THE FILAMENT LATTICE OF COCKROACH THORACIC MUSCLE

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ABSTRACT

The fine structure of the tergo-coxal muscle of the cockroach, *Leucophaea maderae*, has been studied with the electron microscope. This muscle differs from some other types of insect flight muscles inasmuch as the ratio of thin to thick filaments is 4 instead of the characteristic 3. The cockroach flight muscle also differs from the cockroach femoral muscle in thin to thick filament ratios and diameters and in lengths of thick filaments. A comparison of these latter three parameters in a number of vertebrate and invertebrate muscles suggests in general that the diameters and lengths of the thick filaments and thin to thick filament ratios are related.

INTRODUCTION

In previous studies of the femoral muscle of cockroach, *Leucophaea maderae* (Hagopian, 1966; Hagopian and Spiro, 1967), 10–12 actin filaments were found encircling each myosin filament. Other salient features such as long sarcomeres and a sarcoplasmic reticulum which is continuous longitudinally as well as laterally across the fibers are unusual. Since this insect is a member of the most primitive surviving winged insects belonging to the order Orthoptera, and since Tiegs (1955) has stated that the thoracic musculature in Orthoptera has undergone a minimal structural adaptation to flight, it was deemed advisable to compare the structure of the thoracic muscle to that of the femoral muscle. The present study shows that the filament lattice of this indirect flight muscle differs from that reported for most insect flight muscles (Auber and Couteaux, 1963; Huxley and Hanson, 1957; Shafiq, 1963; Smith, 1961, 1962, 1965, 1966 *a*). The filament lattice of this cockroach flight muscle is also compared to that of other types of striated muscle in a variety of species.

MATERIALS AND METHODS

The metathoracic tergo-coxal indirect flight muscle of the insect, *Leucophaea maderae*, was selected because of its location, its large size, and its insertion on the tergite. Tying the wings of the ether-anesthetized insects in an upright position deformed the tergites so that muscle fibers with varying sarcomere lengths could be obtained.

For light microscopy, conventional methods including Best's carmine and PAS (periodic acid-Schiff) staining (with and without diastase) were used for glycogen granules, and van Gieson's and Masson's trichrome stains were utilized for connective tissue.

For electron microscopy, the opened thoraces were immersed in 6% glutaraldehyde (room temperature) buffered with 0.2 M phosphate at pH 7.4 for 3 hr. The tergo-coxal muscle was then removed and was placed in several changes of phosphate buffer with 0.2 M sucrose for 12–15 hr at 4°C. The tissue was then postfixed at the same temperature in 2% osmium tetroxide (phosphate buffered at pH 7.4) at 4°C for 1 hr. After dehydration in various grades of acetone, the muscle was embedded in Araldite, and thin sections were cut, stained with phosphotungstic acid or uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I and a Philips EM 200 microscope. Longitudinal sections of the muscle were cut with the knife edge parallel to the long axis of the muscle fiber.

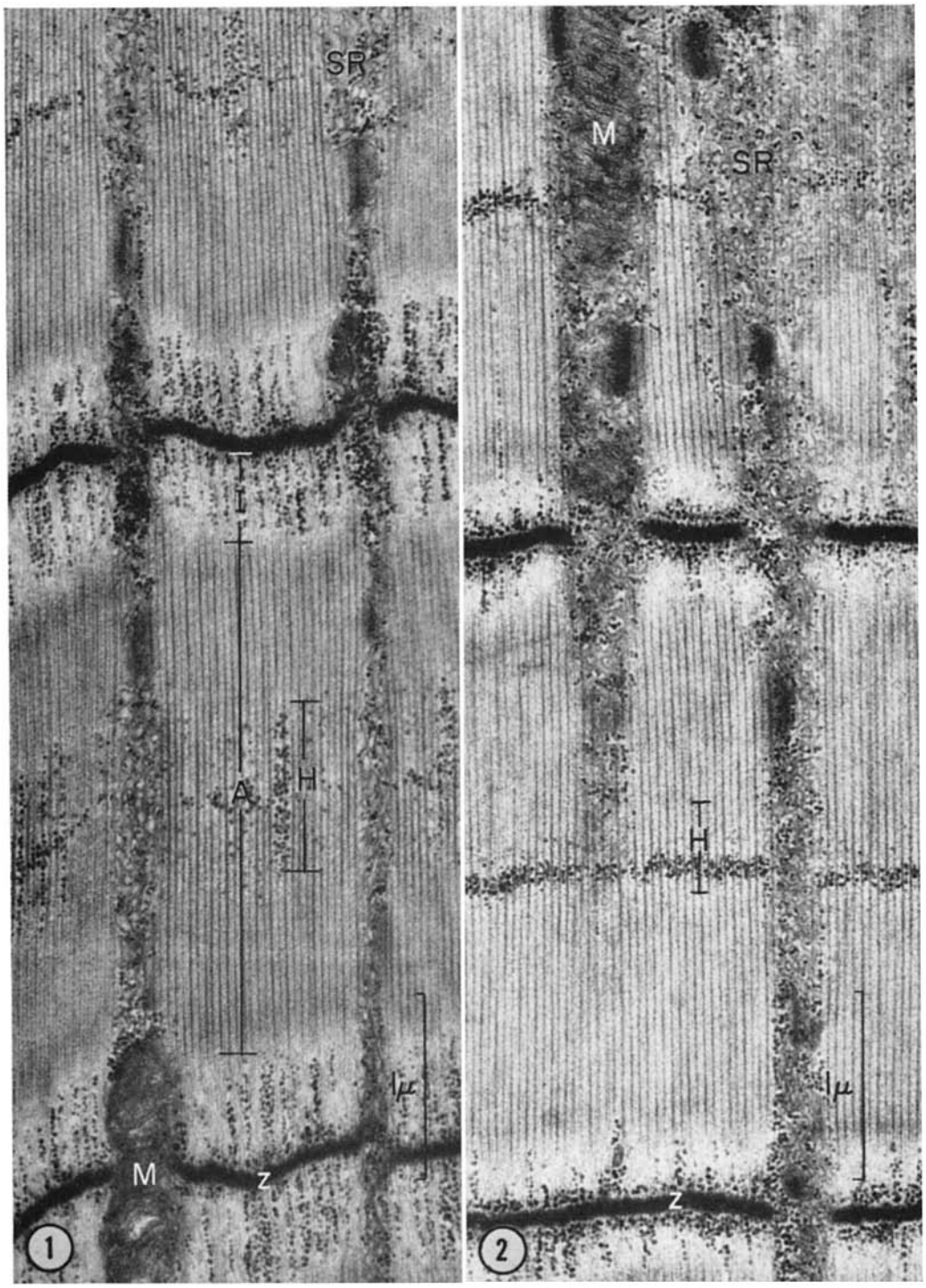

FIGURE 1 Electron micrograph of a portion of stretched metathoracic tergo-coxal, muscle from a cockroach. The sarcomeres (Z line, *Z*, to Z line) are about 4.0 μ long. The various band patterns have the following measurements: the A band (*A*), about 2.7 μ long; the somewhat irregular H zone (*H*), about 1.0 μ; the I band ($\frac{1}{2}$) (*I*), about 0.6 μ; the I filaments (from Z line to the margin of the nearest H zone), about 1.5 μ. Glycogen granules in stretched thoracic muscle fibers are generally dispersed in H zone and I bands. *m*, mitochondrion; *SR*, sarcoplasmic reticulum. × 28,000.

FIGURE 2 Micrograph of cockroach thoracic muscle at same magnification as Fig. 1. Sarcomere length (Z line, *Z*, to Z line) is now about 3.5 μ; I band and irregular H zone (*H*) have accordingly reduced in size. Glycogen-like granules are limited to center of the sarcomere and each side of Z line. *m*, mitochondrion; *SR*, sarcoplasmic reticulum. × 28,000.

RESULTS

The tergo-coxal muscle is a large muscle composed of several hundred fibers. The epoxy-embedded fibers are polygonal in shape and measure about 40–60 μ in thickness and about 7 mm in length. The multiple nuclei of the fibers are always subsarcolemmal. The myofibrils also have polygonal profiles and measure 1–2 μ in width. Mitochondria up to 5 μ in length are found in the intermyofibrillar space. Longitudinal sections show that the sarcomeres of the myofibrils consist of typical interdigitating arrays of thick and thin filaments (Figs. 1 and 2). Frequently nerve endings contact the muscle fiber at depressed areas on the sarcolemma. These neuromuscular junctions are similar to those found in the femoral muscle of this species (Hagopian, 1966). The cell membrane invaginates at regular intervals to form transverse tubules (T system) which make dyadic contacts with the unfenestrated areas of the sarcoplasmic reticulum (SR). The SR is a well developed envelope surrounding the myofibril and consists of fenestrated and nonfenestrated portions which extend both laterally and longitudinally along the myofibrils. The T-system tubules make dyadic contacts with the unfenestrated sites of the SR at the lateral region of the A band, as is the case in all the reported arthropod synchronous muscles (for tabular listing see Smith, 1965). Dyadic contacts are also formed between the SR and cell surface membrane.

Unlike the cockroach femoral muscle, the thoracic muscle contains numerous cell membrane invaginations which do not form junctions with the SR. These invaginations further differ from those of the T system inasmuch as they contain tracheoles (Fig. 6) as described in other insect flight muscles (Shafiq, 1963; Smith, 1961, 1962, 1965). Variable amounts of amorphous substance of moderate electron opacity which may be ground substance are present between the tracheoles and the invaginated membranes of the muscle fibers. This amorphous substance is also seen between muscle fibers where it often contains randomly oriented fibrils measuring about 200–600 A in diameter (Fig. 5). These regions also have staining characteristics of connective tissue with the Masson's trichrome stain. Fibrils of larger diameter exhibit a regularly repeating band pattern of about 550 A with up to six intraperiod bands. The periodicity is approximately that of vertebrate collagen fibrils as observed in thin sections. In addition the subbands resemble those of collagen. The fibrils of smaller diameter fail to show a periodic structure. No collagen fibrils are seen within the invaginations containing tracheoles (Fig. 6).

In longitudinal sections the myofibrils exhibit the usual bands including an H zone at longer sarcomere lengths. M lines, however, are not present. The length of the A band and of the thick or myosin filaments is usually 2.7 μ, with a range of 2.5–2.8 μ, in well-oriented longitudinal sections. The thick filaments taper at their extremities. The thin or actin filaments are usually 1.5 μ in length with a range of 1.4–1.7 μ. Sarcomeres varying from 3.0–4.0 μ in length are observed, and the widths of the I band and H zone vary directly with sarcomere length in accord with the sliding filament model. In sarcomeres measuring 4 μ in length, an H zone which measures about 1 μ in length is seen, whereas in shorter sarcomeres measuring 3 μ in length the thin filaments meet in the center of the sarcomere. Contraction bands in the center of the sarcomere owing to a double overlap of thin filaments were not observed in this muscle which had been fixed *in situ* in the thorax. (Fig. 3).

Transverse sections reveal that the myosin filaments are hexagonally arrayed with a center-to-center spacing of about 400 A. These thick filaments measure 150–160 A in diameter and appear to have substructure (Fig. 4). The thin filaments measure 60 A in diameter. Nine of these filaments usually surround each thick filament in the A band exclusive of the H zone. Counts of filaments in favorably oriented transverse sections reveal a ratio of about 4 (actually 3.8) thin filaments to 1 thick filament.

PAS and Best's carmine stains indicate the presence of glycogen in the center of the sarcomere. These observations are further confirmed in electron micrographs which disclose numerous glycogen granules in the center of the A band as well as additional glycogen deposits adjacent to the Z line in short sarcomeres (Fig. 2). In stretched sarcomeres these glycogen deposits are more dispersed (Fig. 1).

DISCUSSION

This thoracic muscle of cockroach has shorter and thinner myosin filaments and a smaller ratio of thin to thick filaments than does the femoral muscle of the same species. It would appear that the diameter of thick filaments and ratios of thick

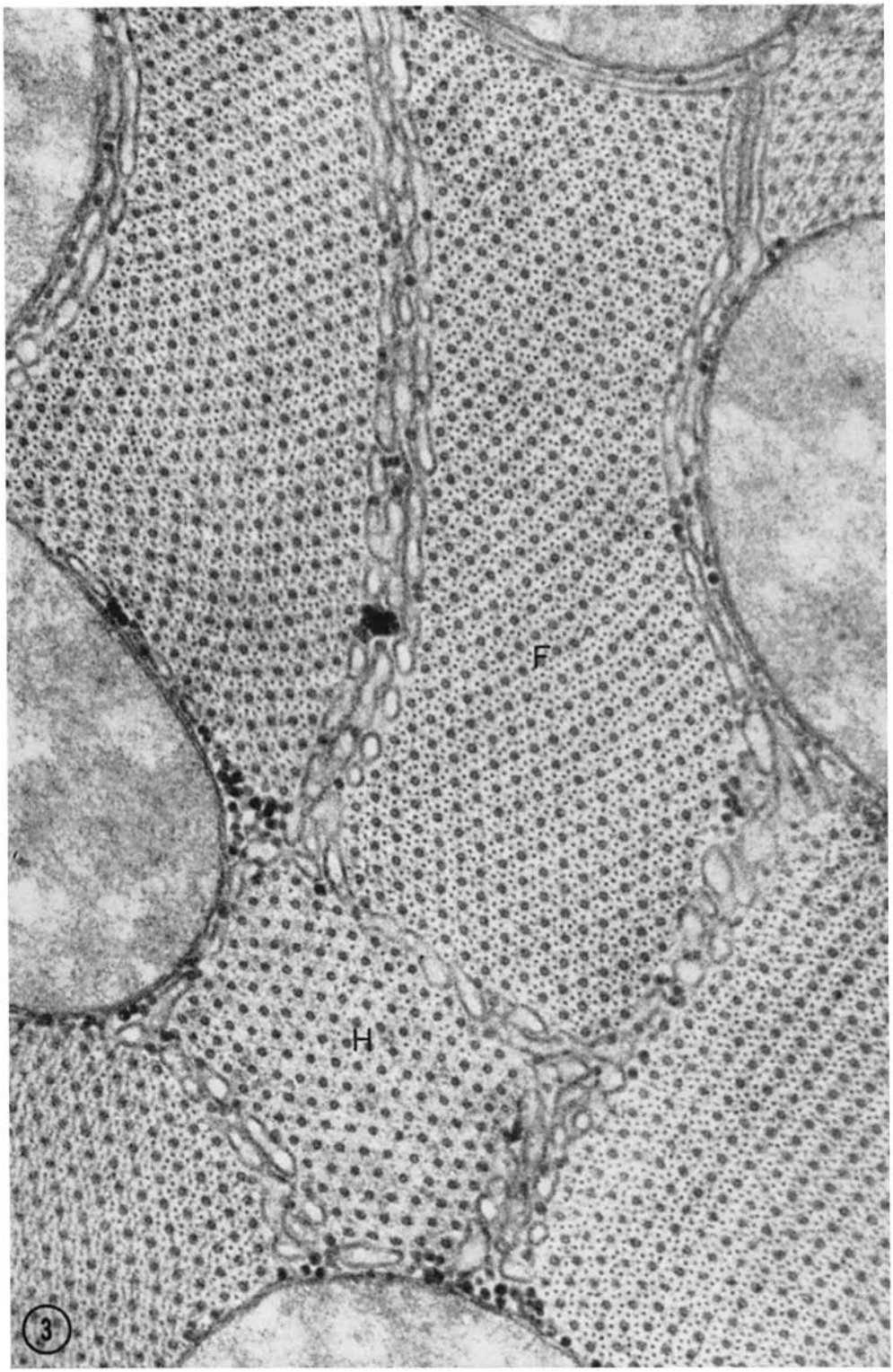

FIGURE 3 A micrograph of a transverse section of the cockroach thoracic muscle. One myofibril (*H*) is at the level of the H zone. The adjacent myofibril (*F*) has a single overlap of thick and thin filaments in the A band. In this region of an overlap there are generally eight or nine thin filaments around each thick filament. × 108,000.

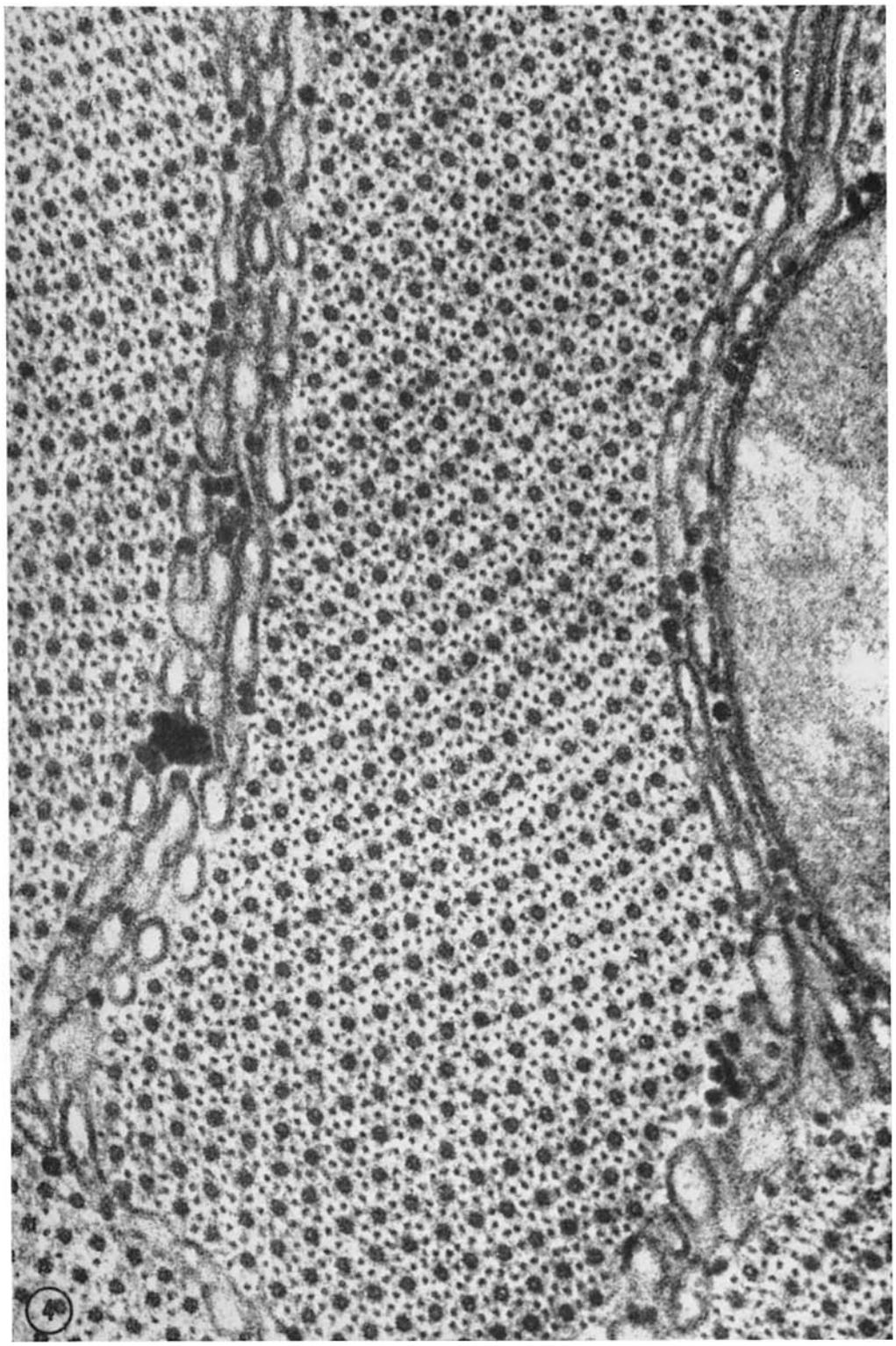

FIGURE 4 A high power micrograph of a portion of Fig. 3 depicting the eight or nine thin filaments surrounding the thick filament. × 180,000.

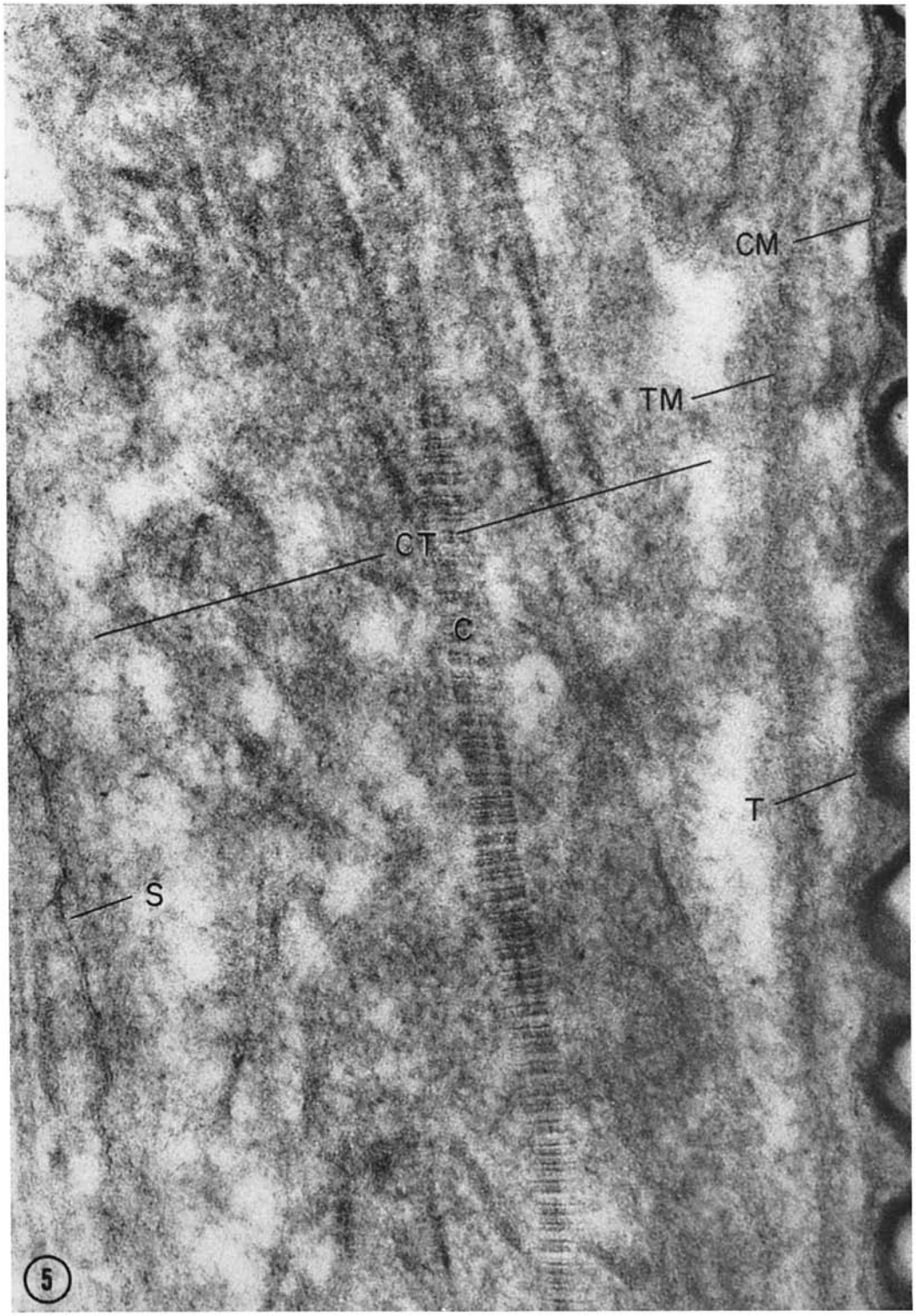

FIGURE 5 A high power micrograph in the area between the tracheole (*T*) and the sarcolemma (*S*) of the thoracic muscle fiber. The connective tissue-like substance (*CT*) is composed of an amorphous ground substance and a few collagen-like fibrils (*C*). The collagen-like fibrils have a periodicity of about 550 A and subbands similar to those of vertebrate collagen. *CM*, plasma membrane of the tracheolar cell adjacent to the cuticulin of the tracheole; *CT*, connective tissue; *TM*, outer plasma membrane of the tracheolar cell. × 116,000.

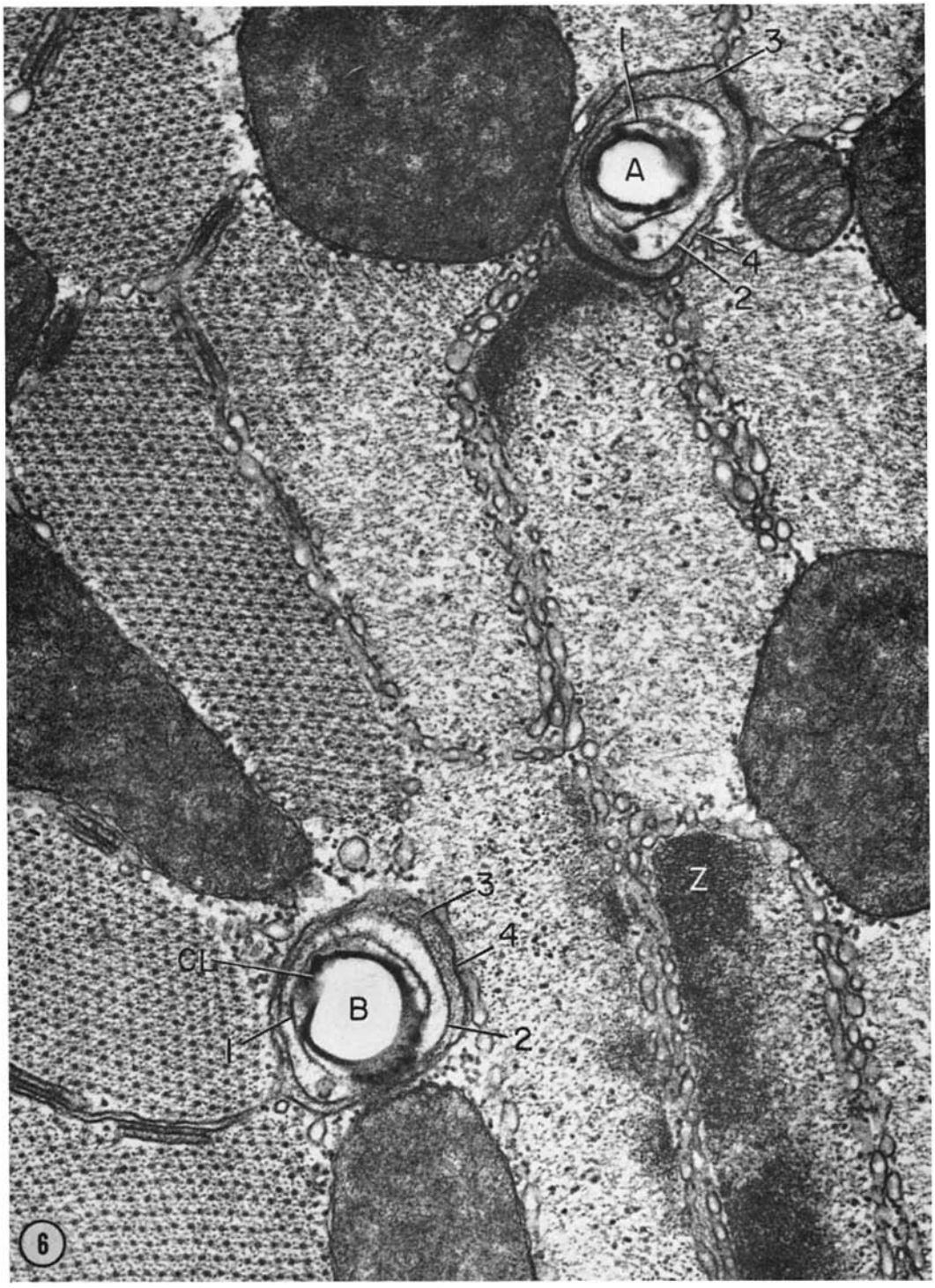

FIGURE 6 A transverse section of the thoracic muscle fiber showing two tracheoles (*A*, *B*) within sarcolemmal invaginations (*4*). This section exhibits moderately electron-opaque, amorphous material (*3*) between tracheoles and the membranes of the transverse tubules. *CL*, cuticulin lining of the tracheole; *Z*, Z line; *1*, tracheolar cell plasma membrane; *2*, outer plasma membrane of tracheolar cell; *3*, amorphous material; *4*, membrane of transverse tubule. × 70,000.

TABLE I

Muscles Initially Fixed in Glutaraldehyde Unless Otherwise Stated

	Length of thick filaments	Diameter of thick filament	Ratio of numbers of thin filaments to thick filaments	
	μ	A		
Vertebrate				
Human flexor carpi radialis	1.5	100–120	2:1	Schotland (1967) (Data in preparation)
Dog heart	1.5		2:1	Spotnitz et al. (1966)
Cat heart	1.5	100–120 (Spiro unpublished)	2:1	Spiro and Sonnenblick (1964)
Chicken heart	1.5	110	2:1	Hagopian and Spiro (1967) (Data in preparation)
Chicken breast	1.6		2:1	Page (1964)
Frog sartorius	1.5–1.6		2:1	Page and Huxley (1963) Spiro and Sonnenblick (1964)
Frog semitendinosus	1.6		2:1	Page and Huxley (1963)
Rabbit psoas*		100–120		Huxley (1963)
Fish muscle		150	2:1	Franzini-Armstrong and Porter (1964)
Invertebrate				
Dragonfly flight	2.2‡		3:1	Smith (1966 *a*)
Butterfly flight (*Phytometra*, *Minucia*, and *Abraxas*)		140	3:1	Auber (1967)
Blowfly flight§	3.0		3:1	Hanson (1956)
Butterfly flight (*Vanessa*, *Pieris*)		140	4:1	Auber (1967)
Cockroach flight	2.7	150–160	4:1	Hagopian and Spiro (1967) (Data in preparation)
Cockroach intersegmental	3.6–4.1	160–180	6:1	Smith (1966 *b*)
Cockroach femoral	4.5	180–200	6:1	Hagopian (1966)
Insect visceral		160–180	6:1	Smith et al. (1966)
Walking leg muscle of a crayfish	3.0–6.0	200	6:1	Swan (1963)
Somatic musculature of a nematode	6.0	230	6:1	Rosenbluth (1965)

* Fixed in formaldehyde and negatively stained.

‡ Measured from published electron micrograph.

§ Unfixed and measured under the phase contrast microscope (it is assumed that section was cut with the fiber axis parallel to the knife edge).

to thin filaments are related, if one compares striated muscles of various species. In addition the thicker myosin filaments are generally longer. Table I indicates reported values for the diameters and lengths of thick filaments and the thin to thick filament ratios in a number of vertebrate and invertebrate muscles, most of which were fixed in glutaraldehyde. As noted, these values are smallest for vertebrate muscles and largest for a number of invertebrate muscles. Such measurements are not easy to make accurately and could depend on preparative techniques, intensity of staining, etc.

There is general agreement, however, that in glutaraldehyde-fixed vertebrate muscle the length of the thick filaments is about 1.5–1.6 μ and that the thin to thick filament ratio is 2.

Recent measurements of the diameter of thick filaments in similarly fixed flexor carpi radialis muscle of human and heart muscle of several species indicate that the diameter is about 120 A. In addition, Huxley (1963) observed a thick filament diameter of 120 A in rabbit psoas muscle fixed in formaldehyde and negatively stained. It should be noted, however, that thick-filament diameters of 150 A have been reported in a fish muscle (Franzini-Armstrong and Porter, 1964). The thoracic muscle of the cockroach differs from some other insect flight muscles in that it appears to have longer and thicker myosin filaments and a larger ratio of thin to thick filaments. In insect flight muscle in which the thin to thick filament ratio is 3, the reported value of the diameter of thick filaments is about 140 A (Auber, 1967). The length of the thick filaments in insect flight muscle, however, may vary from species to species. Hanson (1956) has reported an A-band length of 3 μ in a blowfly; whereas, in the dragonfly the sarcomeres are 2.3 μ in length (Smith, 1966*a*), and the thick filaments appear to be 2.2 μ in length. Thus, while the length of the thick filaments may not invariably reflect the diameter of the thick filaments and the thin to thick filament ratio, in general these three parameters seem to be related. The difficulty in comparing thick filament lengths and diameters and thin to thick filament ratios in various types of flight muscles stems from lack of published data on these parameters. In addition, differences in thick filament diameters in this group of muscles are small and hence difficult to quantitate.

It might be that thicker filaments with larger numbers of myosin monomers and therefore more reactive sites per unit length can interact with greater numbers of thin filaments even though the cross-bridge pattern of the sarcomere is obscure. The relationship between greater filament diameter and length is not so clear. It is possible that either the larger numbers of myosin molecules or the differences in their packing or stagger with respect to another copolymer results in a stable structure and a longer filament length (Huxley, 1963). It should be noted that the thick filaments in many arthropod muscles show a lower electron opacity than the thick filaments in vertebrate muscle; this might indicate that the packing of myosin molecules in arthropod muscle is different from that in vertebrate muscle. The diameters and number of myosin monomers are therefore not necessarily straightforwardly related. The functional implications of these varying filament arrays as regards active tension per unit area of contractile substance cannot be assessed at present, although Lowy et al. (1964) speculate on this matter with regard to fast and slow muscles. In accord with the studies of Hanson and Lowy (1964), it is noted that the diameters of the thin filaments are the same in all species although there is variation in thin filament length.

It is thus suggestive that the sarcomere structure in the cockroach flight muscle is in several ways between that in other flight muscles and that in cockroach femoral muscles as regards thick filament dimensions and thin to thick filament ratios. This fine structural organization may be a correlate of the lack of adaptation for flight which characterizes most thoracic muscles in Orthoptera (Tiegs, 1955). The more numerous mitochondria, glycogen granules, and tracheoles which are found in the thoracic muscle of the cockroach may indicate a higher metabolic activity as compared with the femoral muscle of the same species.

Collagen fibrils are fairly numerous in the cockroach flight muscle. Numerous collagen-like fibrils have been observed in the neural sheath of insects by several investigators (Ashhurst, 1959, 1961, 1965; Ashhurst and Richards, 1964; Baccetti, 1955, 1957, 1961; Gray, 1959; Hess, 1958; Richards and Schneider, 1958; Smith and Wigglesworth, 1959). More recent papers have shown collagen-like fibrils in cells lining the ejaculatory canal in a grasshopper (Martoja and Bassot, 1965), around the rectal papillae of a blowfly and in the corpus allatum and prothoracic gland of a cockroach (Harper et al., 1967). The insect class is supposedly characterized by a scarcity of collagen fibrils despite the widespread occurrence of such fibrils in the Arthropods. It would thus appear that collagen in insects is not so scanty as previously suspected from electron microscopic studies.

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