A COMPARATIVE STUDY ON THE FINE STRUCTURE OF THE BASALAR MUSCLE OF THE WING AND THE TIBIAL EXTENSOR MUSCLE OF THE LEG OF THE LEPIDOPTERAN *ACHALARUS LYCIADES*

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ABSTRACT

Basalar and tibial extensor muscle fibers of *Achalarus lyciades* were examined with light and electron microscopes. Basalar muscle fibers are 100–150 μ in diameter. T-system membranes and sarcoplasmic reticulum make triadic contacts midway between Z lines and the middle of each sarcomere. The sarcoplasmic reticulum is characterized by a transverse element situated among myofilaments halfway between Z lines in every sarcomere. The morphology of Z lines, hexagonal packing of thin and thick myofilaments, and thin/thick myofilament ratios are similar to those of fast-acting insect muscles. Tibial extensor muscle fibers are 50–100 μ in diameter. Except for a lack of the transverse element, the T system and sarcoplasmic reticulum are similar to those of basalar muscle. Wavy Z lines, lack of a hexagonal packing of myofilaments, and larger thin/thick myofilament ratios are similar to those of other postural muscles of insects. The morphology of basalar and tibial extensor muscle is compared to that of other insect muscle with known functions, and reference is made to the possible contribution of the transverse element of sarcoplasmic reticulum in basalar flight muscle to speed and synchrony in this muscle.

INTRODUCTION

Morphological, physiological, and biochemical characteristics which distinguish wing and leg muscles of insects are known for only a few insects (Chadwick, 1953 *a,b*; Prosser and Brown, 1961; Hughes, 1965; Pringle, 1965; Hoyle, 1965; Maruyama, 1965; Sacktor, 1965). All insect locomotor muscles are capable of a wide range of contraction speeds, but wing and leg muscles are usually markedly different in terms of speed and duration of sustained activity (Hughes, 1965; Pringle, 1965). The most striking example is found in insects from the orders Ephemeroptera, Thysanoptera, Coleoptera, Hymenoptera, Hemiptera, and Diptera (Pringle, 1965), in which asynchronous flight muscle is much faster than the corresponding leg muscle of the same species (Hughes, 1965). Only a few fine structure studies have been made comparing wing and leg muscle from a single species. Studies have been made on the cockroach, *Periplaneta americana* (Edwards et al., 1956), the water bug, *Belostoma* sp. (Edwards et al., 1956), and the locust, *Locusta migratoria* (Vogell et al., 1959).

In a preliminary report (Reger, 1967) an unusual organization of sarcoplasmic reticulum in basalar wing muscle of the skipper, *Achalarus lyciades*, was described wherein repeating, transverse elements of sarcoplasmic reticulum were seen among myofilaments half-way between Z lines in every sarcomere. Such transverse elements

have since been seen in wing muscles of another skipper, *Erynnis martialis*, and two moths, *Agrotis* sp. and *Thyridopteryx ephemeraeformis*.[1] Since the leg muscles of these species and the tibial extensor muscle of *Achalarus lyciades* lack this transverse element, and since this morphology of sarcoplasmic reticulum has not been described in other muscles, it seemed desirable to compare all of the fine structure characteristics which distinguish a fast-acting wing from a postural leg muscle in this animal.

MATERIAL AND METHODS

The basalar muscle and the tibial extensor muscle of living *Achalarus lyciades* were exposed by incisions through the thoracic and limb cuticles, immersed in cold (0–5°C) 4% glutaraldehyde (pH 7.6; 0.1 M phosphate buffer), and cut into small pieces. The resulting pieces were immediately immersed in fresh, cold (0.5°C), buffered, 4% glutaraldehyde (pH 7.6; 0.1 M phosphate buffer) containing 1.5 mg of $CaCl_2$ per 5 ml of fixative, and fixed for 1 hr. After fixation in glutaraldehyde the tissue was immediately washed for 1 hr in a solution containing the above buffering system and $CaCl_2$ and was subsequently postfixed in cold (0–5°C), buffered OsO_4 (pH 7.6; 0.1 M phosphate buffer) containing 1.5 mg of $CaCl_2$ per 5 ml of fixative. After fixation the tissue was rinsed for 5 min in buffer, dehydrated in 5-min changes of successive 10% grades of methanol (beginning with 50%), and embedded in Epon 812). Sections 1–2 μ thick were made of the Epon-embedded tissue and were stained with Mallory azure II-methylene blue (P. R. Mallory & Co., Inc., Indianapolis, Ind.) for purposes of orientation before thin sectioning.

Thin sections were cut with a diamond knife fitted to an LKB Ultratome, floated on distilled water, mounted on carbon-coated grids, stained with uranyl acetate and lead citrate, and examined with an RCA 3F or a Hitachii 11A electron microscope. Micrographs, at initial magnifications of 7500–40,000 at exposures of 3–5 sec, were made on Cronar, Ortholitho, Type A sheet-film (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.) and were photographically enlarged up to seven times.

[1] Reger, J. F. 1966. Uupublished observations.

OBSERVATIONS

Basalar flight and tibial extensor muscle fibers of *Achalarus lyciades* show certain structural similarities and differences (Figs. 1–4). Similarities include myofibrillar diameters and sarcomere lengths (Figs. 1, 2). Differences include diameter of muscle fibers, amount of tracheolar penetration within muscle fibers (Figs. 3, 4), size and distribution of mitochondria (Figs. 3, 4), morphology of Z lines (*Z*, Figs. 1, 2), relative number of triads and dyads (Figs. 5, 6), and geometrical organization of myofilaments (Figs. 10–12).

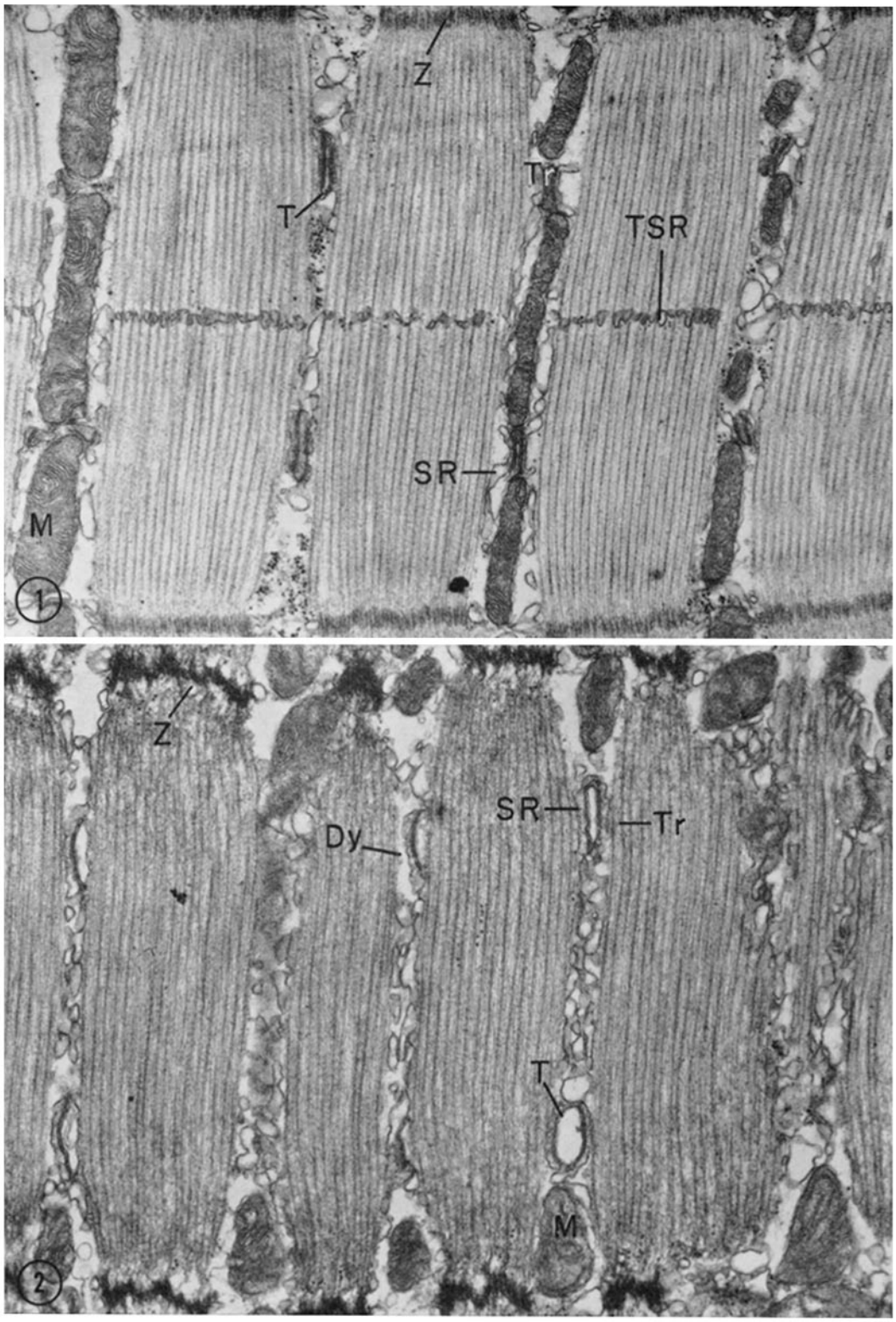
Muscle fibers in basalar flight muscle are 100–150 μ in diameter, whereas muscle fibers in tibial extensor muscle are 50–100 μ in diameter. The diameters and sarcomere lengths of myofibrillae in both muscles (Figs. 1, 2) are 1.0–1.5 and 3–4 μ, respectively. The amount of tracheolar penetration within muscle fibers is slightly greater in basalar flight muscle (Figs. 3, 4).

Mitochondria (*M*, Figs. 1, 3) contain a large number of cristae (*M*, Fig. 3) and are located the full myofibrillar length of muscle fibers (*M*, Figs. 1, 3, 9, 10) in basalar flight muscle. Mitochondria in tibial extensor muscle fibers (*M*, Figs. 2, 6), on the other hand, are usually located only on either side of Z lines (*M*, Figs. 2, 6).

Membranes of the T system (*T*, Figs. 1, 2) and cisternae of the sarcoplasmic reticulum (*SR*, Figs. 1, 2) are in contact principally at triadic junctions (*Tr*, Figs. 1, 5) in basalar muscle and principally at dyadic junctions (*Tr*, *Dy*, Figs. 2, 6) in tibial extensor muscle. Such junctions lie midway between Z lines and the middle of each sarcomere in both basalar and tibial extensor muscles. At regions of triadic and dyadic contact infolded plasma

FIGURE 1 Basalar flight muscle. This figure shows portions of five myofibrils in longitudinal section. Z lines (*Z*), T-system membranes (*T*) and associated sarcoplasmic reticulum cisternae (*SR*) at triadic (*Tr*) junctions may also be seen. Repeating, transverse elements of sarcoplasmic reticulum (*TSR*) are present in the middle of each sarcomere. *M*, mitochondrion. × 26,000.

FIGURE 2 Tibial extensor muscle. This figure shows portions of six myofibrils in longitudinal section. Z-lines (*Z*), T-system membranes (*T*), and associated sarcoplasmic reticulum cisternae (*SR*) at dyadic (*Dy*) and triadic (*Tr*) contacts may be seen. *M*, mitochondrion. × 26,000.


Z
Tr
T
TSR
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M
1
Z
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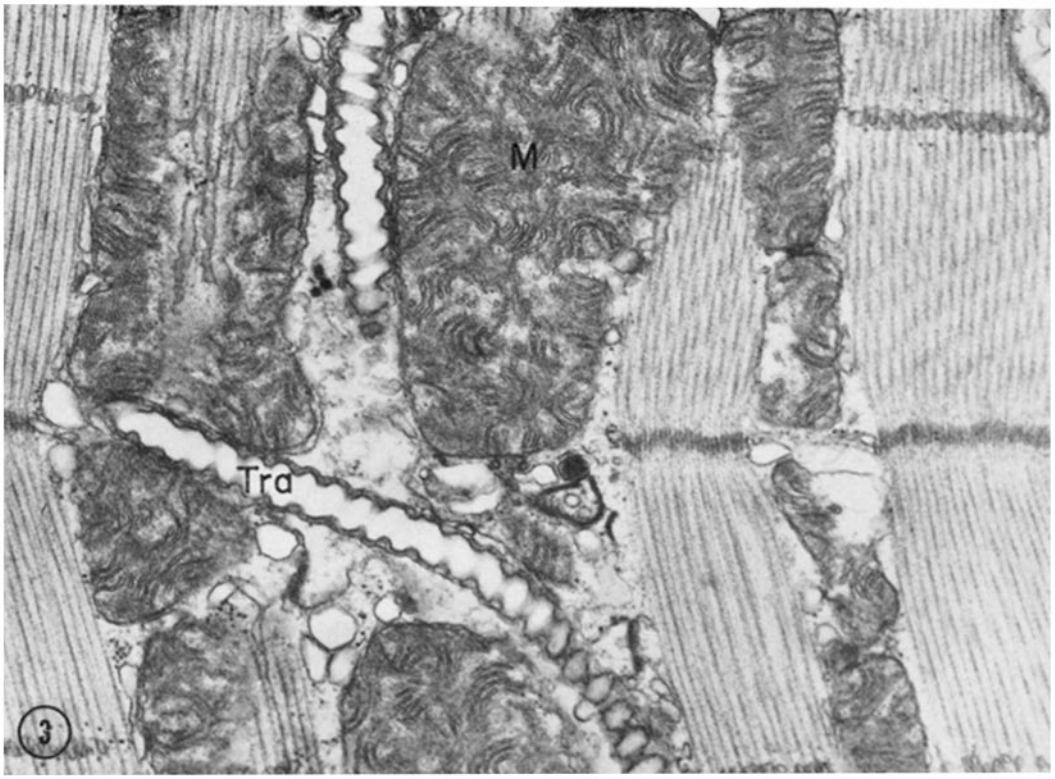


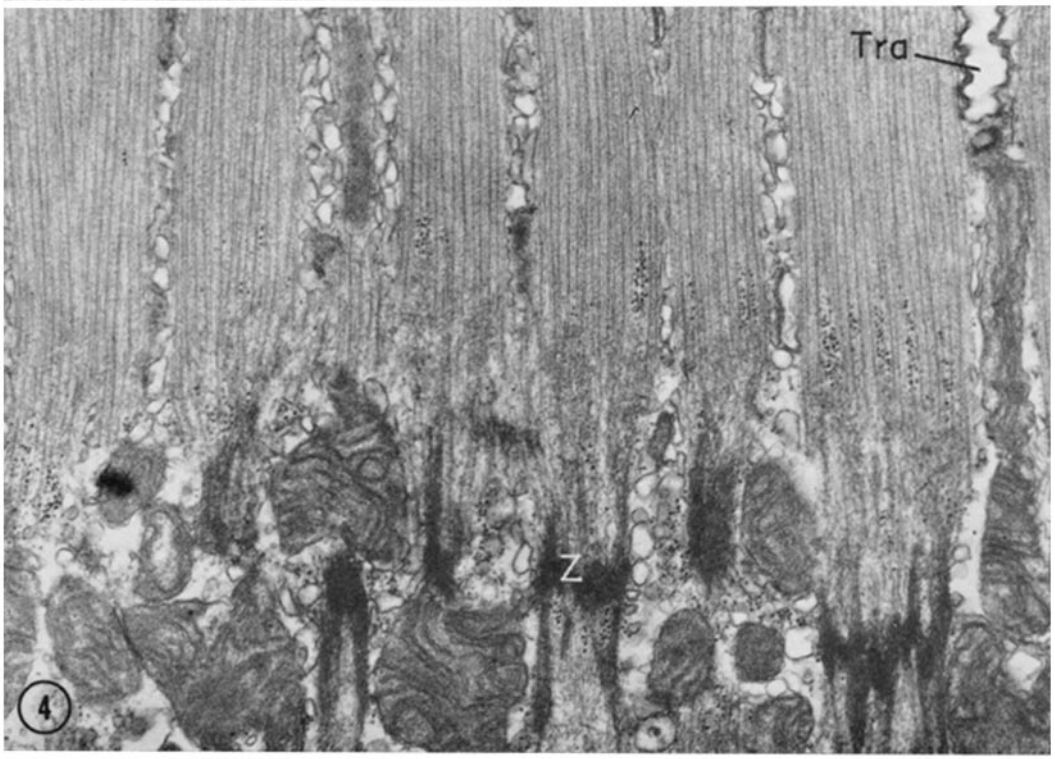


FIGURE 3 Basalar flight muscle. This figure shows a section oblique to the long axis of myofibrils cut so that profiles of mitochondria (*M*) with abundant cristae are evident. *Tra*, tracheole. × 18,500.

FIGURE 4 Tibial extensor muscle. This figure shows sinuous Z lines (*Z*) typical of the tibial extensor muscle. *Tra*, portion of tracheole. × 26,000.

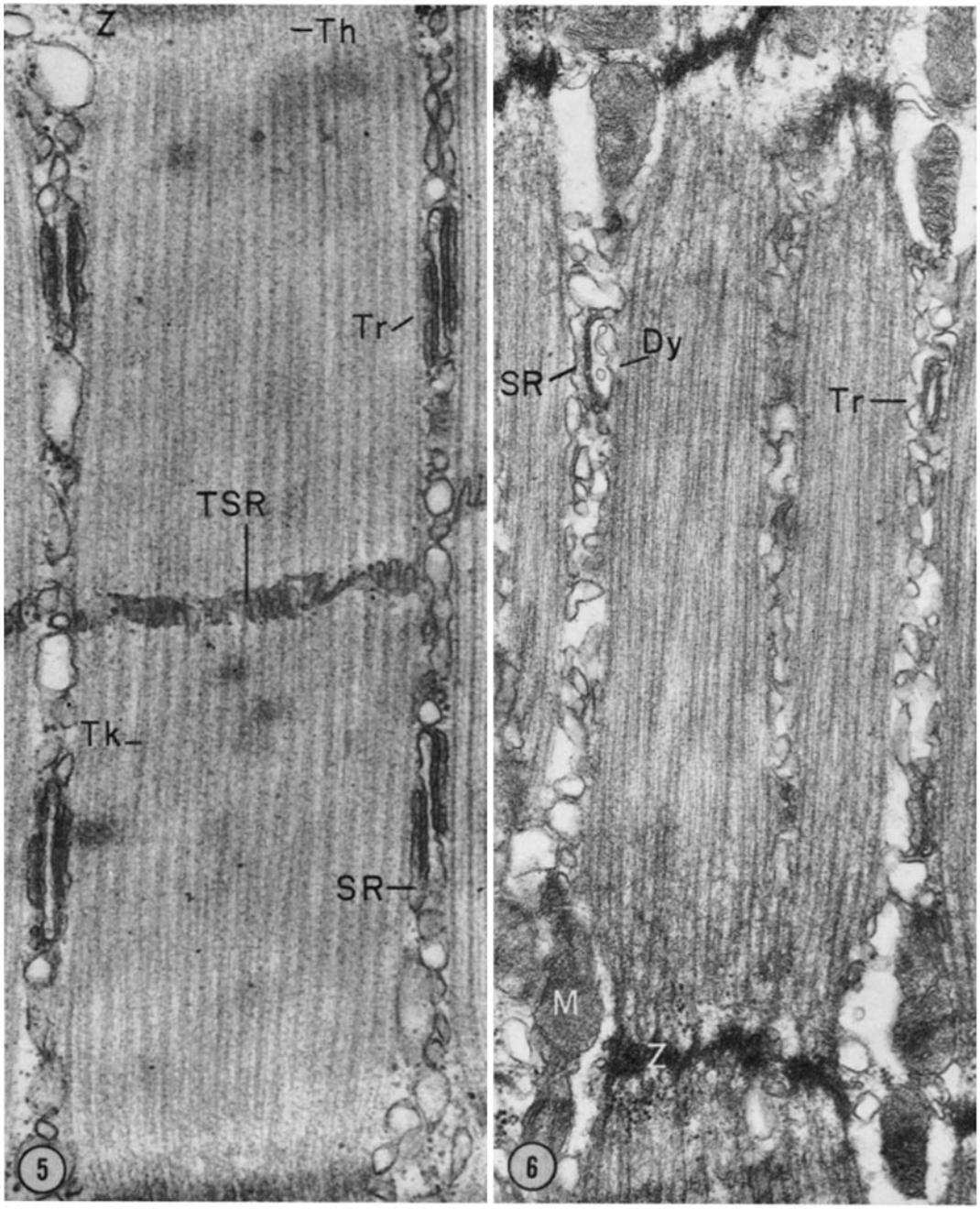


FIGURE 5 Basalar flight muscle. This figure shows one sarcomere. Triads (*Tr*), sarcoplasmic reticulum cisternae (*SR*), and transverse elements of sarcoplasmic reticulum (*TSR*) are also evident. *Th*, thin filaments. *Tk*, thick filaments. *Z*, Z lines. × 56,500.

FIGURE 6 Tibial extensor muscle. This figure shows sarcoplasmic reticulum cisternae (*SR*) in dyadic (*Dy*) and triadic (*Tr*) contact with T-system membranes. Mitochondria (*M*) are seen on either side of Z lines (*Z*). Notice that thin filaments appear to extend from Z lines in bundles rather than in the highly ordered array typical of basalar flight muscle (See Fig. 5). × 42,000.

membranes (*T*, Fig. 12) composing the T system are 400–500 A apart and are in juxtaposition with sarcoplasmic reticulum cisternae for approximate lengths of 0.5–0.6 μ. The amount of longitudinally disposed sarcoplasmic reticulum (*SR*, Figs. 5, 6) situated between myofibrillae is similar in both muscles. However, the sarcoplasmic reticulum in basalar flight muscle (Figs. 1, 5, 7–10) is characterized by a transversely oriented element situated among myofilaments in the center of each sarcomere (*TSR*, Figs. 7–10). Continuity between the transverse elements (arrows, Fig. 8), from one myofibril to the next, is frequently seen. Continuity between longitudinal (*LSR*, Figs. 7, 9) and transverse (*TSR*, Figs. 7, 9) sarcoplasmic reticulum is also frequently seen. When myofibrils are viewed in transverse section, the transverse elements of sarcoplasmic reticulum (*TSR*, Fig. 10) appear as a network across the myofibril. Membranes of the sarcoplasmic reticulum (*TSR*, Fig. 10) are here only 80–120 A (Fig. 10) from thick myofilaments in the middle of each sarcomere. The thick myofilaments in this region, when examined at high magnification (arrows, Fig. 11), are seen to be composed of two subunits, each 60–80 A in diameter.

Z lines, in both basalar flight (*Z*, Fig. 1) and tibial extensor (*Z*, Fig. 2) myofibrils, are in register from one myofibril to the next. However, Z lines of basalar flight myofibrils are straight across the myofibril (*Z*, Figs. 1, 5), whereas *Z* lines in tibial extensor muscle are sinuous (*Z*, Figs. 2, 4, 6). Thin myofilaments which extend from Z lines in basalar flight muscle (*Th*, Fig. 5) are in parallel array, whereas those in tibial extensor muscle (Figs. 4, 6) are not.

The degree of ordering and type of packing of thin and thick myofilaments in basalar flight and tibial extensor muscles are distinctly different (Figs. 5, 6 and Figs. 10–12). Typical of other fast-acting insect flight muscles, both thin and thick myofilaments in basalar flight myofibrils (Fig. 10) are packed in hexagonal patterns, and each thick myofilament is surrounded by six thin myofilaments (*Th*, Fig. 10). On the other hand, thin and thick myofilaments in tibial extensor myofibrils (Fig. 12) are not seen in hexagonal array, and each thick myofilament is surrounded by 10–12 thin myofilaments, as in many other slow-acting invertebrate muscles.

DISCUSSION

The foregoing results which show differences in the fine structure of basalar and tibial extensor muscle fibers of the skipper, *Achalarus lyciades*, may aid in explaining functional differences between the two muscles. Much evidence already exists relating the amount of tracheolar penetration and the number of mitochondria directly to insect muscle speeds (Pringle, 1965). Therefore, the following will be limited to a discussion of the organization and disposition of myofilaments, T-system membranes, and sarcoplasmic reticulum, since more recent evidence indicates their importance in contributing to different functional properties of muscle.

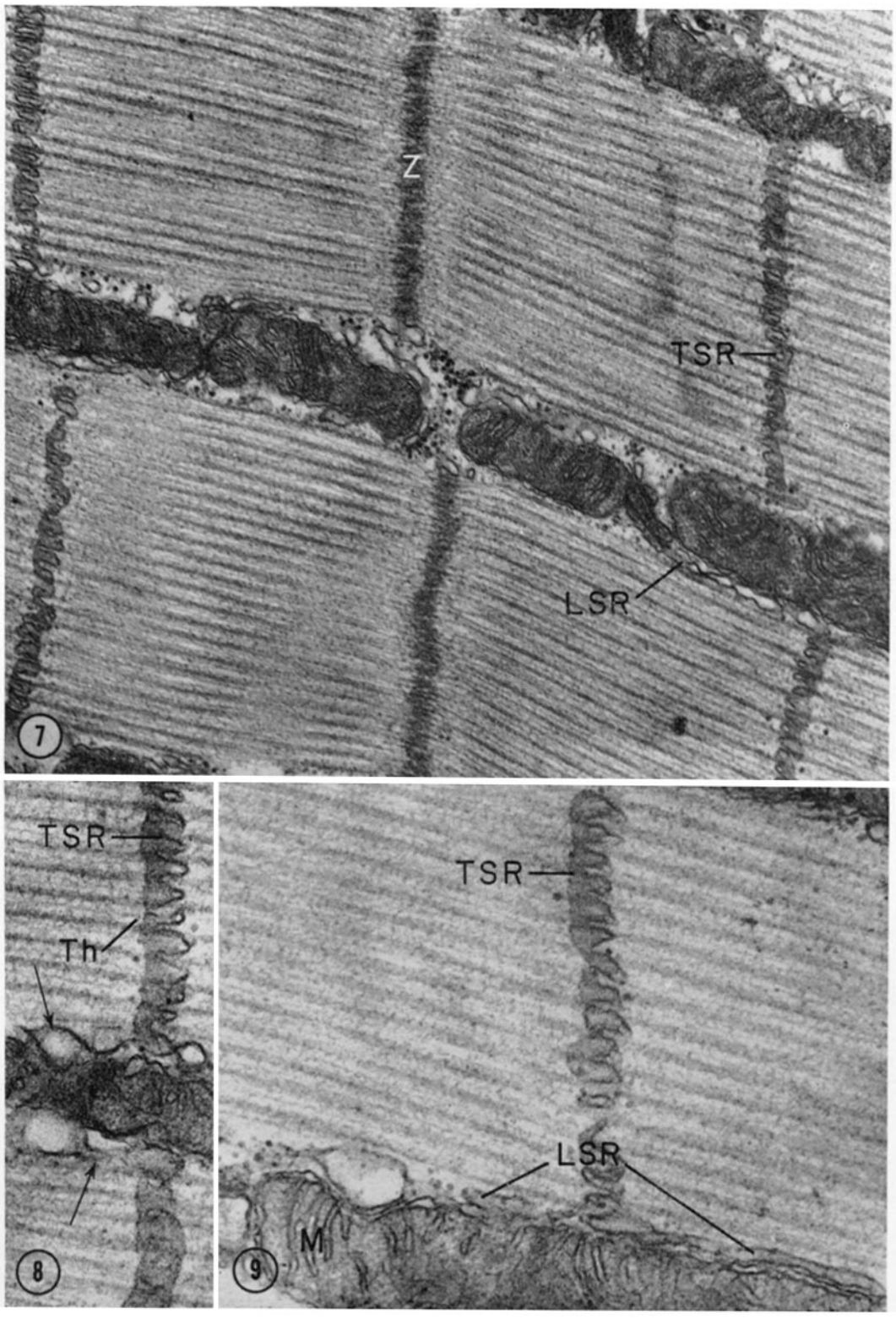
Myofilament Organization

The significance of the disposition and organization of myofilaments in insect muscle with different functional characteristics is becoming increasingly clear (Auber and Couteaux, 1963; Smith, 1966 *b*; Smith et al., 1966). Structural appearance of Z lines, disposition and packing of thin and thick myofilaments, and thin/thick myofilament ratios in basalar flight muscle are similar to those re-

FIGURE 7 Basalar flight muscle. This micrograph shows the typical transverse alignment and repeat order of Z lines (*Z*) and transverse elements (*TSR*) of sarcoplasmic, reticulum. Notice the continuity of longitudinal (*LSR*) with transverse (*TSR*) elements of sarcoplasmic reticulum. × 42,000.

FIGURE 8 Basalar flight muscle. This figure shows the continuity of transverse elements of sarcoplasmic reticulum (*TSR*) from one myofibril to the next (arrows), and the thin myofilaments (*Th*) as they terminate near transverse sarcoplasmic reticulum. × 56,500.

FIGURE 9 Basalar flight muscle. This figure shows longitudinal elements of sarcoplasmic reticulum (*LSR*) as they extend from transverse elements (*TSR*). *M*, mitochondrion. × 56,500.


Z
TSR
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7
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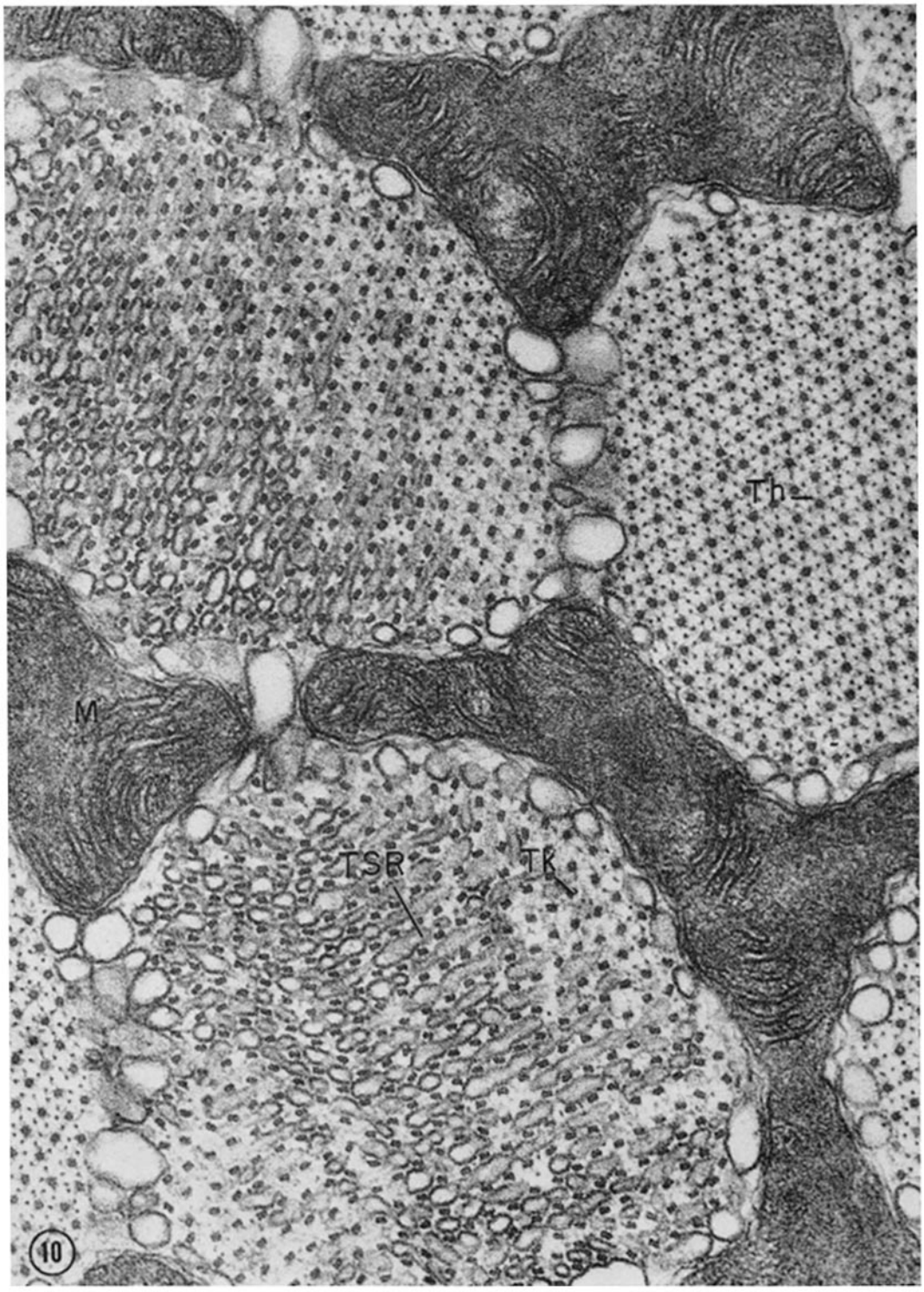

FIGURE 10 Basalar flight muscle. This electron micrograph shows a transverse section through portions of six myofibrils and surrounding mitochondria. Sections at the middle of sarcomeres show transverse elements of sarcoplasmic reticulum (*TSR*) as an extensive network which penetrates between rows of thick myofilaments (*Tk*). Thick myofilaments, when examined at higher magnification (Fig. 11), are seen to be composed of two subunits, each 60–80 A in diameter. Also notice the hexagonal packing of the thick and thin (*Th*) myofilaments. *M*, mitochondrion. × 79,500.

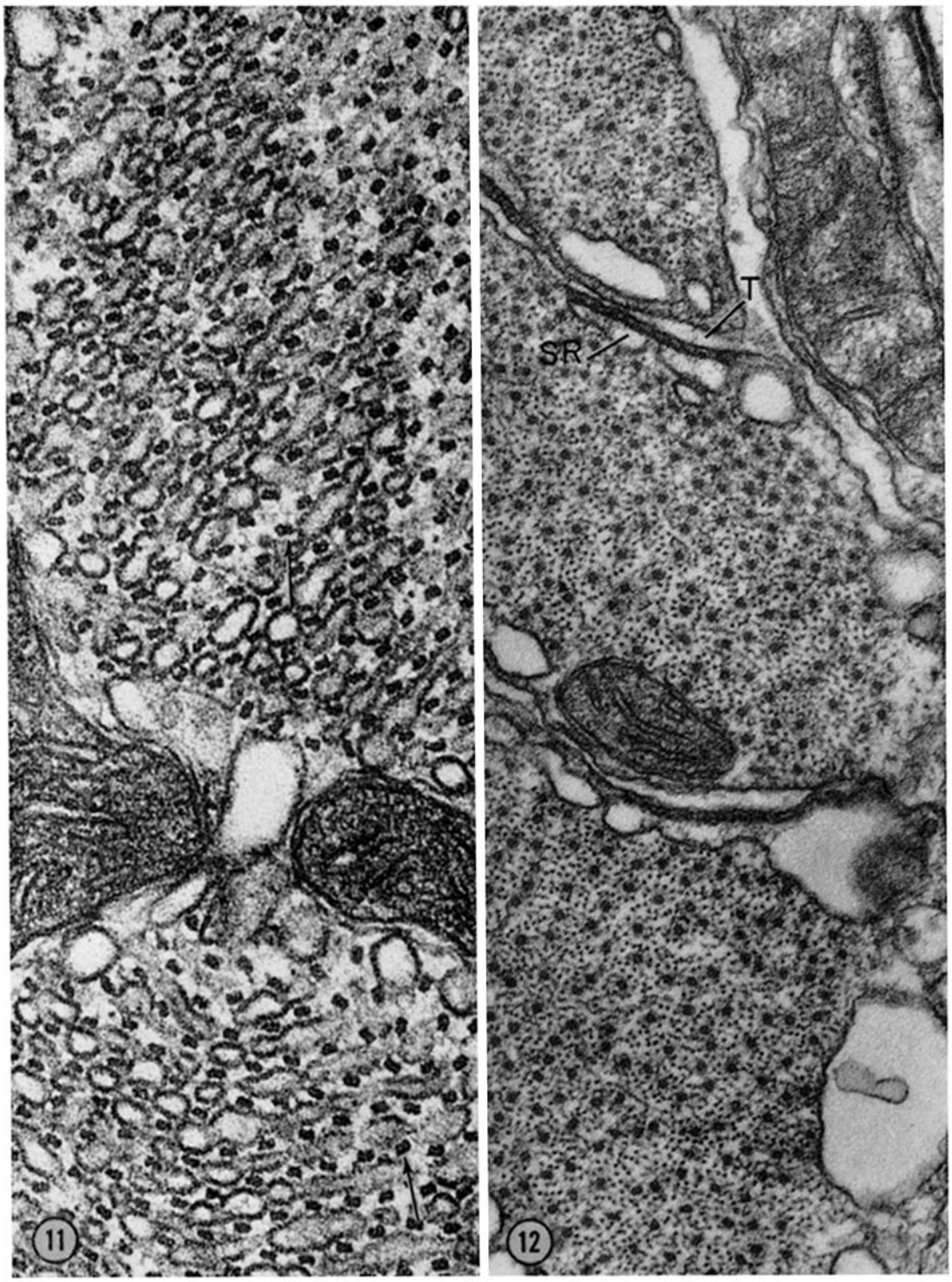

FIGURE 11 This is an enlarged view of a portion of Fig. 10 to show the subunit structure (arrows) of thick myofilaments in the middle of sarcomeres. × 125,000.

FIGURE 12 Tibial extensor muscle. This figure shows a cross-section of three adjacent myofibrils at the surface of the muscle fiber. Notice the infolded T-system membranes (*T*) and associated sarcoplasmic reticulum cisternae (*SR*). Also notice the lack of hexagonal packing of thin and thick myofilaments and the increased thin/thick myofilament ratio compared to basalar flight muscle. × 79,500.

ported for synchronous flight muscle in the dragonfly *Aeshna* sp. (Smith, 1966 *a*) and asynchronous flight muscle in the Diptera, *Drosophila melanogaster* (Shafiq, 1963) and *Calliphora erythrocephala* Mg. (Auber and Couteaux, 1963), and in the Hemipteran, *Megoura viciae* (Smith, 1965 a). The structural appearance of Z lines, disposition and packing of thin and thick myofilaments, and thin/thick myofilament ratios in tibial extensor muscle are similar to those reported in the femoral muscle of a cockroach, *Leucophaea maderae* F. (Hagopian, 1966), the tibial muscle of the scorpion, *Buthus occitanus* (Auber, 1963), and other invertebrate muscles known to be slow acting, such as esophageal myoepithelium (Reger, 1966 *a*) and body wall muscle (Rosenbluth, 1963, 1965; Reger, 1963, 1964) of *Ascaris lumbricoides* and pharyngeal muscle of the planarian, *Dugesia* sp. (Mac Rae, 1963). The precise reason why muscles with larger thin/thick myofilament ratios are slow acting relative to wing muscles and yet have similar T systems and sarcoplasmic reticulum is no doubt related to the mechanical role played by the muscle rather than characteristics of speed and synchrony of response following stimulation (Smith, 1966 b).

T System and Sarcoplasmic Reticulum

The importance of the number and repeat order of triads and dyads as well as the disposition and amount of sarcoplasmic reticulum in functionally different muscles is becoming increasingly clear (Smith 1966 *c*). There was some evidence from this study that the number of regularly arranged, repeating, triadic contacts are more numerous in basalar flight muscle than the number of dyadic and triadic contacts found in tibial extensor muscle. However, the most obvious difference between the two muscles is the presence in basalar flight muscle of a transverse element of sarcoplasmic reticulum which penetrates every myofibril midway between the Z lines. In continuing studies we have now observed such transverse elements of sarcoplasmic reticulum in wing muscles of another skipper, *Erynnis martialis*, and the moths, *Agrotis* sp. and *Thyridopteryx ephemeraeformis*.[1] The significance of these transverse elements in wing muscles is not clear but may be related to speed and synchrony in these flight muscles.

The organization and disposition of the T system and sarcoplasmic reticulum in basalar flight muscle shows both similarities and differences from that in other synchronous flight muscles examined in the Lepidopteran, *Pieris* sp. (butterfly) (Smith, 1962), and the Odonata, *Aeshna* sp. (Smith, 1961, 1966 a) and *Enallagma* sp. (Smith, 1965 *b*). Similarities include the position of triads between Z lines and the center of sarcomeres. Differences include triadic rather than dyadic (Smith, 1965 *b*, 1966 *c*) contacts of T-system membranes with sarcoplasmic reticulum and the presence of a transverse element of sarcoplasmic reticulum in the middle of each sarcomere.

While the basalar flight muscle of *Achalarus lyciades* is no doubt synchronous, as are other Lepidopteran muscles so far studied (Pringle, 1965), wing-beat frequencies may be higher than those of the synchronous muscles which have been previously examined with the electron microscope. It should be noted here that wing-beat frequencies of skippers and moths may be as high as 50–104/sec (Sotavalta, 1947). Although the precise wing-beat frequency range of *Achalarus lyciades* is not known, skippers and moths have similar flight characteristics and speeds (Sotavalta, 1947). For example, wing-beat frequencies of species of *Agrotis* moths range from 30 to 62/sec (Sotavalta, 1947) and that of the moth, *Trochilium tipuliforme* Cl., ranges from 98 to 104/sec (Sotavalta, 1947). The synchronous flight muscles studied by Smith (1961, 1962, 1965 *b*, 1966 *a*) probably have wing-beat frequencies which range from 6 to 52/sec since the wing-beat frequencies of similar species of the same order of insects as studied by Smith (1961, 1962, 1965 *b*, 1966 *a*) have been reported to be only 6/sec for the butterfly, *Pieris napi* (Sotavalta, 1947), and 35–52/sec for other species of Odonata (Sotavalta, 1947).

If the transverse element of sarcoplasmic reticulum found in basalar fllight muscle of *Achalarus lyciades* is of functional significance, how may it be related to what is already known about the sarcoplasmic reticulum in other muscle? Correlated radioautographic and tension-development studies on frog semitendinosus muscle (Winegrad, 1965) have shown that calcium shifts from the sarcoplasmic reticulum cisternae, located at the I band, to regions of the A band during contraction. This may reflect that calcium is released from sarcoplasmic reticulum cisternae during activation and subsequently migrates to actomyosin ATPase sites at myofilament cross-bridges (Winegrad, 1965) in

[1] Reger, J. F. 1966. Unpublished observations.

the A band. If the transverse element of sarcoplasmic reticulum in *Achalarus lyciades* flight muscle contains calcium and serves a function in excitation-contraction coupling, the repeat order and penetration to within 80–120 A of myofilaments at the mid-region of sarcomeres could have important functional implications in terms of this muscle's speed and synchrony, either by releasing calcium prior to contraction or by serving as a calcium sink during relaxation phases of muscle activity. While it is not known whether calcium is present in any portions of the sarcoplasmic reticulum of *Achalarus lyciades* basalar muscle, calcium has been localized in the sarcoplasmic reticulum of several vertebrate muscles (Hasselbach, 1966), including both the longitudinal and cisternal elements of rabbit psoas (Pease et al., 1965). If this transverse element is assumed to function in excitation-contraction coupling in this fast-acting wing muscle, its absence in the tibial extensor muscle of the same species certainly offers an opportunity to compare physiological and biochemical characteristics of two widely different sarcoplasmic reticulum organizations in the same animal.

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