

# THE ORGANIZATION OF FLIGHT MUSCLE FIBERS IN THE ODONATA

DAVID S. SMITH

From the Department of Biology, University of Virginia, Charlottesville, Virginia

## ABSTRACT

The cytological organization of flight muscle fibers of Odonata has been investigated. These fibers, in representatives of the Zygoptera and Anisoptera, have been compared and found to be similar, except that, in the former, pairs of lamellar fibrils, rather than single fibrils, alternate with the mitochondria. In each instance, in these synchronous muscles, the actin filaments of the myofibrils are found to lie opposite to and midway between pairs of myosin filaments—a configuration previously reported in asynchronous flight muscle fibers. The disposition of the T system and sarcoplasmic reticulum membranes in glutaraldehyde-fixed anisopteran muscle is described in detail: the T system tubules are shown to be radially continuous across the fiber, and are derived as openmouthed invaginations from the surface cell-membrane. The detailed organization of the dyad junctions between these tubules and the adjoining cisternae of the sarcoplasmic reticulum is described. The accessibility of the T system interior to diffusion exchange with the general extracellular milieu has been investigated by studies on the penetration of ferritin into the fiber: molecules of this marker have been found to diffuse solely along the T system tubules, and their presence in the tubule extremities adjoining the centrally placed nuclei confirms the morphological evidence suggesting that these tubules provide open diffusion channels extending across the radius of the fiber. The possible physiological role of these membrane components and their distribution in synchronous muscles of insects and vertebrates and in asynchronous insect flight muscle are discussed.

## INTRODUCTION

Insects within the order Odonata are rather sharply demarcated into two groups, the Anisoptera and Zygoptera. The former are generally strongly flying and often large insects (the “dragonflies”), while the latter (the “damselflies”) are slender insects with a relatively weak flight capacity. In each instance (Pringle, 1957) the flight muscles operate under nearly isometric conditions, and the amplitude of the resulting wing beat is small.

Light microscopic studies (*see* Pringle, 1957) indicated that the flight muscles of the Odonata have preserved the primitive radial arrangement

of lamellar fibrils, and a superficial tracheal supply, characteristic of typical leg and trunk muscles of insects. An electron microscopic study of these fibers in the dragonfly *Aeshna* (Smith, 1961 *b*) demonstrated that, as in other insects (Smith, 1962), they are specialized to meet the increased metabolic demands imposed by flight activity, notably by hypertrophy of the mitochondrial supply: in *Aeshna* these organelles are large structures inserted between the fibrils, accounting for up to 40% of the fiber volume, and arranged in precise register with the sarcomere repeat of the myofibrillar striations.

It is known that the flight muscles of Odonata have retained the synchronous relationship between motor nerve stimulation and contraction, as opposed to the "asynchronous" condition that has developed in Diptera, Hymenoptera, Coleoptera, and Hemiptera other than Cicadidae, in which the frequency of rhythmic alterations in fiber length does not correspond to, and may greatly exceed, that of the motor impulse train (Boettiger, 1957; Pringle, 1957, 1965). Electron microscopic studies on a variety of insect orders (Smith, 1961 *a* and *b*, 1962, 1963, 1965 *a* and *b*) have shown that the flight muscles of Odonata, and indeed all synchronous flight muscles hitherto examined, are not only clearly demarcated cytologically from asynchronous fibers, but have important structural features in common with similarly synchronous muscles of vertebrates; particularly as regards the distribution of the sarcoplasmic membranes of the fiber. Considerable attention has recently been focused upon the possible role of the longitudinally oriented cisternae of the sarcoplasmic reticulum and the closely associated tubules of the transversely arranged T system, in the mediation of the phases of the activity cycle of striated muscle. This subject will be discussed more fully in due course, and it may be stated at this point that recent studies on Odonata flight muscle have established a precise correspondence between the derivation and distribution of these elements in an insect muscle, and in physiologically analogous fibers of vertebrate muscle.

#### MATERIALS AND METHODS

Metathoracic dorsoventral flight muscles of *Enallagma ebrium* (Hagen) (Zygoptera) and *Sympetrum rubicundulum* (Say) (Anisoptera) were employed in this study. The former were fixed *in situ* in the bisected thorax in ice-cold 1% osmium tetroxide buffered at pH 7.4 with veronal-acetate containing 0.15 M sucrose.

Fixation was continued for 90 min; then the material was transferred to 70% ethanol, in which anatomical muscle attached to their dorsal skeletal supports were removed. Dehydration was completed in an ethanol series, and the material was embedded in Araldite. Anisopteran flight muscle was fixed for 3 hr in ice-cold 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 0.18 M sucrose, then washed overnight in several changes of cold cacodylate buffer with 0.3 M sucrose, placed in buffered 1% osmium tetroxide for 1 hr, dehydrated in ethanol and embedded in Araldite, in the usual way.

For observations on the penetration of ferritin into the fiber, the method described by H. E. Huxley (1964) in a study on frog skeletal muscle was employed. A solution containing a final concentration of ca. 40% horse spleen ferritin (Sigma Chemical Co., St. Louis) was prepared by sedimenting the commercial solution at 50,000 RPM in a Spinco model L centrifuge for 2 hr, and resuspending the resulting pellet in an insect Ringer's containing Na 167 mM, K 13.4 mM, Ca 4.5 mM (as chlorides), NaHCO<sub>3</sub> 2.38 mM, and NaH<sub>2</sub>PO<sub>4</sub> 0.87 mM. The thorax of *Sympetrum* was bisected medially and immersed in the ferritin solution for 1 hr, and transferred to buffered glutaraldehyde for 3 hr, after which the outermost muscles in the preparation were dissected out. Subsequent washing, postfixation in osmium tetroxide, dehydration, and embedding were carried out as described above.

Sections were cut on a Huxley microtome and examined in a Philips EM 200. Specimen contrast was enhanced by staining either with lead (Reynolds, 1963) (Figs. 1, 2, 7 to 10) or with 50% ethanolic uranyl acetate followed by lead (Figs. 3 to 6).

#### RESULTS

The chief cytological features of flight muscle of a dragonfly have been described in an earlier paper (Smith, 1961 *b*). The purpose of the present investigation has been to compare the organizations of flight muscle in representatives of the two divisions of the Odonata, and in particular to reexamine

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FIGURE 1 A low power electron micrograph of portions of three transversely sectioned flight muscle fibers of the damselfly *Enallagma*. Both the fibrils (*fi*) and the large mitochondria (*m*) are radially disposed around the centrally placed nuclei (*n*). The organization of these fibers differs from that of the corresponding muscle fibers in dragonflies (e.g., *Aeshna*, Smith 1961 *b*; *Sympetrum*, Figs. 3 to 10), in that the membranes of the sarcoplasmic reticulum (*sr*) are situated not only between the sheetlike fibrils and the mitochondria (see Fig. 2) but are also interpolated between the inner surfaces of the paired fibrils. Tracheoles or small tracheae (*tr*) lie between the fibers, and the intracellular light areas (*l*) probably represent lipid droplets, lost during specimen preparation.  $\times 11,000$ .



this material, taking advantage of more recent advances in preparative technique (notably glutaraldehyde fixation) in an attempt to obtain an improved evaluation of the relationship between the sarcoplasmic reticulum and T system and the environment external to the fiber. The latter aim was also pursued by the use of ferritin as an electron-opaque diffusible marker.

### *The Disposition and Structure of the Fibrils*

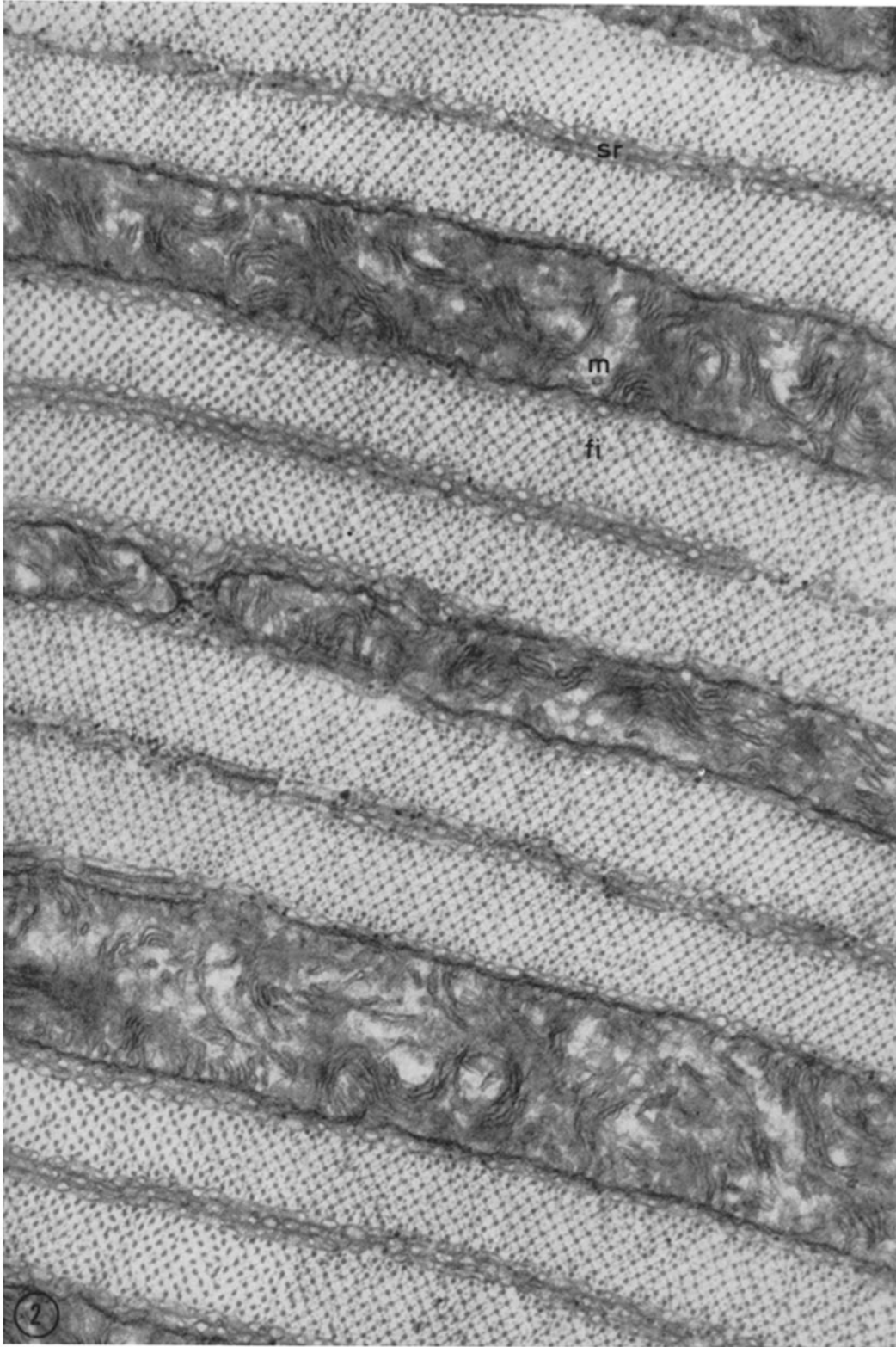
The flight muscles of most insects, whether synchronous or asynchronous in their contraction characteristics, contain a contractile system deployed in cylindrical fibrils, 1 to 2  $\mu$  in diameter. The Odonata, however, are unusual in that they have retained the lamellar fibrils typically present in leg and trunk muscles of insects (*see* Tiegs, 1955). The feature which renders these flight muscles strikingly different from those of the limbs or trunk is the extent of their mitochondrial supply: in Odonata flight muscle the mitochondria or sarcosomes are slablike and very large, and are interposed between the radially arranged myofibrils. In the dragonfly *Aeshna* these structures alternate with the fibrils (Smith, 1961 *b*), and a similar arrangement is met with in *Sympetrum* (Figs. 3 to 6). In the corresponding muscle from a zygopteran, however, a variant on this plan is found. A survey micrograph of flight muscle of the damselfly *Enallagma* is shown in Fig. 1, and it is evident that in this case two fibrils alternate with the mitochondria; the inner surfaces of each fibril pair being separated by elements of the sarcoplasmic reticulum, a feature seen to better advantage at higher magnification (Fig. 2). Similar cisternae are also inserted between the outer surface of each lamella of the contractile system and adjoining mitochondria. The fibril width in damselfly and dragonfly flight muscle is in each instance ca. 0.4 to 0.6  $\mu$ , and it is interesting to note that in the latter, possessed by powerfully flying insects, each lateral surface of the fibrils adjoins mitochondrial membranes, whereas in the more weakly flying damselfly fibril-mitochondrion and fibril-fibril juxtaposition occurs alternately.

In both Anisoptera (Smith, 1961 *b*) and Zygoptera (Fig. 1), the central core of each fiber is occupied by a chain of nuclei closely adjoining the inner extremities of the fibrils. The diameter of the flight muscle fibers in representatives of each of these suborders is similar—in the range of 15 to 25  $\mu$ .

The relaxed sarcomere length of flight muscle fibers of Odonata (as judged by the presence of I bands in fibers that operate in vivo almost isometrically) is ca. 2.3  $\mu$  (Fig. 3). Transverse sections of the fiber (Figs. 2, 7, 10) indicate that the actin filaments are situated opposite to and between pairs of myosin filaments—a configuration described in *Calliphora* indirect flight muscle (H. E. Huxley and Hanson, 1957), and differing from the trigonal disposition of actin filaments of vertebrate skeletal muscle fibers (H. E. Huxley, 1957). *Calliphora* flight muscle is asynchronous, whereas the Odonata possess synchronous fibers, and it is thus clear that the physiological peculiarities of the former type of muscle are not accompanied by any unusual disposition of the myofilaments, at least in the region of overlap in the A band of the sarcomere.

### *The T System and the Sarcoplasmic Reticulum*

It is now generally recognized that the fibrils of almost all vertebrate skeletal muscle fibers are invested with two distinct series of membrane-limited components: a longitudinal system of cisternae and a separate but intimately associated transversely oriented tubular system—the two components comprising the “sarcoplasmic reticulum” of earlier studies (Porter and Palade, 1957). This dual system has been described in a wide variety of vertebrate striated muscles, amongst which may be mentioned the work of Andersson-Cedergren (1959), Fawcett and Revel (1961), Revel (1962), and more recently, Franzini-Armstrong and Porter (1964) and Peachey (1965). Although earlier studies on vertebrate fibers (Andersson-Cedergren, 1959; Revel, 1962) strongly suggested that the transverse tubular system is radially continuous across the fiber, cytological evidence suggested that these tubules end blindly just beneath the surface plasma membrane of the muscle cell. However, Franzini-Armstrong and Porter (1964) showed that in glutaraldehyde-fixed fish myotome muscle, the T system tubules are derived as invaginations from the surface membrane, and that, morphologically at least, the interior of these tubules is confluent with the general extracellular space. In no instance, however, has any evidence been found of linkage between the longitudinally oriented cisternae accompanying the T system elements and the plasma membrane; these cisternae appear to be strictly intracellular, and if considered to be analogous with the endo-



**FIGURE 2** Portion of a transversely sectioned flight muscle fiber of the damselfly *Enallagma* (see Fig. 1). Note the parallel sheetlike fibrils (*fi*), mitochondria (*m*), and the cisternae of the sarcoplasmic reticulum (*sr*). These last lie between the paired fibrils and between the fibrils and the adjoining mitochondria. This section passes through the A band region of the sarcomere and to one side of the tubules of the T system which are, therefore, not included.  $\times 33,000$ .

plasmic reticulum of other cell types, may be termed the sarcoplasmic reticulum (*sensu stricto* of the muscle cell. The possible physiological role of these two components will be considered in the discussion.

The general organization and disposition of the sarcoplasmic reticulum and T system in dragonfly flight muscle have been described earlier (Smith, 1961 *b*); at that time, the sarcoplasmic reticulum was identified as a series of fenestrated cisternae investing the fibrils, and it was suggested that the T system elements are convoluted, but probably continuous across the fiber radius, and furthermore, some evidence was offered indicating their origin as invaginations from the cell surface. Improvements in preparative methods now permit a fuller structural analysis of these components, the disposition of which is unusually regular in these radially symmetrical fibers.

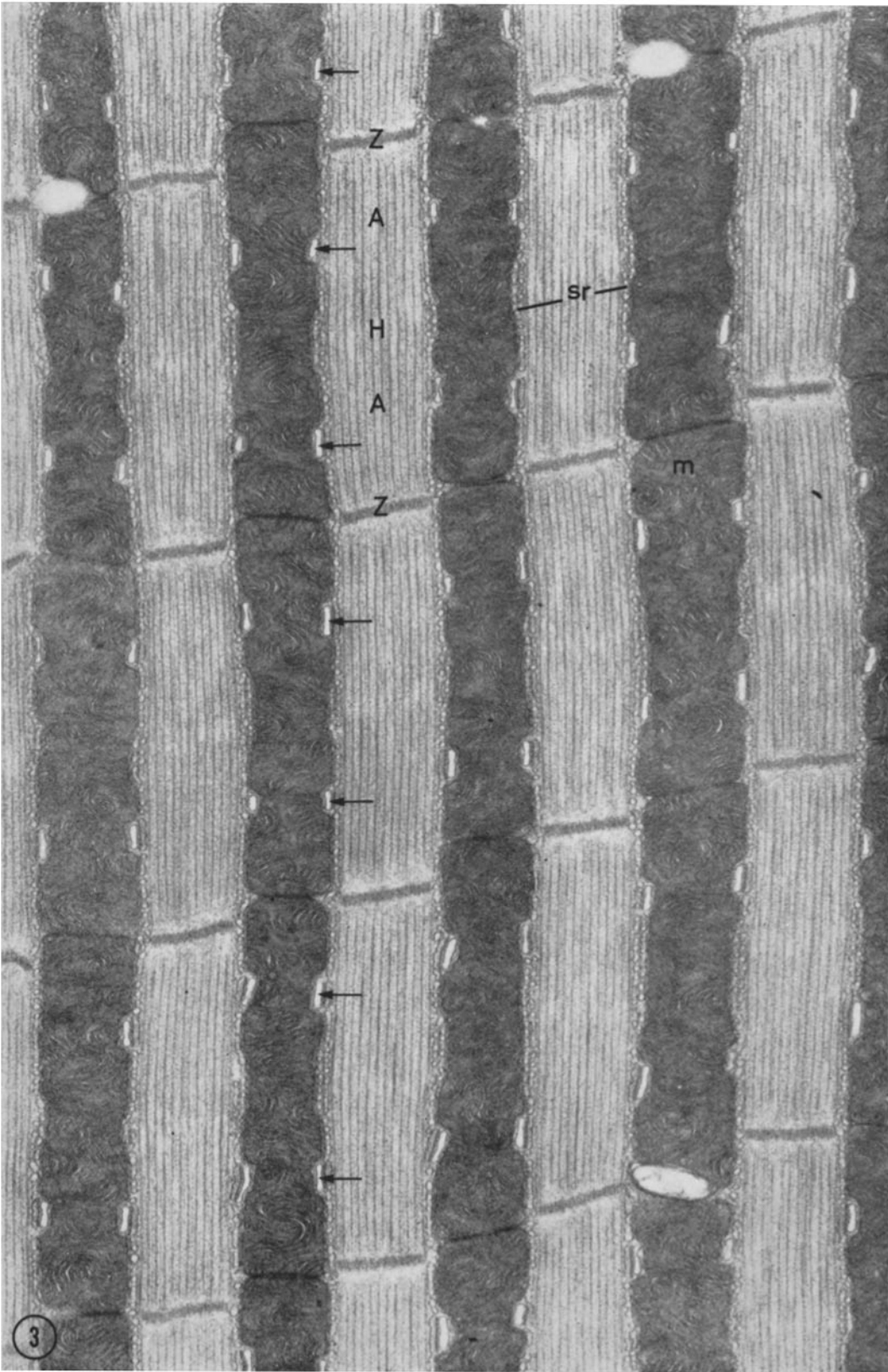
Fig. 3 illustrates the appearance of the T system and sarcoplasmic reticulum in longitudinal sections of *Sympetrum* flight muscle. The characteristic feature of the former is its preferred orientation with the myofibrillar striations (Porter and Palade, 1957), usually lying, in vertebrate fibers, at the level of either the Z band or the A-I junction. In *Sympetrum* muscle, as in other synchronous insect muscles (whether associated with the wings or the legs), the radially oriented T system tubules traverse the fiber midway between the Z level and the H zone in the middle of the sarcomere. Each T system tubule, seen in transverse profile in longitudinal sections, is elongated or approximately rectangular and ca.  $0.04 \times 0.16 \mu$  in size, lying in an indentation or groove in the surface of the adjoining mitochondrion (Figs. 3, 4 *a*). Each tubule is separated from the fibril surface by a cisterna of the longitudinally oriented sarcoplasmic reticulum, which extends as a continuous

sheet, though bearing frequent fenestrations (see Smith, 1961 *b*), alongside successive sarcomeres of the fibrils (Figs. 3, 4 *a*). That the sarcoplasmic reticulum enters into an intimate association with the T system tubules, as in other muscles, is illustrated at higher magnification in Figs. 4 *a* and *b*. The adjacent membrane surfaces of these two components appear to be almost or actually in contact via a series of minute "foot processes" or thickenings, apparently produced from the surface of the reticulum cisterna, and directed towards the adjoining tubule of the T system. These processes are located at intervals of ca. 250 A and project ca. 100 A from the underlying membrane surface. These two-membered *dyads* evidently correspond to the three-membered *triads*, consisting of a central T system tubule and a pair of adjoining terminal cisternae of the sarcoplasmic reticulum, characteristic of many vertebrate skeletal muscles (Porter and Palade, 1957; Fawcett and Revel, 1961; Peachey, 1965). The contents of the two members of the dyad in *Sympetrum* muscle differ markedly; the interior of the T system appears to be entirely devoid of content, while electron-opaque material is present in the adjoining cisterna, and elsewhere within the sarcoplasmic reticulum.

The radial continuity of the T system tubules and their relationship with the surface cell-membrane discussed earlier (Smith, 1961 *b*) is convincingly demonstrated in the present material: as in vertebrate muscle (Franzini-Armstrong and Porter, 1964) glutaraldehyde here appears to afford more satisfactory preservation of the T system structure than does osmium tetroxide. Fig. 5 illustrates a transversely sectioned field of *Sympetrum* flight muscle, traversing the level of laterally aligned T system tubules. This micrograph includes alternating myofibrils and mitochondria

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FIGURE 3 Longitudinally sectioned flight muscle of the dragonfly *Sympetrum*. The sarcomere striations are indicated; narrow light I bands flank the Z bands (see Fig. 4 *a*), and the A bands are divided medially by faintly demarcated H zones, while M bands are lacking. As in the allied genus *Aeshna* (Smith, 1961 *b*), the mitochondria (*m*) are arranged in register with the sarcomeres. Membranes of the sarcoplasmic reticulum (*sr*) lie between the fibrils and the mitochondria, and transversely sectioned profiles of the T system tubules are conspicuous (arrows) in indentations of the surface of the mitochondria, midway between the Z and H levels of each sarcomere. The relationship between the sarcoplasmic reticulum and T system in this muscle is seen to better advantage at higher magnification, in Figs. 4 *a* and *b*.  $\times 26,000$ .



and between these structures lie extensive elongated profiles of the tubules, closely flanked in the dyad configuration, on the side adjoining the fibrils, by cisternae of the sarcoplasmic reticulum. The width of these tubular channels, in this plane of section, is ca. 0.03 to 0.05  $\mu$ , as in transversely sectioned profiles, except where the T system interior is occasionally considerably dilated to conform to the contours of the adjacent mitochondria. In this field, the tubules extend without interruption for up to ca. 7  $\mu$ , and each probably traverses the entire fiber radius (ca. 10  $\mu$ ), ending blindly close to the surface of the centrally located nuclei (Fig. 10).

A similar field, but at the periphery of the fiber, is reproduced in Fig. 6. In this field, the continuity between the membranes defining two T system tubules and the cell membrane is clearly established, while in another instance this link probably lies just out of the plane of section. Through these openmouthed invaginations, the lumen of each T system tubule is seen to be morphologically confluent with the space surrounding the entire fiber, in this case, with the hemolymph. In  $\text{OsO}_4$ -fixed material, this continuity was occasionally observed in synchronous insect muscle (Smith, 1961 *b*, 1962), but after glutaraldehyde fixation it is of such general occurrence as to indicate that most, if not all, of these tubules are derived from the cell surface.

Thus, recent electron microscopic evidence suggests that synchronous striated muscles (with the exception of frog "slow" fibers; Peachey and A. F. Huxley, 1962) are permeated with a pre-

cisely oriented system of open tubular invaginations passing radially across the fiber. In the flight muscle of dragonflies and indeed, it appears, in all synchronous muscles of insects, the surface of each sarcomere throughout the fiber is supplied with two pairs of these invaginations, evenly spaced, and equidistant from each successive Z and H band.

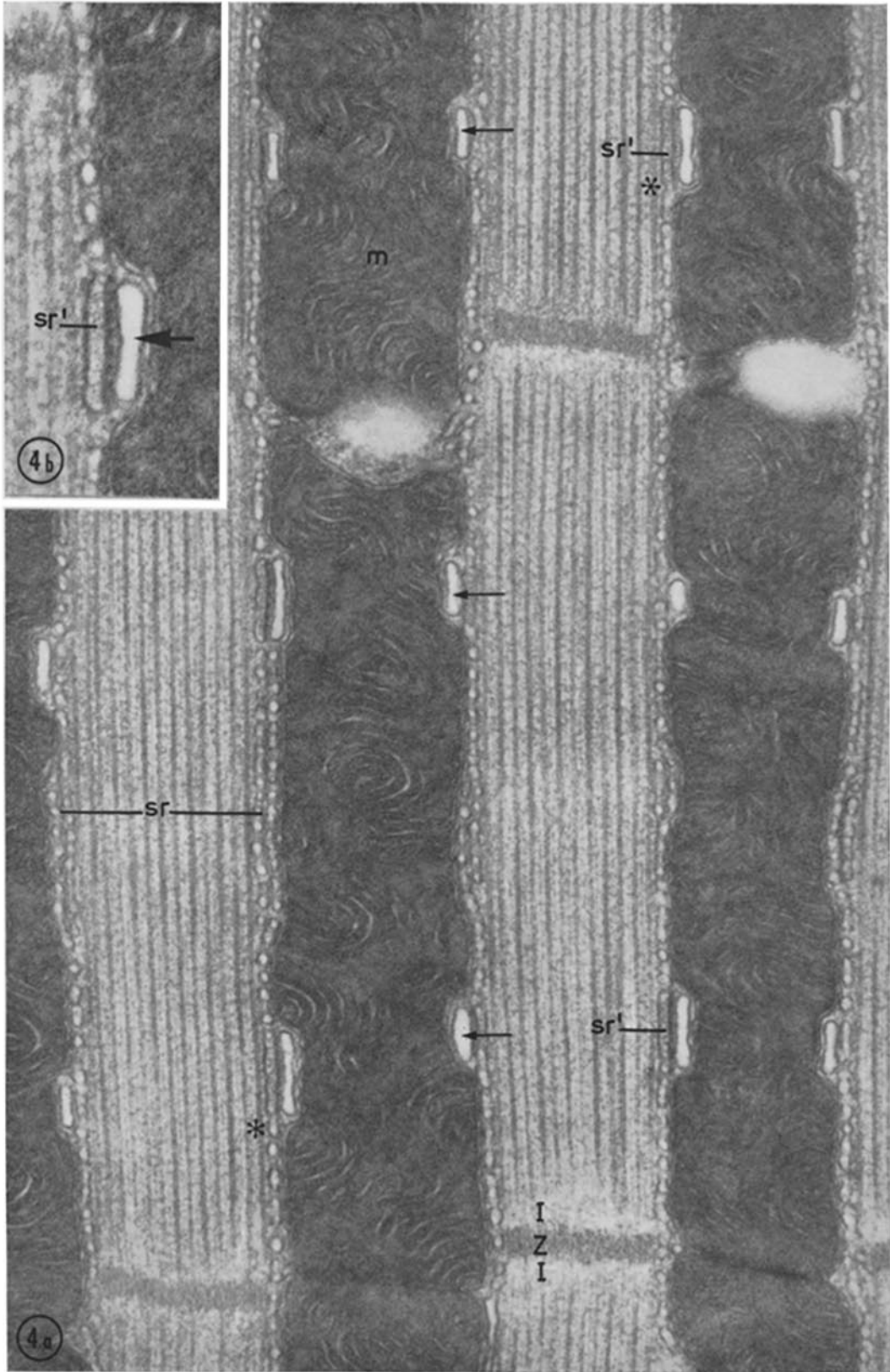
The possible physiological role of the T system tubules in muscular contraction has recently been fully considered and discussed elsewhere (A. F. Huxley, 1959; Franzini-Armstrong and Porter, 1964; Peachey, 1965; Smith, 1965 *a*). In brief, it has been suggested by several workers that these tubules may act as the pathway along which excitation, initiated at the periphery of the fiber by the arrival of the nerve impulse at the fiber surface, may be channeled into the fiber, reducing the distance over which excitation must operate from that of the radius of the fiber to less than 1  $\mu$ —the distance separating the fibrillar material from the closest T system tubule. The identification of the structural excitation-conduction pathway, as Peachey and Porter (1959), Smith (1961 *a*), Franzini-Armstrong and Porter (1964), Peachey (1965) and others have pointed out, may account for the rapid onset of contraction in a striated muscle fiber, following the establishment of peripheral membrane depolarization. A. F. Huxley (1959) pointed out that the simplest model of a structural excitation-conduction pathway electrically coupled with the fiber surface would be met by openmouthed tubules traversing the fiber, and affording low-resistance channels for the

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FIGURE 4 *a* A field similar to that reproduced in Fig. 3, at higher magnification. Note the narrow I bands flanking the dense Z bands. The T system tubules are included (arrows) as elongated profiles associated, in two-membered dyad configurations, with adjoining regions of the sarcoplasmic reticulum (*sr*). The fenestrations of the sarcoplasmic reticulum (see Smith, 1961 *b*) present elsewhere along the sarcomere afford the "beaded" appearance seen here (*sr*), but in the region of the dyad these fenestrations are absent. Confluence between these portions of the sarcoplasmic reticulum is frequently noted (\*).  $\times 63,000$ .

FIGURE 4 *b* Illustrating the structural details of a dyad association between an element of the sarcoplasmic reticulum (*sr*) and a T system tubule (arrow) in *Sympetrum* flight muscle. These two membrane components are separated by a gap of ca. 100 A, almost or completely bridged by regular thickenings of the reticulum membrane, occurring at intervals of ca. 250 A. Note the electron-opaque material in the reticulum cisterna, and the absence of such material from the interior of the T system tubule.  $\times 100,000$ .





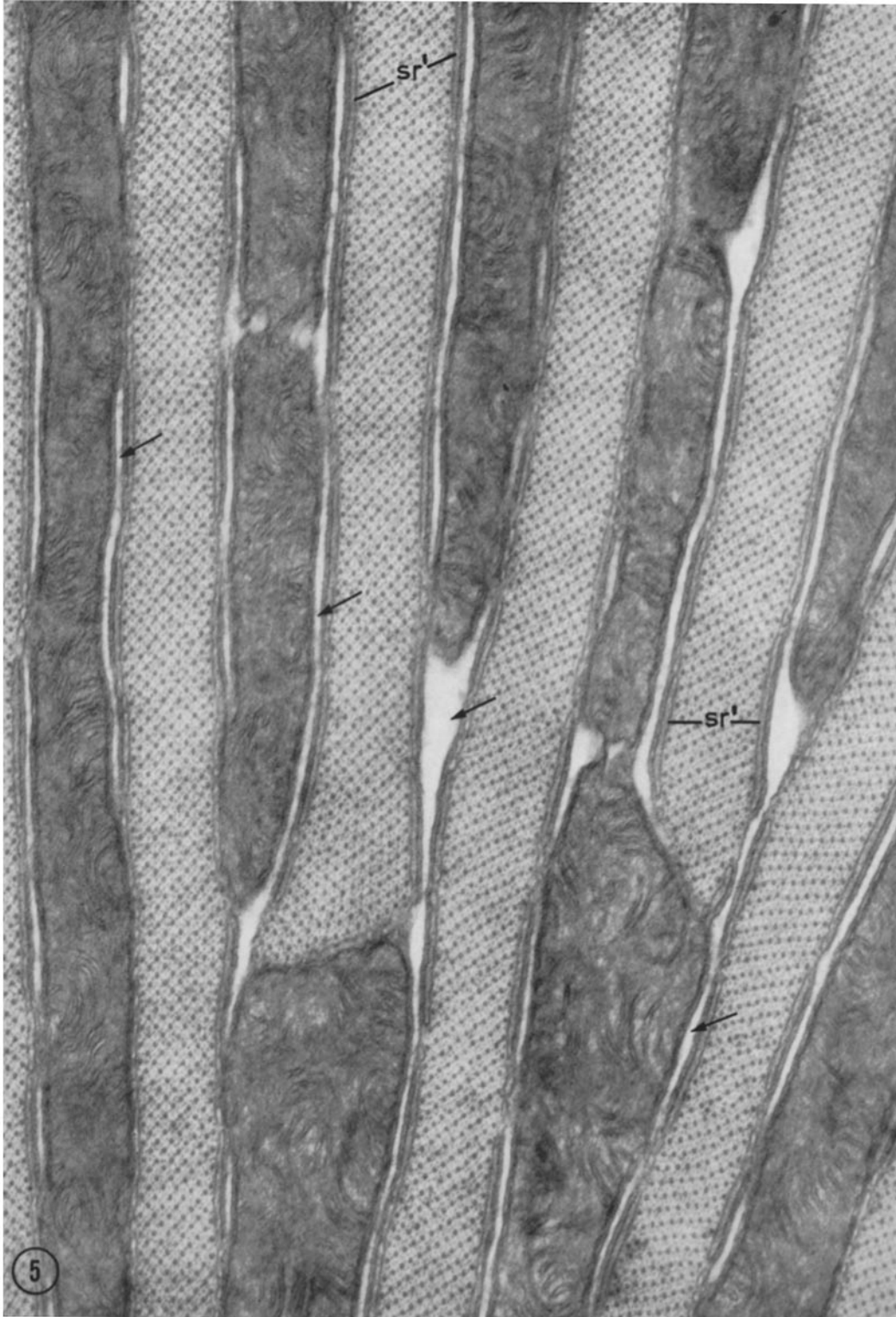


FIGURE 5 Transversely sectioned *Sympetrum* flight muscle; plane of section passes through the level of T system tubules. These tubules (arrows) are interposed between cisternae of the sarcoplasmic reticulum (*sr'*) and the mitochondria (see Fig. 3) and, as may be seen in longitudinal sections of the fiber (Figs. 3, 4 *a*), are oriented with precision between the Z and H band levels of the sarcomere. In this micrograph, the T system tubules continue within the plane of section for up to 7  $\mu$ , and are derived as open invaginations of the surface cell-membrane (Figs. 6 to 8), probably penetrating as continuous tubes along the entire fiber radius at each locus.  $\times 30,000$ .

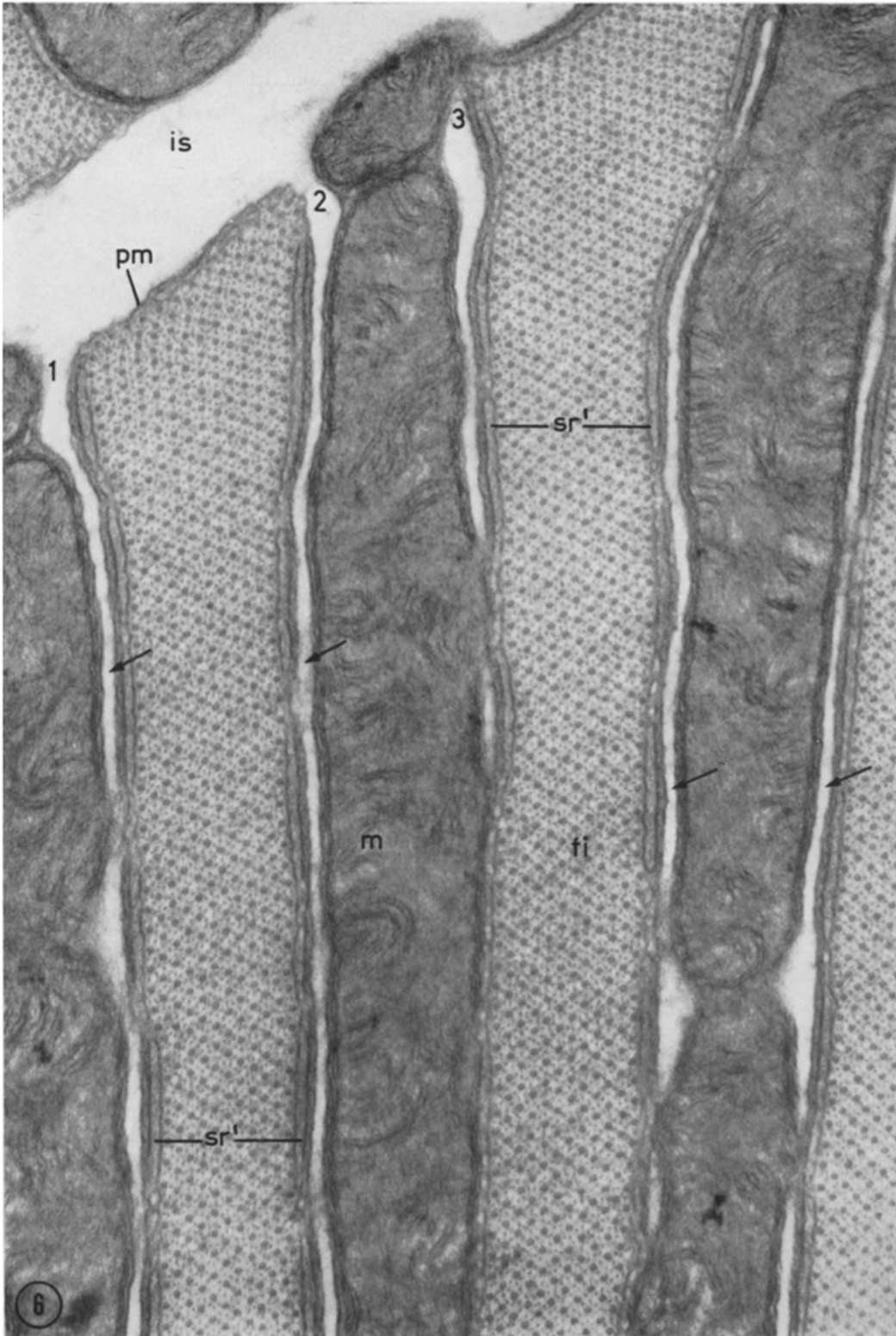


FIGURE 6 Showing derivation of the T system tubules, in flight muscle of the dragonfly *Sympetrum*, as direct invaginations of the plasma membrane at the surface of the fiber (*pm*). The disposition of the T system tubules (arrows), adjoining the sarcoplasmic reticulum (*sr'*) and mitochondria (*m*), is as shown in Fig. 5. At 1 and 2 the tubules open directly to the interfiber (*is*) space, while at 3 the origin of the invaginated tubule appears to lie just out of the plane of section.  $\times 55,000$ .

movement of ions between the tubules and the exterior of the fiber. Microdepolarization experiments carried out by A. F. Huxley and colleagues (Huxley and Taylor, 1958; Huxley, 1959) implicated the level of the triad, in vertebrate muscles, with radial conduction of excitation, and the recognition of the invaginated nature of the T system tubules strengthened the likelihood that they form at least part of the pathway intervening between surface excitation and activation of the contractile apparatus. The question of whether the T system tubules are not only morphologically "open," but also accessible to diffusion exchange of ions and molecules with the extracellular space, has recently been investigated by the use of markers. Endo (1964) observed that entry of a fluorescent dye, Lissamine-rhodamine B, took place in frog skeletal fibers only at the level of the Z bands—the location, in this muscle, of the triads. Furthermore, H. E. Huxley (1964) and Page (1964), in electron microscopic studies, described the ingress of the electron-opaque protein-ferric hydroxide complex ferritin into frog muscle fibers. They found that the marker molecules, applied in an external bathing solution, penetrated along the T system tubules but were not found either in the adjoining terminal cisternae of the sarcoplasmic reticulum or in any other compartment of the fiber. These studies did not, however, provide clear morphological evidence of the continuity between the tubules and the surface cell-membrane in the experimental material. The flight muscle fibers of Odonata, by virtue of their radial symmetry, afford the simplest possible arrangement of the T system tubules and the sarcoplasmic

reticulum, and moreover, the exclusively central location of the nuclei facilitate identification and illustration of marker substances that have penetrated along the entire fiber radius. Therefore, these muscles were employed, in the present study, for a similar study on the space available to ferritin diffusion into the fiber.

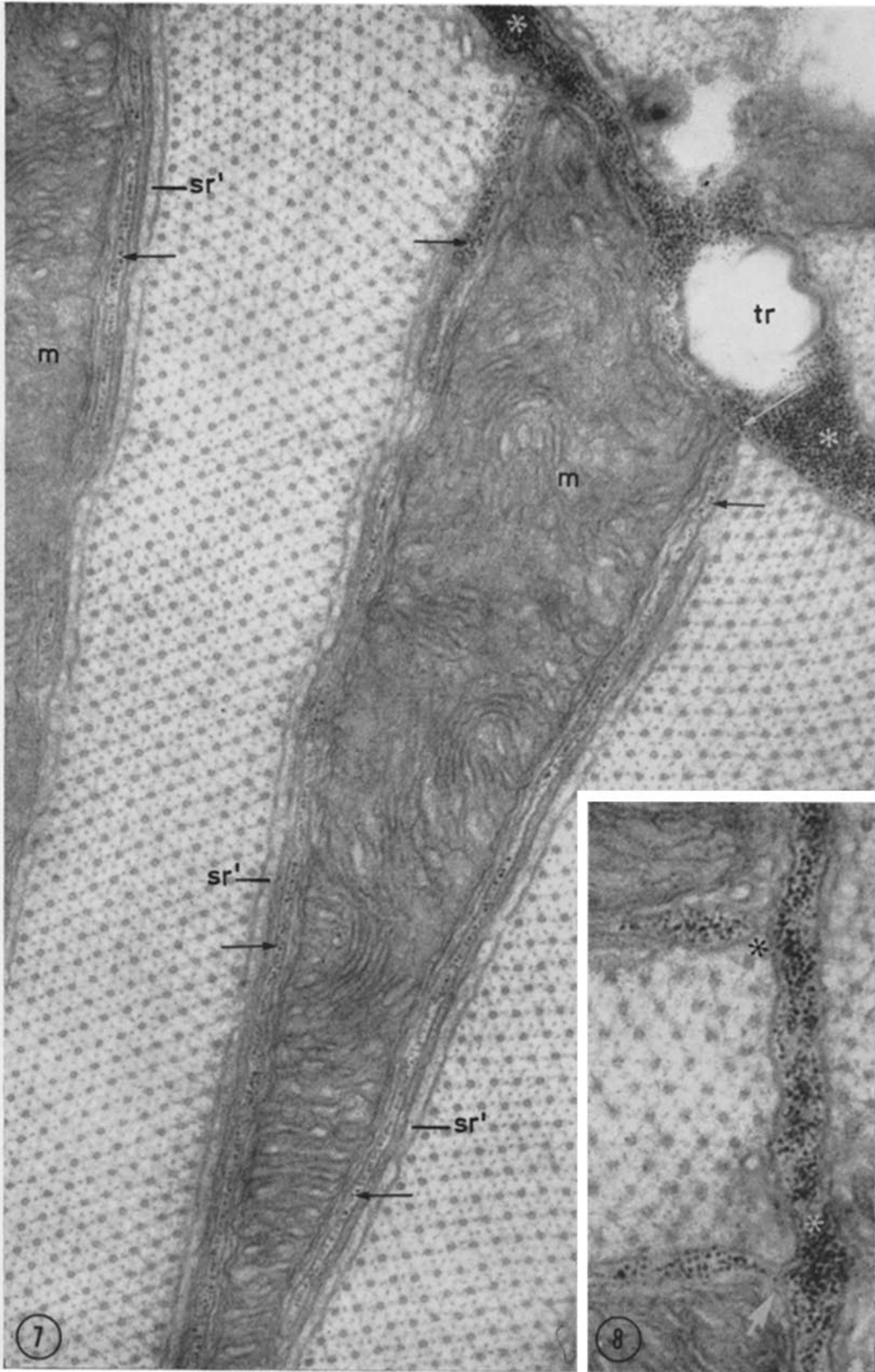
#### *Studies on the Penetration of Ferritin*

Flight muscles of the dragonfly *Sympetrum* were selected for this portion of the work. Although the ability of the fibers to contract after immersion in the ferritin solution was not tested, this treatment, as judged from the resulting micrographs, does not seriously damage the general organization of the muscle. The results obtained with this material are illustrated in Figs. 7 through 10. Fig. 7 represents a field similar to that shown in Fig. 6, including a peripheral region of a fiber. Densely packed molecules of ferritin are present in the extracellular space between the surfaces of two adjoining fibers, and elsewhere ferritin is present within the fiber exclusively in one location—within the radially oriented tubules of the T system. As in the untreated material (Fig. 6), cisternae of the sarcoplasmic reticulum accompany the T system tubules, but have never been observed to contain ferritin. In this micrograph (Fig. 7), and also in Fig. 8, continuity between a T system tubule and the surface cell-membrane is retained: this feature is, however, considerably less frequently met with than in material routinely fixed in glutaraldehyde, and in Figs. 7 and 8 the tubules appear to be sealed off from the cell membrane just short of the periphery of the fiber. Even in

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FIGURE 7 Transverse section of *Sympetrum* flight muscle, soaked in ferritin solution prior to fixation in glutaraldehyde. The arrangement of the fiber components is as in Fig. 6. Note the tracheole (*tr*) and electron-opaque particles of ferritin present in the interfiber space (white asterisks); these particles extend down the T system tubules (black arrows) but are not present in the adjoining cisternae of the sarcoplasmic reticulum (*sr'*). An open T system tubule is preserved (white arrow); this tubule is continuous for some distance within the plane of section.  $\times 75,000$ .

FIGURE 8 A field similar to that shown in Fig. 7, further illustrating the derivation of the channels along which ferritin passes into the fiber. Note the ferritin situated in the space between two fibers (white asterisk), and along the interior of an open T system tubule (white arrow). The profile of the upper T system tubule contains ferritin, but ends short of the surface cell-membrane (black asterisk); in such instances, the open neck of the tubule presumably either lies out of the plane of section, or has become closed during the ferritin treatment.  $\times 105,000$ .



these instances, however, the T system contains large amounts of ferritin, suggesting that this substance penetrates rapidly at the start of immersion, and that subsequently the tubule necks become sealed off from the cell membrane (as in OsO<sub>4</sub>-fixed vertebrate muscle), presumably as a result of exposure to the ferritin-containing Ringer's solution.

Fig. 9 illustrates another field of transversely sectioned *Sympetrum* muscle, after a similar immersion in ferritin solution. Here, the relationship between the T system tubules and the sarcoplasmic reticulum (seen as dyads in longitudinal sections of the fiber; see Fig. 3) is more clearly distinguished, and a T system dilatation, often occurring at the end of a mitochondrion (see Fig. 5), is also included. Again, the T system tubules, in one instance continuous across the micrograph (see Fig. 5), and between 0.025 and 0.05  $\mu$  in width, are filled with large numbers of electron opaque ferritin molecules, which are excluded from the adjoining cisternae of the sarcoplasmic reticulum. In Fig. 10, a region of transversely sectioned *Sympetrum* flight muscle, including the centrally placed nucleus, is shown. Ferritin particles are also evident in this region, within the lumina of T system tubules between the fibrils, and also closely adjoining the surface of the nucleus. Although it is possible that ferritin molecules could be transferred from one segment to another along a discontinuous T system tubule, it is more likely that this substance is able to diffuse to the center of the fiber along continuous channels; certainly, the appearance of sections of muscle fixed directly in

glutaraldehyde (Fig. 5) strengthen this conclusion. These results strongly support the supposition that the T system tubules are open for diffusion interchange between the surface of the fiber and the center of the fiber. That they are not continuous across the diameter of the fiber is indicated by invariable closure of the tubule profiles in the vicinity of the nuclei (Fig. 10). Huxley (1964) and Page (1964) noted that much of the ferritin that diffuses into the fibers of frog muscle is lost during subsequent washing in ferritin-free Ringer's solution. In the *Sympetrum* material, this effect was less marked, and after washing 1 hr considerable amounts of ferritin still remained in the T system tubules. This observation is consistent with the suggestion that sealing of the tubules from the general extracellular space occurs soon after immersion in the experimental ferritin solution.

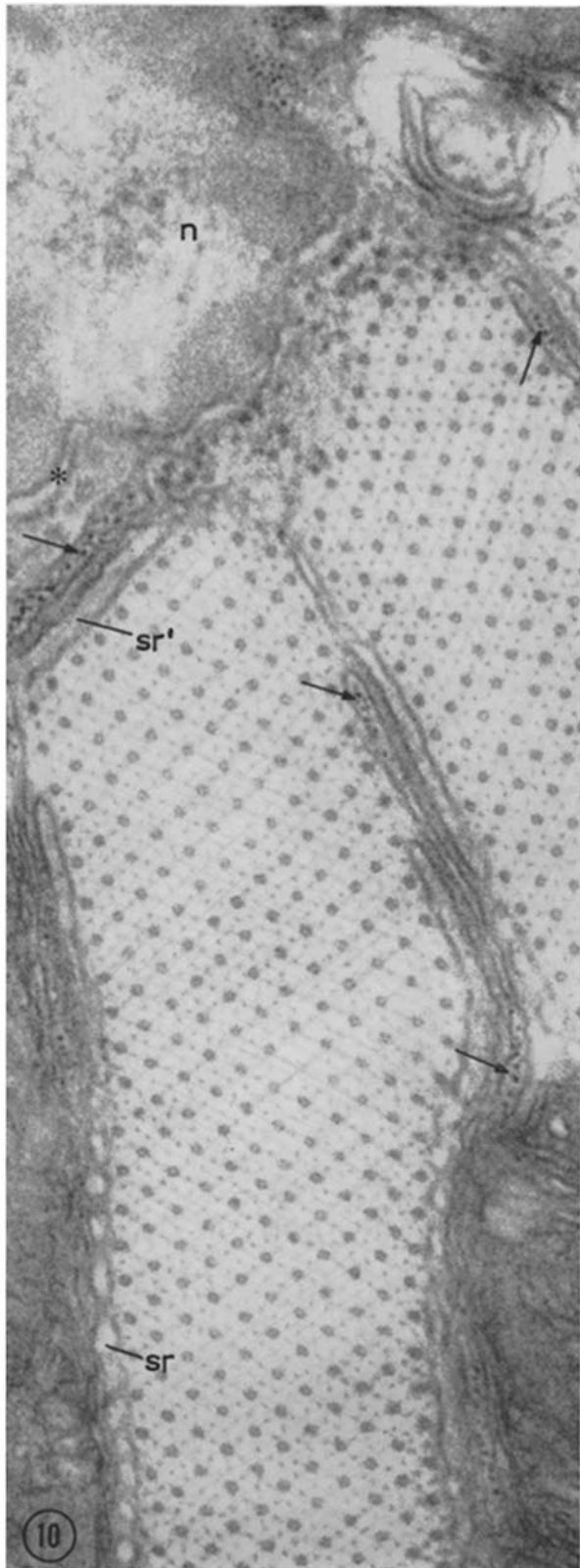
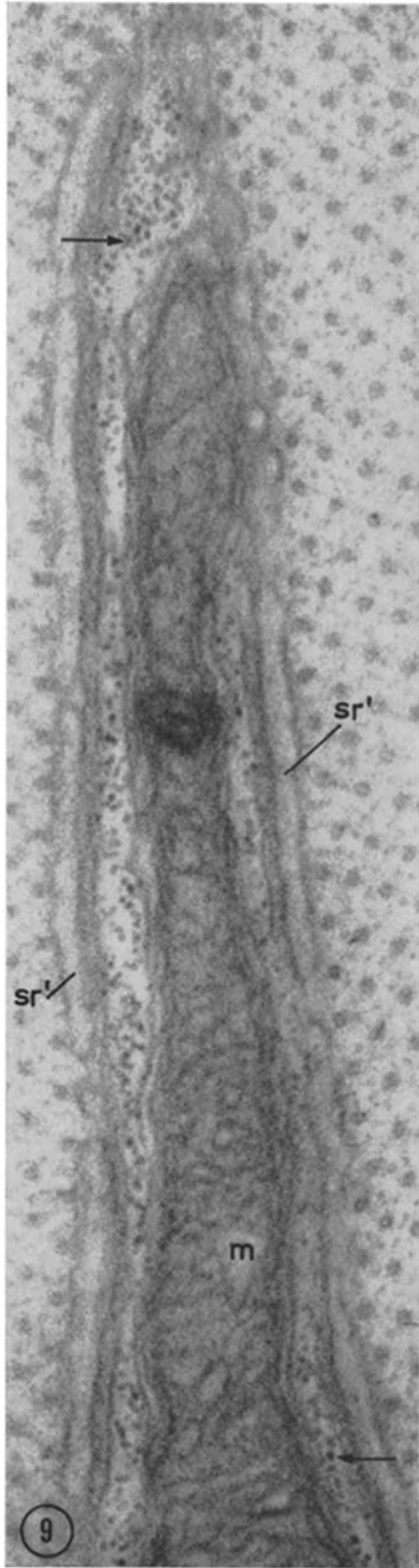
#### DISCUSSION

Physiological, biochemical, and structural observations have recently been integrated into a model which suggests that successive ranks of aligned T system tubules, in most synchronous models, act as channels for the internal conduction of an electrical signal (coupled with the passage of decremental or nondecremental neurally mediated depolarization along the fiber surface) which permits contraction by inducing the release of calcium ions from the sarcoplasmic reticulum or some part of this system. On this model, relaxation is believed to result from curtailment of the calcium-dependent myofibrillar ATPase activity by subsequent active reabsorption of calcium ions

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FIGURE 9 Another field of transversely sectioned ferritin-treated *Sympetrum* flight muscle. Note the ferritin molecules within the T system tubules (arrows) branching on either side of a mitochondrion (*m*): in the upper portion of the micrograph, the T system is dilated, a feature often seen also in glutaraldehyde-fixed material that has not been pretreated with ferritin solution (see Fig. 5). Note the complete absence of ferritin in the cisternae of the sarcoplasmic reticulum (*sr'*) forming dyads with the T system tubules.  $\times 125,000$ .

FIGURE 10 Illustrating the penetration of ferritin to the center of the fiber in *Sympetrum* flight muscle. In Odonata flight muscles, the nuclei are situated only in the central core of the fiber; this field includes a portion of a nucleus (*n*) limited by a typical double nuclear envelope (\*). Note the ferritin molecules within the inner extremities of T system tubules (arrows) approaching and adjoining the surface of the nucleus. These profiles are flanked, as elsewhere, by cisternae of the sarcoplasmic reticulum (as at *sr'*), while the "beaded" membrane profile seen at *sr* represents a region of the fenestrated sarcoplasmic reticulum present along the fibrils elsewhere than at the dyad levels.  $\times 105,000$ .



by the reticulum cisternae (Porter, 1961; Ebashi, 1961; Hasselbach, 1964; Constantin et al., 1965; Franzini-Armstrong and Porter, 1964; Lee, 1965; Peachey, 1965; Smith, 1965 *a*). The bulk of observations pointing to the role of the sarcoplasmic reticulum in cyclic calcium release and uptake has been carried out on mammalian skeletal muscle fibers, but it has recently been shown by Tsukamoto and coworkers (cited by Maruyama, 1965) that calcium binding may similarly induce relaxation in insect synchronous fibers and that relaxing factor preparations from these cells are also effective in presence of fibrils of mammalian muscle, an observation strongly suggesting that a common biochemical control of relaxation (and hence, probably, of contraction) is present in insect and mammalian synchronous muscle fibers.

Peachey (1965) has carried out a detailed study of the organization and extent of the T system tubules and the various regions of the sarcoplasmic reticulum in sartorius muscle of the frog. In this tissue, the T system tubules traverse the sarcoplasm between the cylindrical fibrils at the level of the Z bands, and each is flanked, in triad configurations, by clearly defined dilated terminal cisternae of the sarcoplasmic reticulum, ensheathing the fibrils. In this instance, the reticulum is rather sharply divided into three morphologically distinct portions: the wide terminal cisternae are continuous, via narrow necks, with tubular "intermediate cisternae," which coalesce to form a well demarcated "fenestrated collar" in the center of the A band level. Peachey has estimated that in this muscle the surface area of T system tubules in a fiber 100  $\mu$  in diameter is seven times that of the superficial plasma membrane, and, further, that the T system tubules and the terminal cisternae, respectively, account for about 0.3 and 5% of the fiber volume. The significance of these measurements in an analysis of the physiology of the fiber is fully discussed by Peachey, and two salient points may here be briefly alluded to. First, Peachey points out that the presence of transversely oriented tubules, connected with the surface plasma membrane, may account for the very high membrane capacitance values obtained in muscles with a well developed T system (against a standard provided by nerve cell-membranes with uninflated surface contours). Secondly, the estimated volume of the fiber occupied by the T system tubules (0.3%; Peachey, 1965 or less than 0.5%; H. E. Huxley, 1964) appears to be

small enough to preclude the possibility that myofibrillar activation is triggered by calcium release from the T system tubules per se, and the logical alternative for the location of the labile sump of calcium ions was considered to be the adjoining cisternae of the sarcoplasmic reticulum. In frog sartorius fibers, however, it seems possible that the terminal cisternae of the reticulum, closely adjoining the T system tubules, may contain sufficient calcium to effect contraction, if it is assumed that they contain calcium ions in the same concentration as in Ringer's solution (ca. 2 mM) in the relaxed muscle, and that this calcium is released and equilibrated throughout the fiber upon excitation (H. E. Huxley, 1964; Peachey, 1965).

It is interesting to consider the corresponding values, estimated from electron micrographs of *Sympetrum* flight muscle. In this tissue, the transverse tubular surface area is ca. 6  $\mu^2$  per 1  $\mu^2$  of surface cell-membrane<sup>1</sup>—somewhat less, that

<sup>1</sup> For these calculations the following estimates and measurements, based on electron micrographs of glutaraldehyde-fixed *Sympetrum* flight muscle fibers, were employed: 1. A mean fiber radius of 10  $\mu$ ; 2. A sarcomere length of 2.3  $\mu$ , with two T system tubules arising from the plasma membrane and traversing each side of each sarcomere, for a distance of 10  $\mu$ ; 3. A mean circumferential separation of 0.57  $\mu$  between vertical rows of T system tubules (0.46  $\mu$  across the mitochondria and 0.69  $\mu$  across the fibrils); 4. The T system tubules were assumed to have a rectangular cross-section, of side 0.04  $\times$  0.16  $\mu$  (mean estimate of 100 measurements), and were assumed to be 10  $\mu$  in length; 5. The dimensions of the portion of the sarcoplasmic reticulum immediately adjoining the T system tubules (entering into the dyad associations) were estimated to be similar to those of the T system tubules themselves; 6. The volume of the remaining portions of the sarcoplasmic reticulum cisternae was estimated, very approximately, to be halved by the presence of fenestrations, occurring between the dyad levels. It should be pointed out that (2) and (4) introduce a slight overestimate into the calculation of the area and volume occupied by the T system tubules, since (Fig. 5) the number of tubules stemming from the peripheral plasma membrane is somewhat reduced by tubule coalescence. This coalescence generally takes place close to the center of the fiber (see the similar muscle shown in Fig. 1), and probably reduces the initial number of tubules by 20 to 30% at the perinuclear surface. This natural deviation from the geometrical model introduces a corresponding diminution in estimates of the total volume of the accompanying cisternae of the sarcoplasmic reticulum.



is, than the corresponding value arrived at by Peachey (1965) in frog sartorius muscle. However, the latter calculations were based on a model fiber diameter of 100  $\mu$ , while the mean fiber diameter in the case of the dragonfly muscle described here is only 20  $\mu$ . Furthermore, the volume occupied by the T system tubules in *Sympetrum* muscle is relatively considerably greater than in frog muscle—ca. 2%, compared with ca. 0.3%. In *Sympetrum* fibers, terminal cisternae are not clearly differentiated, although each T system tubule is laterally associated with a region of the sheetlike reticulum cisternae which, unlike that occurring elsewhere, does not bear fenestrations. These specializations of the sarcoplasmic reticulum involved in the dyads account for about the same percentage of the total fiber volume as do the T system tubules themselves (ca. 2%), while a very approximate estimate of the volume of the remainder of the sarcoplasmic reticulum (necessitated by the extensive fenestration of these cisternae) indicates that it occupies about 5% of the fiber volume. In short, while the combined volumes (expressed as percentages of the fiber volume) of the T system tubules and the sarcoplasmic reticulum in dragonfly and frog sartorius muscle appear to be of the same order (respectively, 9 and 13%), it is clear that the distribution of size of the internal compartments in these two muscles is strikingly different. In the present absence of physiological data on the quantitative aspects of fibrillar activation in this insect muscle, it is perhaps unrewarding to speculate further on these values, but they bring to mind two possibilities which may be considered on the basis of the assumption that calcium-mediated control of activity occurs as in vertebrate fibers. (1) In this insect muscle the terminal cisternae are not well differentiated, but the entire sarcoplasmic reticulum (ca. 7% of the fiber volume) is similar in extent to the volume (ca. 5%) of the terminal cisternae of frog sartorius muscle, and it seems possible that in the former calcium release may be triggered by a signal transmitted via the T system tubules but may take place generally from the surfaces of the reticulum cisternae. On the other hand (2), the relatively large volume of the T system tubules in *Sympetrum* flight muscle conceivably reflects a substantial calcium exchange directly between

this compartment and the sarcoplasm, during activation.

The organization of the T system and sarcoplasmic reticulum described in detail in Odonata muscle appears to be representative of synchronous muscles of insects in general. An invaginated T system at the level of the midpoint between Z and H bands was described in *Periplaneta* leg muscle by Smith (1962), and later studies have shown (Smith, 1965 *b*) that a similar configuration is present in the synchronous flight muscle fibers of the Orders Ephemeroptera (mayflies), Trichoptera (caddis flies), Neuroptera (Lacewings, Antlions), Mecoptera (Scorpion flies), Orthoptera (cockroaches, grasshoppers), Lepidoptera (moths and butterflies) and cicadid Hemiptera. A striking cytological difference is found in insect asynchronous flight muscles, in which the rate of muscle contraction (and wing beat) may greatly exceed the rate of motor nervous stimulation. In these instances (e.g., Diptera (Smith, 1963); Coleoptera, Smith, (1961 *a*); Hymenoptera (Smith, 1962) and most Hemiptera (Smith, 1965 *b*)), an extensive T system is retained, invaginated, as in synchronous muscles, from the cell membrane, but accompanied by a very restricted sarcoplasmic reticulum system. In these fibers, the latter is represented by reduced and isolated flattened vesicles, nevertheless associated with the T system tubules in dyad configurations. It is likely (Pringle, 1965) that these asynchronous muscles may rely on normal T system-conducted signals for the initiation and maintenance of contraction, but that the phasing of length changes may be determined by the contractile fibrils themselves, governed by the mechanics of the thoracic segment and the loading imposed by the wings. This clear-cut cytological difference between synchronous and asynchronous fibers in insects, involving reduction of the sarcoplasmic compartment believed to be the site of calcium release and uptake, supports the conclusion that asynchronous muscles have evolved an unusual mechanism for the control of the frequency of contraction.

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