

THE STRUCTURE OF INTERSEGMENTAL MUSCLE FIBERS IN AN INSECT, *PERIPLANETA AMERICANA* L.

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ABSTRACT

The organization of intersegmental muscle fibers associated with the dorsal abdominal sclerites of the cockroach is described. These fibers correspond closely, in the disposition and derivation of the membranes of the transverse tubular system and sarcoplasmic reticulum cisternae, with insect synchronous flight muscle fibers, but differ markedly from these in their fibrillar architecture and mitochondrial content. The mitochondria are small and generally aligned alongside the prominent I bands of the sarcomere, and, in the best-oriented profiles of the A bands, thick filaments are associated with orbitals of twelve thin filaments, a configuration that has also been observed in striated fibers of insect visceral muscle. These structural features of insect muscles are compared and discussed in terms of possible variations in the control of contraction and relaxation, and in the nature of their mechanical role.

INTRODUCTION

The muscle system of insects (or other arthropods) may be generally divided into "visceral fibers" which control movement of the gut and other organs lying within the haemocoel and "skeletal fibers" which control movement of the cuticle-bearing plates of the exoskeleton limiting the surface of the body. The latter category includes fibers responsible for a considerable number of mechanically distinct actions, involving varying metabolic outputs.

Electron micrographic studies on insect skeletal muscle have thus far been generally concerned with the distribution of membrane systems and mitochondria in fibers associated with the wing or leg mechanisms. These studies have indicated that, as might be expected, the mitochondrial content of flight muscles is greater than in fibers capable of less sustained activity, and that in synchronous skeletal fibers of insects and vertebrates the distribution of the T system tubules and associated cisternae of the sarcoplasmic reticulum is generally comparable (see references in Smith, 1966 *b*).

Detailed analysis of the contractile apparatus of insect muscles has been less extensive. Huxley and Hanson (1957) pointed out that, in the A band array (of actin and myosin myofilaments) in asynchronous flight muscle fibers, each actin filament is "shared" by two myosin filaments, in contrast to the trigonal disposition of actin filaments in mammalian skeletal muscle fibers. This feature is now known to be common to both synchronous and asynchronous flight muscle fibers of insects (Smith, 1966 *a*) and also occurs in the skeletal muscle fibers of a cyclopoid copepod (Fahrenbach, 1963). It has recently been shown (Smith et al., 1966) that insect visceral muscle fibers (from three locations in the insect body) are clearly distinct from insect skeletal muscle fibers, hitherto described, in possessing an orbital of up to twelve actin filaments surrounding each myosin filament in the A band region of the sarcomere. These visceral muscle fibers, however, differ from other synchronous muscle fibers in exhibiting a very reduced sarcoplasmic reticulum.

In insects, the movement of each abdominal

segment, with respect to its neighbors, is controlled by small straplike intersegmental muscles, typically arranged in parallel series and lying parallel with the long axis of the body. As in all other insect muscles, visceral or skeletal, these fibers are striated. The organization of the myofibrils and membrane systems of these muscles associated with the dorsal sclerites of the cockroach are described in this report.

MATERIALS AND METHODS

Abdominal intersegmental muscles of adult male *Periplaneta americana* were employed in this study. Muscles were fixed in situ (attached to the tergal sclerites) in cold 2.5% glutaraldehyde, maintained at pH 7.4 with 0.05 M cacodylate buffer containing 0.17 M sucrose. They were fixed for 3 hr, then washed for 24 hr in several changes of cacodylate-buffered 0.34 M sucrose, postfixed in 1% OsO₄ in Veronal-acetate at pH 7.4, dehydrated in an ethanol series, and embedded in Araldite. Sections were cut with glass knives on a Huxley microtome, mounted on collodion-carbon coated grids, and examined in a Phillips EM 200. Sections were stained first with saturated uranyl acetate in 50% ethanol for 10 min and subsequently with lead citrate (Reynolds, 1963) for 2 min.

RESULTS

The dorsal intersegmental muscles in the cockroach *Periplaneta americana* traverse each of the abdominal segments. Each anatomical muscle is ca. 0.2 to 0.25 mm in width, and is composed of fibers of rectangular or polygonal cross-section, generally of the order of 10 × 25 μ but sometimes attaining a width of ca. 40 μ. In longitudinal sections of relaxed fibers (Figs. 1 and 2) the general striation pattern made familiar by studies of vertebrate muscles is evident; in particular, the I bands are of far greater extent than in insect flight

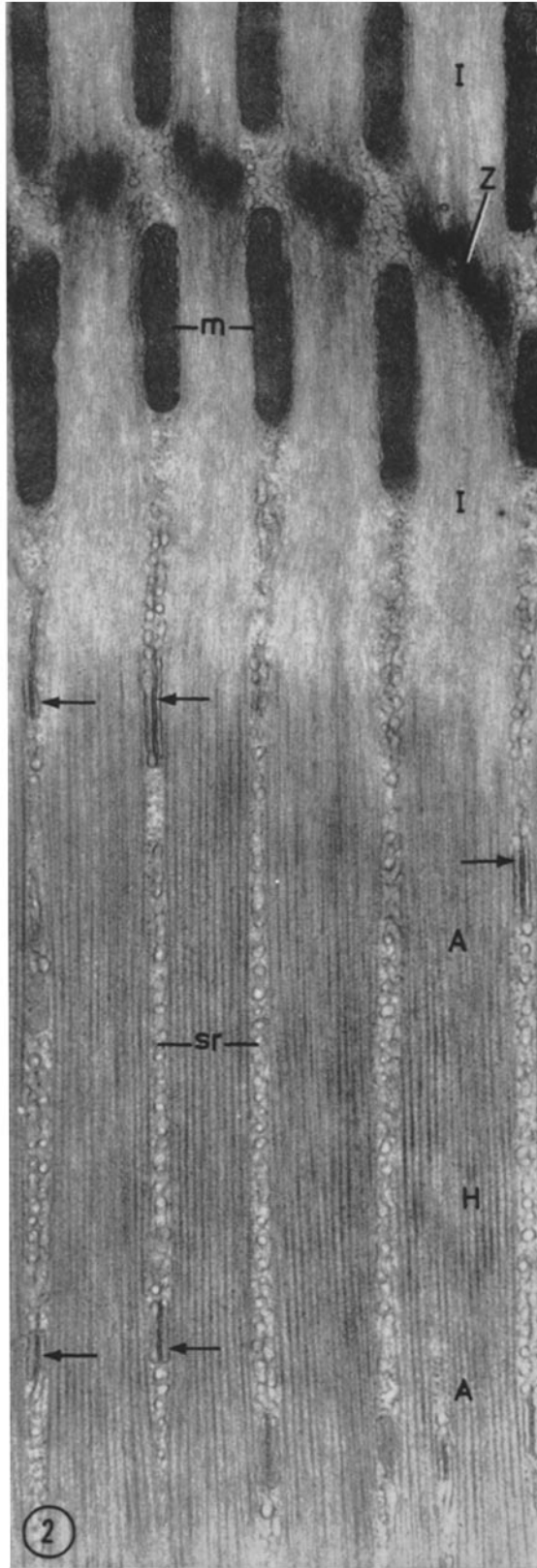
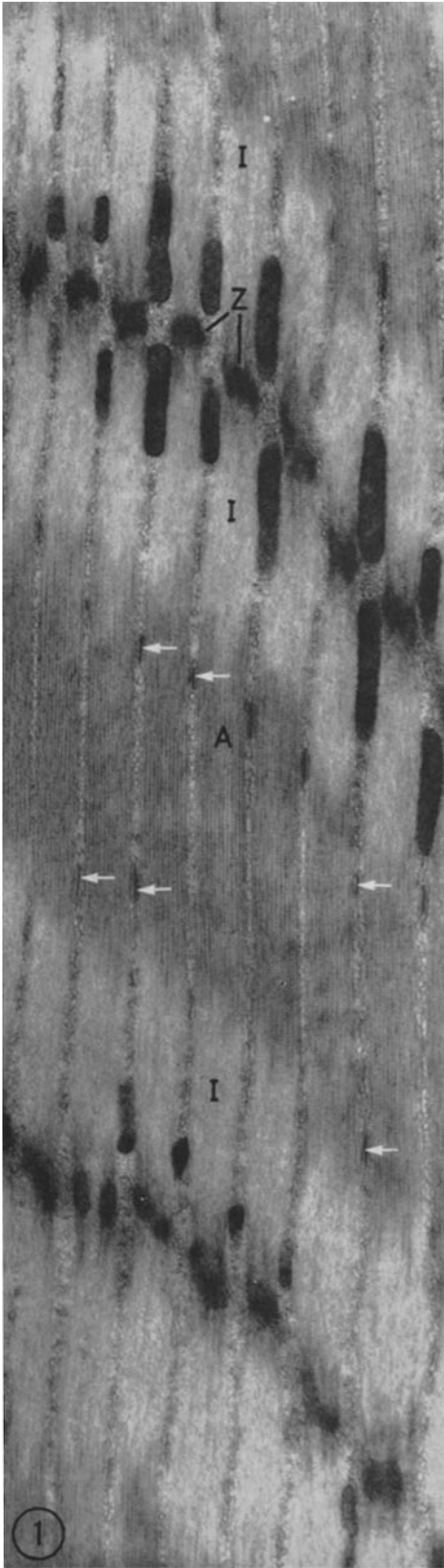
muscles. In these sections, the presumably relaxed sarcomere length is ca. 7.8 to 8.3 μ, a value similar to that observed in insect visceral fibers. In these profiles, the I and A bands are respectively ca. 1.8 to 2.0 μ and ca. 3.6 to 4.1 μ in length; sarcomeres ca. 6 μ in length, exhibiting reduced I bands, apparently fixed during contraction, have been observed. The alignment of the Z bands across the fiber is more precise than in insect visceral muscles (Smith et al., 1966), but falls short of the precise register attained by insect flight muscles. H bands are generally poorly defined in the longitudinal plane of section but, as will be described later, are clearly evident in transverse transects of the sarcomere.

The distribution of mitochondria within the intersegmental muscle fiber is strikingly different from that encountered in flight muscle fibers. In the latter, the mitochondria may be precisely oriented with respect to the sarcomere divisions (Smith, 1962) and occupy ca. 40% of the fiber volume in both synchronous and asynchronous muscles; in intersegmental muscles, the mitochondria are generally confined to the I band level and account for a far smaller proportion of the fiber volume. This reduced level of mitochondrial content presumably reflects the mechanical role of the intersegmental muscles, which are not called upon to produce rapid phasic contractions and whose main role in the adult appears to be concerned with small abdominal movements.

The fibrillar division of the muscle is illustrated in transverse section in Figs. 3, 4, and 6. The contractile material is disposed in radial lamellae, incompletely divided by cisternae of the sarcoplasmic reticulum into polygonal fibrils. In this plane of section, also, the accumulation of mitochondria straddling the Z band and the disposition

FIGURE 1 Low power longitudinal section of *Periplaneta* abdominal intersegmental muscle. Note the sarcomere striations (Z, I, A) of the fibrils, and the presence of dyad associations between T system tubules and sarcoplasmic reticulum cisternae (arrows) just inside the A band levels. × 14,000.

FIGURE 2 A field similar to Fig. 1, at higher magnification. Note the dense Z bands, flanked by extensive I bands, and the A bands exhibiting a median H zone, more clearly seen in transverse section (cf. Fig. 4) (Z, I, A, H). The mitochondria (m) in this muscle are predominantly located within the I band level. Note the dyads (arrows) situated inside the A-I junction level, and the profiles of the sarcoplasmic reticulum (sr) virtually filling the interfibrillar sarcoplasm. × 28,000.



of dyads inside the A-I junction are evident (Fig. 3). The relationship between the tubules of the T system, the cell membrane, and the sarcoplasmic reticulum cisternae is made clear by transversely sectioned peripheral fields (Figs. 4 and 5). The topography of the T system tubules and the cisternae of the sarcoplasmic reticulum conforms to the pattern described in synchronous flight muscles and other skeletal muscles of insects. As has been shown in fish muscle (Franzini-Armstrong and Porter, 1964) and dragonfly muscle (Smith, 1966 *a*), the T system tubules represent radially oriented open-mouthed invaginations of the surface membrane; in this instance the lumen is ca. 150 to 200 Å in width. These are flanked, across a gap of ca. 100 Å, by specialized cisternae of the sarcoplasmic reticulum (Fig. 5), in which the membrane facing the T system surface bears periodic thickenings which traverse the dyad gap at intervals of ca. 250 to 300 Å. A similar morphology has been described in insect leg and flight muscle (Hoyle, 1965; Smith, 1966 *a*), and is reminiscent of the "foot processes" borne on the terminal cisternae of the triad in bat cricothyroid muscle (Revel, 1962). In these intersegmental muscles the maximum T system-fibril distance is ca. 2.5 μ .

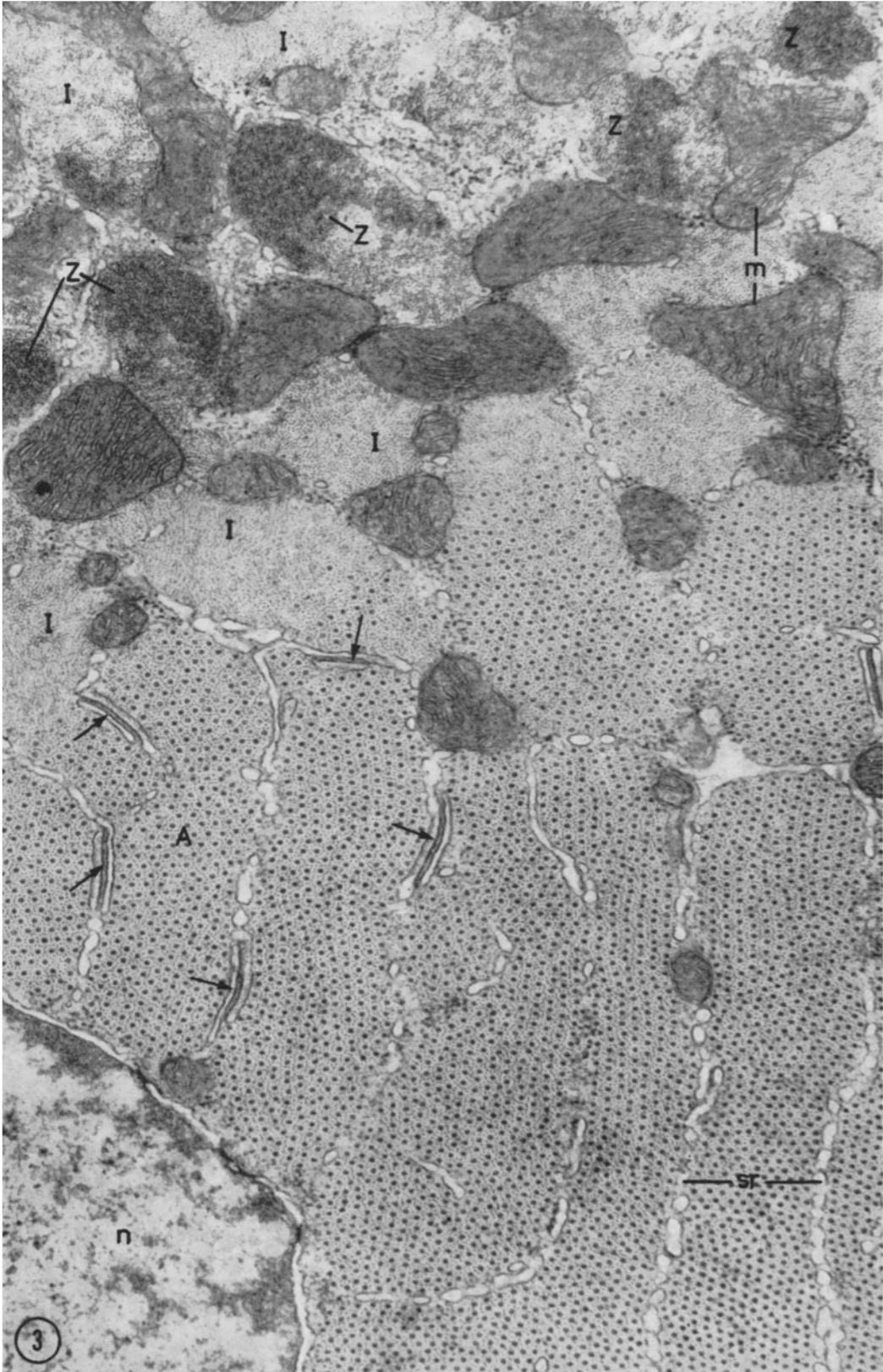
As has been mentioned previously, longitudinal sections of intersegmental muscle reveal a striation pattern similar to that of vertebrate striated muscle, and a common basis for this striation, in terms of disposition of thick and thin myofilaments, is demonstrated in transverse profiles passing through successive sarcomere divisions (Figs. 3 and 4). Whereas in longitudinal sections the H bands are generally indistinct, the presence of thick filament arrays from which thin filaments are excluded are well distinguished in the transverse plane of section. Of particular interest is the organization of the myofilament array in the A band region in these fibers (Fig. 7). In place of the

familiar pattern in vertebrate muscle or insect flight muscles, in which in this region of overlap the actin filaments are shared respectively by three and two myosin filaments, in the present case the thick filaments are surrounded by orbitals of more than six thin filaments. In regions of a section which appear to present the best orientation, twelve-membered thin filament orbitals occur, though elsewhere the array may be either intrinsically less precise, or less well preserved. This configuration appears to be identical with that described in insect visceral muscles (Smith et al., 1966), and in each instance, it is equivalent to a doubling of each thin filament in the flight muscle array, in which each pair of thin filaments is shared by two thick filaments. It must be stressed that at present no information on the topography of interfilament cross-bridging is available. In this glutaraldehyde-fixed material, the diameter of the thick and thin filaments is respectively ca. 160 to 180 Å and ca. 40 to 50 Å, with the center-to-center spacing of the thick filament array being ca. 410 to 430 Å. These values are in general agreement with those observed in similarly prepared insect visceral muscle (Smith et al., 1966). Franzini-Armstrong and Porter (1964) also noted an increase in the diameter of myosin filaments in glutaraldehyde-fixed fish muscle, to 150 Å, considerably greater than the value of 110 Å generally obtained in osmium tetroxide-fixed preparations of vertebrate muscle.

DISCUSSION

The architecture of intersegmental muscles of the cockroach, described here, adds a further facet to our knowledge of the comparative morphology of insect muscles. This muscle exhibits the myofilament array that occurs in visceral muscles of insects and perhaps in other invertebrate fibers, but is nevertheless equipped with well developed T system and sarcoplasmic reticulum membranes,

FIGURE 3 Low power micrograph of the transversely sectioned *Periplaneta* abdominal intersegmental muscle. Note the A and I band profiles (*A*, *I*) and the blocks of dense Z material (*Z*). Further details of the myofilament array in this plane of section are shown in Fig. 6. Mitochondria (*m*) are located mainly at the level of the I band (cf. Figs. 1 and 2). The fibrils are defined by cisternae of the sarcoplasmic reticulum (*sr*) and several dyads are included (arrows), located inside the limit of the A band (cf. Fig. 2). A portion of a nucleus appears at the lower left (*n*). $\times 40,000$.



both of which are reduced in insect visceral fibers. In most skeletal muscle fibers of vertebrates and in synchronous flight muscle fibers of insects, the transverse tubules are extensive, regularly disposed with respect to the myofibrillar striations, and intimately associated (via dyads or triads) with specialized regions of the cisternal reticulum. Much evidence favors the implication of the latter in the control of myofibrillar activation and deactivation, through cyclic exchange of calcium ions between the cavities of the sarcoplasmic reticulum and the fibrils, occurring in phase with the train of excitatory signals distributed, via the cell membrane, along the open-mouthed T system tubules, and transferred to the reticulum across the dyad or triad gap. This subject has been discussed, in the context of vertebrate skeletal muscle fibers, by Porter (1961), Ebashi (1961), Huxley (1964), Hasselbach (1964) and Peachey (1965). The effectiveness of a "relaxing factor" fraction with calcium-binding properties, isolated from locust synchronous muscle (Tsukamoto et al., 1965), on both mammalian and insect myofibrillar preparations, together with the comparable membrane distribution in these muscles, points to a common mode of activation control in synchronously responding skeletal muscle fibers.

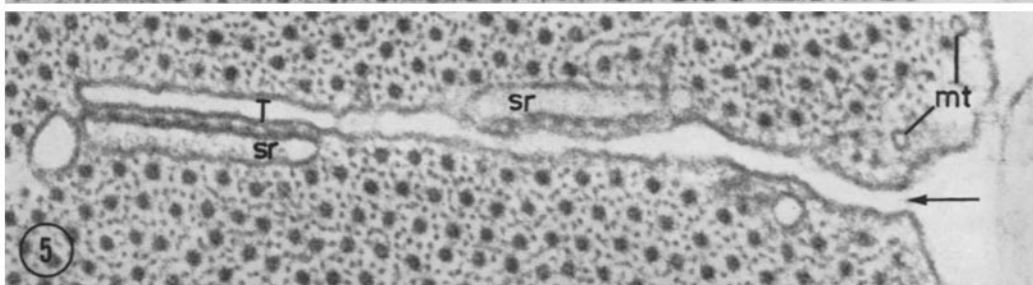
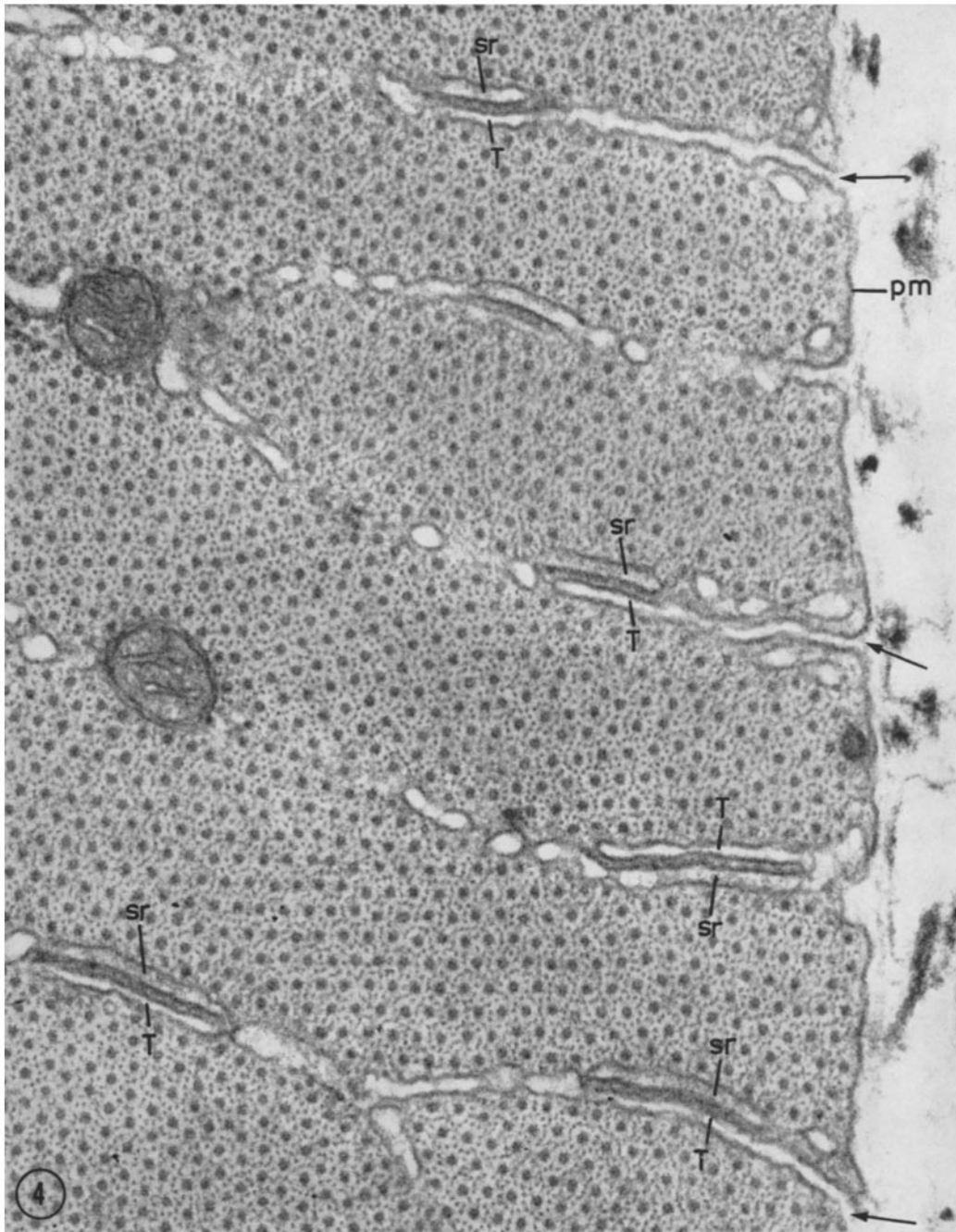
In asynchronous flight muscle of insects, in which the fiber diameter is generally large and in which length changes occur very rapidly (Pringle, 1965; Smith, 1966 *b*), the T system is extensive but is associated with a much reduced sarcoplasmic reticulum. Here, it seems probable that, while the onset of contraction may occur normally via T system excitation, subsequent myogenic length changes may not be coordinated with syn-

chronized cyclic supply of calcium ions to the myofibrils (cf. Pringle, 1965). On the other hand, insect visceral muscle fibers have a very small diameter (less than that of most vertebrate smooth muscle fibers) and exhibit great reduction of the transverse tubular system and the sarcoplasmic reticulum. These muscle fibers effect slow peristaltic contractions comparable with those of mammalian visceral muscle fibers, in which lack of internal membranes appears to be correlated with direct activation of the contractile system via calcium exchange taking place directly across the peripheral cell membrane (cf. Peachey and Porter, 1959; Hasselbach, 1964). It is possible (Smith et al., 1966) that insect visceral muscles reflect an intermediate condition in which both a reduced intracellular compartment and the cell membrane may be involved in calcium movements.

The present observations on intersegmental muscles demonstrate that an increase in the actin:myosin ratio of the contractile system is not a special feature, in insects, of the small-fiber visceral muscles. The degree of development and alignment of the internal membranes of the intersegmental muscles, on the basis of the above discussion, is consistent with their greater fiber diameter and synchronous contractile response. The functional importance of the filament array in these fibers must be sought elsewhere, probably at the level of the mechanical role played by the muscle. In this connection, it is interesting to note that while vertebrate skeletal muscles and insect flight muscles display precise orbitals of six actin filaments around each myosin filament in the overlap regions of the sarcomere, orbitals of more than six thin filaments have been reported in the

FIGURE 4 In this micrograph the arrows indicate the derivation of T system tubules (*T*) in intersegmental muscle as invaginations of the plasma membrane (*pm*). The T system tubules are closely associated with flattened cisternae of the sarcoplasmic reticulum (*sr*). $\times 83,000$.

FIGURE 5 A similarly labeled field, further illustrating the relationship between the T system tubules and the sarcoplasmic reticulum. Note the "clear" lumen of the T system invaginations (*T*) and the presence of electron-opaque material in the cisternae of the sarcoplasmic reticulum (*sr*) involved in the dyads; also the regular thickenings along the surface of the sarcoplasmic reticulum membrane adjoining the invaginated cell membrane surface (cf. Smith, 1966 *a*). In this muscle, peripherally located microtubules (*mt*) are present in small numbers and are oriented parallel with the fiber long axis. $\times 98,000$.



muscles of widely diverse animal phyla. In unstriated pharyngeal muscle fibers of a planarian, MacRae (1963) noted that the single fibril of each cell appears to contain generally randomly disposed thin filaments which are occasionally organized into orbitals of ten to twelve around the thick filaments. Rosenbluth (1965) described ten to twelve-membered orbitals within the A region in the unusual striated somatic muscle of the nematode *Ascaris*, and it seems possible to discern in these micrographs the thin filament pattern illustrated here in insect intersegmental muscle. In skeletal muscle fibers of the crayfish *Orconectes*, Swan (1963) found that the thick filaments are associated with groups of nine to twelve thin filaments, and, from the presence around the former of hexagonally arranged "dense structures," Swan suggested that each thick filament may interact with its own set of six thin filaments instead of sharing these, as in vertebrate skeletal and insect flight muscle fibers. The present results cannot contribute evidence relating to this interesting suggestion, and it must be stressed that the possibility that in insect intersegmental muscle fibers each pair of thin filaments is shared by adjoining pairs of thick filaments is prompted by analogy with insect flight muscles, rather than by observation of interfilament cross-linkages.

It has been pointed out that in *Periplaneta* intersegmental muscle fibers the regular twelve-membered orbital is most apparent in regions

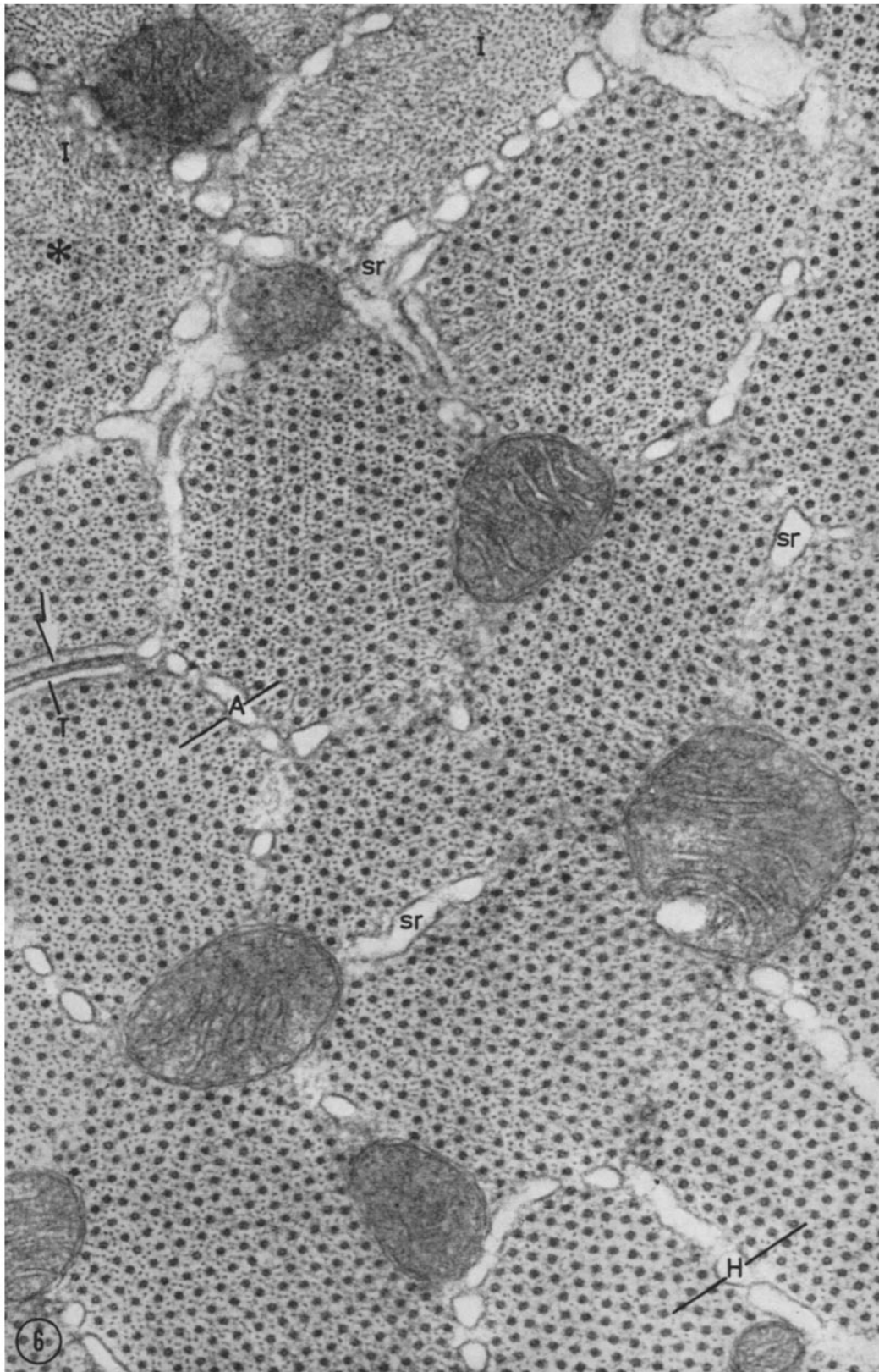
where the hexagonal thick filament disposition is most evident. Here, and in the invertebrate fibers mentioned above, one is faced with the problem of whether a fiber is intrinsically variable or undetermined in its actin:myosin ratio, or whether the mode of preparation introduces some disorder.

Recently, many electron microscope studies have been concerned primarily with the disposition of the membrane systems involved in activation and the control of the contraction-relaxation cycle. Sufficient evidence is now available suggesting that muscle fibers display significant inter- and intraphyletic variation in myofibrillar topography, uncorrelated with distribution of T system and sarcoplasmic reticulum membranes. More rigorous examination of the contractile system of phyletically diverse muscles would not only contribute to our knowledge of the evolution of muscle tissue, but also might throw some light on a somewhat neglected problem—the possible relationship between myofibrillar disposition of a muscle and its mechanical role in the animal body.

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FIGURE 6 Transversely sectioned intersegmental muscle, further illustrating the filament disposition in the various sarcomere regions. The plane of the section passes through an I band (*I*) (at upper left) containing thin filaments, and the appearance of thick filaments (*) delimits the edge of the A band (*A*). A band profiles occupy much of the field, but the section includes H band profiles (*H*) at lower right, characterized by the thick filament array. The fibrils are incompletely demarcated by tubules of the sarcoplasmic reticulum (*sr*), and a dyad profile, comprising a T system tubule (*T*) and accompanying reticulum element (arrow), is included. $\times 80,000$.



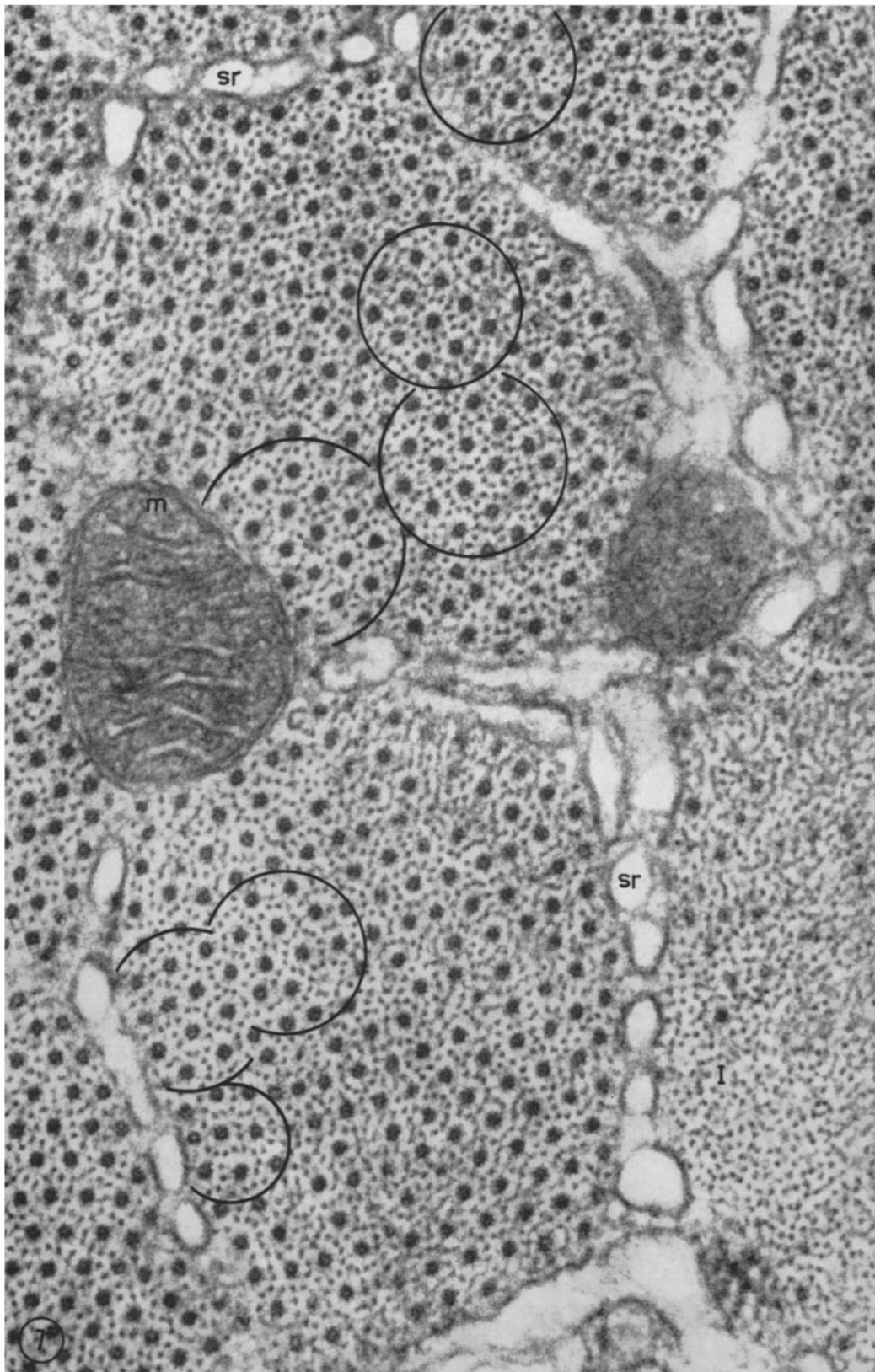


FIGURE 7 Electron micrograph illustrating the configuration of thick and thin filaments within the A band in *Periplaneta* intersegmental muscle. Areas in which the twelve-membered orbitals of thin filaments are well defined are encircled. Note the mitochondria (*m*) and cisternae of the sarcoplasmic reticulum (*sr*) between the polygonal fibrils. At the lower right, the plane of section passes through an I band (*I*), in which thin filaments are alone present. $\times 136,000$.

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