THE MYOFILAMENT ARRANGEMENT IN THE FEMORAL MUSCLE OF THE COCKROACH, *LEUCOPHAEA MADERAE* FABRICIUS

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ABSTRACT

The structure of the femoral muscle of the cockroach, *Leucophaea maderae*, was investigated by light and electron microscopy. The several hundred fibers of either the extensor or flexor muscle are 20 to 40 μ in diameter in transverse sections and are subdivided into closely packed myofibrils. In glutaraldehyde-fixed and epoxy resin-embedded material of stretched fibers, the A band is about 4.5 μ long, the thin filaments are about 2.3 μ in length, the H zone and I band vary with the amount of stretch, and the M band is absent. The transverse sections of the filaments reveal in the area of a single overlap of thick and thin filaments an array of 10 to 12 thin filaments encircling each thick filament; whereas, in the area of double overlap in which the thin filaments interdigitate from opposite ends of the A band, the thin filaments show a twofold increase in number. The thick filament is approximately 205 to 185 A in diameter along most of its length, but at about 0.2 μ from the end it tapers to a point. Furthermore, some well oriented, very thin transverse sections show these filaments to have electron-transparent cores. The diameter of the thin filament is about 70 A. Transverse sections exhibit the sarcolemma invaginating clearly at regular intervals into the lateral regions of the A band. Three distinct types of mitochondria are associated with the muscle: an oval, an elongate, and a type with three processes. It is evident, in this muscle, that the sliding filament hypothesis is valid, and that perhaps the function of the extra thin filaments is to increase the tensile strength of the fiber and to create additional reactive sites between the thick and thin filaments. These sites are probably required for the functioning of the long sarcomeres.

INTRODUCTION

Electron microscopy of insect striated muscle has revealed that, as in vertebrates, sarcomeres are composed of regular arrays of thick and thin filaments. In the A-band region of interdigitation of thick and thin filaments, however, a basic morphologic difference between vertebrate and insect striated muscles has been recognized (1, 2). In vertebrates, each thin (actin) filament is parallel to and equidistant from 3 adjacent thick (myosin) filaments. During contraction, the actin filament apparently interacts with each of the 3 myosin filaments. Previous studies of insect muscle have revealed that each thin filament is equidistant from only 2 adjacent thick filaments. Thus, the actin filament may interact, during contraction, only with these 2 thick filaments. Whereas in vertebrates the ratio of actin to myosin filaments in an A-band region of interdigitation is 2:1, in in-

sect muscles so far adequately studied that ratio is 3:1. Prior studies of insect muscle, in this regard, have generally been conducted on thoracic and abdominal muscles (2, 3, 4). This study of transverse and longitudinal sections of the femoral muscle of the cockroach reveals a ratio of thin to thick filaments of approximately 5:1 in sarcomeres stretched sufficiently to generate an H band, and of approximately 11:1 in sarcomeres shortened to the extent where H bands are abolished.

MATERIALS AND METHODS

The metathoracic (hind) leg (Fig. 1) of the cockroach, *Leucophaea maderae*, was severed between the thorax and the coxa and pinned onto cork so that the tibia was either fully flexed or fully extended with respect to the femur. The degree of tibial flexure determined both the amount of shortening in the femoral flexor muscle and the amount of stretching in the femoral extensor muscle. The fixatives for both light and electron microscopy were initially perfused into the distal end of the tibia and permitted to emerge at the trochanter. After 1 hr of perfusion, the hind leg (minus the coxa, metatarsus, and tarsus) was immersed directly into the fixative for another hour. This was followed by removal of pieces of exoskeleton from both sides of the femur under a dissecting microscope while the femur was still immersed in the fixative, and by reimmersion of the femur into fresh fixative. The femoral flexor and extensor muscles were dissected free just prior to the end of fixation.

Tissues prepared for light microscopy were fixed with Bouin's or Stieve's solution, dehydrated, embedded in paraffin, and sectioned at 7 and 4 μ, respectively. Bullard's hematoxylin and eosin and a modification of Masson's trichrome stains were employed expressly to permit study of histologic detail.

Tissues for electron microscopy were prefixed with 5% glutaraldehyde (5) or 6% glutaraldehyde plus 2% acrolein at 4°C for 4 hr, and in each case the fixative was buffered with phosphate at pH 7.4. After prefixation, the tissue was immersed into 0.2 M sucrose solution for 18 hr at 4°C. It was then transferred at the same temperature into a 1% solution of osmium tetroxide (phosphate buffered at pH 7.4 and containing 0.11 M sucrose; reference 6) and kept there for 1 hr. Tissue blocks were then dehydrated in a graded ethanol series and embedded in Epon 812 (7) or Maraglas (8). Thick sections (0.5 μ) were cut with glass knives on the Porter-Blum or Huxley microtome and placed on glass slides for determination of orientation by phase-contrast microscopy. Thick sections were also stained with basic fuchsin, toluidine blue, and malachite green for future use in interpreting and orientating thin sections. To minimize the errors in measurements of filament length, longitudinal

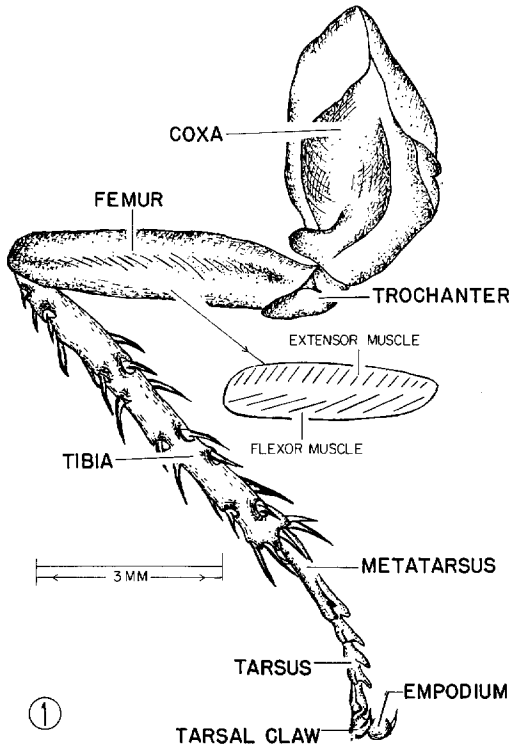


FIGURE 1 Diagram of the metathoracic leg of the tropical cockroach, *Leucophaea maderae*, illustrating a cutaway view of the femur with its extensor and flexor muscles.

sections were cut with the knife-edge parallel to the fiber axis. Thin sections were mounted on carbon-stabilized and parlodion-covered grids, stained with lead hydroxide (9), and examined at 50 kv in an RCA EMU 3B or D microscope. Micrographs were made on Kodak contrast lantern slide plates at initial magnification of 6000 to 20,000 at exposures of 2 to 5 sec.

OBSERVATIONS

The metathoracic femur encloses an extensor muscle and a flexor muscle (Fig. 1). Each is bipinnate, has an extensive origin from the femoral cuticle, and inserts into its corresponding apodeme. The fixed individual fibers, numbering several hundred in each muscle, are 20 to 40 μ in diameter and about 1000 μ long when the muscle is stretched to its physiologic limit. The myofibrils of the fibers are primarily straplike at the periphery of the fibers and have minimum and maximum diameters of 0.5 μ and 4.0 μ in transverse sections. The central regions of the fibers are composed of predomi-

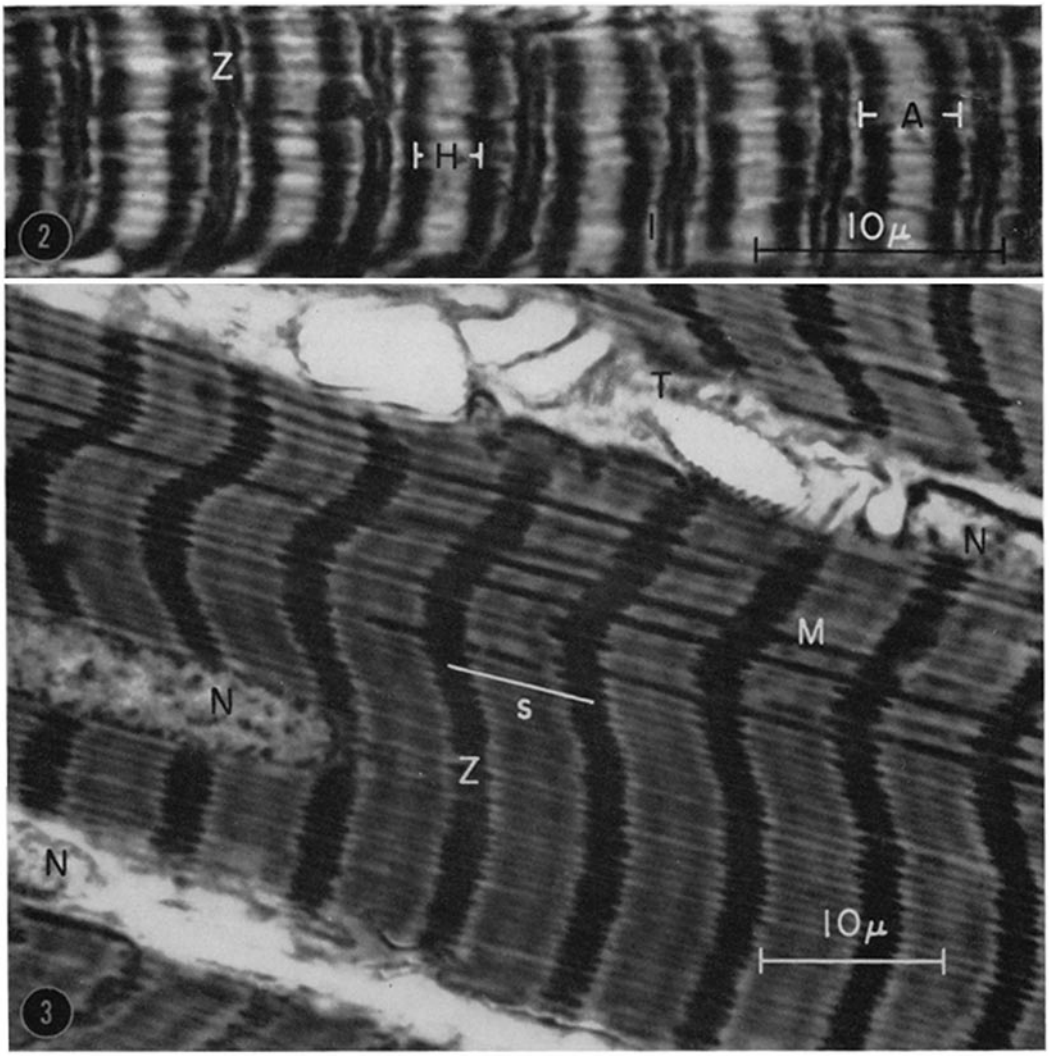


FIGURE 2 A paraffin-embedded longitudinal section of a stretched extensor muscle fiber depicting the various bands. The A band (*A*) is about 4.0 μ long, the I filaments (*I*) (refers to the length from the boundary of one H band to the nearest Z disc) is approximately 2.3 μ long, and the H band (*H*) is about 1.6 μ long. The sarcomere length (Z disc (*Z*) to Z disc) is about 6.3 μ. The wide Z disc is due to the mitochondria on each side. *A*, A band; *H*, H band; *I*, I band; and *Z*, Z disc. × 3200.

FIGURE 3 Survey view of an epoxy resin-embedded stretched femoral muscle. In this longitudinal section most of the mitochondria (dense transverse lines) are oriented on each side of the Z disc (*Z*), while others (long dense lines, M) are wedged between the myofibrils. Also present are tracheoles (*T*) and three nuclei (*N*). The sarcomere length (*s*) is approximately 8.2 μ. × 2150.

nately polygonal myofibrils which measure 0.5 to 2.0 μ in diameter. In both types of myofibrils, however, the sarcomeres consist of interdigitating arrays of thick and thin filaments. At numerous points, nerve endings contact the muscle fiber at slight depressions on the sarcolemma to form neuromuscular junctions (Fig. 9). The sarcolemma invaginates into the fiber at regular distances (Fig. 10), and frequently the invagination of the resultant transverse tubules extends for a long distance, as reported for many other arthropods. The transverse tubules are opposite the lateral

regions of the A band. In view of the fact that each transverse tubule is associated with a cisterna of the sarcoplasmic reticulum, it is referred to as a "dyad" (10). Occasionally, the transverse tubules branch and then associate with the sarcoplasmic reticulum. Since the sarcoplasmic reticulum was not pertinent to the main subject of this investigation, it was only superficially studied; however, a tentative tridimensional reconstruction would primarily show a fenestrated envelope surrounding each myofibril. It is a well developed system, as can be seen in Figs. 4 through 8.

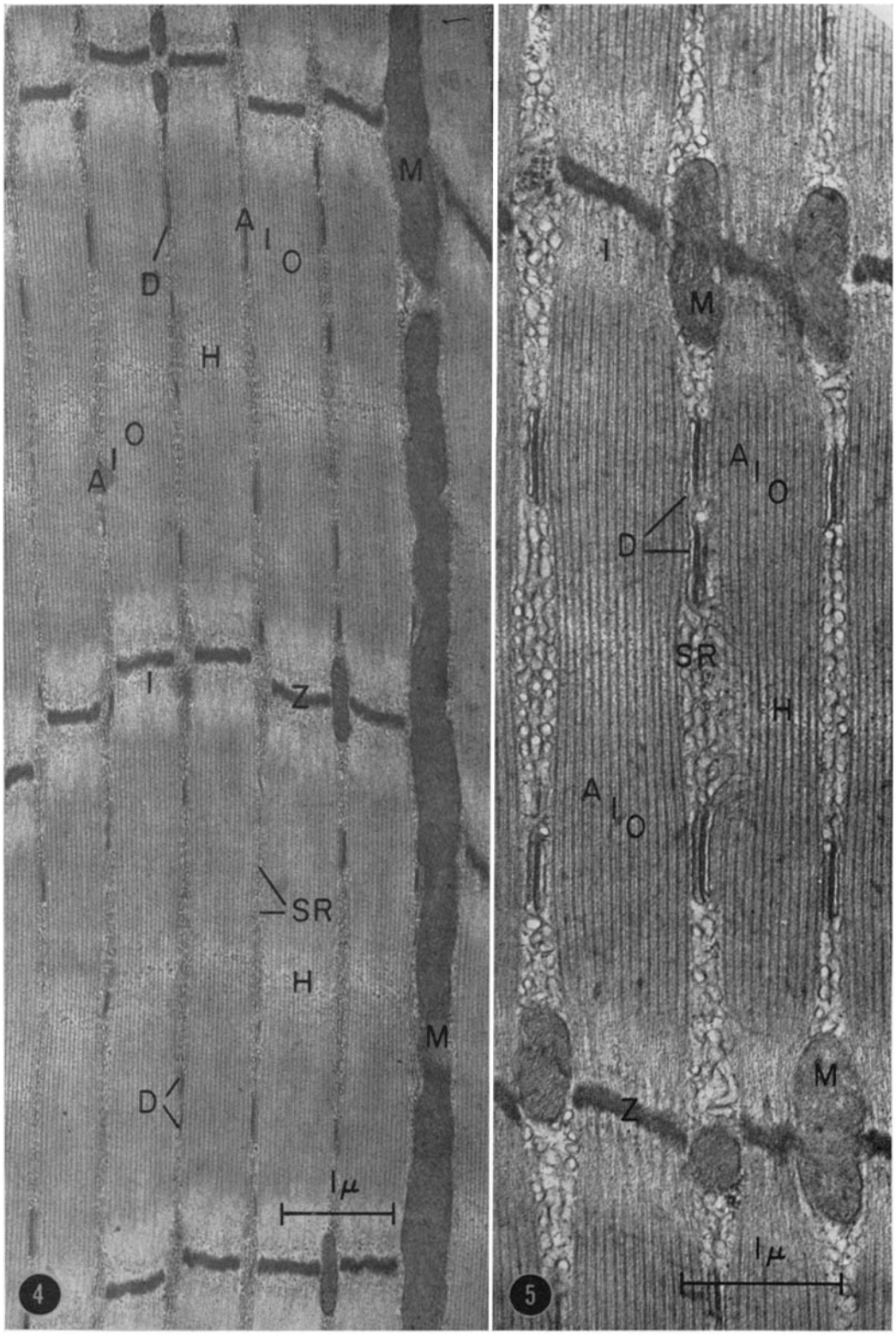
The three types of mitochondria observed in these fibers will be considered only briefly here, since they are now being studied in greater detail. The oval mitochondria are situated under the sarcolemma and are about 2 μ long and about 0.6 μ in diameter (Fig. 6). The elongated mitochondria (Figs. 3 and 4), which are wedged between myofibrils, vary in length from 5 to 25 μ and are approximately 0.5 μ in diameter. A third type of mitochondrion has a striking association with the Z disc (Figs. 3 through 10): these mitochondria appear in the interfibrillar sarcoplasm, and serial sections show them to have three processes. All three groups of mitochondria display a tightly packed and complex arrangement of cristae. The nuclei of the fibers are located peripherally and are usually near the surface.

Longitudinal sections of tissue embedded in paraffin and epoxy resin show A, I, H, and Z transverse striations, although, as is well recognized, they are larger in arthropods than in vertebrates. The thick filaments (prefixed in glutaraldehyde) in longitudinal sections measure approximately 4.5 μ (Figs. 4 and 5), whereas the thin filaments, which extend from the boundary of one H band to the nearest Z disc in well oriented sections, are about 2.3 μ long. All the material embedded in epoxy resin was prefixed in glutaraldehyde, since Page and Huxley (11) investigated the effects of different fixatives and found that glutaraldehyde did not cause any shrinkage of filament length. The width of the I band varies with the sarcomere length, while that of the A band (4.5 μ) remains constant. The H band, bisecting the A band, is observed only in sections of relatively stretched muscles, being replaced by a dense broad "contraction" band in sections of muscles fixed at the physiologic extreme of shortening. The M band, typical of vertebrate striated muscle, is not evident.

Fixing the muscles at various degrees of stretching and shortening provides evidence to support the Huxley and Hanson hypothesis of sliding filaments. In Figs. 2 to 5, the sarcomeres of the stretched muscle range from 8.2 to 5.3 μ in length and exhibit H bands, whereas in Figs. 6 and 7 the sarcomeres of a slightly shortened muscle range from 4.9 to 4.4 μ in length and do not reveal any H bands. When a sarcomere is shortened to a greater degree, a double overlap or contraction band is produced in its central region (Fig. 9). The contraction band is manifested because the thin filaments interdigitate from opposite ends of the A band and partially overlap. This interdigitation is due to the fact that the sarcomeres are shortened to about 3.0 μ and the thin filaments, on both sides of the A band, total about 4.6 μ in length. The difference between these lengths, 1.6 μ, is the width of the midcontraction band. The boundaries of the midcontraction band can be confirmed in Fig. 9 by measuring the distance of the thin filament at one end of the band to its respective Z disc (long arrow). This distance of about 2.3 μ is in accord with that of the thin

FIGURE 4 An electron micrograph showing a portion of a stretched extensor muscle. The H band (*H*) is not well defined here. The overlap of A band and I filaments (*AIO*) is discernible. The A band is about 4.5 μ, the I filament (*I*) about 2.3 μ, and the sarcomere about 5.7 μ. *D*, dyad; *M*, mitochondria; *SR*, sarcoplasmic reticulum; and *Z*, Z disc. × 16,000.

FIGURE 5 Micrograph of four longitudinally sectioned extensor myofibrils. The myofibrils are barely stretched, and thus the sarcomere (5.3 μ) is shorter than that in any of the previous figures. The A band is about 4.4 μ long, and the interdigitation of the A band with the I filaments (*I*) is quite clear. *AIO*, A band and I filaments overlap; *D*, dyad; *H*, H band; *M*, mitochondria; *SR*, sarcoplasmic reticulum; and *Z*, Z disc. × 24,500.


M
A
I
O
D
H
A
I
O
I
Z
SR
H
M
D
1μ
4
I
M
A
I
O
D
SR
H
A
I
O
M
Z
1μ
5

filament length. Another contraction band near the Z disc (Fig. 9, *CZ*) is due to the presence of the A band being pushed up against the Z disc. Additional proof of the double overlap of thin filaments is furnished by reorienting the same tissue block in order to make transverse sections and by finding an area with the interdigitation of thin filaments (Fig. 13).

The appropriate transverse sections show that the thick filaments are about 205 to 185 A in diameter for most of their length, but that they taper to a point approximately 0.2 μ from their ends. In many well oriented, very thin transverse sections, the thick filaments appear electron-transparent in their centers (Fig. 11), as reported for other arthropod muscles (Hodge (12), Huxley and Hanson (1), Auber and Couteaux (13), and Shafiq (14) in Dipterans; Smith (15) in a dragonfly; and Bouligand (16) and Fahrenbach (17) in copepods). Center-to-center spacing between well oriented and uncompressed thick filaments in cross-sections is about 415 A. Moreover, 10 to 12 thin filaments encircle each thick filament (Figs. 11 and 12). This is unlike the reported arthropodan hexagonal array, in which each thick filament is surrounded by 6 thin ones (2–4, 12, 13). Since this femoral muscle fiber has about twice the usual number of thin filaments, the possibility of a double overlap (interdigitation of thin filaments from opposite ends of the A band) had to be considered. Since this possibility could only occur in sections of shortened fibers, the filament count could be checked by observing sections from stretched muscle fibers.

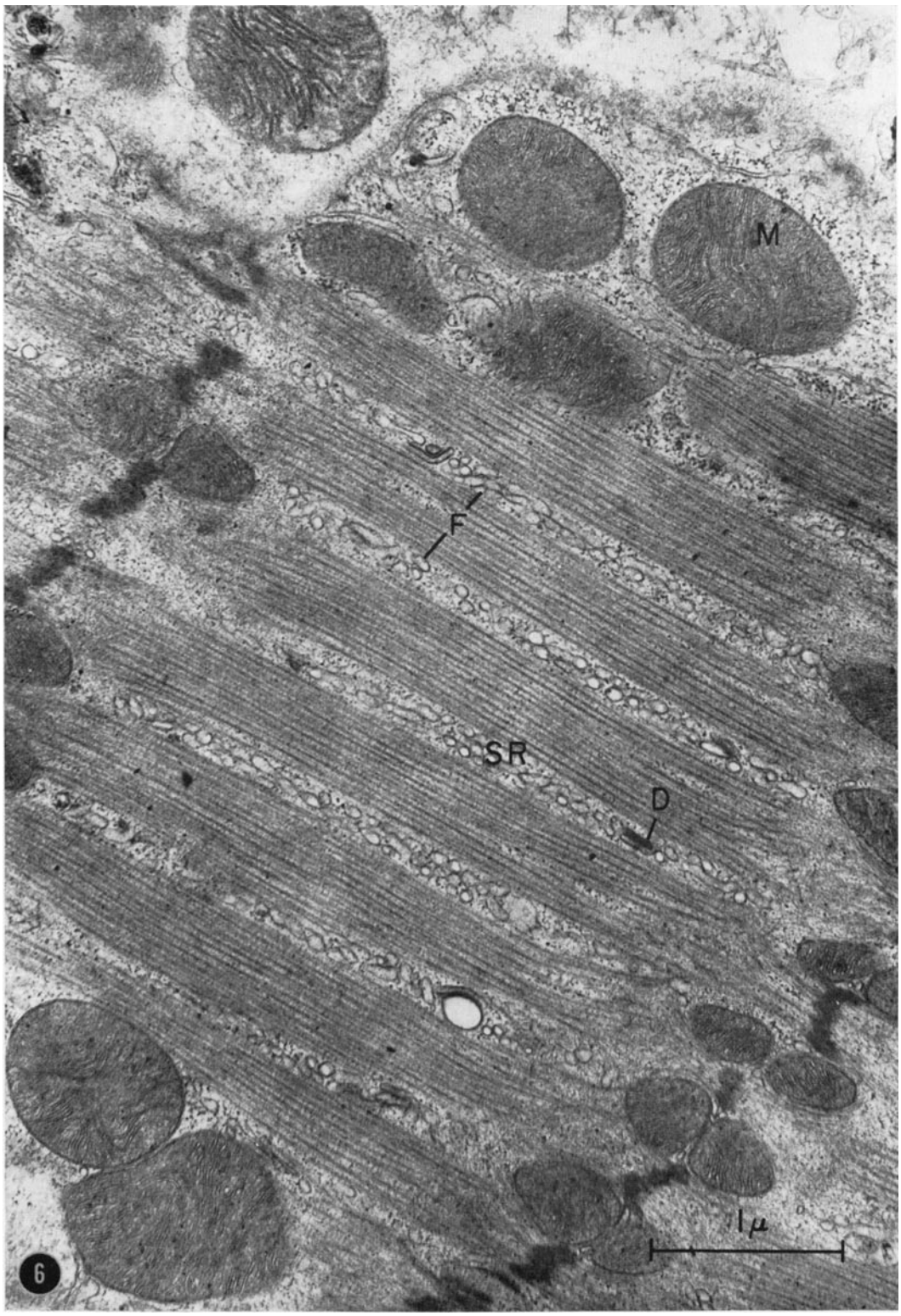
The first approach was to oppose the tibia fully against the femur and fix the femoral muscle by perfusion. In this position the flexor muscle is shortened and the extensor muscle is stretched. Thus, if observation of micrographs of stretched longitudinal extensor muscles discloses H bands and correspondingly long sarcomeres, then the chances are good that cross-sections will be of sarcomeres with H bands. When the same tissue block is reoriented to make transverse sections, the H bands are revealed by the absence of thin filaments or by the predominance of thick filaments in a myofibril (Fig. 11, large arrow). The adjacent myofibril in the same figure (small arrow) shows some thick filaments with 12 thin filaments encircling them. Fig. 11 also demonstrates that the plane of sectioning passes through the edge of an A band bordering on an I band. This does not rule out double overlap, since there is still a remote chance that one myofibril is shortened and out of register with another which is stretched. Furthermore, when a tissue block is reoriented, it is never certain that the same myofibril is being examined. Therefore, reorientation is solely an adjunct in the verification of a single or double overlap of thin filaments.

The thin filaments measure about 70 A in diameter. In favorably oriented sections in the region of a single overlap (interdigitation of thick and thin filaments), there are row upon row of circles (composed of thin filaments) with thick filaments as their center points (Fig. 12). In favorably oriented cross-sections in the region of a double overlap (interdigitation of thin filaments from opposite ends of the A band), the number of thin filaments doubles and the interfilamentous spacing becomes less regular (Fig. 13). Filament counts in stretched muscle in the region of single overlap show a ratio of 1 thick to 5.4 thin filaments (SD = 0.3), while counts in areas of shortened muscles in the region of double overlap show a ratio of 1 to 11.4 (SD = 0.6).

DISCUSSION

The most remarkable fine structural feature of the cockroach femoral muscle is the relatively high ratio of thin to thick filaments in the sarcomeres. In transverse sections of the lateral regions of the A band, where thin and thick filaments interdigitate, the ratio of thin to thick filaments in vertebrates is 2:1, but in insects, 3:1, and in the femoral muscle studied here, about 5.4:1. That this high ratio is not the result of extreme shortening of sarcomeres and interdigitation of thin filaments

FIGURE 6 Micrograph of a longitudinally sectioned flexor muscle fiber which is slightly shortened. The upper right corner and the bottom area are grazing sections of the fiber surface. The H band is no longer present. Sarcomere length is about 4.7 μ. *D*, dyad; *F*, myofibril; *M*, mitochondria; and *SR*, sarcoplasmic reticulum. × 27,500.


M
F
SR
D
1μ
6

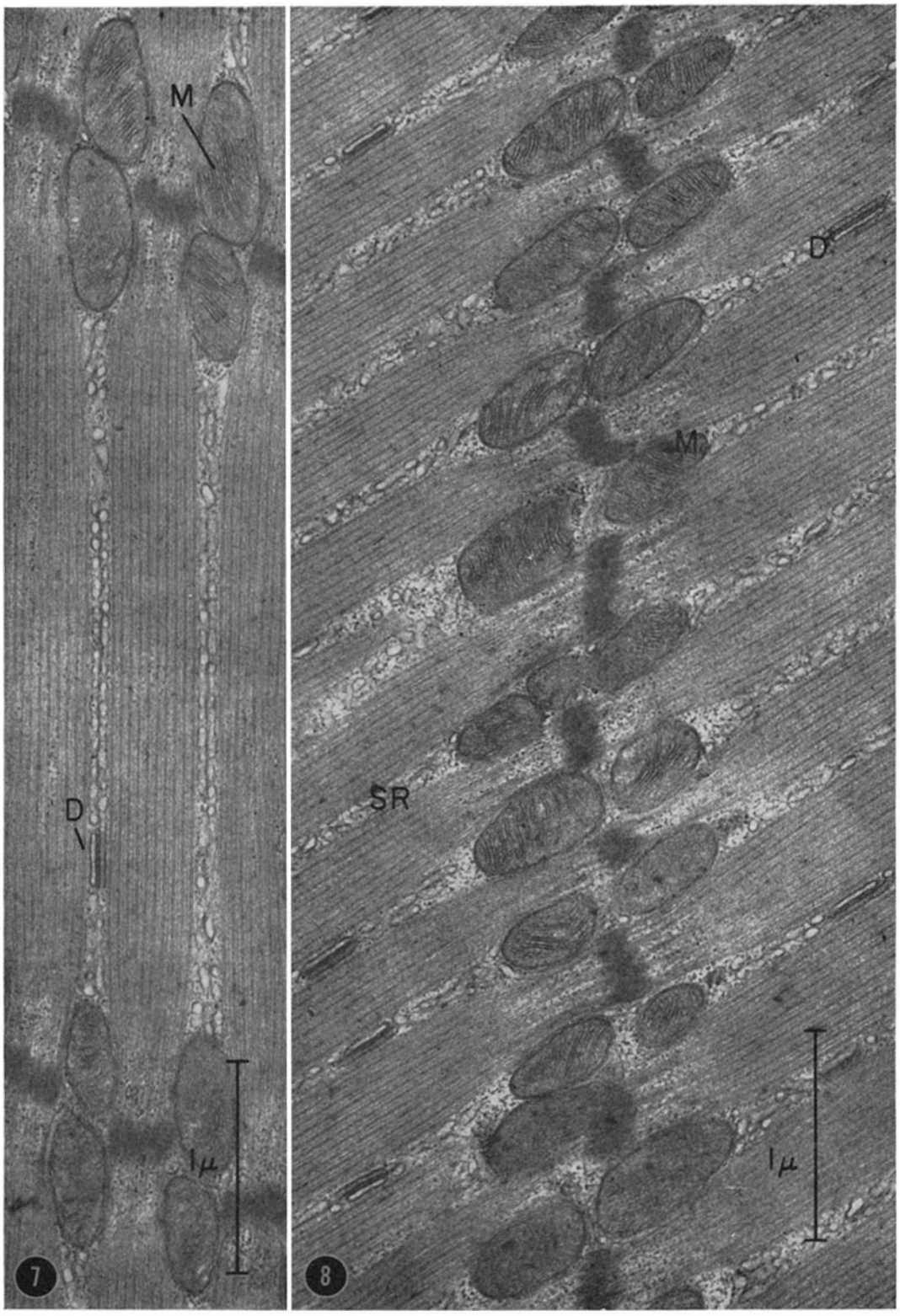
from opposite ends of the sarcomere is confirmed by the fact that the ratio was determined only in stretched sarcomeres. This ratio in stretched sarcomeres (Fig. 12) is further confirmed by the fact that the ratio is doubled in transverse section counts in an area of shortened sarcomeres (Fig. 13) where the thin filaments interdigitate. Examination of favorably oriented sections of stretched sarcomeres frequently showed 12 thin filaments encircling 1 thick filament. Occasionally, there were 10 or 11 encircling thin filaments, a finding perhaps primarily attributable to the oblique plane in which some of them were sectioned. Thus, instead of being revealed as an entity, each filament may appear to merge with its neighbor, thereby presenting a false, single profile. The only known micrographs demonstrating 10 to 12 thin filaments around a thick one were recently published by Auber-Thomay (18) and by Rosenbluth (19) in the muscles of a nematode, by Auber (20) in the tibia muscles of a scorpion, by Hanson and Lowy (21) in the oyster adductor muscles, by Bouligand (22) in the appendicular muscles of the mouth parts and mid gut muscles of a copepod, and by Brandt and coworkers (23) (6 to 8 thin filaments) in the walking leg muscles of a crayfish. In other studies, Sanger and Szent-Györgyi (24) reported 10 to 12 thin filaments surrounding a thick filament in a scallop muscle; Swan (25) noted 9 to 12 thin filaments grouped around one thick filament in a walking leg muscle of a crayfish; and Toselli (26) counted 12 thin filaments around 1 thick filament in the abdominal muscles of the hemipteran insect, *Rhodnius*. Although some of the high counts may be attributed to double overlap of thin filaments, it is still interesting to note that only the invertebrates of the phyla Nematoda, Mollusca, and Arthropoda are represented. Examination of femoral muscle cross-sections showed no fibers composed of thick filaments each surrounded by 6 thin filaments as is found in some vertebrates and invertebrates. Although no such filament arrangements were encountered in this study, the possibility still remains that they exist. The thick and thin filaments of the femoral muscle in this study appear to be greater in both length and diameter than those in any other electron microscope studies on insect filaments. Moreover, the extraordinary sizes of the various band patterns of a fully stretched fiber are unusual for an insect. Cross-bridges or projections from the thick filaments were not observed at these relatively low magnifications, but their existence is possible since the longitudinally oriented fibers were sectioned with their axes parallel to the edge of the knife. Consequently, this tends to compress the arrays of interdigitating filaments and perhaps to obscure the bridges. High power micrographs of transverse sections of the fibers, however, indicate bridges on the thick filaments (Fig. 12, inset).

The femoral muscles are also unusual in another respect. Tiegs believed that an arthropod leg muscle fiber has a basic arrangement in which straplike myofibrils are radially disposed around a central core of nuclei (27). He reported these tubular fibers in the limbs of spiders, in insect leg fibers of the order Orthoptera (cockroaches belong to this group), and in many other species of higher and lower orders. This is not, however, the structure of the cockroach's femoral muscles, in which the nuclei are subsarcolemmal and the myofibrils in transverse sections appear to be polygonal for some and straplike for others.

To the author's knowledge, all previous studies which describe more than 6 thin filaments grouped around a thick filament and which record sarcomere lengths have revealed that sarcomere lengths (stretched or shortened) are longer than those in muscle fibers where 6 thin filaments surround 1 thick filament. Therefore, a functional and purely physical interpretation of the extra number of thin filaments might be that the extra number would augment the tensile strength of the

FIGURE 7 Micrograph of three myofibrils of the shortened flexor muscle. The length of the sarcomere (4.4 μ) approaches that of the A band. *M*, mitochondria; and *D*, dyad. × 32,000.

FIGURE 8 Micrograph of a longitudinal section of a shortened flexor muscle fiber in the area of the Z disc. This mitochondrial pattern is seen very often. *D*, dyad; *M*, mitochondria; and *SR*, sarcoplasmic reticulum. × 32,000.


M
D
1μ
7
D
M
SR
1μ
8

fibers and would also create more reactive sites between the thick and thin filaments. These additional sites are probably necessary for the functioning of the long sarcomeres. Not enough is yet known about the reactive sites between the two types of filaments in this muscle to even attempt speculation on the interaction of filaments.

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FIGURE 9 Micrograph of a longitudinal section of a shortened flexor muscle in which a nerve ending appears. The sarcomere length is about 3.0 μ, and the area of double overlap (*DO*) (interdigitation of thin filaments) is discernible. The contraction band (*CZ*) is due to the thick filaments of the A band pushing up against the Z disc. The length of the I filaments (*I*, 2.3 μ) is shown. A transverse section of the double overlap from the same tissue block is shown in Fig. 13. *CZ*, contraction band; *DO*, double overlap; *SV*, synaptic vesicles. × 48,000.


SV
DO
I
CZ
1μ
9

FIGURE 10 Survey view micrograph of a transverse section of two stretched extensor muscle fibers, illustrating the various band areas as well as the transverse tubules (*TT*) invaginating from the sarcolemma. *AIO*, A band and I filaments overlap; *H*, H band; *I*, I band; *M*, mitochondria; *N*, nucleus; *S*, sarcolemma; *SR*, sarcoplasmic reticulum; *TT*, transverse tubule; and *Z*, Z disc. × 39,000.

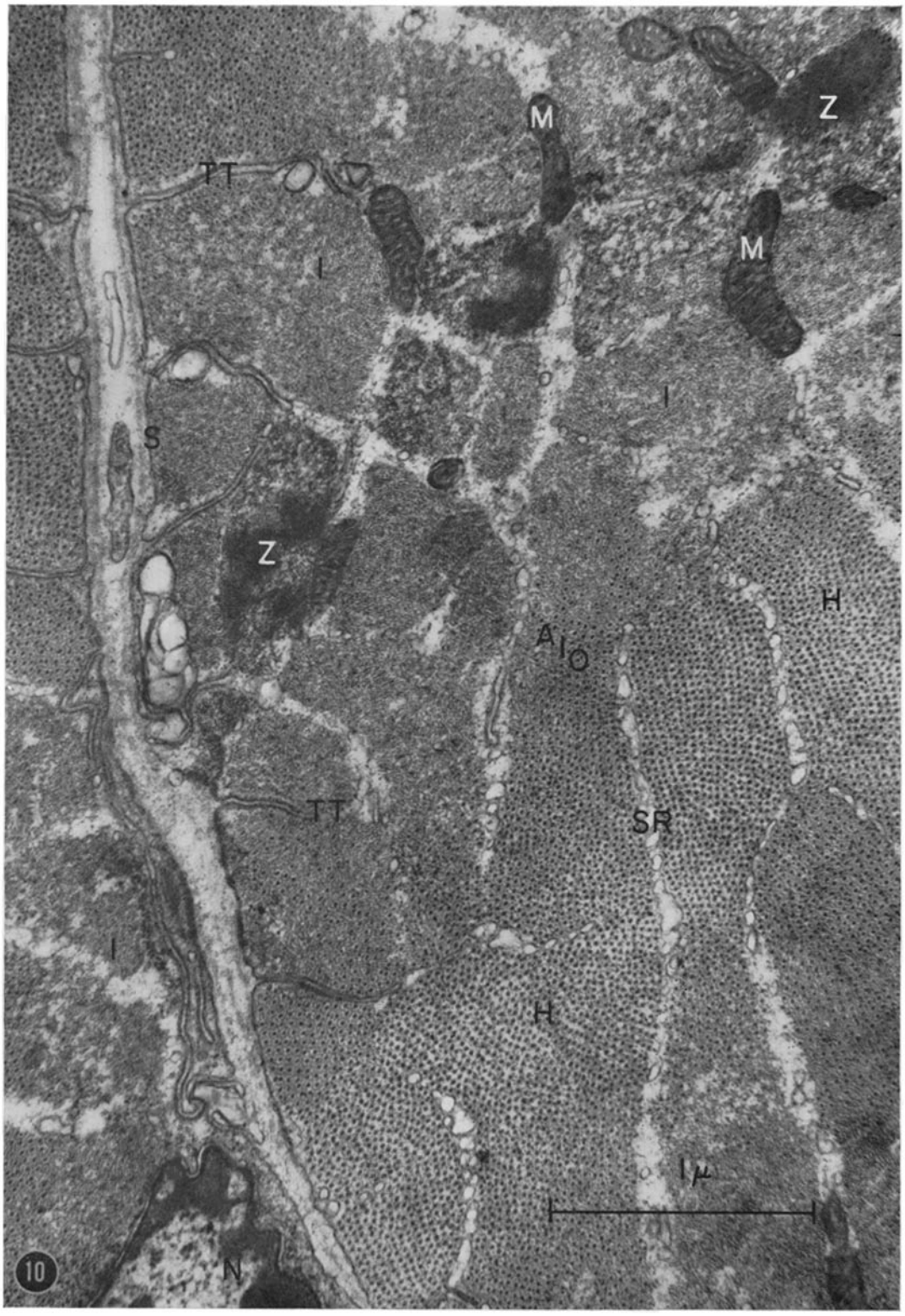

M
Z
TT
M
I
I
S
Z
H
A
I
O
TT
SR
I
H
1μ
10
N

FIGURE 11 High power micrograph of a transverse section of a stretched extensor muscle fiber showing the arrangement of 12 thin filaments around each thick filament (short arrow). The possibility of a double overlap of thin filaments seems unlikely, since the myofibril (long arrow) to the right of it is predominately composed of thick filaments, thus designating the H band region. The plane of sectioning at the left side of the micrograph passes through the edge of an A band bordering on an I band. The thick filaments (large dots) appear hollow while the thin filaments (small dots) seem electron-opaque in this section. This is not always the case. *I*, I band; and *M*, mitochondria. × 109,000.

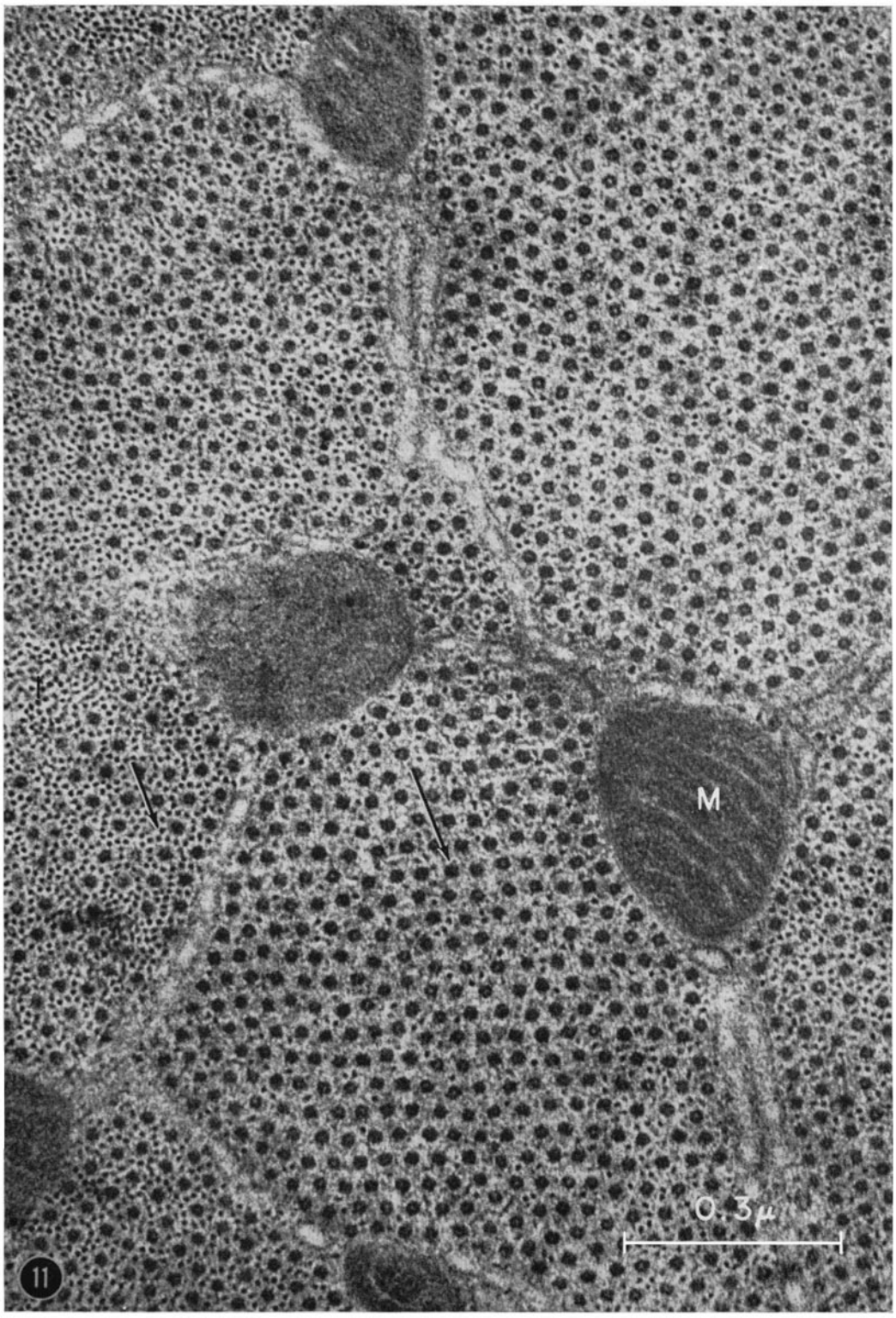
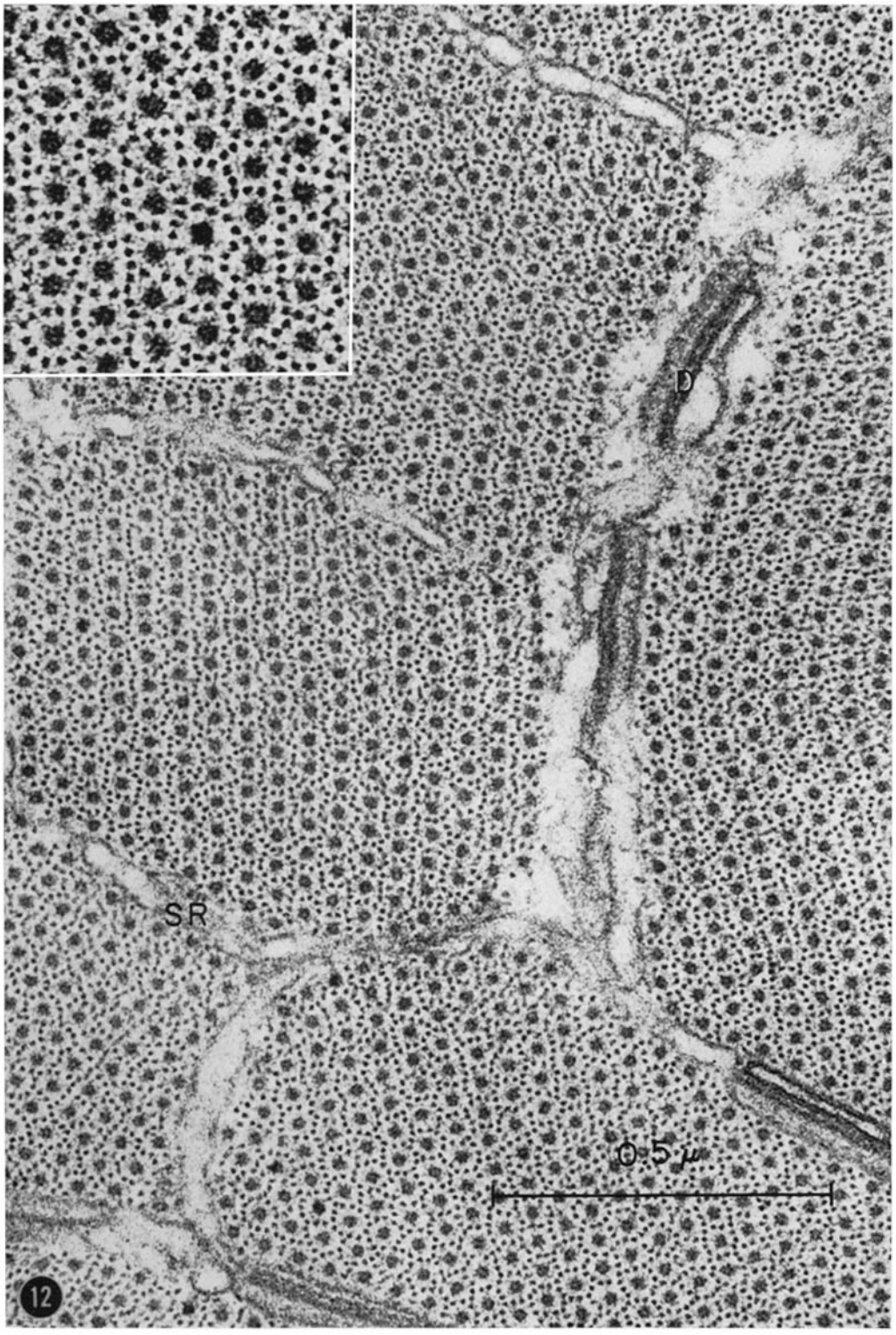

M
0.3 μ
11

FIGURE 12 Micrograph at a high magnification of a transverse section of a stretched flexor muscle fiber. The plane of sectioning is in the region of a single overlap of the thick filaments (large dots) and the thin filaments (small dots). There are 10 to 12 thin filaments around each thick filament. The thick filaments are symmetrically spaced in a hexagonal array. *D*, dyad; and *SR*, sarcoplasmic reticulum. × 102,000. Inset: High power view of cross-bridges on the thick filaments (large dots). × 150,000.


D
SR
0·5 μ
12

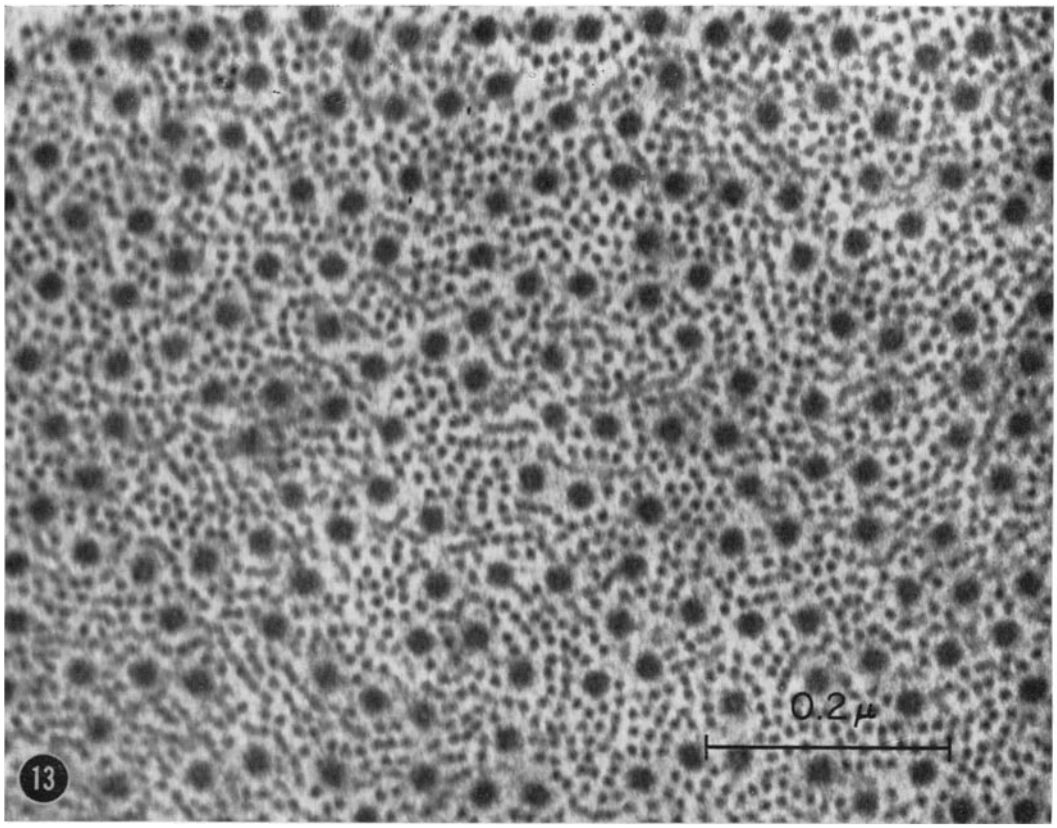


FIGURE 13 High power micrograph of a transverse section of a shortened flexor muscle fiber. It depicts the double overlap of thin filaments (small dots) from opposite ends of the A band. Note the double number of thin filaments and the disruption of the interfilamentous spacing. A longitudinal section of the same tissue block is shown in Fig. 9. The thick filaments (large dots) have a diameter of about 205 A, while the thin filaments (small dots) are approximately 70 A in diameter. × 155,000.