A light and electron microscopic examination of muscles in the walking legs of the horseshoe crab, *Limulus polyphemus* (L.)[1]

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An examination was made of the gross morphology and ultrastructure of skeletal muscles in the legs of the horseshoe crab, *Limulus polyphemus* (L.). The origins, insertions, actions, and innervation of each head of the seven distal leg muscles are described. A detailed description of the transverse tubular system and the sarcoplasmic reticulum is also given. Of special interest is the possibility that the muscle fibers may be split into "units" of different diameters.

The sarcomere length of fibers in the seven muscles was determined to see if both long and short sarcomere length fibers are present. The distribution of fiber sarcomere lengths in each muscle is unimodal, and mean values for each of the different muscles range from 7.6 to 8.5 μ. The ultrastructural features of fibers in each muscle also are similar. Therefore, it appears that horseshoe crab leg skeletal muscles are composed structurally of a fairly uniform population of fibers and that fast and slow categories of fibers, at least as defined for other arthropods, are not present in the horseshoe crab muscles studied here.

Introduction

Present knowledge of arthropod skeletal muscle comes almost entirely from studies on crustaceans and insects. Undoubtedly the best studied are the crustaceans. Here, muscle fibers show a wide range in properties, and on the basis of several different criteria, they often can be grouped into two main categories: fast and slow. In comparison with the fast fibers, the slow fibers have much slower contraction and relaxation times, longer sarcomeres, higher thin to thick myofilament ratios, and fewer dyads per unit length of muscle fiber (reviewed by Atwood 1967). Both fast and slow fibers occur within some crustacean muscles (Dorai Raj 1964; Atwood and Dorai Raj 1964; Jahromi and Atwood 1971*a*), while others appear to contain a uniform population of fibers (Sherman and Atwood 1971).

There have been relatively few studies of other arthropod skeletal muscles, and more are needed to provide a better general understanding of skeletal muscles in arthropods. One class of arthropods that is particularly interesting to study because of its long evolutionary history is the class Merostomata, which consists of the horseshoe crabs. A number of studies using modern techniques have been made of horseshoe crab opisthosomal (abdominal) muscle in regard to myofilament organization and function (de Villafranca *et al.* 1959; de Villafranca 1961, 1967, 1968; de Villafranca and Philpott 1961; de Villafranca and Marschhaus 1963; de Villafranca and Naumann 1964; Ikemoto and Kawaguti 1967; de Villafranca and Campbell 1969; de Villafranca and Waksmonski 1970; Stanley 1970; Stanley and de Villafranca 1970). However, no detailed examination has been made of other structural features, such as the transverse tubular system and the sarcoplasmic reticulum. Furthermore, there has been no examination of horseshoe crab muscles for possible differences in structural features.

For these reasons, the present study was undertaken to describe some of the ultrastructural features of skeletal muscles in the walking legs of the horseshoe crab *Limulus polyphemus* (L.) with special emphasis on the transverse tubular system and the sarcoplasmic reticulum. Another objective was to determine if muscle

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fibers differing in important structural features occur in *L. polyphemus*; in this regard, seven different muscles in the legs were examined for possible differences in sarcomere length, and four of these were also examined with the electron microscope for possible differences in ultrastructural features. During the study, it was noted that certain of the gross morphological features of the distal seven leg muscles were somewhat different than reported previously. Therefore, we also have included a description of the gross morphology of these muscles and their innervation.

Materials and Methods

Specimens of *Limulus polyphemus* ranging in carapace width from 18 to 23 cm were obtained from Gulf Specimens Co., Panacea, Florida, U.S.A. They were kept until use in artificial seawater at a temperature of 13 to 17°C.

The second, third, and fourth pairs of legs were studied. Segments of the leg containing the muscle to be examined were pinned out on a piece of balsa wood in such a way that the muscle was held at its normal *in situ* resting length. As much of the exoskeleton as possible was removed from the segment to facilitate penetration of the fixative.

Two different procedures were used to fix and prepare tissue for light microscopy. In the first, the preparation was fixed in Carnoy's solution, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Paraffin sections were cut at a thickness of 6 μ, mounted on glass slides, and stained with Delafield's hematoxylin and aqueous eosin.

In the second procedure, which was used for most of the sarcomere length measurements, muscles were fixed for 2 h with 4% glutaraldehyde in artificial seawater; they were then rinsed several times in artificial seawater and placed in 70% ethanol. A number of muscle fibers were then dissected from different regions of the muscle and teased apart into small myofibril bundles. The bundles of myofibrils, along with a drop of 70% ethanol, were placed on a glass slide, and covered with a cover slip. At least five myofibrils from each fiber were examined with a light microscope at a magnification of 430× and representative sarcomeres measured with an eyepiece micrometer.

For electron microscopy, muscles were fixed for 2 h in a solution containing 4% glutaraldehyde, 0.1 *M* sodium cacodylate buffer (pH 7.2), 2.0% sodium chloride, and 0.2% calcium chloride. They were then washed for 30 min in a solution containing only the buffer and salts. After the wash, the muscles were postfixed for 1 h in 1% osmium tetroxide buffered as above and again washed for 30 min in the buffer–salts solution. A number of small pieces of tissue (3 × 1 × 1 mm) were then removed from different regions of the muscles, prestained for 2 h in a saturated solution of uranyl acetate in 50% ethanol, dehydrated in a graded ethanol series, and embedded in either Spurr embedding material (Polysciences, Inc., Warrington, Pennsylvania, U.S.A.) or an araldite–epon mixture. Thin sections were obtained using a LKB Ultratome and placed onto 200-mesh copper grids that had been coated with a 2% collodion solution. The sections were stained with ethanolic uranyl acetate and lead citrate (Reynolds 1963) and examined with a Phillips EM 200 electron microscope.

The gross morphological studies were performed on both living and preserved (Carnoy's fixative) muscles. Innervation was determined both by vital staining with methylene blue (1% methylene blue in seawater) and electrical stimulation of the main leg nerves and their branches using either platinum hook electrodes or suction electrodes.

Results and Discussion

Gross Morphology

The gross anatomy, innervation, and function of the musculature of the walking legs of *L. polyphemus* were first described by Patten and Redenbaugh (1899) and later by Vachon (1945). These investigators give a general overview of the walking leg musculature: origins, insertions, functions, and innervation. Three recent studies have reported similar observations of a few of the walking leg muscles (Pringle 1956; Hayes and Barber 1967; Ward 1969). However, all of these investigators considered most of the muscles as single morphological units and gave little or no description of the different heads of each of the muscles. There are also several discrepancies in their reports. Therefore, a description will be made of the morphology and innervation of each of the muscles in the distal segments, the basipodite to the dactylopodite. (See Fig. 1.) The terminology used by Patten and Redenbaugh (1899) in their treatise on the gross morphology of *L. polyphemus* will be used throughout this description.

The extensor of the dactylopodite (claw opener) is a small muscle composed of two distinct muscle heads. Group I originates along the dorsal surface of the propodite and group II along the anterior surface near the large condyles of the propodite. Both heads insert on a single apodeme which is attached near the posterior proximal margin of the dactylopodite. Hayes and Barber (1967) using vital staining showed that this muscle was innervated by both the internal pedal nerve (IPN) and the external pedal nerve (EPN), which is the smaller of the two leg nerves. Pringle (1956) and Patten and Redenbaugh (1899) state that only the EPN innervates this muscle. In our experiments, stimulation of both the EPN and the IPN produced recordable tension of the dactylopodite extensor; however, the tension produced by stimulation of the IPN was very small,

indicating that most of the motor innervation is provided by the EPN. (See Table 1.)

The flexor of the dactylopodite (claw closer) is also composed of two heads. However, these are not as clearly defined as those of the extensor. Head I originates on the ventral surface of the propodite and head II on the posterior surface of the propodite. Both heads insert on a single apodeme which is joined to the anterior proximal margin of the dactylopodite. Physiological evidence (Parnas *et al.* 1968), as well as methylene blue studies (Hayes and Barber 1967), have shown that this muscle is innervated solely by the IPN; we agree with their findings.

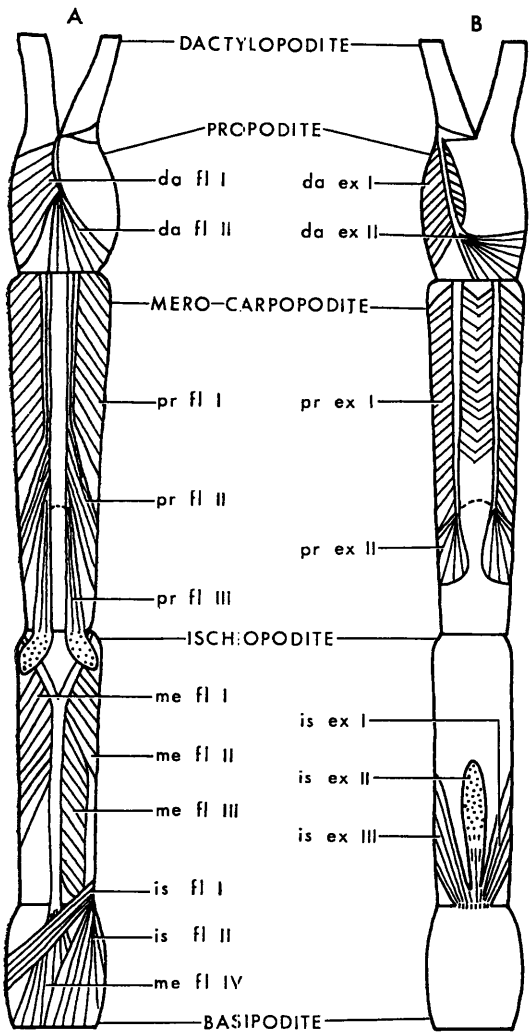

FIG. 1. Schematic representation of the seven distal muscles in the fourth walking leg of *L. polyphemus* (not drawn to scale). In A, the extensor muscles have been removed to show the dorsal aspect of the four flexor muscles. In B, the flexors have been removed to show the ventral aspect of the three extensor muscles. The posterior surface of the leg is to the right in A and to the left in B. The broken line in the mero–carpopodite represents the mero–carpopodite groove. The abbreviations are dactylopodite, *da*; propodite, *pr*; mero–carpopodite, *me*; ischiopodite, *is*; flexor, *fl*; extensor, *ex*. Roman numerals denote the different heads of each muscle. See text for details.

The extensor musculature of the propodite is composed of two synergistic muscles. The two muscles are symmetrical, one located in the anterior half of the mero–carpopodite and the other in the posterior half. A description of the anterior extensor will hold for its synergist. The anterior extensor consists of two muscle heads. Head I is a thin mass of tissue which originates along the dorsal and anteriodorsal surfaces of the mero–carpopodite distal to the mero–carpopodite (M–C) groove, which is the region of fusion between two embryonic segments: the meropodite and the carpopodite (Patten and Redenbaugh 1899). Head II is much thicker than head I and originates on the anterior surface of the mero–carpopodite proximal to the M–C groove. Both insert on a large single apodeme which is attached near the distal edge of the dorsal surface of the propodite. Pringle (1956) reported that the EPN did not innervate the extensor muscle of the propodite; however, Hayes and Barber (1967) stated that this muscle is innervated solely by the EPN. The results of our methylene blue studies revealed that the extensor muscle is innervated by branches of both the EPN and the IPN. Stimulation of the EPN evoked contractions of the extensor, but stimulation of the IPN produced no measurable tension development. This, however, does not rule out the possibility of inhibitory input to the propodite extensor from the IPN.

TABLE 1

Gross innervation of walking leg muscles in *Limulus polyphemus*

Muscle	Internal pedal nerve (IPN)	External pedal nerve (EPN)
Dactylopodite extensor	X	X
Dactylopodite flexor	X	
Propodite extensor	X*	X
Propodite flexor	X	
Mero–carpopodite flexor	X	
Ischiopodite extensor		X
Ischiopodite flexor	X	

*Based on methylene blue studies; not confirmed by electrical stimulation studies (see text).

The flexor musculature of the propodite also consists of two synergistic, symmetrical muscles, an anterior muscle and a posterior muscle (Fig. 1). Each of these muscles is composed of three distinct, large muscle heads. The most distal head (I) originates along the entire ventral surface of the mero–carpopodite distal to the M–C groove. Head II originates on the dorsolateral surface of the mero–carpopodite proximal to the M–C groove. Head III originates on the dorsal surface of the distal portion of the ischiopodite. Both II and III insert near the large condyles of the propodite via several long apodemes. Head I also inserts on the propodite by way of these apodemes; in addition, this particular head also inserts along the entire lateral surface of the propodite. Therefore, contraction of head I of the anterior or posterior muscle will produce either promotion or remotion respectively. Vachon (1945) stated that this joint was capable of only extension and flection, but Patten and Redenbaugh (1899) earlier had stated correctly that the propodite could be both promoted and remoted although they claimed these actions were due to coordinated contractions of the extensors and flexors.

The innervation of the propodite flexor is exclusively via branches of the IPN. Two large nerve branches (one to each synergist) emerge from the IPN at the proximal margin of the mero–carpopodite; these nerves traverse the surface of the flexor sending branches to each of the three heads. Stimulation of the nerve branch which innervates head I confirms the promotor and remotor capabilities of these heads; however, it is not possible at this time to state that the motor axons innervating head I do not also innervate heads II and III.

Patten and Redenbaugh (1899) claimed that extension of the mero–carpopodite was accomplished by the part of the propodite flexor, which originates in the ischiopodite. Vachon (1945) disputed this, stating that there was no functional muscle that extended the propodite and that extension was achieved by means of a mechanical process. Ward (1969) has further demonstrated that extension of the propodite is a mechanical process involving three different parameters: weight of the distal segments, contraction of the flexor muscles of the ischiopodite and basipodite, and contact between the claw and a substrate.

Flection of the mero–carpopodite is accomplished by four muscle heads: heads I, II, and III originate in the ischiopodite, forming the distal part of the mero–carpopodite flexor; head IV originates in the basipodite, forming the proximal part of the mero–carpopodite flexor. Head I originates on the anteriodorsal surface of the ischiopodite, and head II originates on the posteriodorsal surface of the ischiopodite. Head III originates on the posterioventral surface of the ischiopodite, while head IV originates on the ventral surface of the basipodite. All four heads insert on a single large apodeme which runs from the basipodite through the ischiopodite and attaches via soft cuticle to the mero–carpopodite. The distal part of this muscle was described by Patten and Redenbaugh (1899) and Vachon (1945) as composed of only two heads, but this is incorrect. Innervation of the four muscle heads of the mero–carpopodite flexor is solely via the IPN (Hayes and Barber 1967; Fourtner and Pax 1972).

Extension of the ischiopodite is accomplished by a muscle composed of three distinct heads. Patten and Redenbaugh (1899) and Vachon (1945) have described these as individual muscles. However, all three heads have the same insertion on the distal dorsal margin of the basipodite. Therefore, we consider these as one functional muscle. Heads I and III are symmetrical and originate on the anterior and posterior surfaces of the ischiopodite respectively. Head II originates all along the ventral surface of the ischiopodite. Pringle (1956) apparently interpreted this muscle to be the flexor instead of the extensor of the ischiopodite; however, the ischiopodite flexor is located in the basipodite. Our results concerning its innervation agree with those of Pringle (1956) and Hayes and Barber (1967), who demonstrated that the EPN innervates this muscle.

The flexor of the ischiopodite is a very large muscle and consists of two heads. Head I originates on the anterior surface of the basipodite and inserts on the posterioventral surface of the ischiopodite. Head II originates on both the anterior and posterior surfaces of the basipodite and inserts on the large condyle of the posterior proximal margin of the ischiopodite. Our results confirm those of other workers (Hayes and Barber 1967; Ward 1969) that inner-

vation of this muscle is accomplished solely by the IPN.

Sarcomere Lengths

Studies of crustacean skeletal muscle fibers have shown that they often can be categorized both morphologically and functionally on the basis of their sarcomere length (Atwood 1967; Jahromi and Atwood 1969). Crustacean fast fibers have 2–5 μ sarcomeres, while those of slow fibers range from 6 to 14 μ. An examination was made of sarcomere lengths of fibers in the seven distal walking leg muscles of *L. polyphemus* to see if similar morphological categories of fibers are present.

Muscle fibers were examined from every muscle group of the seven muscles. The sarcomere length of a given fiber was determined by selecting a 100-μ length of the fiber, counting the number of sarcomeres present, and then dividing 100 μ by the number of sarcomeres. The results of these studies are given in Table 2. The total range of sarcomere lengths observed was 5.9 to 10.5 μ. However, the greatest range encountered for a single muscle head was 3.1 μ. Some of the variability in values is probably the result of the difficulty in fixing a given muscle at the same initial length from one preparation to the next and to naturally occurring variations in sarcomere lengths (cf. Franzini-Armstrong 1970). Nevertheless, the range of the mean values of sarcomere length for the seven muscles is less than 1 μ.

To insure that by taking a mean value for each muscle we were not averaging out two different populations of fibers at the extremes of the range, we constructed frequency histograms for each of the different muscles. In each case, the histograms were unimodal, indicating that each muscle consists of a single population of fibers in regard to sarcomere length.

Comparing these results with those reported for crustaceans, it appears that—at least in regard to sarcomere length—the muscles examined in *L. polyphemus* contain a fairly uniform population of fibers.

Other Microscopical Features

Muscle fibers from four different muscles were examined for possible differences in other structural details using both light and electron microscopes. These were the mero–carpopodite flexor, the propodite flexor, the propodite extensor, and the dactylopodite flexor. The structural details of fibers from all of these muscles were identical; therefore, the following description is applicable to fibers of each of the muscles examined.

Examination of transverse sections of paraffin-embedded material revealed that each muscle consists of fibers having a wide range of diameters (Fig. 2). Very small fibers (10–20 μ) occurred in close association with large fibers (40–60 μ). The small fibers appeared to be separated from the larger ones by a rather thin sarcolemma, but it was uncertain whether the small fibers actually should be considered as individual fibers or if a group of the large and small "units" really represent a single fiber. As a result of this uncertainty, it was not deemed possible to determine accurately the total number of fibers in each muscle, nor was it possible to obtain definite values of fiber dimensions.

The relationship between the large and small fibers was examined more closely with the electron microscope. A number of observations indicate that the small units may have arisen from the large units by a process of fiber splitting, and that the small units may be anatomically and

TABLE 2

Sarcomere lengths in microns of fibers in the walking leg muscles of *Limulus polyphemus*

Muscle	Mean (S.E.)	Range	No. fibers	No. muscles
Dactylopodite extensor	7.6 (0.3)	6.3 to 9.5	175	3
Dactylopodite flexor	7.7 (0.5)	6.3 to 10.5	294	4
Propodite extensor	7.7 (0.5)	6.1 to 10.0	289	4
Propodite flexor	8.5 (0.5)	6.5 to 10.5	214	3
Mero–carpopodite flexor	8.1 (0.3)	5.9 to 10.5	485	6
Ischiopodite extensor	7.7 (0.3)	6.3 to 9.5	135	3
Ischiopodite flexor	8.3 (0.6)	6.3 to 10.5	140	3

functionally separate from the larger units. The overall shape of the closely associated units and the very thin sarcolemma between them are suggestive of fiber splitting (Fig. 3). A further indication of possible fiber splitting comes from the different densities of myofilaments seen in the large and small units. Myofilaments invariably are more densely packed in the large units. Finally, both the large and small units contain a full complement of subcellular structures, including nuclei.

The possibility of fiber splitting in *L. polyphemus* is particularly interesting in light of a recent study of the lobster stretcher muscle (Jahromi and Atwood 1971*b*). It was discovered that the fibers in this muscle were often subdivided into "subunits" and that these subunits were electrically and anatomically coupled by small sarcoplasmic bridges. Furthermore, the subunits increased in number as the muscles grew in size. These results indicated that fiber splitting may occur during growth in lobsters, perhaps to maintain reasonably sized fibers (Jahromi and Atwood 1971*b*). Horseshoe crabs, like the lobsters, are amongst the largest of the arthropods, and although no clear demonstration of fiber splitting was made in the present study, our observations indicate the possibility of fiber splitting in *L. polyphemus* as well.

Many of the ultrastructural features of horseshoe crab skeletal muscle sarcomeres have been described by de Villafranca (1961), de Villafranca and Philpott (1961) for *L. polyphemus*, and Ikemoto and Kawaguti (1968) for *Tachypleus tridentatus*. The former authors used primarily the abdominal extensor muscle, although in the latter paper they mentioned that leg muscles were also studied. Unfortunately, it was not stated which leg muscles were used; therefore, a direct comparison cannot be made between their work and the results of the present study.

The ultrastructural features of sarcomeres in the leg muscles are shown in Figs. 4–7. Features typical of striated muscle are present. Most notable are the presence of wide A bands (about 5 μ in Figs. 4 and 5), thick Z lines, and H zones. A pseudo H zone is present in the middle of the A band and appears to contain an M line (Fig. 5). The details of the M line were not well preserved by our methods. Well-developed N lines are not present in our material, although in some areas within the I band the thin filaments have a beaded appearance (Fig. 5).

The previous studies of the ultrastructural features of horseshoe crab skeletal muscles failed to find H zones, pseudo H zones, and M lines (de Villafranca and Philpott 1961; Ikemoto and Kawaguti 1968). This is somewhat surprising, for all of the horseshoe crab skeletal muscles examined to date have sarcomeres of similar length.

The number of thin filaments around each thick filament ranges from 8 to 12 in regions of maximal overlap of thick and thin filaments (Fig. 6). This figure is similar to that reported for the opisthosomal extensor muscle of *T. tridentatus* (Ikemoto and Kawaguti 1968).

The sarcoplasmic reticulum (SR) consists of a series of tubules which lie between the myofibrils (Fig. 7). These tubules often divide into numerous branches to form a fenestrated collar around the myofibrils (Figs. 8 and 9). The SR forms dyads with the transverse tubular system (TTS) in the intermyofibrillar spaces (Figs. 5 and 7). Dyads occur in both A and I band regions, and they are present in variable numbers from one sarcomere to the next (Figs. 4 and 5). At the dyad, the SR forms broad cisternae which are usually 0.3 to 0.5 μ wide (Figs. 6, 8, and 10). The cisternae are curved slightly and appear saucer-shaped in transverse view (Fig. 10). The T-tubule member of the dyad is situated on the concave surface of the curved SR tubule, at a distance of about 100 Å from the inner surface of the SR cisternae (Figs. 6, 8, and 10). Periodic thickenings occur along the SR membrane facing the T-tubule (Figs. 6 and 8). These thickenings are about 200 Å wide, and they lie about 100 Å apart from one another. This arrangement imparts a lattice-like appearance to the dyadic junction when observed in face view (Fig. 9). In some sections, the periodic thickenings on the SR surface appear to bridge the dyadic junctional gap completely, while in other sections they appear to form an incomplete linkage between the SR and TTS. This apparent difference in dyadic structure may have resulted from a nonuniform preservation of material for electron microscopic examination.

The TTS arises as invaginations of the plasma membrane either along the muscle fiber surface, or more commonly, from a sarcolemmal invagi-

nation (Figs. 7 and 10). The T-system consists of a number of individual tubules which are situated at right angles to the main axis of the fiber and between the myofibril bundles. Each T-tubule expands as it approaches the SR to form dyadic junctions. Tubules of the TTS and SR appear to weave through the intermyofibrillar spaces in such a way that they form dyads in an alternating fashion (Fig. 10). The T-tubules are smaller than the SR tubules and have denser membranes.

In addition to the T-tubules, another type of membrane invagination occurs along the Z-line material. These invaginations consist of short indentations of the sarcolemma (Fig. 11). They resemble T-tubules, but they do not form dyads. Because of their short incursion of the fiber, these invaginations do not appear to correspond to the Z-tubules reported present in certain crustacean muscles (Peachey 1967; Atwood 1971).

From the results of the present study, it appears that muscle fibers in *L. polyphemus* walking legs are quite similar to one another in structural features. This is not at all like the situation in insect and crustacean muscles where the fibers in the various muscles often have quite different morphological features (Atwood 1967; Usherwood 1967; Hoyle and McNeill 1968; Franzini-Armstrong 1970; Cochrane *et al.* 1972). In certain important morphological respects, the fibers in *L. polyphemus* are similar to crustacean slow fibers. Both have long sarcomeres, large numbers of thin myofilaments around each thick myofilament, and thick Z lines. On the other hand, there appear to be more dyads per unit length of fiber in *L. polyphemus* than are reported for most crustacean slow fibers. In addition, H and pseudo H zones and M lines are present in *L. polyphemus* fibers, while these features are indistinct or absent entirely from crustacean slow fibers and perhaps from slow fibers of animals in general (Page 1965; Hess 1970). Therefore, *L. polyphemus* leg muscles do not clearly fit into either the fast or slow categories as defined for crustacean muscles.

This suggestion is supported by the results of physiological studies of two of the walking leg muscles of *L. polyphemus*, the dactylopodite flexor (closer) and the mero–carpopodite flexor (Parnas *et al.* 1968; Fourtner and Pax 1972). From these studies, it appears that the physiological properties tend to be intermediate between crustacean fast and slow muscles. All of the fibers examined gave graded spikes to single or two closely paired stimuli applied to leg motor neurons (Fourtner and Pax 1972). All fibers are polyneuronally innervated and all of the various axons produce excitatory postsynaptic potentials of similar duration (Fourtner and Pax 1972; Sherman and Fourtner 1972). Furthermore, all of the axons elicited a mechanical response of the muscle upon a single stimulation, and each axon could produce twitch contractions upon appropriate stimulation (Parnas *et al.* 1968; Fourtner and Pax 1972).

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EXPLANATION OF FIGS. 2–11

FIG. 2. Light micrograph of a transverse paraffin section through a surface region of the mero-carpopodite flexor. Note the presence of very small fibers along with the much larger ones. Magnification: 200.

FIG. 3. Electron micrograph showing in transverse view two small fibers in close association with a single large fiber in the propodite flexor. A thin sarcolemma (arrows) separates the small fibers from the larger one. Note the loosely packed myofilaments in the small fibers as compared to the myofilament density in the larger fiber. Magnification: 4000.

FIGS. 4 and 5. Electron micrographs showing the ultrastructural features of sarcomeres in the propodite flexor in longitudinal view. The sarcomeres are in good register as shown at low magnification (Fig. 4). The details of the sarcomeres are designated in the enlarged view of Fig. 5. The typical A and I bands and Z lines are present, as are dyads (D) and elements of the sarcoplasmic reticulum (SR). An H zone (H) is evident in the middle of the A band. A pseudo H zone traverses the center of the H zone. The dark material in the center of the pseudo H zone (unlabeled arrow) may represent an M line. M, mitochondria. Magnification: 5000 in Fig. 4 and 11 000 in Fig. 5.

FIG. 6. Electron micrograph of the A band region in the dactylopodite flexor to show the relationship in numbers between thick and thin filaments. There are about 8 to 12 thin filaments around each thick filament. Also shown is an enlarged view of a dyad. The T-tubule (T) lies on the concave side of the curved surface of the sarcoplasmic reticular element (SR). Note the periodic thickenings (unlabeled arrows) along the inner surface of the SR element which imparts a septate appearance to the dyadic junction. Magnification: 120 000.

FIG. 7. Electron micrograph of the propodite flexor to show the general ultrastructural features of the leg muscles in transverse view. The myofibrils (MF) are strap-shaped and are delineated by elements of the sarcoplasmic reticulum (SR) and the transverse tubular system (T), which form numerous dyads (D). Invaginations of the plasma membrane (P) form the T-system. A, A band; I, I band; M, mitochondria; N, nucleus. Magnification: 8000.

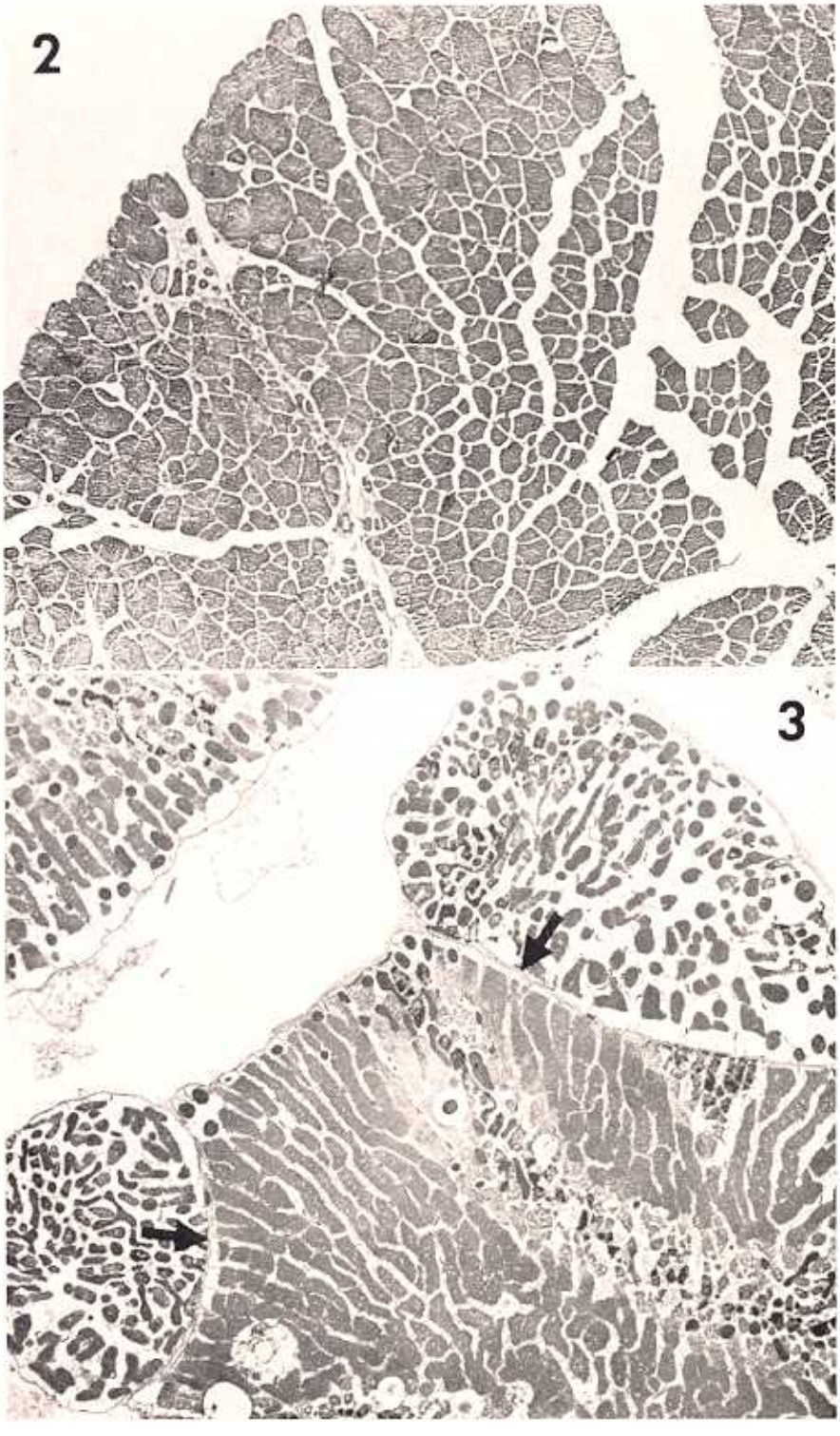
FIG. 8. Electron micrograph of a region in the propodite flexor to show the fenestrated nature of the sarcoplasmic reticulum (SR) formed by numerous branchings of the SR tubules. A dyad also is shown in the upper right. MF, myofilaments; T, T-tubule. Magnification: 80 000.

FIG. 9. Electron micrograph of a region in the propodite flexor to show the lattice-like appearance of the dyads (D) in face view. MF, myofilaments; SR, sarcoplasmic reticulum. Magnification: 33 000.

FIG. 10. Enlarged view of a region in the dactylopodite flexor to show the alternating fashion by which the T-tubules (T) and sarcoplasmic reticulum (SR) form dyads. The arrows indicate the alternate positions of the T-system element at successive dyads. Note that the T-tubule arises as an invagination of the plasma membrane. SI, sarcolemmal invagination. Magnification: 94 000.

FIG. 11. Electron micrograph of a region in the dactylopodite flexor to show the plasma membrane invaginations (arrows) which occur along the Z lines (Z). These invaginations do not form dyads (D), in contrast to those plasma membrane invaginations occurring in the A and I band regions. M, mitochondria; SI, sarcolemmal invagination. Magnification: 20 000.

NOTE: Figs. 2–11 follow.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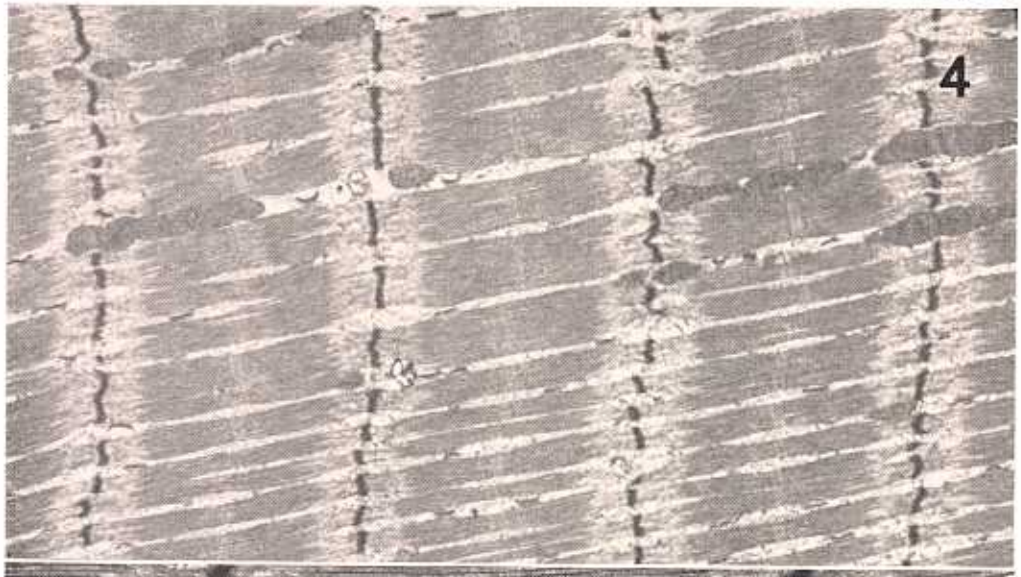
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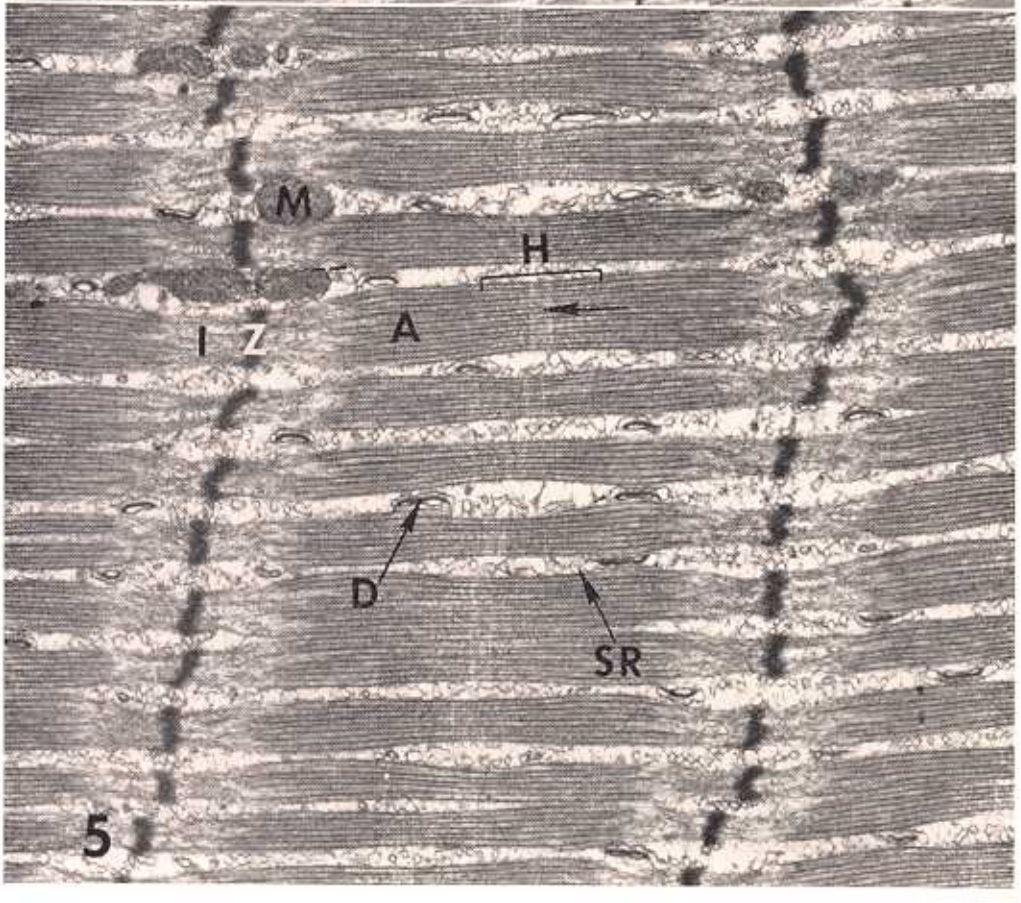
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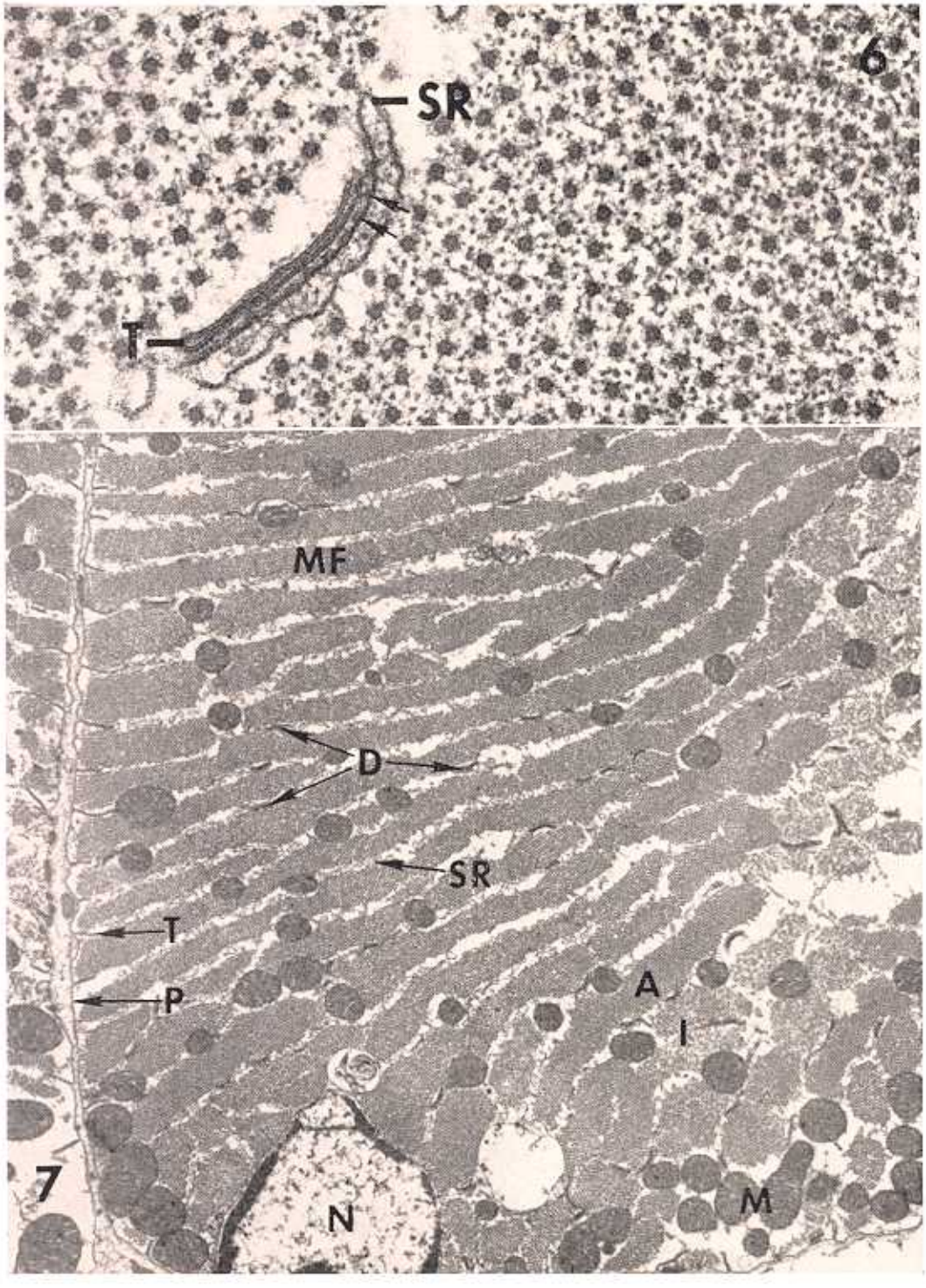
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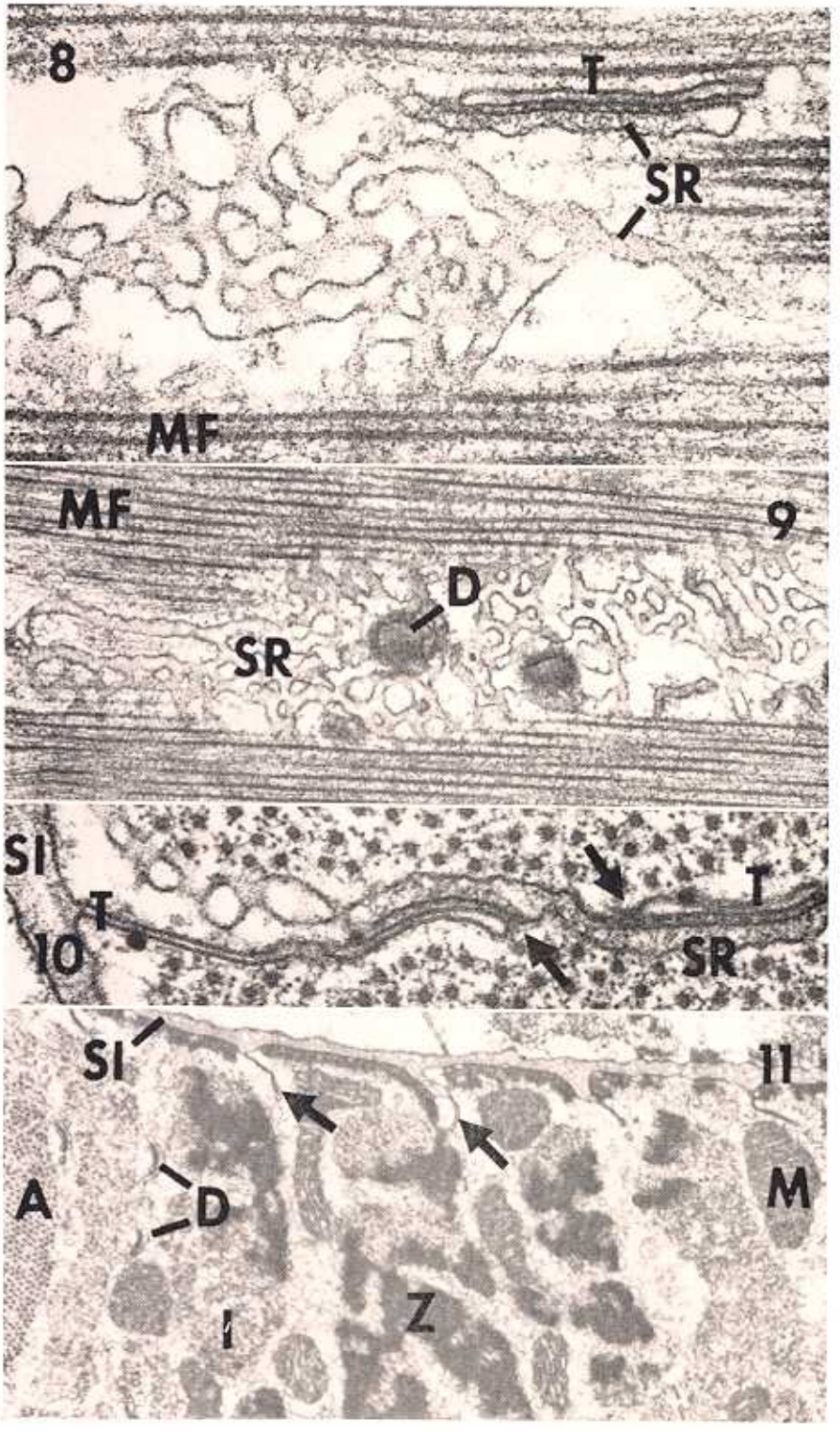
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