THE FINE STRUCTURE OF FAST AND SLOW CRUSTACEAN MUSCLES

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ABSTRACT

Known phasic and tonic muscle fibers of the crab *Cancer magister* were studied by electron microscopy. Phasic fibers have sarcomeres about 4.5 μ long, small polygonal myofibrils, and a well-developed sarcoplasmic reticulum. The thick myofilaments, disposed in hexagonal array, are each surrounded by six thin filaments. The tonic fibers have a sarcomere length of about 12 μ, larger myofibrils, a poorly developed sarcoplasmic reticulum, and a disorderly array of myofilaments. Each thick myofilament is surrounded by 10–12 thin filaments. The same morphological type of slow muscle has been found in the crustaceans, *Macrocyclops albidus*, *Cypridopsis vidua*, and *Balanus cariosus*, in each case in an anatomical location consistent with tonic action. A search of the literature indicates that this type of muscle is found in all classes of arthropods and is confined to visceral and postural muscles or specializations of these.

INTRODUCTION

In recent years two disciplines have accumulated information pertaining to certain aspects of arthropodan muscles. On the one hand, a number of reports in the electron microscopy literature describe or illustrate deviant myofibrillar arrangements, in crustaceans (14, 15, 50), insects (27, 47, 48, 51) and chelicerates (11, 12, 22), in which one thick filament is surrounded by more than six thin filaments, generally 10–12. In a study of the femoral muscles of the cockroach, *Leucophaea maderae*, Hagopian (27) has convincingly demonstrated that this higher number of thin filaments exists in stretched fibers and that it is not the result of double overlap but indicates a fundamentally different arthropodan muscle. Relatively little speculation concerning the function of the muscles accompanied these primarily descriptive papers.

On the other hand, neurophysiologists have devoted considerable attention to the existence of phasic and tonic fibers in crustaceans (1, 2, 5–10, 16–18, 20, 21, 30, 33–35). These investigations in diverse species usually included some light microscopic studies which agreed that the twitch, or phasic, fibers have a short sarcomere length, while the slow, or tonic, fibers have a much longer one. No electron microscopy has been done on any of the known tonic muscles; hence, the connection between the above mentioned findings has not been established.

The present study was undertaken in order to bridge this gap. To this end, the distal head of the acessory flexor muscle in the meropodite of the walking leg of *Cancer magister* (Crustacea, Decapoda) is eminently suitable. This particular muscle has been subjected to extensive neurophysiological investigation (10, 16–18, 20, 21). Phasic and tonic fibers are located at opposite edges of the straplike muscle; hence, there is no necessity to determine the physiological identity of each fiber before fixation. All fibers of the muscle are innervated by only two common axons, ex-

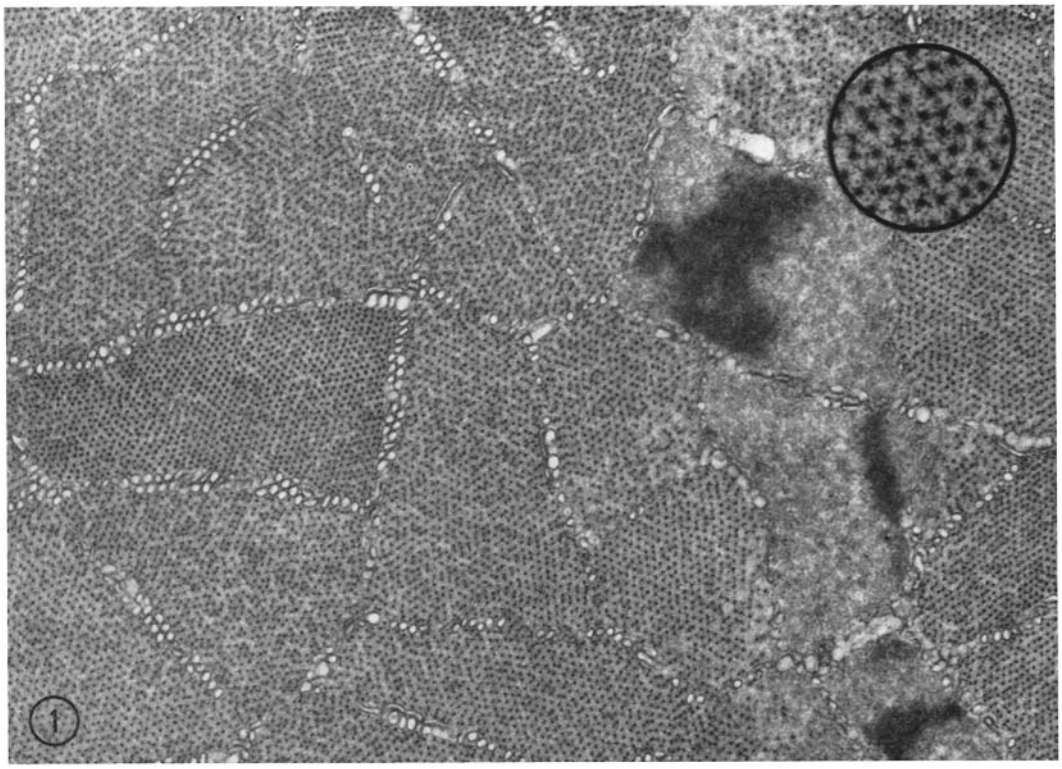

FIGURE 1 Cross-section of a distal (phasic) fiber of the distal accessory flexor muscle of the walking leg of *Cancer magister*. Note small myofibrils, abundant sarcoplasmic reticulum, hexagonal array of myosin filaments, and small number of actin filaments. Strict regularity has been disturbed by fixation. × 17,500; inset × 53,000.

citatory and inhibitory, a feature which precludes interpretative problems encountered with the usual, highly complex polyneuronal innervation of crustacean muscles.

A number of muscles which were suspected of being slow muscles, for purely functional–anatomical reasons, were also studied with the electron microscope. The fibers of these muscles will be referred to as "slow" by reason of their morphological similarity to the known tonic fibers of *Cancer magister*, although their actual physiological characteristics are conjectural. In the following description, only those features pertinent to the correlative aim of the study will be detailed.

MATERIALS AND METHODS

The walking legs of freshly caught crabs (*Cancer magister* Dana, 1852) were dissected as described by Cohen (17, 18) to expose the distal head of the accessory flexor muscle. With the amputated leg fixed in normal ambulatory position, the preparation was submerged in fixative consisting of 1% glutaraldehyde and 5% formalin in *s*-collidine buffer at pH 7.4, and of a variety of other ingredients, usually 3% NaCl, 4.5% sucrose, and 0.05% $CaCl_2$. After 3–6 hr the fixative was replaced by buffer containing salt and sucrose; several fibers from the tonic and phasic regions of the muscle were excised and postfixed in osmium tetroxide for 2 hr. Barnacle (*Balanus cariosus* Pallas, 1788) scutal depressor muscles were fixed in a similar manner except that they were tied in moderately extended condition to wooden sticks before fixation. Copepods [*Macrocyclops albidus* (Jurine) 1820] and ostracods [*Cyridopsis vidua* (O. F. Müller) 1776] were fixed in *s*-collidine-buffered osmium tetroxide.

All tissues were dehydrated in a graded ethanol series, passed through propylene oxide, and embedded in Araldite. Sections were stained with uranyl acetate, lead citrate, potassium permanganate, or a combination of these. Micrographs were taken on an RCA EMU-3F or a Philips EM200 electron microscope. Plastic sections for light microscopy were cut at 0.5–0.75 μ and stained with toluidine blue in 1% Borax.

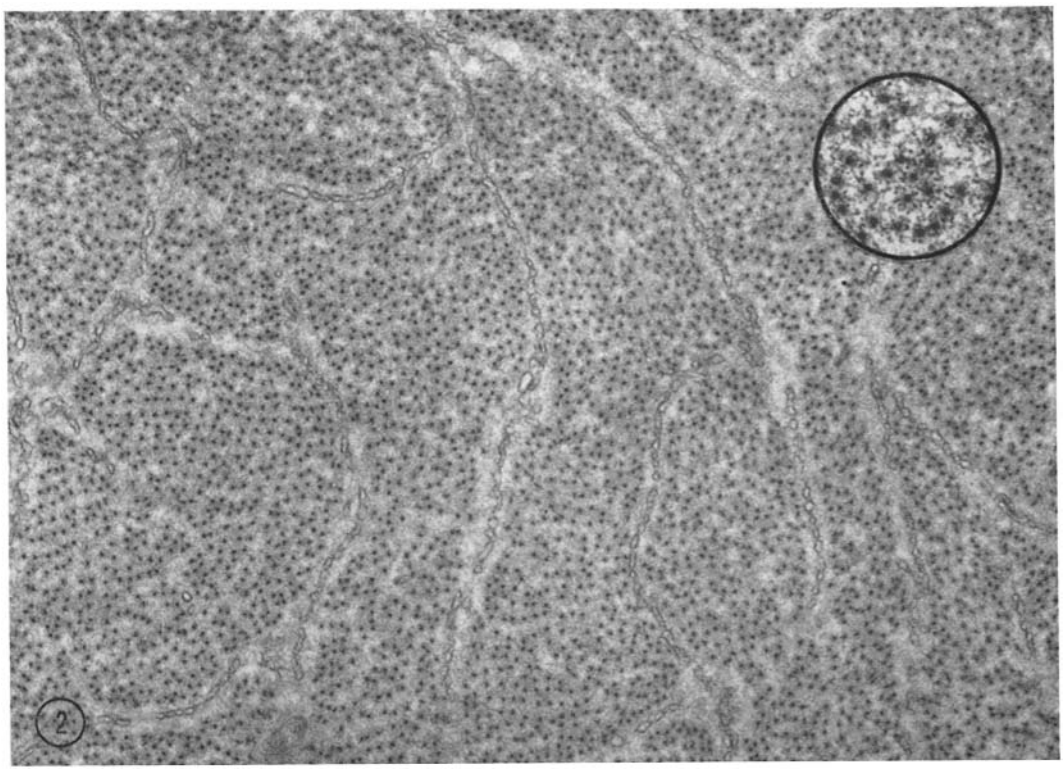

FIGURE 2 Cross-section of a proximal (tonic) fiber of the distal accessory flexor muscle of the walking leg of *Cancer magister* (from the same muscle as Fig. 1). Note larger myofibrils, deficient sarcoplasmic reticulum, irregular distribution of myosin filaments, and abundance of actin filaments. × 17,500; inset × 53,000.

RESULTS

The distal fibers of the distal accessory flexor muscle of *Cancer magister* (Fig. 1) are known to be phasic in nature, giving fast twitch contractions in response to stimulation (10). Each fiber has a diameter of about 0.5 mm and a resting sarcomere length of about 4.5 μ. The fiber is subdivided into polygonal myofibrils with an average diameter of 1.5 μ, a feature which presumably accounts for the "*Fibrillenstruktur*" observed in the fibers by light microscopy (21). From the surface of the fibers a multitude of clefts invaginates into the depth of the cell; from these, the T elements invade the interstices of the myofibrils, which are surrounded by these tubules and by an array of single and double layers of the sarcoplasmic reticulum. It is not clear whether the prominent, beaded disposition of the sarcotubules is due to artifactual disruption of originally continuous elements or to a regular longitudinal alignment. The thick myofilaments have a diameter of about 190 A and an average interfilamentous spacing of 580 A. Each thick filament is surrounded by six thin filaments in a reasonably hexagonal lattice, although fixation problems have not permitted a diagrammatic demonstration of this pattern in the crab.

The proximal fibers of the same muscle (Fig. 2) are tonic in their physiological response, and produce slow tension development on sustained stimulation (10). The fibers have an average diameter of 100 μ and their resting sarcomere length measures about 12 μ. The shape of the myofibrils tends to be platelike, common cross-sectional dimensions being 1 × 5 μ or more. The somewhat radial arrangement of the myofibrils with respect to the center of the fiber gives rise to the appearance of *Felderstruktur*, as seen in paraffin sections (21). The rather incomplete, single-layered sheet of membranous elements between adjacent myofibrils appears to

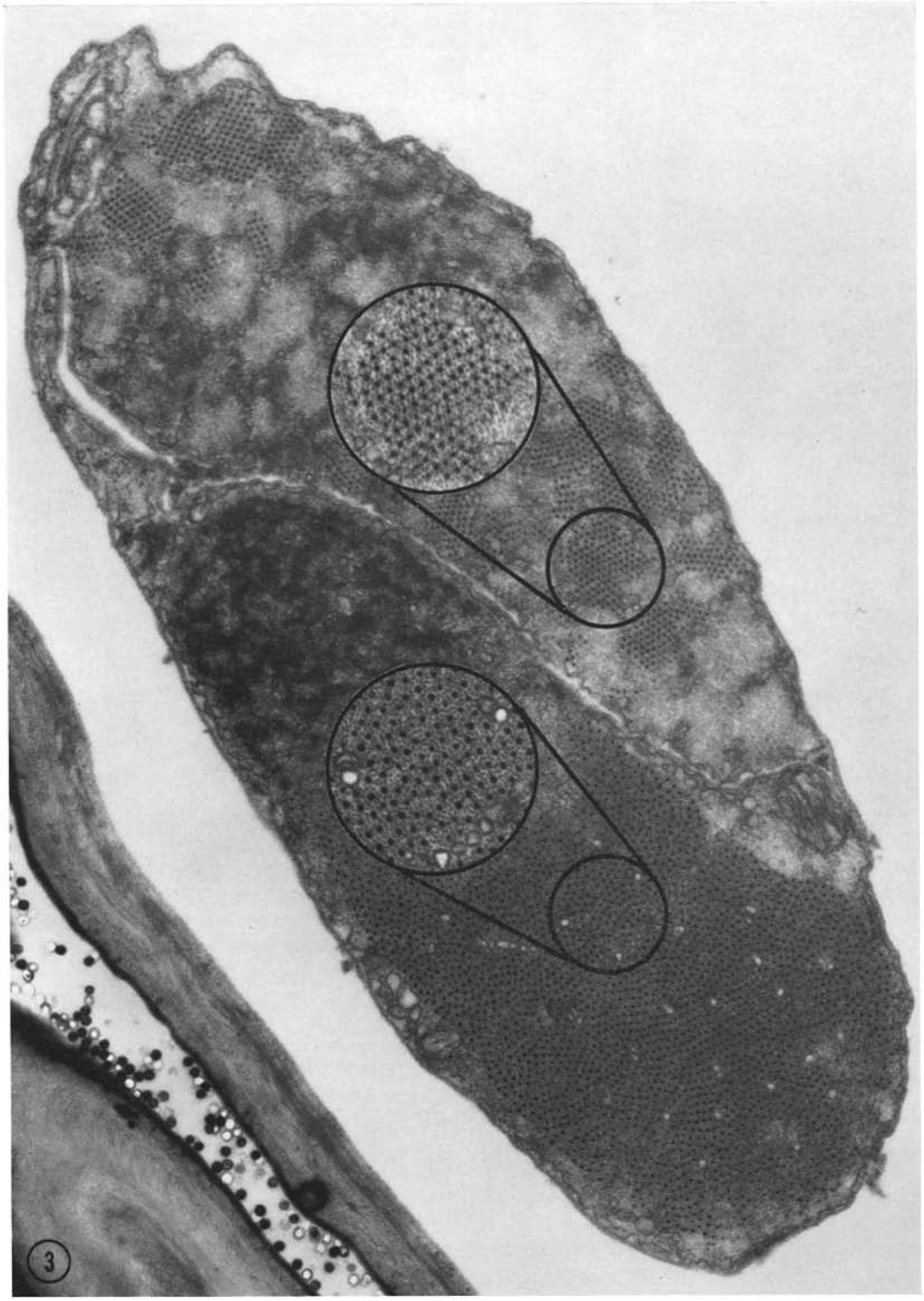

FIGURE 3 Oviducal dilatator muscle of *Macrocyclops albidus*, composed of a fast and a slow fiber. The substance of the Z line, visible in both fibers, is conspicuously more electron-opaque in the slow fiber (below). × 26,000; insets × 59,000.

consist of pure sarcolemmal clefts or T tubules, diads being extremely rare. The thick myofilaments are arrayed in a markedly disorderly fashion with hardly a suggestion of hexagonal grouping. Center-to-center spacing between them is about 750–900 A, values which are possibly exaggerated though not caused by fixation artifact. The diameter of the thick myofilaments averages 240 A. The most pronounced feature of the tonic fibers is the abundance of thin filaments, forming dense orbits around each thick filament in highly contracted muscle, but remaining at an average of 12 per thick filament when the muscle is fixed in a moderately relaxed position.

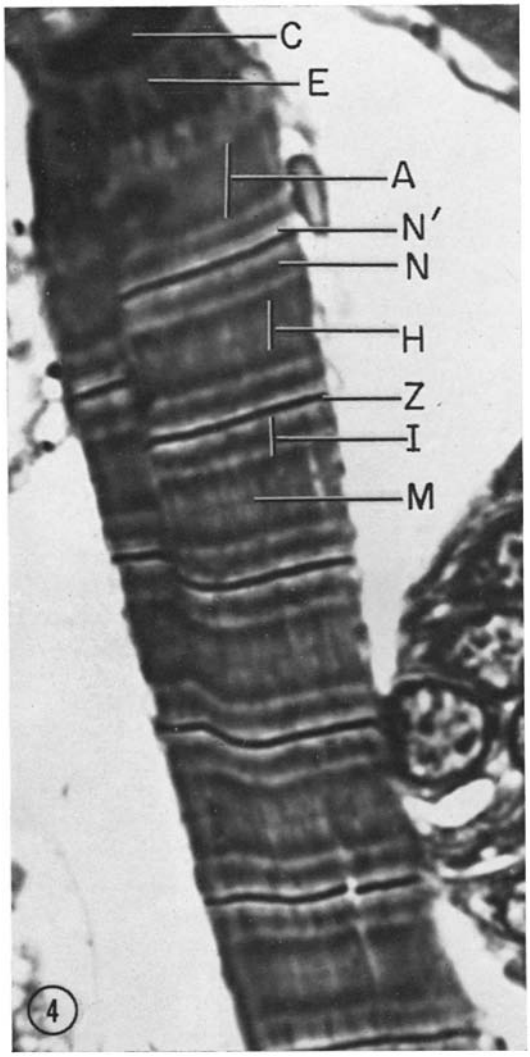

FIGURE 4. Light micrograph of an esophageal dilatator muscle of *Macrocyclops albidus*, a muscle showing the ultrastructural features of slow muscle fibers. Note long sarcomere length and detailed banding pattern. *C*, cuticle; *E*, epithelium—myocuticular junction; *A*, *N′*, *N*, *H*, *Z*, *I*, *M*, muscle striations. × 2,700.

Similar morphological differences exist in *Macrocyclops albidus*, in which slow muscles are found in association with the esophagus, midgut, hindgut, and the openings of the oviduct. Two solitary slow fibers are located in the urosome in a midventral position. A common disposition of these fibers consists of an association of a slow and a fast fiber as one small muscle (Fig. 3), a situation analogous to the assemblage of slow and fast fibers in the accessory flexor muscle of the crab. Both types of fibers have the same range of diameter, but the fast fibers have a sarcomere length of 1.5–3 μ, while the slow fibers have 8–12-μ cross-striations. Fast fibers have an elaborate sarcoplasmic reticulum, described in a previous paper (23), which divides the fiber into myofibrils of an average diameter of 1.5 μ. Slow fibers, on the other hand, have diadic elements situated within the array of myofilaments without giving rise to myofibrillar subdivisions. Fast and slow fibers have thick filaments with average diameters of 110 and 155 A, respectively, spaced about 300 and 415 A, respectively, from each other. In the hexagonal array of myofilaments in the fast fiber, each thick filament is accompanied by six thin ones, whereas in the slow fiber no rigid pattern is apparent and each thick filament is surrounded by about 12 thin myofilaments. The banding pattern of slow fibers is rather indistinct in the electron microscope. In thicker plastic sections, however, many of these slow fibers show exquisitely detailed cross-striations (Fig. 4). Of these bands, *M*, *N*, and *N′* are virtually invisible in electron micrographs of the same fiber, and the H band is quite indistinct.

The fast fibers of *Cypridopsis vidua* have been described in a previous paper (24). Unlike the fibers described above, they are not subdivided into myofibrils by the sarcoplasmic reticulum; instead the reticulum is disposed in the substance of the fiber in a predominantly longitudinal direction as complex multiple aggregates of T tubules and accompanying cisternae, an architecture somewhat similar to that of slow copepod fibers described above. Sarcomere length averages 2.5 μ. Thick myofilaments measure about 130 A

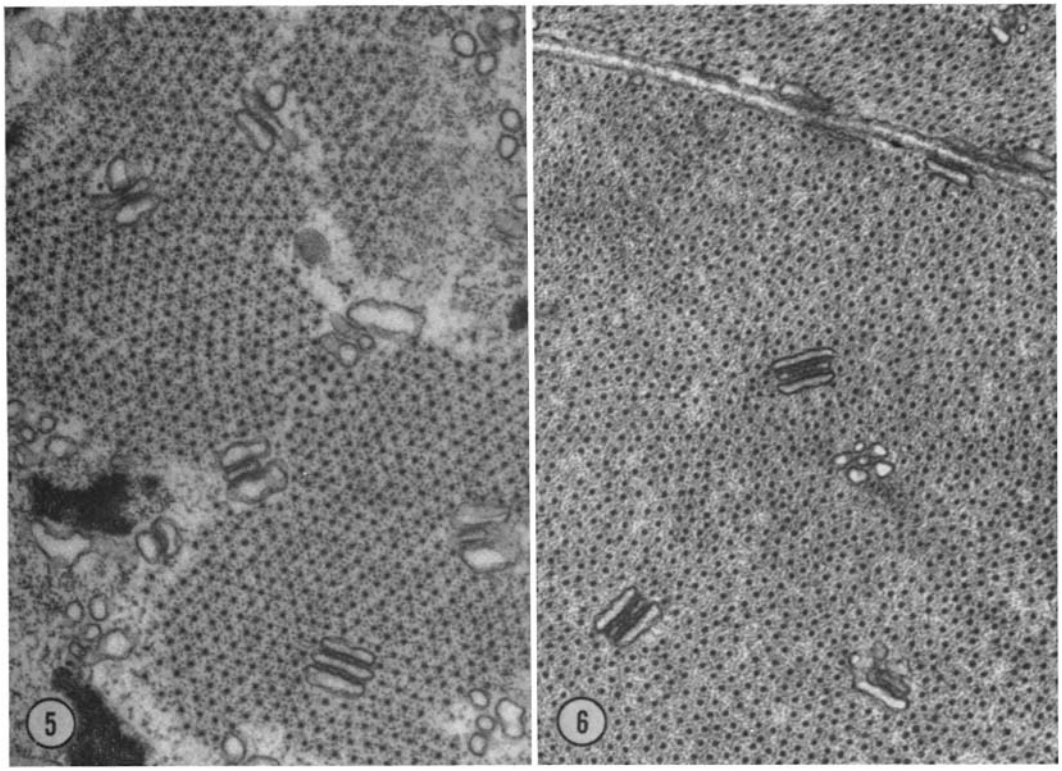

FIGURE 5 Cross-section of an appendicular (fast) muscle fiber of *Cypridopsis vidua*. Note hexagonal disposition of myofilaments and relative abundance of sarcoplasmic reticulum. × 45,000.

FIGURE 6 Cross-section of the carapace adductor muscle of *Cypridopsis vidua*. Elements of the sarcoplasmic reticulum are more widely spaced, the array of myofilaments is irregular, and there is an abundance of actin filaments. × 41,000.

in diameter and 480 A in center-to-center spacing, each being surrounded by six thin filaments (Fig. 5). Slow fibers occur in association with the digestive tract, as in copepods; in addition, the massive muscle which serves as the adductor of the bivalved carapace is composed entirely of slow fibers (Fig. 6). These fibers have an average diameter of 25 μ, a diameter two to four times that of fast fibers. The length of sarcomeres amounts to 7–10 μ. In cross-section the fibers have the expected irregular array of thick filaments, 175 A in diameter and 460 A in center-to-center spacing, each being surrounded by about 12 thin filaments. The sarcoplasmic reticulum is similar to that in the fast fibers, although more widely spaced.

The fibers of the scutal depressor muscle of *Balanus cariosus* (Fig. 7) have an average diameter of 100 μ and a sarcomere length of about 8 μ. By contrast, the fibers of the appendicular muscles have a sarcomere length of about 2.5 μ and an average diameter of 25 μ, the latter value being highly variable. In the scutal depressor muscles numerous deep infoldings of the sarcolemma penetrate into the fiber, but the sarcoplasmic reticulum is meagerly developed, with distantly spaced diads or occasional triads. The thick filaments are disposed 450 A apart in an irregular hexagonal array and measure about 160 A in diameter, each surrounded by about 12 thin filaments. Rotation printing (38) of thick filaments at 60° increments (n = 6) yields a distinct hexagonal appearance (inset, Fig. 7), whereas printing at n = 9, 10, or 12 produces increasingly circular images without selective enhancement. The corners of the hexagon, formed by a rota-

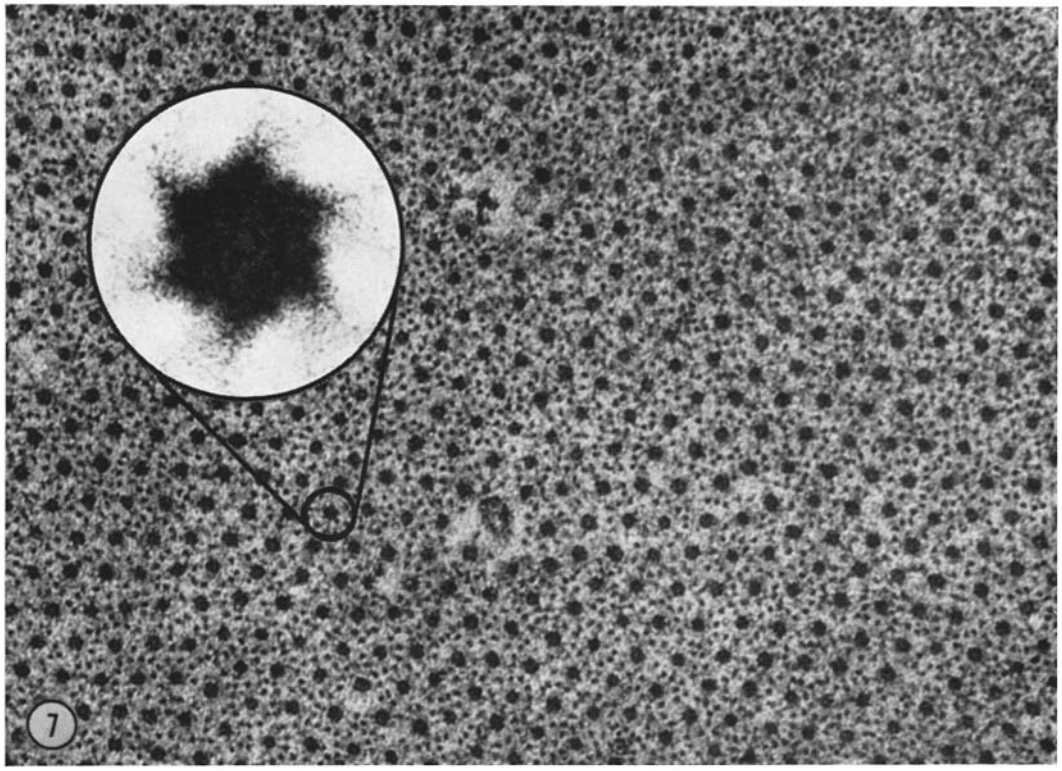

FIGURE 7 Cross-section of the scutal depressor muscle of *Balanus cariosus*. A semblance of hexagonal symmetry is maintained. The edgeof the I band is visible at the upper right hand corner, where the myosin filaments are seen to taper before terminating (arrow). The encircled myosin filament was rotation printed ($n = 6$) to yield the inset. × 115,000; inset × 1,500,000.

tionally printed thick filament, do not always point toward the adjacent myofilaments of the lattice, but may deviate as much as 30° from that direction.

DISCUSSION

The principal interest of the present study lies in the distinctive difference in the light- and electron microscopic structure of the distal and proximal fibers of the accessory flexor muscle of *Cancer magister*. The physiology of these fibers is known (10, 20, 21) in detail and merits recapitulation. The distal fibers give twitches and rapid tension development in response to brief stimuli, but relax rapidly on sustained depolarization. Membrane response to depolarization consists of graded responses or large spikes with a length constant of 1–2 mm and a time constant of 5–20 msec. These phasic, or fast, fibers have been characterized in the present study as having short sarcomeres, a highly developed sarcoplasmic reticulum, small myofibrils, and a hexagonal array of thick myofilaments, each surrounded by six thin filaments.

The proximal fibers, on the other hand, develop tension only in response to sustained depolarization, and they relax very slowly. Brief stimulation produces no contraction at all, but recruitment of the fibers occurs only at stimulation frequencies of 25–35 times per second. Depolarization of the membrane results in a passive response with a length constant of 2.0–8.5 mm and a time constant of 50–800 msec. The fibers maintain contractions over long periods of time during sustained depolarization. These tonic, or slow, fibers have long sarcomeres, a minimal sarcoplasmic reticulum, relatively large myofibrils, and an irregular array of thick filaments, each being associated with 10–12 thin filaments.

The light microscopic resemblance of fast and

slow fibers of crabs to these fibers of vertebrates has frequently been commented upon. These similarities include *Fibrillenstruktur* and *Felderstruktur*, features which are a function of the differing myofibrillar size and shape in fast and slow fibers, respectively. Further liknesses consist of the relative abundance of the sarcoplasmic reticulum and the similar contractile responses to stimulation. Surprising as this resemblance may be, it is without doubt an instance of independently and convergently evolved muscular systems whose structural similarities are dictated by functional factors, such as distance of diffusion of calcium ion into the myofibrils as related to contraction speed.

A search of the literature reveals many reports of known or presumptive, slow fibers, suggested either by their long sarcomeres or by the high number of thin filaments. The location of these fibers suggests that the muscles containing them can be grouped into several functional–anatomical categories: I. postural musculature including (*a*) body wall muscles, (*b*) attitudinal appendicular muscles, (*c*) "locking" muscles, and (*d*) proprioceptor muscles; II. visceral musculature including (*a*) digestive system muscles, (*b*) reproductive system muscles, and (*c*) circulatory system muscles.

Excellent examples of slow body wall muscles are the ventral superficial abdominal flexor muscles of the crayfish, *Procambarus clarkii*, the fibers of which have a sarcomere length of 10 μ and electrophysiological characteristics of slow fibers, although governed by complex innervation (35). These muscles are responsible for delicate postural control of the abdomen in contrast to the powerful twitch of the tail which is mediated by physiologically fast muscles the fibers of which have sarcomere length of 2–3 μ (34). One can attribute similar function to the dorsal abdominal intersegmental musculature of the cockroach, *Periplaneta americana* (sarcomere length about 8 μ) (47), the intersegmental muscles of the bug *Rhodnius prolixus* (51), and the ventral urosomal fibers of *Macrocyclops albidus*; in all of these cases the higher number of thin filaments exists. Intersegmental muscles of the chilopod *Julus* sp. (sarcomere length 8–10 μ)[1] and muscles of the dorsal metapodosoma of the mite *Tarsonemus randsi* (sarcomere length 10 μ) (3) are probably similar to the preceding muscles.

Slow attitudinal appendicular muscles have been abundantly described in the legs of crustaceans, primarily with neurophysiological methods but usually with enough light microscopy to establish the presence of *Felderstruktur* and long sarcomeres (about 10 μ). Included in this group are various claw and leg muscles of the crab *Carcinus maenas*, the lobster *Homarus americanus* (33), the crabs *Pachygrapsus crassipes*, *Chionectes tanneri*, *Cancer magister*, and the shrimp *Nephrops norvegicus* (5-9). These muscles probably function in conjunction with phasic muscles to provide a finer degree of control of ambulatory or manipulative motion, to yield habitually sustained or slow motion, such as is found in the opener of the claw, or to support the animal against gravity. This latter function may be of importance in the femoral muscles of the cockroach *Leucophaea maderae*, in the fibers of which the arrangement of myofilaments corresponds to that of slow fibers (27). Fibers of similar ultrastructural appearance occur in the walkings legs of the crayfishes *Orconectes virilis* (50) and *Procambarus clarkii* (14) and in the buccal appendicular musculature of the copepod *Acanthocyclops viridis* (15). Short and long sarcomeres (3 and 8 μ, respectively) have also been observed in the appendages of the ostracod *Cypria* sp. (4). One would expect slow fibers to be abundant in the legs of arthropods with clasping habits, such as lice or ticks.

Locking muscles might be considered specialized body wall muscles which function only in long-sustained contractions lasting, in the case of the barnacle scutal depressor muscles, for many hours. The adductor muscle of *Cypridopsis vidua* closes the bivalved carapace firmly enough to resist the entrance of fixative and also appears to be able to maintain the contraction for long periods. The term locking muscle is not meant to imply that the physiological mechanism of contraction is different from that of ordinary slow muscles.

Slow fibers occurring in various crustacean proprioceptors have been the subject of extensive neurophysiological studies. Alexandrowicz (1, 2) described the muscles of stretch receptors of the lobster *Homarus vulgaris*, the spiny lobster *Palinurus vulgaris*, and the hermit crabs *Pagurus striatus* and *P. calidus*. The complex stretch receptor of *Cancer magister* has been studied singly or collaboratively by Atwood (10), Cohen (16–18), and Dorai Raj (20, 21) and has provided material for the present investigation. This proprioceptor monitors the position and movement of the meropodite—

[1] Fahrenbach, W. H. Unpublished observations.

carpopodite joint by way of a complicated linkage of a tendon, several accessory muscles, an elastic strand, and associated receptor neurons. The distal head of the accessory flexor muscle used in the present study appears to influence the tension of the receptor organ. Thereby, the central nervous system, via efferent innervation to these muscle fibers, exerts some influence over sensory input from the proprioceptor and, as a consequence, over the setting of postural tone in the leg. The interplay of fast and slow fibers would afford very delicate gradations of tension control. The accessory flexor muscle has fibers which are intermediate between fast and slow fibers, in terms of sarcomere length and physiological response; these fibers have not been studied with the electron microscope.

Slow muscle fibers of the digestive tract have been mentioned, in the present study, in *Marcocyclops albidus* and *Cypridopsis vidua*. The ultrastructure of the fibers of the midgut musculature of the scorpions, *Euscorpius carpathicus* and *Buthus occitanus* (11, 12), and of the larva of the moth, *Ephestia kühniella* (48), corresponds to that of slow fibers.

Slow muscle fibers of the reproductive tract occur as investment of the seminal vesicle of the stick-insect *Carausius morosus* and of the spermatheca of the cockroach *Periplaneta americana* (48). The muscle fibers of the medium septum of the ovary of the mosquito *Aedes aegypti* (25) probably belong to this same category. The oviducal openings in *Macrocyclops albidus* are operated by dilatators in which one fast fiber and one slow fiber frequently form the whole muscle. The large number of eggs and their disproportionately large size probably necessitates maximal dilatation of the cuticle-lined end of the oviduct for a protracted period during oviposition.

A single report illustrates the typical myofilamentous array of slow muscles fibers in the circulatory system of an arthropod, namely in arteries of the horseshoe crab, *Limulus polyphemus* (22). These fibers are responsbile for the slow peristaltic waves of the arteries and are also said to form the musculature of the heart.

This literature survey and the new findings of this study illustrate the existence of slow fibers in all classes of the arthropods, be it by neurophysiology, light microscopy, electron microscopy, or a combination of these techniques. The characteristic array of myofilaments in slow fibers may represent a more primitive arrangement of myofilaments than the rigid hexagonal lattice found in fast fibers. This pattern, subserving to some degree the function of smooth muscle for arthropods, is widely distributed among other invertebrates, although it may occur in association with oblique striations, with paramyosin as the thick filaments, or with ill defined or absent Z-lines. Perusal of the electron microscopy literature reveals this cross-sectional pattern of myofilaments in the platyhelminths *Dugesia tigrina* (36), *D. dorotocephala* (39), *Notoplana acticola* (37); in the aschelminths *Tachygonetria* sp. (13), *Parascaris equorum* (13), *Ascaris lumbricoides* (44), *Capillaria hepatica* (52); in the annelids *Hirudo medicinalis* (42, 43), *Lumbricus terrestris* (49); and in the molluscs *Helix aspersa* (40), *Pecten irradians* (45), *Octopus vulgaris* (26), and *Sepia officinalis* (26). A thorough coverage of the older zoological literature would most likely yield many further examples of striated muscles with very long sarcomeres, and most probably with the other attributes of slow muscles; as an extreme case the 33-μ long sarcomeres found in a syllid polychaete (28) may be cited.

It is apparent that very few morphological generalizations about slow versus fast crustacean fibers can be made beyond the greater sarcomere length, the characteristic arrangement of myofilaments, and the somewhat greater diameter of the thick filaments. With respect to fiber and myofibril size these fibers may be identical or greatly disparate, depending on the species. Conspicuous differences in abundance of the sarcoplasmic reticulum exist in crab muscles, but are hardly noticeable in muscles of the ostracod. Innervation may be simple, as in *Cancer magister*, or polyneuronal and complex, as in *Procambarus clarkii* (34, 35). It remains for neurophysiologists to determine whether these few diagnostic ultrastructural similarities are accompanied without fail by the physiological attributes of slow muscles.

Several aspects of the contractile elements merit discussion. The increase in the number of actin filaments for a given cross-sectional area should be reflected in the architecture of the Z line. No information is available on this point, at the present time, beyond the greater electron opacity of the Z line of slow fibers as compared to that of fast fibers when both are viewed in the same section. The possibility was considered that the 12-fold orbital arrangement of actin filaments might be mirrored by a structural change in the myosin

filaments. The clear enhancement of hexagonal symmetry in rotation-printed myosin filaments (Fig. 7) argues against this possibility, although the 25–35% greater diameter of myosin filaments in slow fibers appears to be a constant and real feature. The increased length of the myosin filaments would increase the number of cross-bridges, i.e. reactive sites, by a factor of three or four, provided the basic postulated molecular makeup (19, 32) is maintained. The apparent freedom of the myosin filaments to undergo longitudinal, rotational displacement, as well as the less than rigid orbits of the actin filaments, might permit all 12 orbital actin filaments to be linked to the central myosin filament, rather than just six of these, as is the case in fast fibers. This less rigid geometry would, on the one hand increase the probability of cross-link formation (19) with respect to time and, on the other hand, would result in the coupling of any given myosin filament to both adjacent Z-lines by all its associated 24 actin filaments. The ability of slow fibers to provide strong, sustained contractions, or possibly even supercontractions (29), is supported by the nearly doubled concentration of Ca^{++} found in the slow fibers of *Cancer magister* as compared to that of the fast fibers (10).

Slow arthropodan muscle fibers warrant further diverse investigations, in that the phenomenon of slow contraction is undoubtedly a function of numerous interacting factors, such as the various ultrastructural differences detailed in the present paper, as well as peculiarities of membrane properties and physiological response.

This study constitutes publication No. 235 from the Oregon Regional Primate Research Center, supported in part by grant FR 00163 from the National Institutes of Health and in part by a grant from the Muscular Dystrophy Associations of America, Inc.

I wish to thank Mrs. Audrey Griffin for excellent technical assistance.

Received for publication 17 February 1967; revision accepted 19 May 1967.

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