine and virtually longitudinal. × 5000.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tochondria, granular endoplasmic reticulum, glycogen, lipid, and fibrillar bundles—reside in tag-like processes which attach along the narrow edges of the fiber and extend out among neighboring cells (Fig. 15). These components are almost completely segregated from the myofibrillar part of the muscle.

Contractile Apparatus

Electron micrographs of transversely sectioned muscle fibers (Figs. 4, 7, 8) reveal the presence of thick and thin myofilaments in a regularly repeating succession of bands which are, however, narrower (0.2–0.3 μ) and, because of the absence of dense bodies, less distinct than the corresponding bands in *Ascaris* muscle fibers. Each band contains an A zone consisting of two to three rows of thick-filament profiles in extended fibers (Fig. 7) and five to six rows in shortened fibers (Fig. 8). The stagger, or displacement, of the adjacent rows of thick filaments is approximately equal to the reciprocal of the number of rows of thick filaments per band in transverse section, and is, therefore,

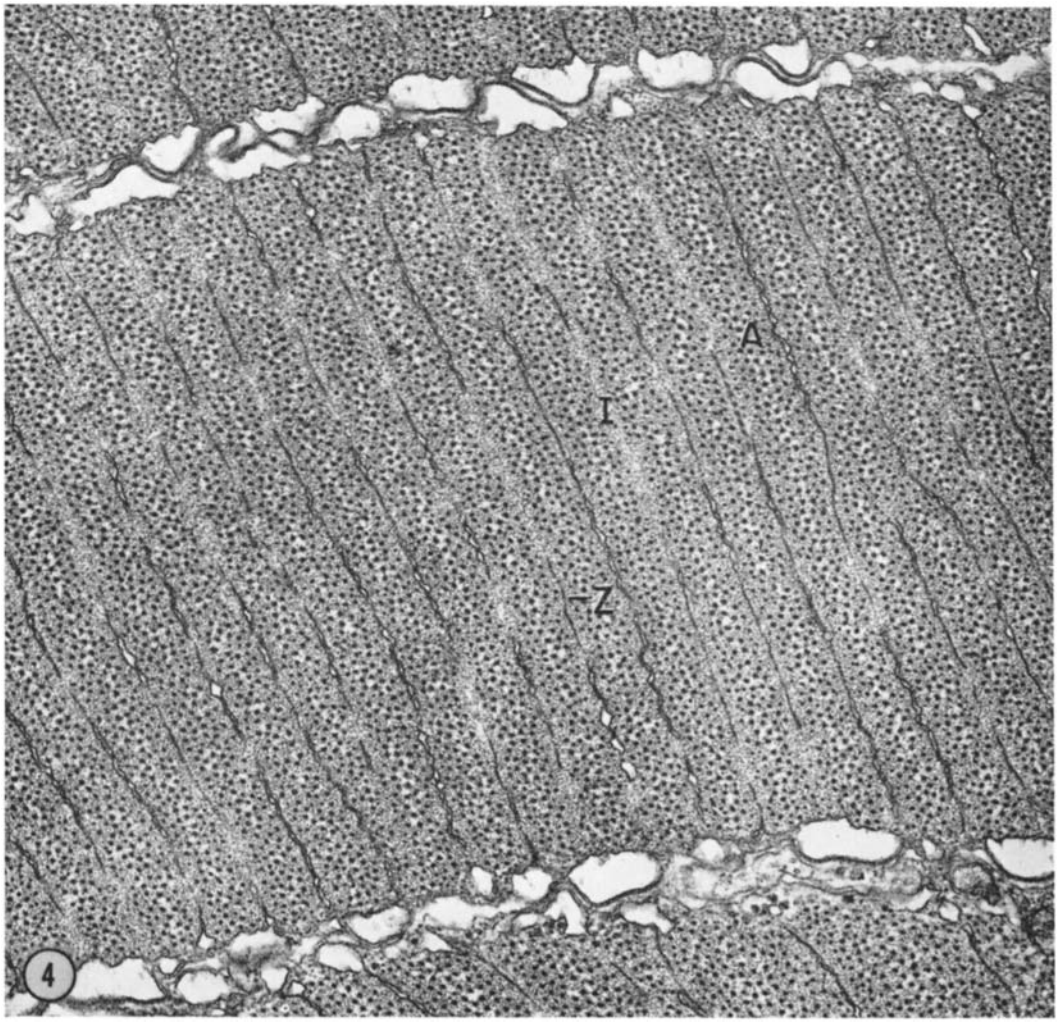


FIGURE 4 Cross-section through three fibers fixed at intermediate length. Their surfaces are marked by prominent vesicular profiles. The striation pattern in this plane is produced by a regular succession of A and I zones. The latter are frequently, but not always, divided in half by either Z lines or transverse sarcotubules. × 24,000.

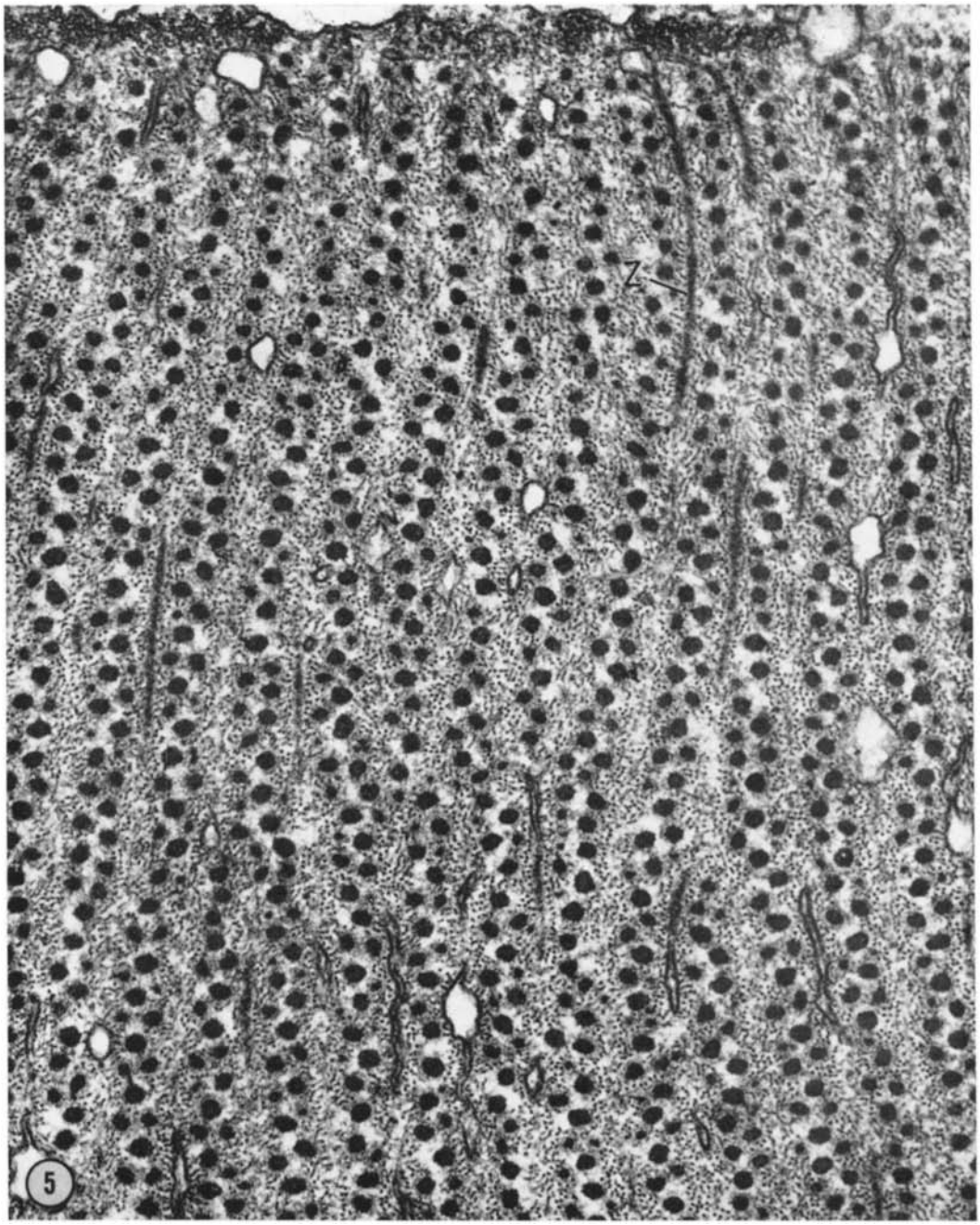


FIGURE 5 Cross-section of a fiber in which the thick filament profiles are approximately 550 μ in maximum diameter. The Z lines are thick; the sarcotubules are sparse; and the striation pattern is somewhat irregular. Profiles of this kind and that shown in Fig. 6 occur among the predominant type shown in Figs. 1–4. × 58,000.

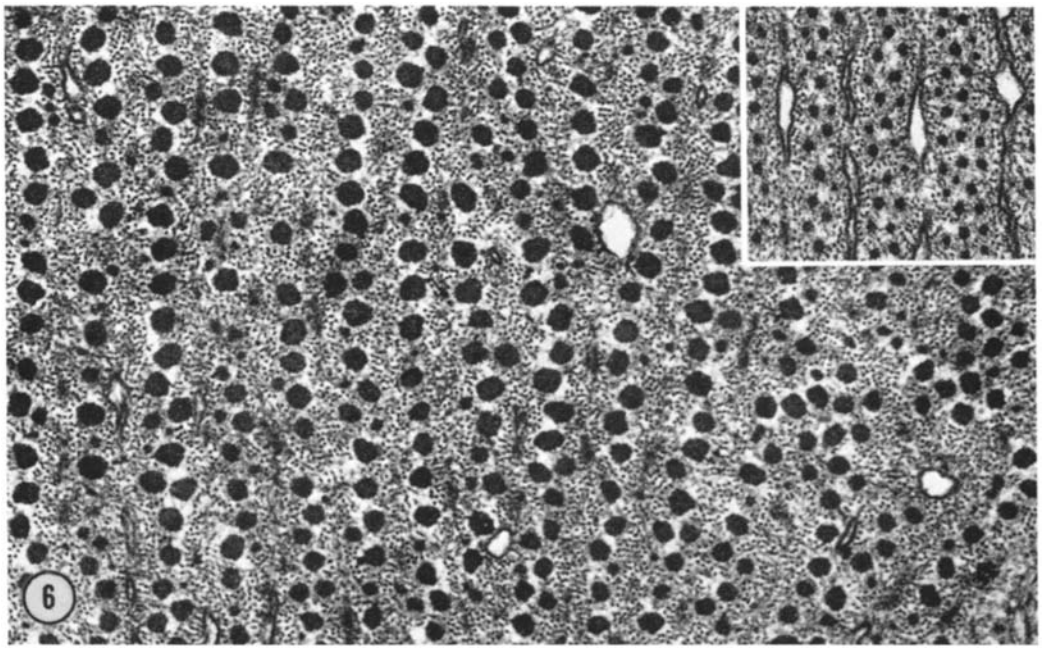


FIGURE 6 Cross-section of a fiber similar to that in Fig. 5, but containing even larger-diameter thick filaments and sparser SR. *Inset:* Fiber of the predominant type, for comparison. × 58,000. Inset, × 58,000.

about 40% of the thick filament length in extended muscle and 20% in shortened muscle. Thus, the stagger in bloodworm muscle is considerably greater than in *Ascaris* muscle, where there are 10–15 rows of thick filaments per band in cross-sections of extended fibers and 20–30 rows in shortened fibers (27), corresponding to displacements of approximately 8 and 4%, respectively.

Thin-filament profiles occur among the thick filaments in an irregular manner and, presumably because of the marked stagger in the bloodworm muscle, H zones or zones of "double overlap" are not clearly defined in transverse sections. Thick filaments are ~320 A in maximum diameter and the A zone width is ~0.1 and 0.25 μ in extended and contracted fibers, respectively.

The thick filaments in *Ascaris* muscle fibers are surrounded, and apparently interconnected, by a moderately dense matrix material which is most prominent in the middle of the A zone (27). Some comparable matrix material also occurs in bloodworm fibers, but it is much sparser and less conspicuous (Figs. 7, 8). On either side of the A zone in extended fibers are very narrow I zones composed of thin filaments alone (Fig. 7), but in shortened fibers the I zone is virtually absent (Fig. 8).

Fibers of the type just described predominate in the muscle, but scattered among them are a smaller number of profiles in which the thick filaments are ~550 A in maximum diameter (Fig. 5) and a few in which the thick-filament diameter reaches ~670 A (Fig. 6). In the latter two types of fiber, the number of rows of thick filaments in each repeating unit is about half that in the predominant fiber type and the thin-to-thick filament ratio is increased considerably. The fibers are less orderly in organization than those of the predominant type and their Z lines are thicker.

Dense bodies, which are so prominent in *Ascaris* fibers, are absent from the bloodworm muscle, and in their place, at the middle of the I bands, are Z lines[1] (Figs. 4–8, 13, 14). These exhibit a fine

[1] The Z lines described here are probably equivalent to the "compact sarcotubules" (21) and "cross-filaments" (23) described in leech muscles, and to the "J-particles" (16) and "J-rods" (15) in earthworm muscles. They are probably also responsible for the "stripes" described in earthworm muscle (8). Z lines have also been reported in other polychaete muscles (2).

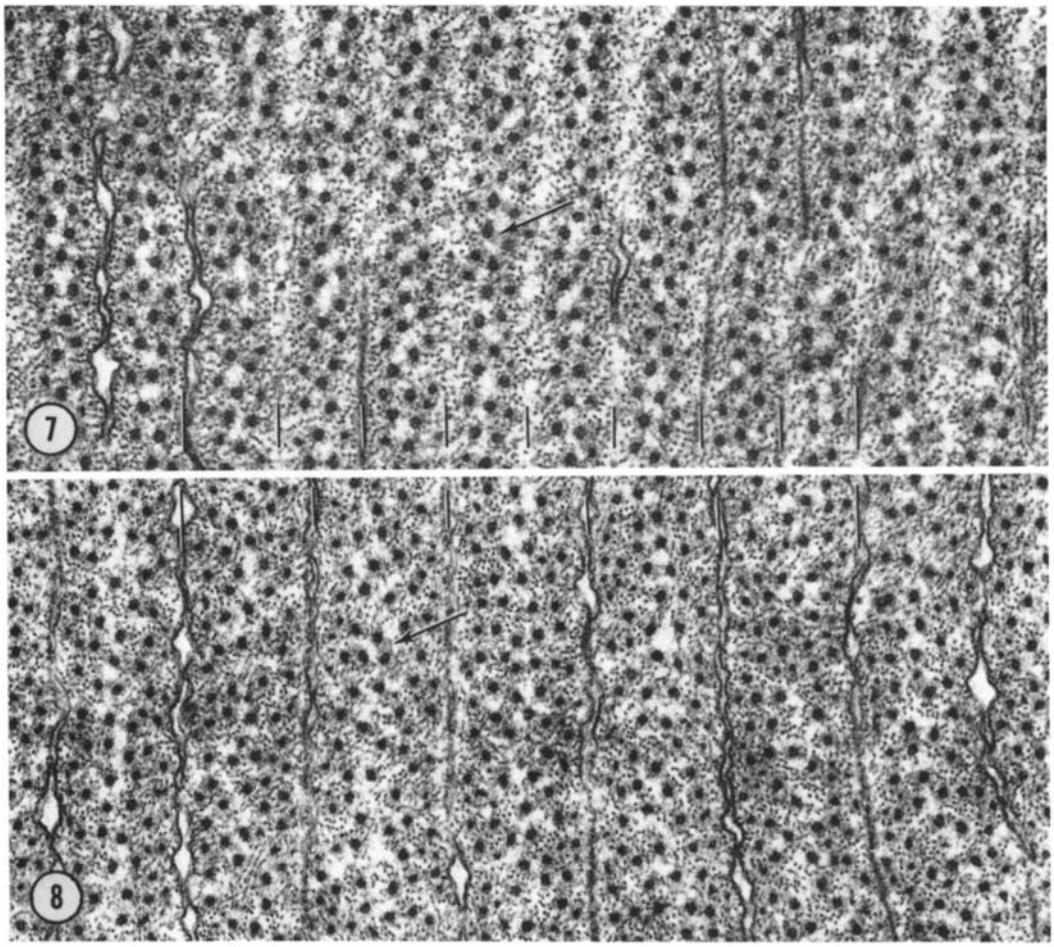
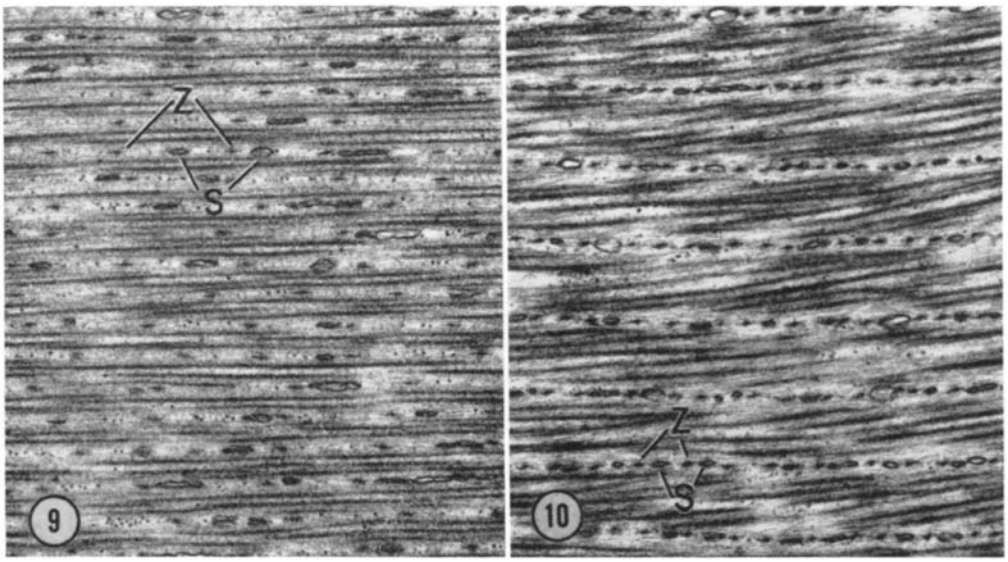


FIGURE 7 Extended. Each repeating unit is ~0.2 μ wide and consists of two to three thick-filament profiles (A zone) flanked by thin-filament profiles (I zone). Some of the I zones are bisected by Z lines or transverse sarcotubules, but most are not. Thin filaments surround thick filaments irregularly in the A zone; the H zone is not well defined. Arrow indicates matrix material between thick filaments. Z lines exhibit a faint axial periodicity (cf. Figs. 5, 13). Eight repeating units are marked off. × 60,000.

FIGURE 8 Shortened. The repeating unit here measures ~0.3 μ and consists of five to six thick-filament profiles surrounded irregularly by thin filaments. I and H zones are virtually absent. Matrix material between thick filaments is still visible (arrow). Adjacent A zones are separated from each other by Z lines or sarcotubules. The basis for the apparent increase in Z lines and SR in comparison with extended muscle is shown in Figs. 9 and 10. Five repeating units are marked off. × 60,000.

cross-striation presumably due to the orderly insertion of thin filaments into them. In transverse sections (Figs. 4–8) and in longitudinal sections cut perpendicular to the long diameter (Figs. 1 and 13), the Z lines are linear but appear not to run all the way across the I zones, and considerable portions of I zones are undivided and extend without interruption from one A zone to the next. The reason for this apparent discontinuity is clear in longitudinal sections cut parallel to the long diameter (Figs. 9, 10, 14). In this plane, each I band is divided in half by a row of sarcotubules in cross-section (see below). Between each two such profiles are one or several nodal densities, marking



FIGURES 9 and 10 Extended vs. shortened muscle fibers in longitudinal section (parallel to the long diameter; cf. Fig. 3).

FIGURE 9 Extended. Each A band consists of about two overlapping thick filaments. The A bands are separated by I bands, each of which is divided in half by a single row of oval sarcotubular profiles (*S*) alternating with cross-sections through Z lines (*Z*), both of which are discontinuous in this plane. Thus, a transverse section is apt to pass between them, resulting in the appearance of undivided I zones as shown in Fig. 7. × 24,000.

FIGURE 10 Shortened. I bands are virtually absent; thick filaments are separated by thin filaments; and sarcotubular (*S*) and Z line (*Z*) profiles, although discontinuous, are much closer together than in Fig. 9. A cross-section is, therefore, much more likely to include either a Z line or a sarcotubule between each pair of A zones (cf. Fig. 8). The angle of striation with respect to thick filament axis is noticeably greater than in Fig. 9, and the A bands are much wider in the vertical direction. × 24,000.

the junctions between thin filaments of the two half I bands. The nodal densities, which are interpreted as cross-sections through Z lines, are, however, disconnected from one another in this plane, and for this reason a section cut in a perpendicular plane may miss a Z line altogether or the Z line may run in the plane of section along part of its length and then pass out of the plane of section again. The Z lines of the bloodworm muscle are, therefore, truly *lines* in contrast to the planar Z *discs* of cross-striated muscle. Thus, whereas the myofilaments of cross-striated muscle are bound together into three-dimensional sheaves by Z discs, in the bloodworm muscle they are bound together into two-dimensional plates by Z lines. Since the plates are not connected to each other, slippage or shearing of the plates with respect to each other is possible and, indeed, occurs in this muscle as well as in *Ascaris* muscle.

Interdigitation of thick and thin myofilaments can be seen in longitudinal sections (Figs. 10, 13), and occasionally one thick filament remains in the plane of section almost all the way from one I band to the next. The maximum observed length of the small-diameter thick filaments is approximately 3 μ, i.e. about half the length of the thick filaments in *Ascaris* muscle fibers. The wider-caliber thick filaments are considerably longer. On the basis of a thick-filament length of 3 μ and an A-band width of 0.1–0.25 μ, the angle of striation to thick-filament axis is approximately 2–5° in the longitudinal plane parallel to the long diameter. (See reference 25 for calculation.) The sarcomeres in the bloodworm muscle are, therefore, virtually longitudinal in orientation.

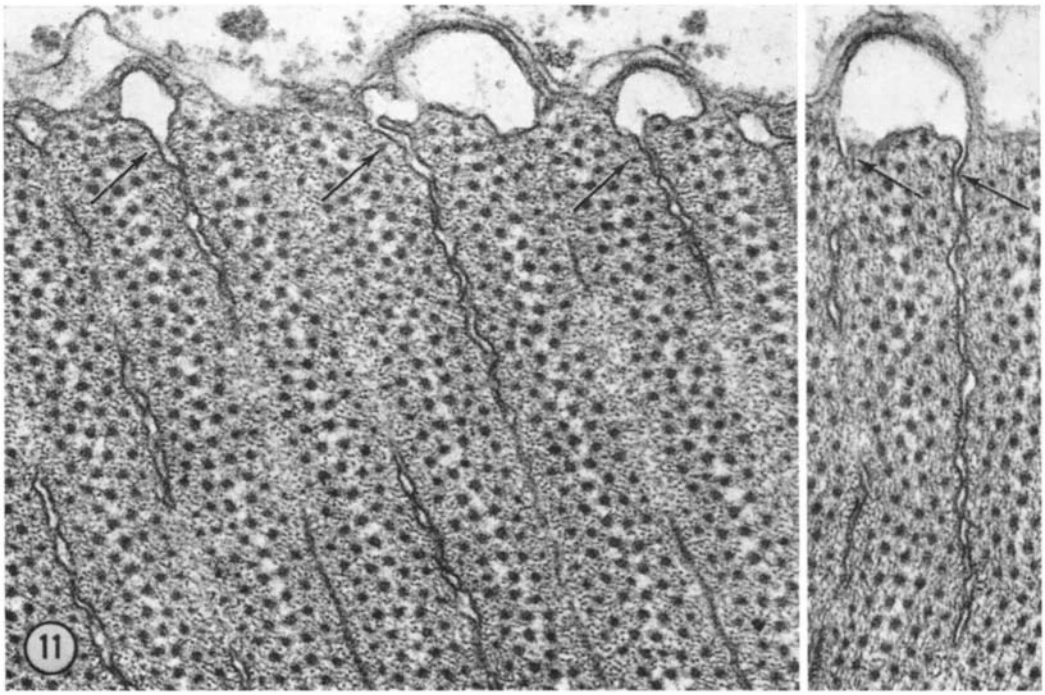


FIGURE 11 Connection of sarcotubules to subsarcolemmal cisternae. The tubules run transversely in I zones to the surface of the fiber where (at arrows) they join cisternae just under the plasma membrane. The cisternae usually extend across two or three I zones in the plane of the section. Fibers fixed at intermediate length. × 58,000.

Sarcoplasmic Reticulum

At the mid-I zone level of a cross-sectioned fiber of the predominant type, single, membrane-limited tubules resembling the "T tubules" (1) of cross-striated muscle extend radially into the fiber from its surface and, in some cases, can be followed across almost the full width of the fiber from one surface to the other (Fig. 13). The tubules are, however, never associated with other tubules or cisternae to form dyads or triads. They differ from T tubules also in that their lumina are very variable in diameter. Moreover, at the periphery of the fiber, these tubules are not continuous with the sarcolemma, as are the T tubules of cross-striated muscle, but rather open into dilated intracellular cisternae which parallel the sarcolemma and are very closely applied to it (Fig. 11). A substantial portion of the cell membrane is associated with such cisternae, each of which may give rise to several tubules.

The relationship between the subsarcolemmal cisternae and the sarcolemma is comparable to that between the lateral and central components of triads in cross-striated muscle (Fig. 12). The space between the respective membranes is relatively uniform in width and it sometimes contains an intermediate line. Usually, it is filled with an amorphous dense material which may be periodically disposed. Such subsarcolemmal cisternae with transversely oriented tubular extensions also occur in other annelids (12, 15), and comparable cisternae have been reported in vertebrate smooth muscles as well, but without tubular extensions (22). The maximum distance between dyads and myofilaments in bloodworm fibers is $\sim 2\ \mu$ (i.e., half the fiber thickness). In fast-acting cross-striated muscles such as those in the swim bladders of certain fish (6), the corresponding separation (i.e., half the distance between successive triads) is about half as much.

In longitudinal sections parallel to the long diameter, the sarcotubules are cut cross-wise and appear at frequent intervals, in the middle of the I band, as small oval profiles. The spacing of these

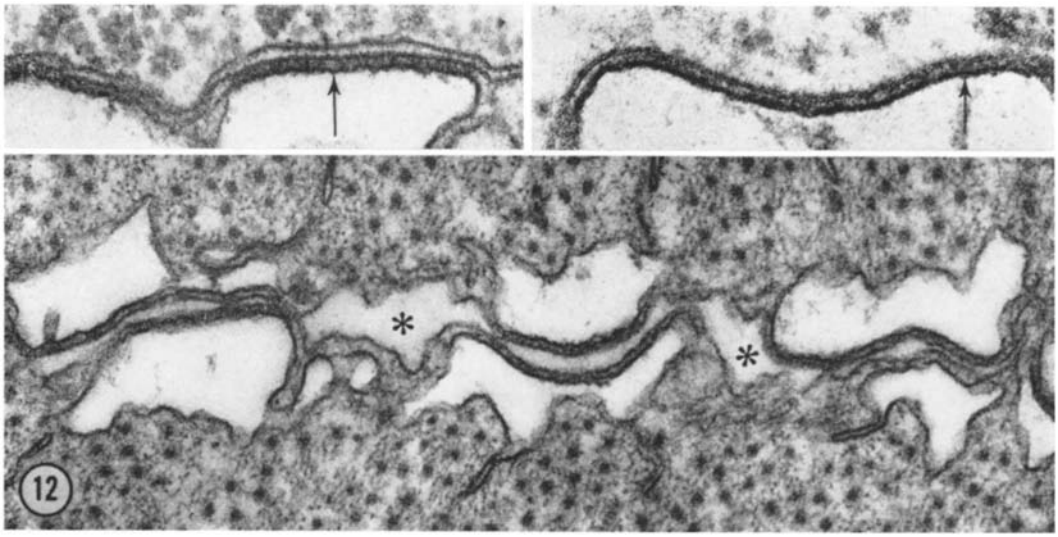


FIGURE 12 Relationship of subsarcolemmal cisternae to sarcolemma. The lower figure shows the surfaces of two adjacent muscle fibers separated by an intercellular space (asterisks). Within each fiber are three cisternae which form "dyads" with the sarcolemma. The apposed membranes at the dyad are separated by a constant interval which contains a material of moderate density. Junctions of this kind occur only at the fiber surface. *Upper left inset:* Detail of two apposed cells. The upper cell contains glycogen particles; the lower cell contains subsarcolemmal cisternae. The space between the cisternal membrane and the sarcolemma is distinctly denser than the intercellular space between the two plasma membranes. An intermediate line appears at the arrow. *Upper right inset:* Detail of a dyad. The cisternal membrane is thinner, but denser than the plasma membrane, and the dense material between the two membranes has a radial component (arrow). × 64,000. Left inset, × 77,000. Right inset, × 96,000.

profiles is much greater in extended fibers (Fig. 9) than in shortened fibers (Fig. 10), thus providing further evidence for shearing within the sarcomeres during length changes. In the longitudinal plane parallel to the short diameter, the sarcotubules are seen as elongated structures parallel to the Z lines (Figs. 1 and 13). The tubules sometimes branch or interconnect in this plane, but by and large, although the sarcomeres are almost longitudinal in orientation, the tubules are transverse. The apparent density of the tubules in this plane of section undoubtedly indicates that they are included entirely within the thickness of the section so that the limiting membrane of the tubule is superimposed on its lumen. Often, parts of a tubule project out of the section, resulting in the spurious appearance of fenestrae (Fig. 13).

As a result of their transverse orientation, the sarcotubules of bloodworm muscle are related only to the midportions of the I bands, in contrast to the sarcotubules of cross-striated muscles which are longitudinally oriented and are related both to A and to I bands. The fact that thick filaments of bloodworm muscle are ~3 μ in length does not mean, however, that the sarcotubules are separated from each other by that great a distance. Because of the marked stagger of adjacent myofibrillar plates, the sarcotubules, like the myofilaments, are staggered in the longitudinal direction. Since the plates are relatively thin, the myofilaments of one plate are still relatively close to the sarcotubules of the next plate and of plates even farther removed. As a result, the maximum distance of thick filaments from sarcotubules is only 0.1–0.2 μ in the fibers containing the small-diameter thick filaments (Figs. 7–10). Fibers containing the thicker (and longer) thick filaments (Figs. 5, 6) have far fewer tubules and fewer subsarcolemmal cisternae, and here the distances are greater.

Endomysium

One of the most striking differences between *Ascaris* muscle and bloodworm muscle is in the amount and distribution of connective tissue ele-

FIGURE 13 Sarcotubules and Z lines in an extended fiber sectioned longitudinally (perpendicular to the long diameter). Both structures are cut lengthwise in this plane, and at least one Z line and one sarcotubule (*S*) extend virtually across the full width of the fiber. The apparent density of the tubules indicates that their entire thickness lies within the section. Thin filaments above and below the tubules appear superimposed on them. In several places, the limiting membrane of a tubule passes out of the section, producing the appearance of clear fenestrae. Some branching occurs within the plane of the section. *Inset:* Sarcotubule joining a subsarcolemmal cisterna (arrow). × 36,000. Inset, × 42,000.

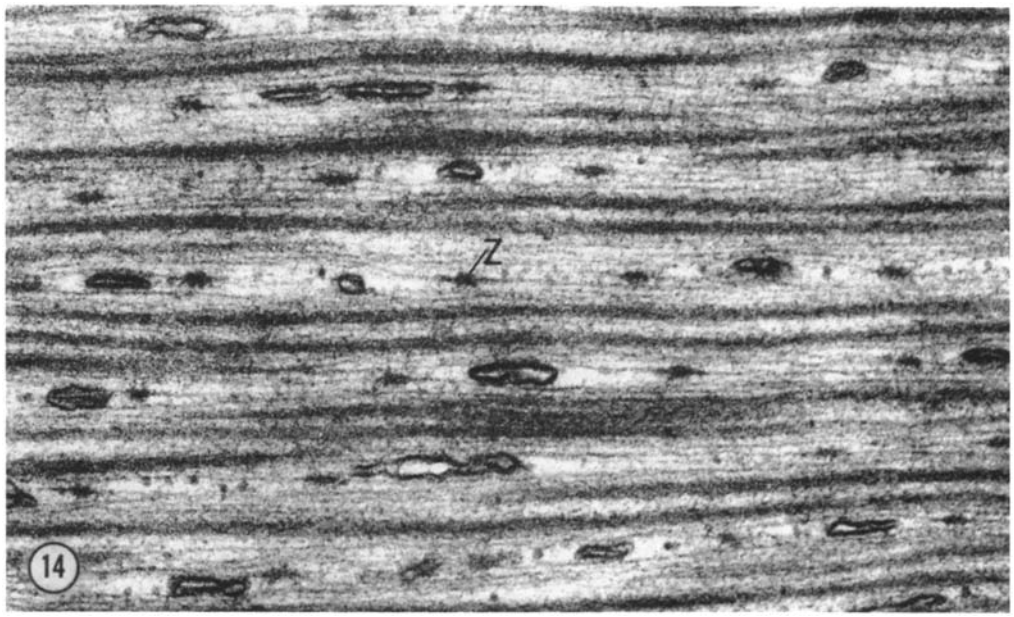


FIGURE 14 Sarcotubules and Z lines in an extended fiber sectioned longitudinally (parallel to the long diameter). Both structures are cut across in this plane. Profiles of the Z lines are spindle-shaped and are joined at both ends by thin myofilaments. The latter appear slightly thicker and denser just before inserting into the Z lines. Sarcotubules tend to be elongated in a direction parallel to the fiber axis. × 68,000.

ments in relationship to the muscle fibers. In *Ascaris* muscle, the fibers are clothed individually by multiple laminae of amorphous connective tissue, attached to the fibers at intervals all along their length. Direct cell-to-cell connections have not been observed.

Bloodworm muscle fibers, in contrast, are almost bare of connective tissue and virtually in contact with one another (Figs. 1–4, 11, 12, 15). Occasionally, desmosomal attachments between adjacent muscle fibers are encountered. These are probably responsible for the syncytial appearance of polychaete muscle noted previously (5). More frequently, desmosomes occur between muscle fibers and slender cellular processes which contain fibrillar bundles (Figs. 2, 16, 17). The latter processes are assumed to be extensions of the muscle cells themselves, but may also represent cells of another type. In either case, it is clear that, whereas the force developed by individual *Ascaris* muscle fibers is transmitted by way of the endomysium, the force developed by bloodworm fibers must be transmitted through cells, for there is virtually no endomysium in this muscle. Similarly, the intracellular skeleton in the *Ascaris* muscle penetrates throughout the contractile apparatus and apparently links the contractile elements to the endomysium all along the sides of the fibers. In the bloodworm muscle, the only possible counterpart to the intracellular skeleton consists of fibrillar bundles, which occur in the sarcoplasmic parts of the cell (Fig. 15), radiate from the desmosomes (Fig. 17), and also occur just under the sarcolemma (Fig. 5), but do not penetrate into the contractile apparatus itself.

DISCUSSION

The basic organization of *Glycera* muscle (summarized in Fig. 18) is comparable to that of *Ascaris* muscle in that the thick filaments are staggered, with the result that the sarcomeres are virtually longitudinal in orientation and the striation angle is only a few degrees. In several respects, however, the predominant type of bloodworm fiber is distinctive: sarcoplasmic reticulum is abundant and consists of numerous subsarcolemmal cisternae from which transversely oriented sarcotubules originate; dyads occur only at the cell surface;

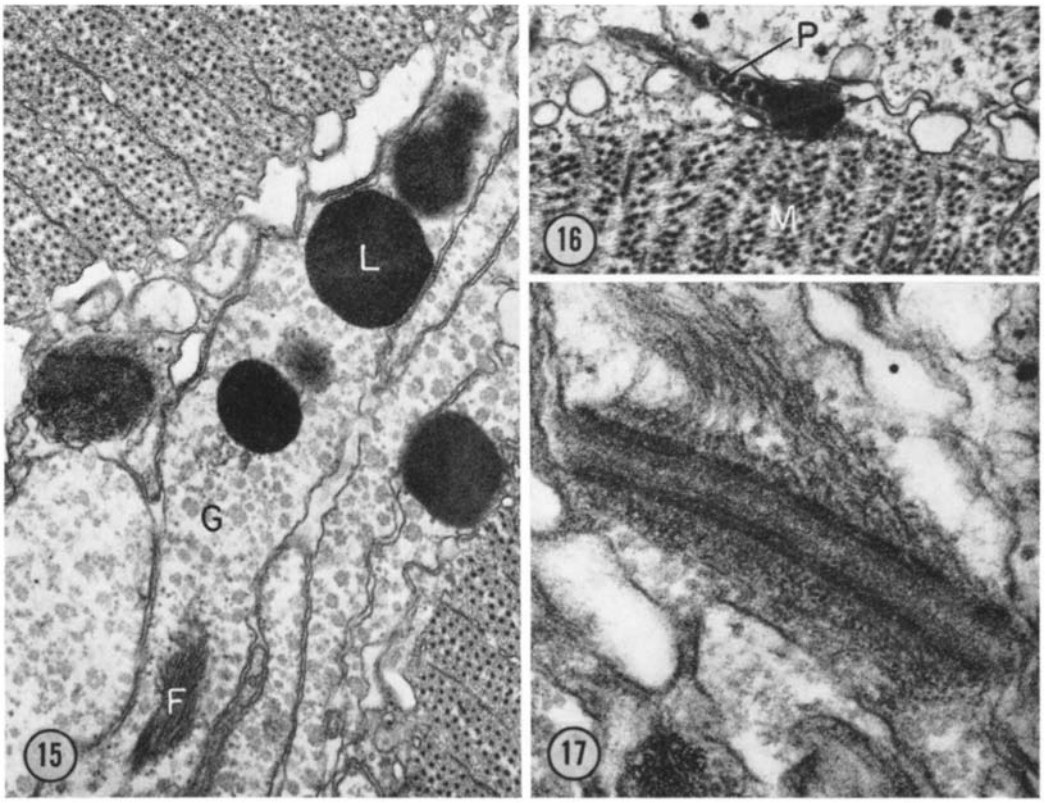


FIGURE 15 Two muscle fibers separated by overlapping cellular processes which contain glycogen particles (*G*), fibrillar bundles (*F*), and large droplets (*L*). These processes are probably parts of other muscle cells. There is virtually no extracellular material. × 30,000.

FIGURE 16 Desmosome linking a muscle fiber (*M*) with a slender, glycogen-containing cell process (*P*). × 25,000.

FIGURE 17 Desmosome linking two cellular processes presumed to be parts of muscle cells. Tonofilaments in the upper process form a bundle similar to the fibrillar bundle shown in Fig. 15. Tonofilaments in the lower process are cut across and resemble the filaments occurring at the periphery of the muscle fiber in Fig. 5. × 69,000.

endomysial connective tissue is virtually absent, and the intracellular skeleton is reduced and confined to the nonmyofibrillar portions of the cells; cells are attached to one another rather than to connective tissue all along their length; thick filaments are shorter and are staggered to a greater degree; and dense bodies are replaced by Z lines which are virtually unidimensional. Several of these differences have functional implications.

Series vs. Parallel Linkages

The mechanical characteristics of a muscle are dependent on the extent to which the contractile elements are connected in series and in parallel. In a fiber with long myofilaments, more actin-myosin cross-links will be in parallel than in a fiber with short myofilaments and, other things being equal, the maximum force developed by the respective fibers will be directly proportional to myofilament length and the velocity will be inversely proportional. Similarly, on a grosser level, long fibers, or short fibers connected in series, will be faster and less powerful than short fibers acting in parallel with one another (26).

The fact that the thick filaments of bloodworm muscle are about half the length of those in *Ascaris*

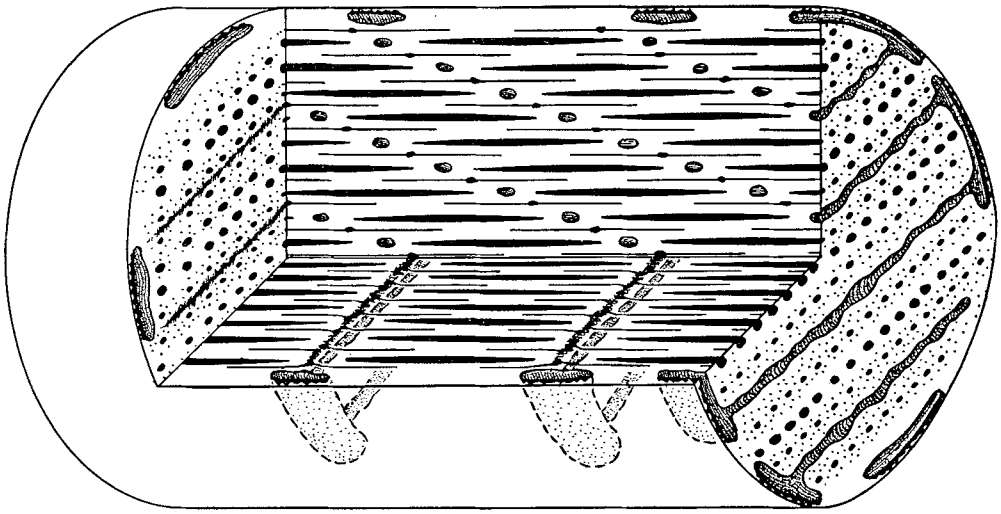


FIGURE 18 Summary diagram showing the arrangement of the myofilaments and their relationship to the sarcoplasmic reticulum in three mutually perpendicular planes. The angle of striation in the vertical longitudinal plane has been exaggerated for the sake of clarity. In reality, the striations are almost longitudinal in this plane, forming an angle of only a few degrees with the filament axis (cf. Figs. 3, 9, 10, 14).

muscle thus provides a basis for increased velocity in bloodworm muscle. An even more important consideration is that the *Ascaris* muscle fibers are connected not to each other, but to the surrounding connective tissue and, therefore, may be acting in parallel to a much greater extent than bloodworm fibers, which are directly connected to each other. Furthermore, because of the relationship between the intracellular skeleton and the contractile apparatus in the *Ascaris* muscle, even parts of the same fiber may be acting in parallel rather than in series (27). Thus, the bloodworm fibers may, in effect, be equivalent to very long fibers by virtue of series linkages between them, while the fibers of *Ascaris* muscle may be equivalent to pennate skeletal muscle fibers which attach to tendinous elements extending along the length of the muscle.

Sarcoplasmic Reticulum

Ascaris muscle fibers are characterized by a T system consisting of radial invaginations of the sarcolemma into the fibers (cf. 7, 28). These clefts are associated with flattened sacs of sarcoplasmic reticulum to form dyads; however, aside from these "terminal cisternae" there is little evidence for longitudinally disposed elements of the sarcoplasmic reticulum within the fibers. Bloodworm muscle fibers, in contrast, have no sarcolemmal invaginations, but the terminal cisternae, or subsarcolemmal cisternae at the surface of the fibers give rise to a profusion of tubular extensions corresponding to the L system of cross-striated muscle (19, 20).

The role of the abundant sarcoplasmic reticulum (SR) in bloodworm muscle is a matter for speculation. Like the SR of cross-striated muscle, it is probably capable of concentrating calcium (4, cf. 12) and thereby regulating the calcium concentration around the myofilaments. In addition, the limiting membrane of the SR may be electrically coupled to the sarcolemma. If, for example, the dense material separating the SR membrane from the sarcolemma had the properties of a dielectric, then the dyads could act as capacitors capable of conveying rapid changes in potential from the cell surface into the sarcotubules. This kind of coupling would in no way prevent the SR from maintaining within its lumen an ionic composition quite independent of that in the extracellular fluid. Whatever the function of the SR, it is clear that the ratio of SR to myofibrils is much higher in bloodworm muscle than in *Ascaris* muscle, and the average distance between contractile elements and the SR is much smaller.

In short, it may be possible to account for physiological differences between bloodworm and *Ascaris* muscles on the basis of differences in thick-

filament length, differences in the relationship of the contractile elements to the endomysium and intracellular skeleton, and differences in the amount and distribution of the sarcoplasmic reticulum. Furthermore, it may also be significant that, because of the shorter length of the thick filaments in the bloodworm muscle and the greater degree of their stagger, the length along which thick filaments overlap is only about one-third as much as in *Ascaris* muscle.[2] The midportions of adjacent thick filaments are also considerably displaced from each other, and the matrix surrounding them is much less prominent in the bloodworm muscle. Because of these structural differences, impedance to shear may, therefore, be substantially lower in the bloodworm muscle, and, depending on when shear occurs during the contraction-relaxation cycle, this may be another factor affecting the time course of length changes and the mechanical properties of the respective muscles.

It is of interest also that, besides the predominant fiber type in the bloodworm muscle, there are fibers which are less orderly in their organization and which have relatively little SR and much larger caliber and longer thick filaments. Fibers of this kind resemble those of paramyosin smooth muscles such as the ABRM (9). Bloodworm muscle is thus composed of a mixture of fiber types which may correspond to the fast and slow fibers of cross-striated muscle (11, 18) or to the translucent and opaque portions of such muscles as the oyster adductor (10). No information is available as yet concerning the innervation of the different fiber types or their myosin ATPase activity.

[2] On the assumption that the thick filaments in bloodworm muscle are 3 μ long and that neighboring rows are displaced by 20–40% of this amount, the length along which adjacent rows of thick filaments overlap is approximately 1.8–2.4 μ. In *Ascaris* muscle, if it is assumed that the thick filament length is 6 μ and the stagger is 4–8%, the overlapped length is approximately 5.5–5.8 μ.

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