STRUCTURE OF *LIMULUS* STRIATED MUSCLE

The Contractile Apparatus at Various Sarcomere Lengths

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ABSTRACT

The musculature of the telson of *Limulus polyphemus* L. consists of three dorsal muscles: the medial and lateral telson levators and the telson abductor, and one large ventral muscle; the telson depressor, which has three major divisions: the dorsal, medioventral, and lateroventral heads. The telson muscles are composed of one type of striated muscle fiber, which has irregularly shaped myofibrils. The sarcomeres are long, with discrete A and I and discontinuous Z bands. M lines are not present. H zones can be identified easily, only in thick (1.0 μm) longitudinal sections or thin cross sections. In lengthened fibers, the Z bands are irregular and the A bands appear very long due to misalignment of constituent thick filaments. As the sarcomeres shorten, the Z lines straighten somewhat and the thick filaments become more aligned within the A band, leading to apparent decrease in A band length. Further A band shortening, seen at sarcomere lengths below 7.4 μm may be a function of conformational changes of the thick filaments, possibly brought about by alterations in the ordering of their paramyosin cores.

INTRODUCTION

Since they were first described (albeit backwards—Clusi, 1605), the distinctive appearance of the *Xiphosura* has excited the curiosity and interest of natural historians (de Laet, 1633; Bernhardi a Berniz, 1671). By virtue of the selective advantage conferred on them by their unique morphology and specialized physiology, horseshoe crabs have been highly successful within their ecological niche. They have changed very little since their first appearance in the fossil record of the Cambrian, and virtually not at all since the Jurassic (Sharov, 1966). The musculature of *Limulus polyphemus* L., the sole western xiphosuran species, was dissected and described by several investigators within the last century (Milne-Edwards, 1873; Owen, 1873; Benham, 1885), in studies that established the lineage and arachnoid affinities of this ancient arthropod.

Renewed interest in *Limulus* skeletal muscle stems from several reports published within recent years indicating anomalous behavior of A bands of glycerinated myofibrils during sarcomere shortening (de Villafranca, 1961; de Villafranca and Marschhaus, 1963). Still more recently, paramyosin has been isolated from extracts of *Limulus* skeletal muscle (de Villafranca and Leitner, 1967).

Paramyosin is a protein found in relatively great amounts in molluscan "catch" muscles (Lowy and Millman, 1963; Lowy and Hanson, 1962; Twarog, 1967; Squire, 1971) and in lesser quantities in some other invertebrate contractile

tissues that do not exhibit catch behavior (Kominz et al., 1957; Hanson and Lowy, 1961; Squire, 1971). Despite the great morphological diversity of these tissues (Hanson and Lowy, 1961; Hoyle, 1969; Perkins et al., 1971), their paramyosin component is believed to constitute the cores of the thick filaments and to be covered by a myosin cortex (Szent-Györgyi et al., 1971; Hardwicke and Hanson, 1971).

The results of our immunohistochemical studies (Levine et al., 1972) indicate that paramyosin and myosin may be similarly disposed in the thick filaments of *Limulus* striated telson muscles. We also noted variations in the pattern of A band staining with antiparamyosin as well as changes in the A band length at different sarcomere lengths. These observations at the light microscope level suggest that repositioning of, and/or conformational changes in, elements of the contractile apparatus may occur during sarcomere shortening. In order to rule out the effects of glycerination (Stephens, 1965; Peachey, 1968) on the observed decrease in A band length and to determine the type and extent of the contribution of the individual components of the contractile apparatus to this phenomenon, we undertook a detailed morphological analysis of freshly fixed *Limulus* telson muscle.

In this paper we describe the gross anatomy, light microscope appearance, and fine structure of *Limulus* telson muscles, with emphasis on the organization of the contractile apparatus at various sarcomere lengths.

MATERIALS AND METHODS

Adult *Limulus* specimens with carapace 20–35 cm in diameter were obtained from both the Marine Biological Laboratories, Woods Hole, Mass. and the Gulf Specimen Co., Inc., Panacea, Fla. Upon arrival they were kept in an aerated sea water tank until used.

Dissection Procedures

For dissection, specimens were placed with their dorsal surfaces up. A segment of the opisthosomal carapace was removed from the telson attachment, posteriorly, to just caudal to the most posterior pair of entapophyseal pits, anteriorly. The underlying connective tissue was dissected away. During the entire dissection period, the animals were perfused with oxygenated sea water, 900–1,000 mosM, isosmotic with *Limulus* hemolymph (Robertson, 1970), via a cannula inserted into a small hole drilled in the opisthosomal carapace at the midline and slightly anterior to the last pair of entapophyseal pits.

The muscles controlling telson movement were exposed. The origin and insertion of each of the muscles on either side of the animal were determined by complete dissection. Maximum, intermediate, and minimum lengths of the most dorsal muscles were measured *in situ* with the telson completely depressed, extended, and elevated. The telson was maintained at any one of these positions to obtain bundles with fibers having respectively long, intermediate, or short sarcomeres.

Preparation for Electron Microscopy

Small fiber bundles of the dorsal or ventral muscles were tied securely at both ends with silk and then tied onto glass rods. When cut free from the animal the tied bundles remain at their *in situ* length. These bundles were fixed by immersion in 5% glutaraldehyde (freshly prepared from 70% purified stock; Ladd Research Industries, Inc., Burlington, Vt.), in 0.2 M s-collidine (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) buffer, pH 7.2 with 0.5 M sucrose, 1.400 mosM, for 6 h at room temperature (23°C), and left in fixative overnight at 4°C. Fixation was followed by rinsing for 24 h in several changes of buffer plus sucrose, 900 mosM.

Determination of Sarcomere Length

After fixation and rinsing, single fibers or bundles of up to 200 fibers (determined by fiber counts) were temporarily placed on glass slides, kept moist with buffer, and covered with plastic wrap. These preparations were placed on a vertically positioned mechanical microscope stage, mounted in series between the light source of a Spectra-Physics helium-neon laser (Spectra-Physics, Inc., Mountain View, Calif.), emitting at 6,328 Å, and a Polaroid sheet film camera back (Polaroid Corp., Cambridge, Mass.). Diffraction patterns photographed on Polaroid 55 P/N sheet film at approximately 2-mm intervals along the lengths of the bundle were used to ascertain the uniformity of sarcomere lengths in a given preparation. Sarcomere lengths were calculated from the diffraction patterns according to the grating equation. Calculations demonstrated variations in sarcomere length as ±5.0% (Dewey et al., 1972). The number of sarcomeres even at maximum in vivo extension that were averaged per diffraction pattern of a small bundle (200 fibers) was well over 100,000, as calculated using a beam width of 0.8 mm, and assuming that there were at least ten myofibrils per fiber. This technique provides a tool for statistical sampling of the sarcomere population

which is much more accurate than measuring sarcomere lengths of single fibers or fibrils by microscopal techniques. Prior determination of sarcomere lengths by laser diffraction enabled the selection of fibers of known sarcomere lengths for longitudinal and transverse sectioning for electron microscope analysis.

Microscopy

After removal from glass slides, the bundles were postfixed in 1:1 (vol/vol) 4% aqueous osmium tetroxide: 0.2 M s-collidine buffer, pH 7.2 with 0.5 M sucrose for 2 h at room temperature, followed by rinsing for 1 h in several changes of buffer. The tissue was stained en bloc in 2% aqueous uranyl acetate (J. T. Baker Chemical Co., Phillipsburg, N. J.; reagent grade), dehydrated in graded acetone or ethanol and propylene oxide, and flat embedded in Epon-Araldite.

Thick (1 μm) and thin (200–500 Å) sections were cut with diamond knives (E. I. DuPont de Nemours & Co., Wilmington, Del.) on LKB Ultrotomes I and III. Longitudinal sections were always cut normal to the axis of the bundle. Thick sections were dried on glass slides, stained with Azure II and Methylene Blue (Allied Chemical Corp., Morristown, N, J.), and mounted in Harleco synthetic resin (Harleco, Philadelphia, Pa.) for light microscopy and photomicrography. Thin sections were collected on Formvar-coated copper-mesh grids and stained with Reynolds' (1963) lead citrate for electron microscopy.

Light micrographs were taken on a Zeiss Ultraphot using Kodak M plates, and electron micrographs were taken on Hitachi HU-11C or HU-12 electron microscopes using Kodak EM plates. Magnifications were calibrated with a stage micrometer for light microscopy and a diffraction grid for electron microscopy.

Measurements

Measurements were made of sarcomere, A band, and thick filament lengths on electron micrographs of longitudinally sectioned fibers. The A band lengths were determined on a total of 232 sarcomeres, ranging from 4.3 to 12.5 μm long. Thick filament lengths were determined on 190 of the above 232 sarcomeres in which the entire lengths of at least ten thick filaments, including their tapered ends, could be traced. These measurements were tabulated and linear regression curves were computed for each parameter as it related to sarcomere length.

OBSERVATIONS

Gross Morphology

Our dissection agrees substantially with that of Benham (1885) as to the origin and insertion of the dorsal, although not of the ventral musculature. We feel that the nomenclature used by Benham (1885) was cumbersome and nonfunctional, so we will refer to the telson muscles by using the earlier nomenclature of Owen (1873) which we have modified according to our more complete dissection (Figs. 1 *a*, *b*).

Each of the telson muscles inserts via a tendon and apodeme through a fibrous connective tissue membrane that is continuous dorsally, and closely follows the contours of the dorsal and lateral processes of the telson base (Figs. 1 *b*, *c*). Ventrally, the membrane is marked by a deep central depression or suture but is not discontinuous (Fig. 1 *d*). We will refer to this membrane as the *articular membrane of the telson*. The telson base has a single median dorsal process which has two lateral faces to which attach the dorsal telson muscles of either side of the opisthosoma. Ventrally, the telson base has two lateral processes to which attach the ventral telson muscles. Between the dorsal and ventral processes of the telson base two conical processes project from the opisthosomal carapace. These processes serve as hinge fulcra and prevent retraction of the telson into the opisthosoma.

Due to the bilateral symmetry of *Limulus*, we will consider the muscles present on one side of the median plane of the opisthosoma.

The musculature exposed by removal of the dorsal opisthosomal carapace posterior to the level of the seventh (metasomatic) entapophyseal pit includes four large muscular masses (Figs. 1 *a*, *b*). The two most medial arise from the opisthosomal carapace and entopophyseal components of the endoskeleton (Fig. 1 *b*). The lateral two muscular masses arise from the ventral carapace which rises abruptly to fuse with the dorsal carapace laterally (metasomatic sternite). Below the dorsal muscles lies a large ventral muscle mass that consists of three muscular bundles arising both dorsally and ventrally, that join before insertion into the lateroventral process of the telson base (Figs. 1 *a*, *b*).

Dorsal Musculature of the Telson

We define as dorsal musculature those muscles which insert on the dorsal facets of the telson. The two more medial dorsal muscles raise the telson; we retain Owen's (1873) nomenclature for these as *telson levators*. The muscle lying closest to the midline is the *medial telson levator* (internal pygotergal of Benham, 1885). It arises as several

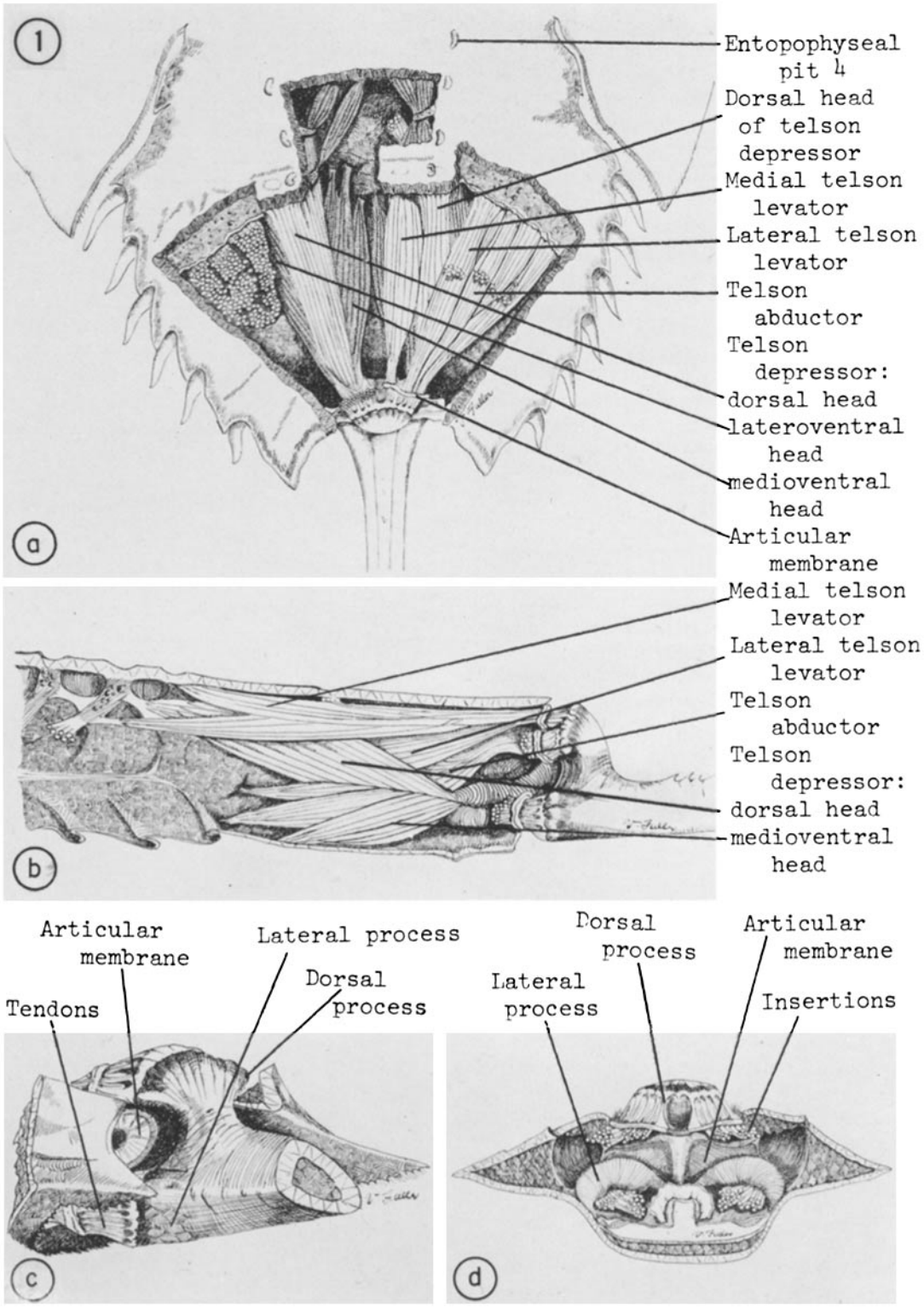


FIGURE 1 Drawings of dissection of *Limulus* telson muscles and attachments. (*a*) Dorsal view. Dorsal carapace removed showing on the right side the dorsal muscles and on the left side (dorsal muscles removed) the ventral muscle. (*b*) Midsagittal view. Left side of animal removed with attachments of muscles severed from left facet of the dorsal and left lateral process of the telson. (*c*) Lateral posterior view of telson and carapace. (*d*) Anterior view of base of telson with muscle insertions.

smaller heads attached to (anteriorly to posteriorly) the inner faces of the fifth, sixth (mesosomatic), and seventh (metasomatic) entapophyses and from the dorsal opisthosomal carapace in the metasomal region. These lateral origins are visible externally as semilunar depressions in the carapace. The medial telson levator inserts into the face of the dorsal process of the telson. Immediately lateral to the medial telson levator lies the medial head of the ventral muscle mass described below. Lateral to this muscle mass is the *lateral telson levator* (middle pygosternal of Benham, 1885). This muscle arises both ventrally, as one head from that part of the metasomatic sternite that rises to join the dorsal carapace, and dorsally, as two heads, one more anterior than the other, from connective tissue underlying the carapace. The lateral telson levator inserts more laterally than its medial neighbor into the face of the dorsal process of the telson.

The most lateral of the exposed dorsal muscles, the *telson abductor* (external pygotergal of Benham, 1885), lies in a slightly more ventral plane than the medial and lateral telson levators. This muscle arises from connective tissue underlying the lateral margins of the opisthosomal carapace, where the metasomatic sternite is continuous with the carapace, and inserts most laterally into the face of the dorsal process of the telson. Shortening of the telson abductor moves the telson away from the medial plane of the body.

Ventral Musculature of the Telson

After removal of the dorsal muscles, a large muscle mass is exposed, the *telson depressor*. This muscle arises as three major heads that join posteriorly to insert, via tendinous connections, into the lateroventral process of the telson.

The *dorsal head* of the telson depressor arises as three separate smaller branches from the outer faces of the fifth, sixth, and seventh entapophyses. It crosses dorsoventrally between levators and then runs below the medial telson levator before joining with the rest of the depressor. The *medioventral head* of the telson depressor arises as three slips from the floor of the metasomatic sternite, ventral to the origin of the medial telson levator, and runs beneath the latter where it joins the other heads of the depressor. The *lateroventral head* is shorter than the other major branches of the telson depressor. This head originates in the connective tissue covering the lateral region of the metasomatic floor and runs beneath the lateral telson levator, before uniting with the medioventral and dorsal heads of the telson depressor.

Between the carapace and the muscles lies a layer of dense, pigmented connective tissue which also contains fatty deposits. Each muscle is completely surrounded by a thin fascia of connective tissue, the epimysium, which is continuous with the epitendineum onto the articular membrane of the telson. This fascia is dissected away to expose the fascicles which, in turn, are surrounded by connective tissue directly continuous with that of the tendinous attachment of each fascicle. The length of individual fascicles varies. The tendons of the fascicles join to form a larger tendon before insertion into the telson base. Small bundles are easily separated from each fascicle.

Morphology of the Muscle Fibers

Individual muscle fibers of the telson muscles are striated, 40–100 μm in diameter, and are surrounded by a dense endomysium (Fig. 2). As seen with the electron microscope the endomysium includes two components. Immediately adjacent to the fiber is a finely fibrillar to homogeneous layer of extracellular material, which contains small collagenous fibrils. Large aggregates of collagenous fibrils occur in angular spaces between fibers (Fig. 3).

Neural elements, to be described in a separate report, vessels of the hemolymphatic circulation, which often contain granular amebocytes, and connective tissue cells are found in the connective tissue spaces (Fig. 5). Individual fibers appear to end at different lengths within a bundle, but their connective tissue covering continues posteriorly to the region of tendinous insertion.

In the electron microscope, the sarcolemma is seen to be a trilaminar membrane, surrounding the periphery of and invaginating into the substance of the fiber in broad clefts and narrow channels. The clefts frequently contain extracellular matrix and often terminate in narrow channels of the transverse tubular system (T tubules) devoid of connective tissue components. T tubules also arise directly from the surface of the fibers (Figs. 3–6).

The fibers are multinucleate and the ellipsoidal nuclei are located both peripherally and centrally within a fiber. Mitochondria are fairly numerous

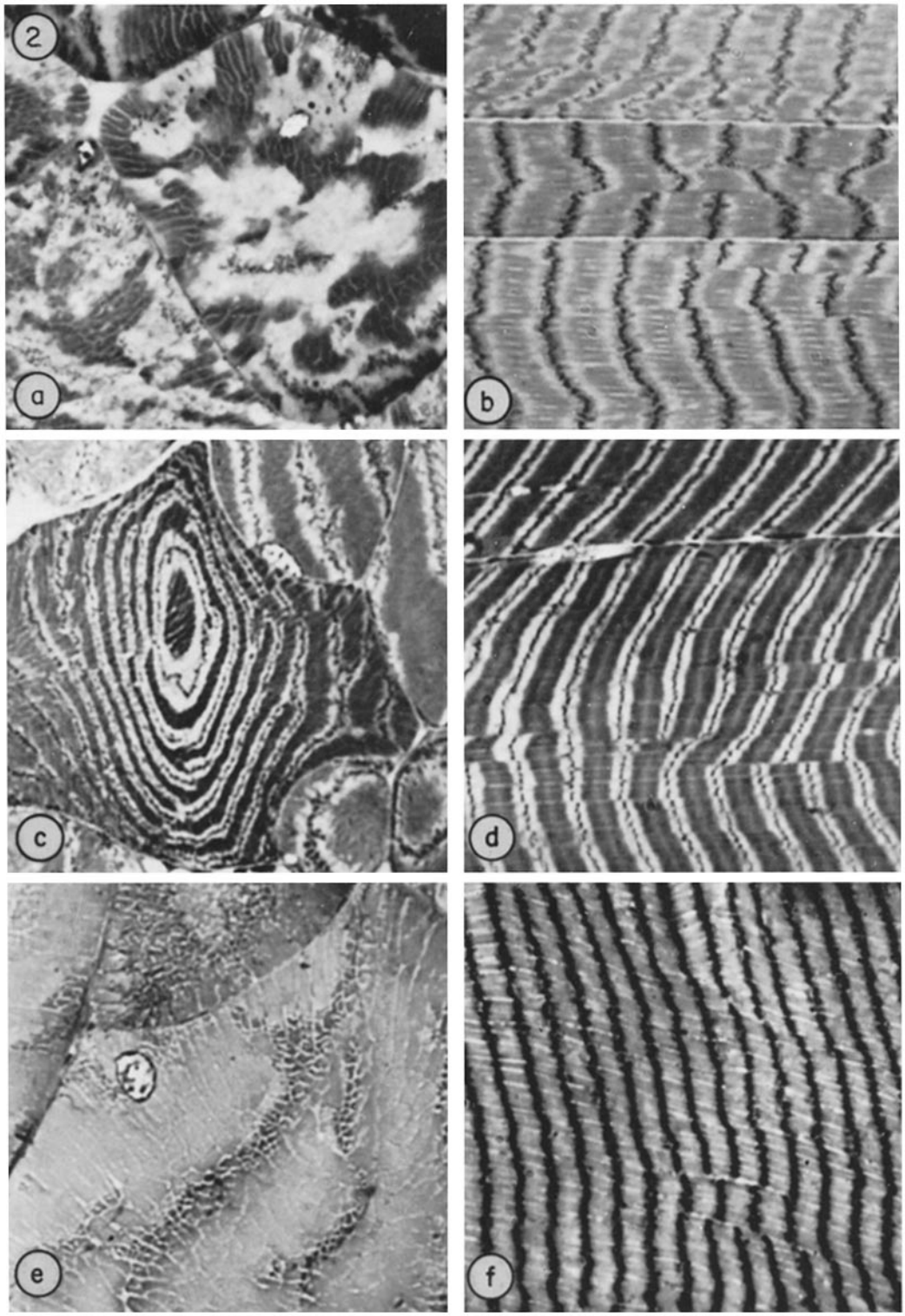


FIGURE 2 Light micrographs of *Limulus* muscle fibers. × 1,250. These light micrographs and the electron micrographs and diffraction patterns (Figs. 11–13) were taken from the same muscle bundles. (*a*) and (*b*) Cross and longitudinal sections of fibers with lengthened sarcomeres. (*c*) and (*d*) Cross and longitudinal sections of fibers with intermediate length sarcomeres. (*c*) Note the apparent "longitudinally" sectioned sarcomeres in Fig. 2 *c*. This pattern results from the misalignment of both sarcomere components and adjacent sarcomeres within one myofibril and on adjacent myofibrils. Electron micrographs show only cross-sectioned profiles of filaments. (*e*) and (*f*) Cross and longitudinal sections of fibers with shortened sarcomeres.

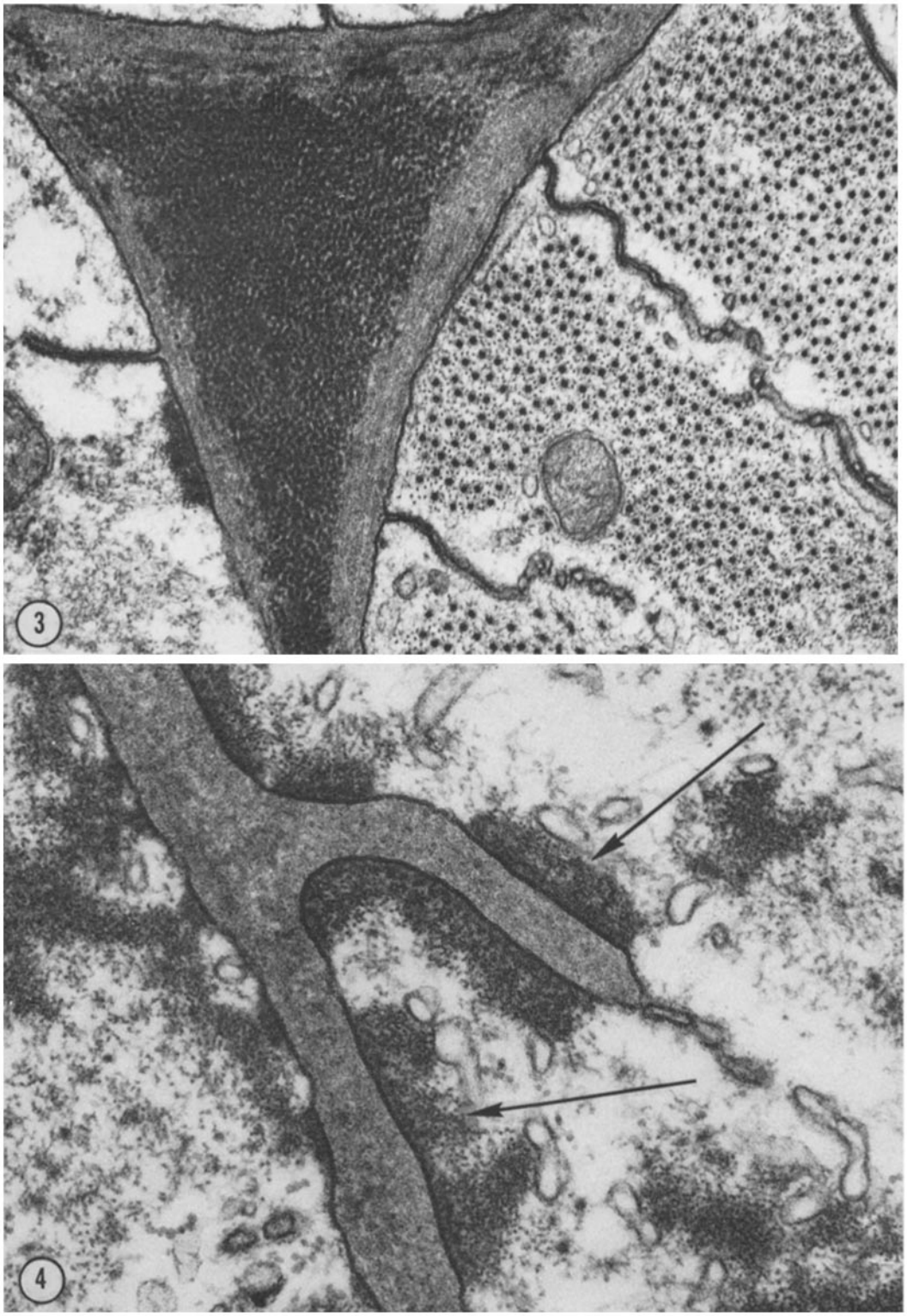


FIGURE 3 Connective tissue between adjacent fibers. Note the finely fibrillar matrix immediately adjacent to the sarcolemma. Bundles of collagen arranged parallel to the axis of muscle fibers occur in angles between fibers. × 60,000.

FIGURE 4 Two adjacent muscle fibers. The sarcolemma appears as a triple-layered membrane. In the right-hand fiber it invaginates into the cell as a cleft filled with connective tissue matrix. Transversely sectioned Z material and regions of insertion of myofibrils into the sarcolemma (arrows) are apparent. × 63,000.

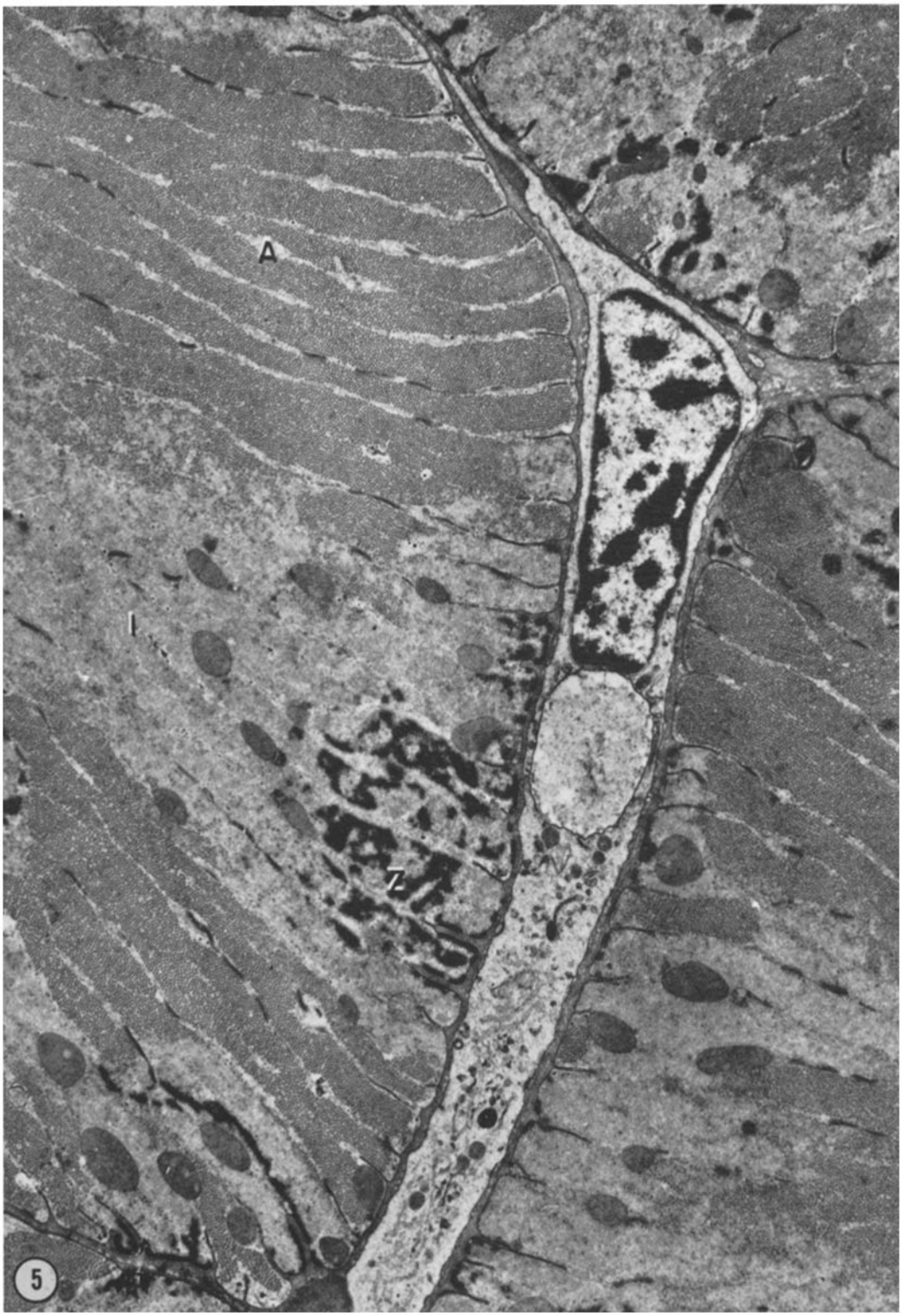


FIGURE 5 Transverse sections of lengthened muscle fibers. Myofibrils are straplike at the periphery of fibers. A, I, and Z bands are apparent. A large connective tissue cell lies between the fibers. × 8,000.

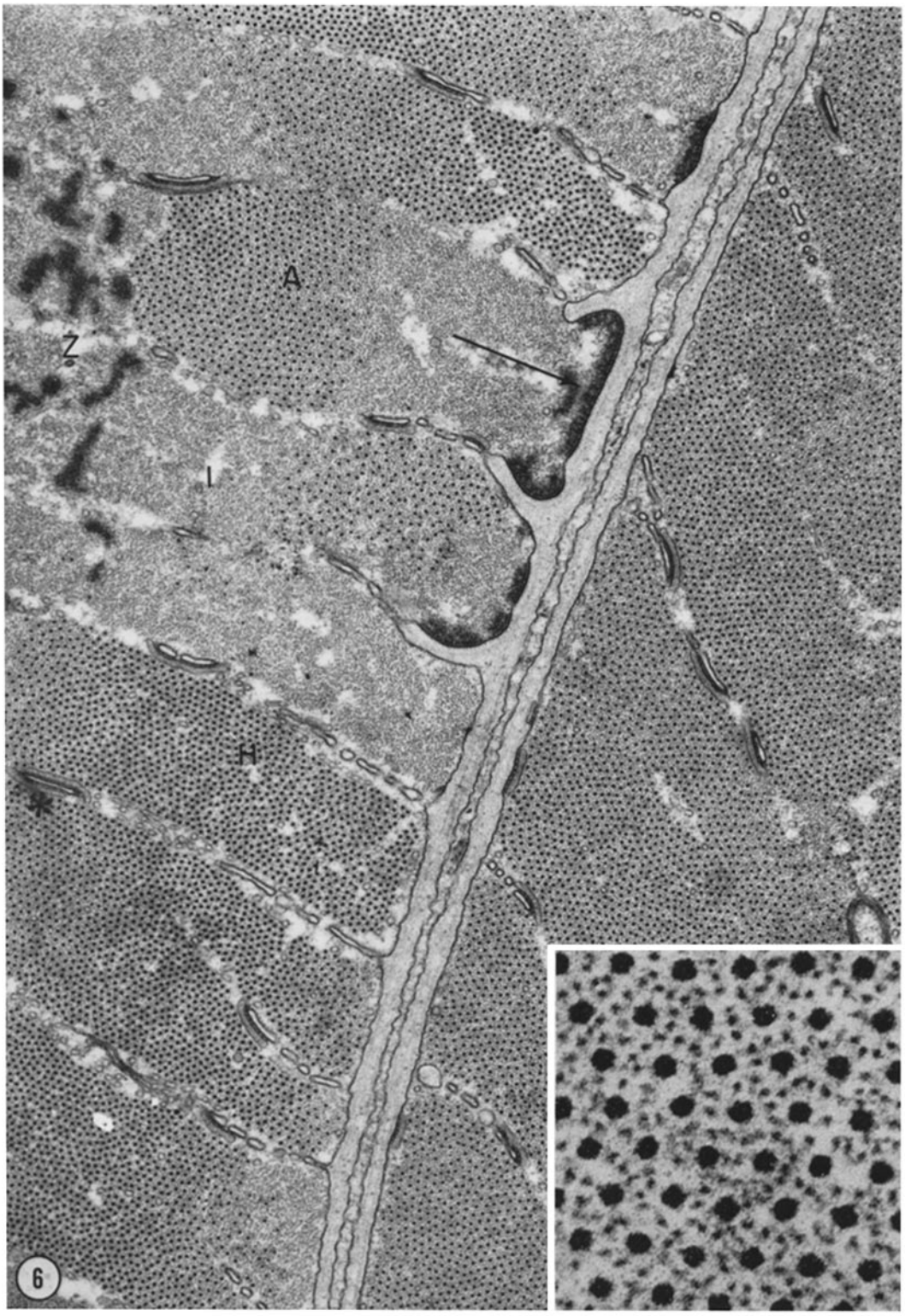


FIGURE 6 Transverse section of lengthened fibers. A, H, I, and Z bands are visible as are myofibrillar attachment regions (arrow). Tubules, arise from both the muscle fiber surfaces and clefts and form dyadic and triadic (*) junctions with elements of the sarcoplasmic reticulum. × 35,000. *Inset.* Region of overlap of thick and thin filaments. × 170,000.

and occur between myofibrils but tend to aggregate near the Z line (Fig. 7).

An extensive system of smooth-surfaced membranes, the sarcoplasmic reticulum, forms dyadic and occasionally triadic junctions with the T tubules (Fig. 6). A detailed description of the T system and sarcoplasmic reticulum in *Limulus* telson levators is forthcoming.

The bulk of the sarcoplasm of the fiber is occupied by the contractile elements, which are longitudinally arranged into myofibrils. The contractile elements of the myofibrils are organized into repeating sarcomeric units common to striated muscles. Z, A, and I bands are prominent and in 1.0 μm thick longitudinal sections an H zone is present in the central A band of most sarcomeres (Fig. 2). The H zone is more difficult to find and may appear of variable length in longitudinal thin sections (Fig. 7), but is readily seen in cross-sectioned material in the electron microscope (Fig. 8). The Z bands appear continuous in the light microscope (Fig. 2) but are seen to be interrupted within myofibrils in thin sections (Fig. 7).

The myofibrils have irregular contours and are out of register. This arrangement is apparent in cross section and gives rise to the various undulating patterns of regions of different staining intensity in the light microscope (Figs. 2 *a*, *c*, *e*). In Fig. 2 *c* the misalignment of sarcomeres is such that the cross-sectioned appearance of the fiber resembles concentric "longitudinally sectioned" sarcomeres. Electron micrographs of similar fibers, however, show that all the filaments are cut in cross section and have round profiles. Longitudinally sectioned fibers often contain Y-shaped arrangements of Z lines belonging to sarcomeres both within one myofibril and in adjacent, out of register, myofibrils (Figs. 2 *b*, *d*, *f*). Electron micrographs demonstrate variations in myofibrillar configuration. Peripherally located myofibrils tend to be straplike (Fig. 5) while those centrally located vary from nearly to irregularly cylindrical (Fig. 9). Particularly in straplike myofibrils the sarcomeres assume a slightly oblique orientation. This is also apparent where all regions of the sarcomere occur in the cross section of a single myofibril (Figs. 6, 8). The obliquity of the striations in *Limulus* telson muscles, however, is not nearly so pronounced as it is in the clearly obliquely striated muscles of other invertebrate groups (Rohlich, 1962; Reger, 1964). Some myofibrils insert into and end in dense accumulations of Z band material which frequently occur on the sarcoplasmic side of the sarcolemma in regions free of invaginations and along the clefts (Figs. 4, 6). Other myofibrils may attach at such regions but continue on along the cell.

We have not been able to find more than one type of muscle fiber present in the telson levators, at either the light or electron microscope levels. We have not as yet employed histochemical techniques (Padykula and Gauthier, 1963) to determine differences among fibers.

Structure of the Contractile Apparatus at Various Sarcomere Lengths

LONG SARCOMERES: Fibers having predominantly long sarcomeres are obtained by fixing small bundles from the telson levators with the telson held in a completely depressed position. Analysis by light diffraction shows that sarcomeres in fibers tied at this length range between animals from 9.0 to 11.0 μm long (Figs. 10, 11 *a*, *b*).

In the light microscope, longitudinal sections of freshly fixed lengthened fibers have broad Z lines which follow a zig-zag course across the myofibril. Both I and A bands appear long, and the boundary between the two is indistinct. A broad H zone occupies the central region of the A band (Fig. 2 *b*). Thin longitudinal sections examined in the electron microscope reveal that the thick filaments of long sarcomeres (>7.5 μm) are not in register within a given A band; rather the tapered ends of the filaments terminate at various regions within the I band thus creating an irregular A-I boundary (Fig. 11 *a*). The individual thick filaments, however, measured in electron micrographs where thick filaments could be traced from tapered end to tapered end, stay at a constant average length of 4.9 μm (Fig. 10). The greater overall length of the A band, which measures up to 6.1 μm in sarcomeres greater than 10.0 μm, is due to the misalignment of the thick filaments within the sarcomere, and decreases in proportion to decreasing sarcomere length.

Cross-sectioned myofibrils (Figs. 2 *a*, 5, 8) having long sarcomeres commonly contain all of the regions of the sarcomeres. H bands are readily discerned in these preparations as areas populated by thick filaments free of surrounding thin filaments. In the region of overlap within the A band 9–13 thin filaments surround each thick filament. The occasional appearance of cross-sectioned thin filaments intruding randomly into the H zone and of the tapering ends of thick filaments

FIGURE 7 Longitudinal section of two muscle fibers with sarcomere length of 7.6 μm. Thick filaments are misaligned giving an irregular A-I boundary. An indistinct H zone is apparent and no M line is present. Z lines are irregular and discontinuous. Mitochondria occur near the Z line. × 65,000.

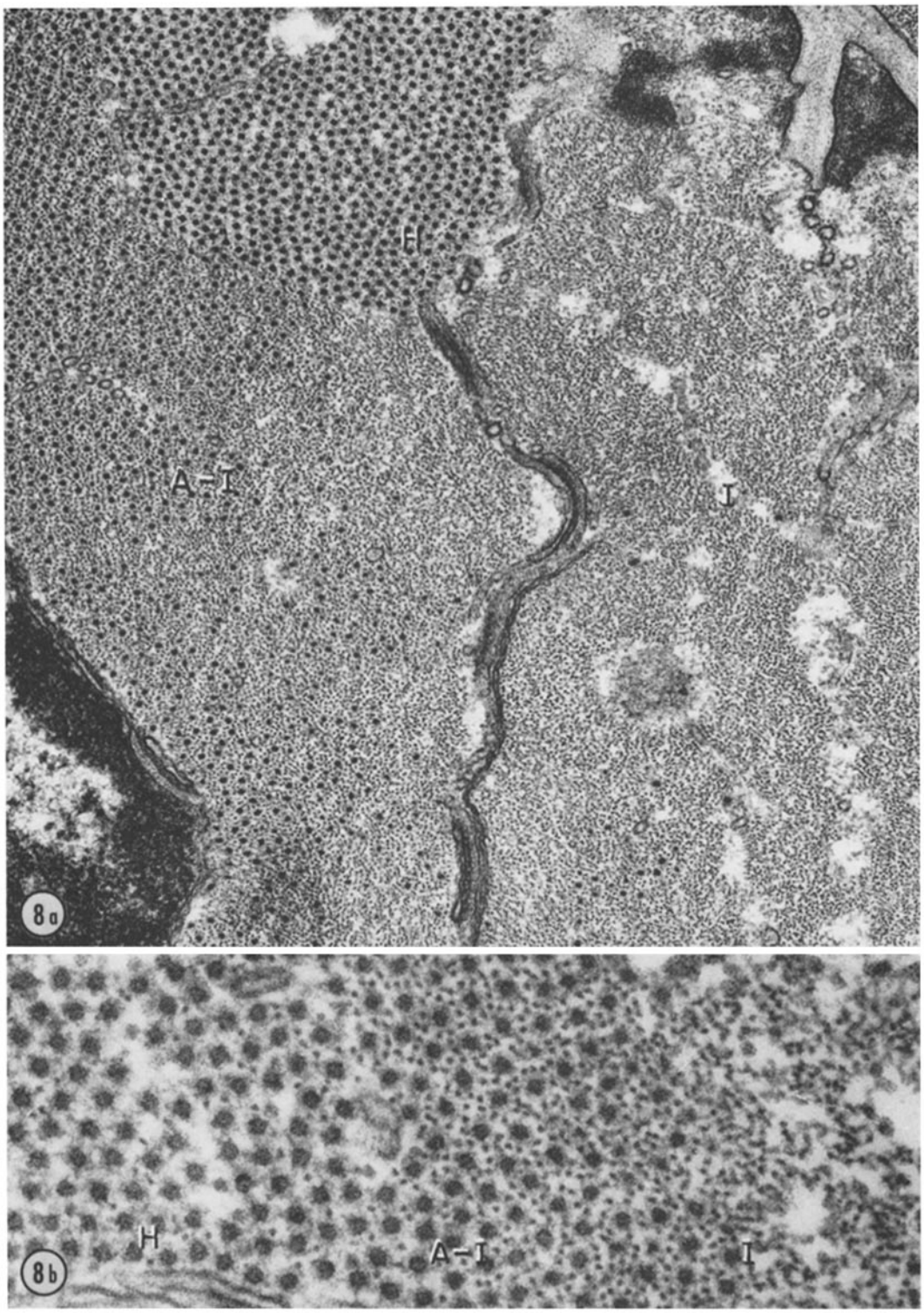


FIGURE 8 Transverse sections of lengthened fibers. (*a*) A fiber with sarcomere length of 9.4 μm. Misalignment of thick filaments is seen as isolated groups occurring in regions otherwise populated mainly by thin filaments. H, A-I, and I bands are apparent. × 40,000. (*b*) Transverse section of portion of a myofibril with sarcomere length of 9.4 μm. H, A-I, and I band regions are seen. Note occasional intrusion of thin filament into H zone. × 130,000.

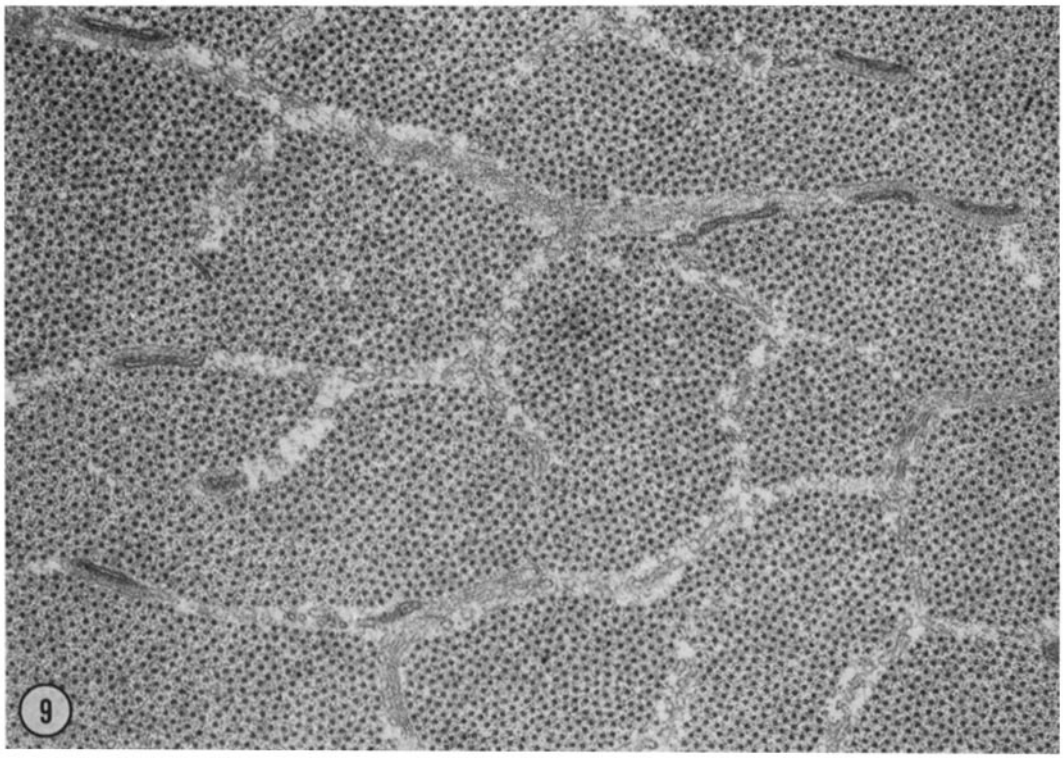


FIGURE 9 Transverse section of central region of a fiber. Myofibrils are irregularly cylindrical and surrounded by elements of the sarcoplasmic reticulum. Dyads are present. Sarcomere length 9.4 μm. × 30,000.

intruding into I band regions gives further indication of filament misalignment (Fig. 8).

INTERMEDIATE LENGTH SARCOMERES: Intermediate length sarcomeres (7.4–7.0 μm) are obtained from fibers of telson levators fixed with the telson extended at an angle of 180° to the body (Figs. 10, 12). Longitudinally sectioned, intermediate length sarcomeres have somewhat straighter and narrower Z lines than long sarcomeres. The A and I bands are more discretely defined, with a definite A–I junction discernible in the light microscope. In the light microscope a distinct H band is still present in the central A band region (Fig. 2 *d*). The lengths of the H and I bands are shorter than in long sarcomeres, as is the length of the A band. In the electron microscope, thick filament misalignment, though still apparent, is much less than that seen in long sarcomeres (Fig. 12). In sarcomeres 7.4 μm long, thick filament length also averages 4.9 μm (Fig. 10). At sarcomere lengths below 7.4 μm, both A band and thick filament length decrease in proportion to sarcomere length. In cross-sectioned material the areas occupied by thin filaments alone (I band) are smaller in extent when compared to similar regions in cross sections of long sarcomeres (Fig. 2 *c*). In overlap zones of the A band 9–13 thin filaments still surround each thick filament. An H band remains discernible.

SHORT SARCOMERES: Sarcomeres of dorsal levator muscle bundles fixed with the telson completely elevated range between animals from 5.9 to 4.4 μm (Figs. 10, 13). The Z lines in longitudinally sectioned short sarcomeres show much greater regularity than those of either long or intermediate length sarcomeres, in both the light and electron microscope (Figs. 2 *f*, 13). While the I band is not always apparent in the shortest sarcomeres in the light micrographs (Fig. 2 *f*), it is always present in the electron micrographs where misalignment of thick filaments appears to be minimal (Fig. 13 *a*). Both the overall A band length and thick filament length (which are essentially the same) are directly proportional to sarcomere length.

In cross section (Fig. 2 *e*) myofibrils having short

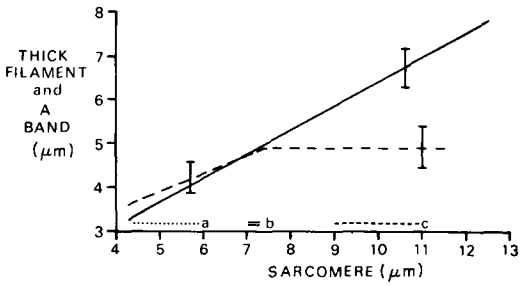


FIGURE 10 Graph showing the relationship of both A band and thick filament length to sarcomere length. Slopes were computed from measurements made on electron micrographs of longitudinally sectioned (sections cut normal to the fiber axis) freshly fixed muscle fibers. The thick filament lengths and overall A band lengths (including misalignment) were measured independently on medium power (approximately × 10,000–20,000) electron micrographs of individual sarcomeres. Thick filament lengths were recorded only from filaments which could be traced entirely through the A band and when at least ten filaments of the same length were measured per sarcomere. *N* in this case refers to sarcomeres having been analyzed, not individual filaments measured. Bars indicate standard errors of slopes. Confidence interval for all slopes: $P < 0.01$. Solid line, overall A band length, $N = 232$; dash line, thick filament length, $N = 190$ (sarcomeres). *a*, *b*, and *c* along the abcissa are ranges of sarcomere lengths of medial telson levator muscles as determined by laser diffraction. Range includes variation among animals: *a*, telson maximally elevated; b, telson at 180° to carapace (straight out); *c*, telson maximally depressed.

sarcomeres appear to be densely populated by overlap zones of the A band. I bands are seen. In short sarcomeres, 9–13 thin filaments appear to surround each thick filament at variable distances and no H zone is found.

At very short sarcomere lengths (3.0 μm long) (Fig. 14) Z lines are still straighter and no I band is present. The thick filaments, however, neither "crumple up" against the Z line nor do they pass through perforations in the latter as has been observed in "supercontracted" barnacle fibers (Hoyle et al., 1965).

DISCUSSION

We find, in agreement with Benham's (1885) description of *Limulus polyphemus* L., two mediodorsal muscles on either side of the midline, which are telson levators. The third dorsal muscle described by Benham (1885) and us, functions as the telson abductor. Contrary to Benham, but in agreement with Owen (1873) we find one ventral muscle mass, the telson depressor, which has three major heads, one of which arises dorsally and two ventrally. We propose to reinstate the older nomenclature of Owen for these muscles with the modification of terming the lateral dorsal muscle "telson abductor" rather than "oblique muscle," and specifying the three divisions of the telson depressor.

Light and electron microscopy show that the telson muscles are composed of multinucleate striated muscle fibers. Similar to Fourtner and Pax's (1972) findings in *Limulus* carpopodite distal flexors but unlike many crustacean muscles (Franzini-Armstrong, 1970; Fahrenbach, 1967), the *Limulus* telson muscles appear to be composed of a single fiber type. The light microscope appearance of these muscles is reminiscent of those of various "tonic or slow muscles." The myofibrils are irregular in shape. Misalignment of neighboring myofibrils is similar to the situation in other arthropod muscles (Cochrane et al., 1972).

The resemblance between *Limulus* telson muscle fibers and slow fibers of arthropodan (Fahrenbach, 1967; Hagopian and Spiro, 1968) and even vertebrate (Page, 1965) muscles is apparent at the electron microscope level as well. The sarcomeres are long with long thick filaments and show irregular configurations, especially in lengthened fibers. H bands are difficult to define in longitudinal sections. Besides their slightly oblique orientation, the sarcomeres of *Limulus* fibers exhibit discontinuous Z lines which, especially in lengthened fibers, follow a jagged course across the myofibril. The Z lines of *Limulus* fibers more closely resemble those of the barnacle, *Balanus* (Hoyle et al., 1965; Leyton and Ullrick, 1970) especially in long sarcomeres than they do the totally separate Z disks found in obliquely striated muscles (Rohlich, 1962; Reger, 1964).

The zig-zag course of the Z lines may account for the difficulty in discerning H bands even in lengthened sarcomeres in longitudinal thin sections. If we assume that all of the thin filaments are of equal length, as found in frog tonic fibers (Page, 1965), and that they originate at the level of the Z line, the irregular morphology of the Z line would cause individual thin filaments to terminate at different regions of the A band. Thus, longitudinal thin sections might not include a sufficient number of thin filaments ending in a specific region within the A band to provide a

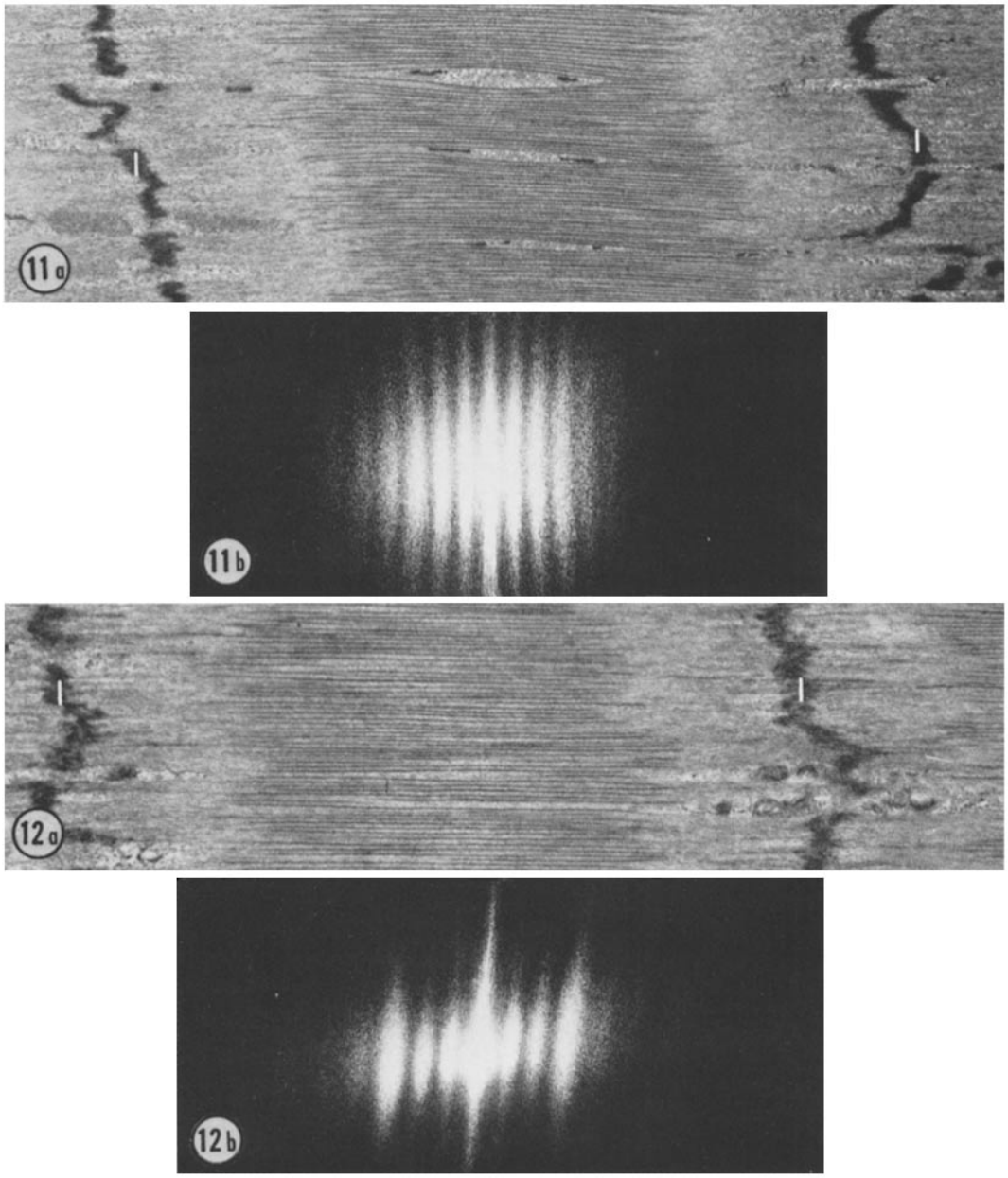
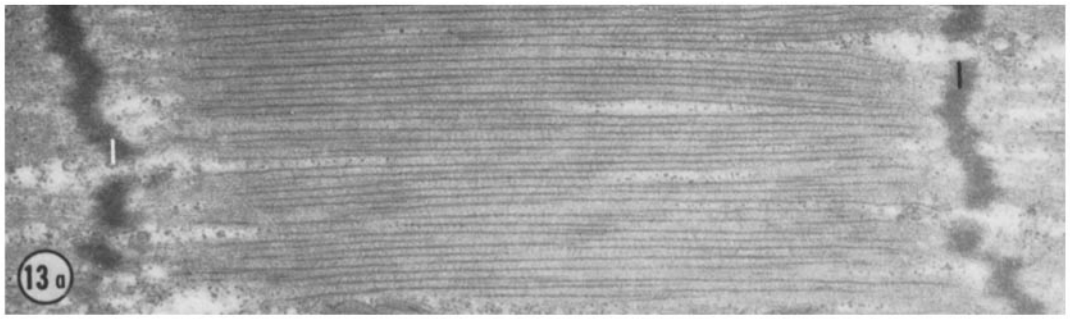
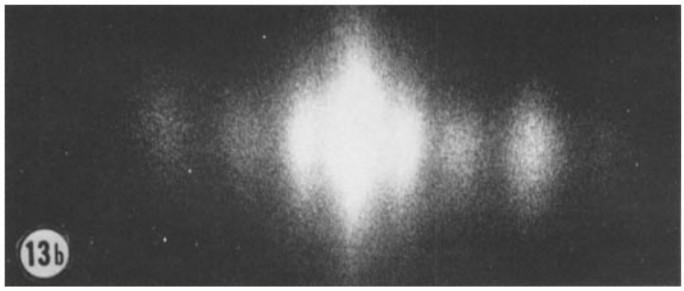


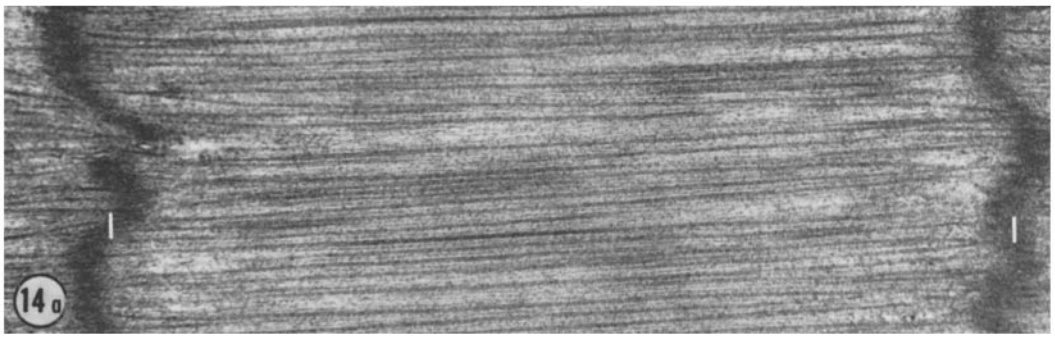
FIGURE 11 Electron micrograph and optical diffraction pattern from bundle of fibers with sarcomeres at 9.4 μm. Bars indicate sites of measurement. Overall A band width 5.5 μm. (*a*) Electron micrograph. × 12,000. (*b*) Specimen-to-film distance, 49 mm.

FIGURE 12 Electron micrograph and optical diffraction pattern from bundle of fibers with sarcomeres at 7.5 μm. Bars indicate sites of measurement. Overall A band width 5.0 μm. (*a*) Electron micrograph. × 13,500. (*b*) Specimen-to-film distance, 50 mm.

recognizable H zone. That this is the case may be seen by the ready identification of H zone regions in thin cross sections of myofibrils. Variations in the degree of penetration into the A band by individual thin filaments is seen as the occasional intrusion of thin filaments into an area otherwise populated solely by thick filament profiles.







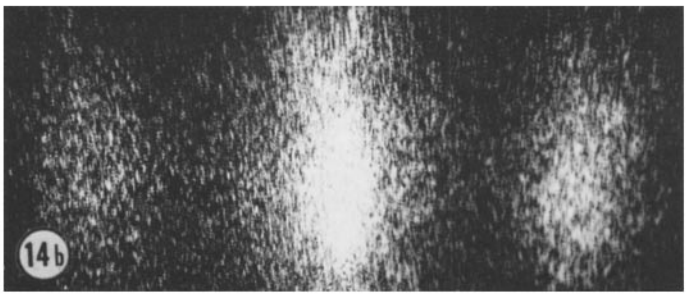


FIGURE 13 Electron micrograph and optical diffraction pattern from bundle of fibers with sarcomeres at 5.0 μm. Bars indicate sites of measurement. Thick filament length 3.5 μm. (*a*) Electron micrograph. × 25,000. (*b*) Specimen-to-film distance, 50 mm.

FIGURE 14 Electron micrograph and optical diffraction pattern from bundle of fibers with sarcomeres at 3.0 μm. Bars indicate sites of measurement. No I band present. (*a*) Electron micrograph. × 40,000. (*b*) Specimen-to-film distance, 140 mm. Although some thick filaments appear to enter the Z line, there are no true discontinuities in the latter structure here, through which the filaments can be seen to clearly pass into the next sarcomere.

The presence of a recognizable H zone in 1.0 μm thick longitudinal sections, on the other hand, is most likely due to the increased thickness of the section, which therefore includes a larger population of thin filaments ending at or near the same region in the A band. Furthermore, light diffraction studies on single glycerinated fibers (Dewey et al., 1972) also show the presence of an H band in the central region of the A band.

The conspicuous absence of M lines in *Limulus* telson muscles is also reminiscent of those of some tonic or slow striated muscles (Fahrenbach, 1967; Hoyle, 1969). In fast striated muscles, M line protein appears to serve as a mechanism to maintain the thick filaments comprising the A bands in an ordered array (Pepe, 1967). As we have postulated previously (Levine et al., 1972), the misalignment of thick filaments seen in long sarcomeres of *Limulus* telson muscles is probably related to the ability of these structures to move in relation to each other when not held in position by M line material. Such an effect of stretching on the thick filament array is commonly seen in obliquely striated muscles and other invertebrate striated muscles that lack M lines (Hoyle, 1969; Cochrane et al., 1972; Franzini-Armstrong, 1970; Leyton and Sonnenblick, 1971; Hagopian, 1966).

Thick filament misalignment in lengthened sarcomeres may be functionally related to the overall extensibility of the muscle fibers and to the changes in sarcomere length seen at various stages of the excursion of the telson levators. A 30% increase in length of these muscles occurs as the telson is moved from "rest" to its full depressed position. This is accompanied by a 33% increase in sarcomere length. Within this range of sarcomere lengths, the thick filament length remains constant at 4.9 μm. Thus far we have been able only to estimate thin filament length as 2.4 μm. This figure is based on evidence of a persistent H zone in 5.0 μm sarcomeres obtained from light diffraction studies (Dewey et al., 1973). If the thick filaments did not become misaligned, the greatest length to which sarcomeres could be extended and still retain any degree of overlap between thick and thin filaments would be less than 9.7 μm. Shortening of sarcomeres at greater lengths would be difficult. Misalignment of thick filaments in long sarcomeres together with decrease in regularity of the Z lines provides a longer A band and maintains overlap even in sarcomeres longer than 10 μm. Thus there is sufficient interaction between thin and thick filaments to permit sarcomere and fiber shortening.

We have previously shown an apparent decrease in A band length and concomitant narrowing of antiparamyosin-binding regions in peroxidase-labeled antibody studies on glycerinated *Limulus* telson muscle myofibrils as sarcomeres shortened from 10.0 to approximately 7.4 μm (Levine et al., 1972). Analysis of light diffraction experiments on single glycerinated *Limulus* fibers by Fourier synthesis reveals prominent "shoulders" on the A bands of long sarcomeres which decrease in breadth as the sarcomeres become shorter (Dewey et al., 1972). The present electron microscope observations confirm that the decrease in apparent A band length as sarcomeres shorten from 10.0 to about 7.4 μm, and associated narrowing both of antiparamyosin-staining bands and of A band shoulders, all reflect the gradual realignment of the thick filaments during sarcomere shortening. It is especially important to note that the length of individual thick filaments remains constant at 4.9 μm throughout the range of sarcomere lengths from >10.0 to about 7.4 μm. The indistinctness of the A–I boundary observed in long sarcomeres with the light microscope is most likely due to the degree of thick filament misalignment.

Interesting questions remain about the mechanisms involved in thick filament realignment during shortening of the sarcomeres and the possible contribution of this phenomenon to the overall tension development of the muscle fibers. Of equal importance is the mechanism of thin filament realignment as related to straightening of the Z line during sarcomere shortening. We envision the existence of a cooperative effect between that population of thin filaments which is maintained in an ordered array within the A band even in very long sarcomeres and the thick filaments between which they interdigitate. Interactions between the filaments would result in both the conventional sliding of the thin filaments toward the sarcomere center and the repositioning of out of register thick filaments and thin filaments into a more orderly array as shortening progresses. As the sarcomeres shorten, and the thick filaments become realigned, the Z lines appear narrower and, although never as straight as in many other striated muscles, they

assume a more regular configuration than is found in very long sarcomeres. This effect probably serves to optimize the degree of interaction between thick and thin filaments within the A band. If the development of maximum tension is determined by the maximum ability of thick filament cross bridges to interact with an optimal number of thin filaments we should be able to predict the L_0 of *Limulus* telson levator sarcomeres as the length at which thick filament realignment and some straightening of the Z band is seen. In support of this, preliminary studies show that maximum isometric tension is reached when the telson is maintained at an angle of 180° to the body, and where the sarcomere length is approximately 7.0 μm (M. Dewey, unpublished observations).

The continued decrease in both A band and thick filament length, with decrease in sarcomere length from 7.4 to less than 5.0 μm, which has been observed using electron microscopy, light microscopy, phase-contrast optics, and fluorescence microscopy on antibody stained myofibrils, is a more complicated issue.

Previous reports demonstrating A band shortening during sarcomere shortening (de Villafranca, 1961; de Villafranca and Marschhaus, 1963; Gilmour and Robinson, 1964) have been criticized on the basis that this phenomenon appeared only in glycerinated preparations and was probably related to the glycerination procedure (Stephens, 1965; Peachey, 1968). The observations on freshly fixed tissue we present here indicate that the decrease in A band length as *Limulus* sarcomeres shorten below 7.4 μm (Levine et al., 1972) is due to changes in thick filament length. Thus, we may discount the effects of glycerination on the myofilaments. As only one type of fiber is present in the telson muscles, neither can we ascribe the differences in filament lengths in sarcomeres of different lengths to inadvertent sampling of different fiber populations, as has been suggested by Franzini-Armstrong (1970).

We believe that such conformational change as may occur within the thick filaments may also be a function of the great excursion exhibited by these fibers and may be related to their paramyosin content. One hypothesis which has been advaned to explain the catch phenomenon in lamellibranch smooth adductors and *Mytilus* anterior byssus retractor muscle is that small changes in the ordering of the paramyosin molecules comprising the cores of the very thick filaments of these muscles impose secondary effects on the cortical myosin molecules. These effects are reflected by the stability of the heavy meromyosin-actin linkages seen in the catch state (Szent-Györgyi et al., 1971). Our previous immunohistochemical study indicates, in agreement with Ikemoto and Kawaguti (1967), that the thick filaments of *Limulus* striated muscle also have paramyosin cores. It is possible that during shortening of the sarcomeres below 7.4 μm rearrangement of the medullary paramyosin molecules imposes a conformational change on the whole thick filament. This, in turn, may permit a decrease in sarcomere length greater than that imposed on the fiber by thick filaments 4.9 μm long.

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