THE ORGANIZATION OF THE FLIGHT MUSCLE IN A DRAGONFLY, *AESHNA* SP. (ODONATA)

DAVID S. SMITH, Ph.D.

From The Rockefeller Institute. Dr. Smith's present address is Department of Zoology, Cambridge, England

ABSTRACT

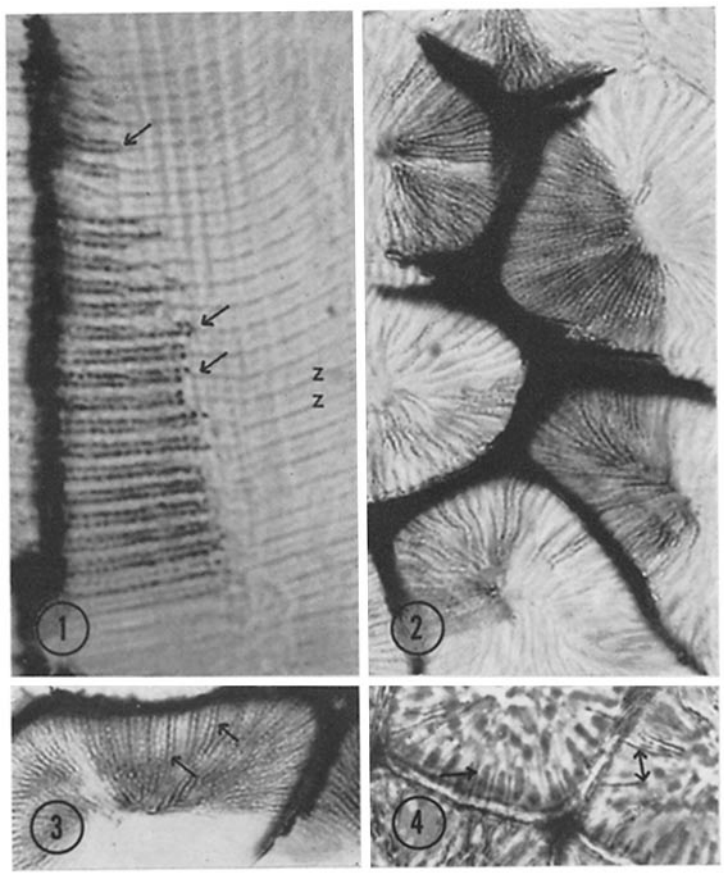
The structure of the flight muscle of a dragonfly (*Aeshna* sp.) has been studied with the light and electron microscopes, and the organization of this specialized tubular muscle is described. This tissue is characterized by the great development of the sarcosomes, which are slab-like and are arranged within the fiber opposite each sarcomere of the radially oriented lamellar myofibrils. A well developed and highly ordered sarcoplasmic reticulum is present, consisting of perforated curtain-like cisternae extending across the face of each fibril, together with tubular invaginations of the fiber plasma membrane situated within indentations in the sarcosomes and traversing the fibril surface midway between the Z and M levels. The structure of these fibers, and notably the organization of the reticulum, is compared with that of other types of muscle, and the possible role of the two components of the sarcoplasmic reticulum in the contraction physiology of the dragonfly muscle fiber is discussed.

INTRODUCTION

It has long been supposed that the striated muscle fiber of an insect is built on essentially the same plan as that of vertebrate and other invertebrate animals, and that these examples differ only through variation in the disposition and relative abundance of similar components, notably the fibrils, nuclei and sarcosomes. It was further realized that within each group of animals variation in detail occurs; for example, in an insect the flight muscle often differs from limb or intersegmental fibers and, again, from analogous muscles in representatives of other orders.

The comparative histology of insect muscle has received much attention; the extensive literature on the structural classification of these fibers has recently been drawn together in the schemas of Tiegs (1955), Pringle (1957), and Boettiger (1960), and it is upon these accounts that the following outline is based. The basic arrangement of arthropod leg or trunk muscle fibers, according to Tiegs, involves a radial disposition of strap-like fibrils surrounding a central core of nuclei.[1] This "tubular" muscle is found, for example, in the limbs of spiders, and among the insects in the leg muscle of many Orthoptera, Odonata, Lepidop-

[1] Tiegs (1955) states that "radial lamellar" fibers were first described by Aubert in 1853. He tentatively suggests that the supposed lamellae may in some instances actually represent rows of small cylindrical myofibrils. This is clearly not the case in dragonfly muscle, nor, for example, in spider leg muscle (Smith, unpublished observations). However, Tiegs illustrates the leg muscle of a grasshopper, *Caedicia*, in which radially arranged rows of cylindrical fibrils in the outer portion of the fiber are replaced in the center by an irregular array, an apparent transition between the radial and close-packed configurations.



FIGURES 1 TO 4

A series of light micrographs illustrating the results obtained by applying Veratti's modification of Golgi's "black reaction" to flight muscle fibers of the dragonfly *Aeshna*. All these figures are of material embedded in Esterwax.

FIGURE 1

A 5 μ longitudinal section. The region between two adjacent fibers has been heavily impregnated, and the sarcoplasmic reticulum is visualized in a portion of one of these. The striation is clearly seen; the Z bands with less dense M bands between them are shown, and the impregnated intramuscular structures appear as double rows of granules straddling the Z bands (arrows). The plane of this section corresponds to that of the electron micrograph (Fig. 9), and it is clear that the impregnated portion of the reticulum is the series of tubular plasma membrane invaginations traversing the surface of each fibril above and below the level of successive Z bands. × 850.

FIGURE 2

A transverse section of the material shown in Fig. 1, likewise showing incomplete impregnation of the reticulum of a group of fibers. In this 8 μ section, radially arranged filaments are seen extending from the fiber periphery to the central axis. During microscopic examination of the specimen, successive series of these filaments are brought into sharp focus, suggesting that they form precisely ordered ranks within the fiber. They are believed to correspond to the stained tubules seen in Fig. 1. The curtain-like cisternae described in the text (and see Fig. 23) are not impregnated, and this specificity may reflect chemical differences between the two components of the entire sarcoplasmic reticulum as defined in this paper. × 1800.

tera, Hymenoptera, and Diptera, a series including both "higher" and "lower" forms. It is believed that the muscles involved in the flight mechanism have been derived in evolution from trunk or leg muscles of the meso- and metathoracic segments of the primitive types. The metabolic demands made of the wing-bearing segments have resulted in the specialization of these muscles as, in the most extreme case, in the fibrillar muscles of Diptera, Hymenoptera, Coleoptera, and some Hemiptera. In certain Orthoptera (*e.g.* roaches, Blattidae) the tubular radial disposition of the fibrils has been preserved in the flight muscles, which do not exhibit the other features associated with active flight: a greatly increased sarcosome content, and an internalized tracheolar supply. These last specializations are met with in the more actively flying Orthoptera (*e.g.* locusts) and in Lepidoptera, where the radial or lamellar fibrils have been replaced by an irregular array of "close-packed" fibrils (somewhat resembling the situation met with in many vertebrate muscles), the diameter of these subcylindrical fibrils being only about one-half of that of true "fibrillar" fibers. Although this close-packed arrangement in some respects resembles the fibrillar type on morphological grounds, it is well known that whereas the close-packed muscles exhibit a *synchronous* (1:1) contraction response to motor nerve stimulation, fibrillar muscle is *asynchronous* and may have an extremely high contraction frequency, far in excess of that of impulses received (Boettiger, 1957; Pringle, 1957; Darwin and Pringle, 1959; etc.).

In this schema the dragonflies occupy a somewhat special position, for although they are powerful fliers, they have retained their basic radial arrangement of the flight muscles, which respond synchronously, and the most striking specialization observed in such fibers is their enormously high content of mitochondria, which are individually of great size.

In a recent paper (Smith, 1960) the structure of fibrillar flight muscle of the beetle *Tenebrio* was described. In this insect, as in bees, wasps, and flies, the high sarcosome content of the tissue reflects its activity and high metabolic rate, which may also be correlated directly with the extensive tracheolar system which invades the fiber. It was shown that in *Tenebrio* the ingrowth of the respiratory exchange system has an important effect upon the topographical relationship between the fiber plasma membrane and the contractile material, since the tracheoles carry in with them sheaths of fiber plasma membrane from which the tracheoles are separated by an extracellular gap. It was suggested that the fiber membrane so internalized may constitute the internal system by which excitation is channeled within these fibers, which, despite their high repetitive frequency, are nevertheless several times the maximum diameter of vertebrate muscle fibers. The flight muscle of the dragonfly, described in the present paper has, for a synchronous muscle, an unusually high rate of contraction, *Aeshna coerulea* having a wing-beat frequency of 25 cycles per second (Heidermanns, 1931) and *A. juncea* and *Sympetrum vulgatum*, 35 cycle/sec and 46 to 52 cycle/sec, respectively (Sotavalta, 1947). However, it shows an essential difference from the asynchronous muscle of the beetle in that *the tracheolar supply to the fibers lies exclusively between them and at no point makes the slightest inroad into them. Thus in the present case, if an internal conduction pathway for the nerve excitatory impulse is present, corresponding to that of fibrillar muscle, then it must be independent of the tracheoles*, and it is with this fact in mind that this example should be compared with fibrillar muscles of other insects, and also with muscle of vertebrates and other forms, in terms both of structure and of physiology.

MATERIALS AND METHODS

The dorsoventral mesothoracic flight muscles of a dragonfly (*Aeshna* sp.) were employed. The thorax

FIGURE 3

A field similar to that shown in Fig. 2, in which pairs of filaments (arrows) are seen, one filament lying on each side of the radially oriented myofibrils. × 1800.

FIGURE 4

A lightly impregnated transverse section of this tissue, viewed in phase contrast. The sarcosomes appear dark, and the fibrils lie between them. The dense filaments (the intermediary tubules) are visible running inward from the surface of the fiber toward its axis (arrows). × 1800.

of the insect was separated from head and abdomen and bisected medially in sucrose-containing 1 per cent OsO_4 buffered with veronal-acetate at pH 7.7. The fixation vessel was surrounded with crushed ice and the material fixed for 90 minutes and then transferred directly to 70 per cent ethanol. Portions of the anatomical muscles (each containing many fibers) were removed at this point and dehydration was completed. Subsequent embedding procedure in Epon was according to Luft (1961). Sections were cut on a Porter-Blum microtome using glass knives, mounted on collodion-coated grids, "stained" with lead hydroxide, and examined in an RCA EMU-2 microscope. Histochemical tests for lipids and carbohydrates (Oil Red O, PAS) were carried out on thick sections of osmium-fixed material embedded in methacrylate.

The "black reaction" of Golgi, employed to impregnate the intermediary tubules of the sarcoplasmic reticulum (Figs. 1 to 4), was carried out according to the modifications suggested by Veratti (1902). Good, though characteristically incomplete, staining of this component was obtained with the following schedule: (i) Fix in a mixture of 1 per cent OsO_4 and 3 per cent $K_2Cr_4O_7$ (1:4) for 10 days. The muscles in each half of the thorax were exposed as fully as possible to the fixative by removal of the surface tracheae and the pleural thoracic wall. (ii) Transfer the muscles to 0.75 per cent $AgNO_3$ (several changes, until the solution is free of precipitate). Leave for 3 days, agitating occasionally. (iii) Replace in the fixative (i) for a further 3 days. (iv) Transfer once more to $AgNO_3$ solution for 3 days. (Note: All these steps are carried out at 15°C.) The muscles were then dehydrated in ethanol, cleared for several days in thick cedarwood oil, embedded in Esterwax (BDH), and sectioned at 5 to 8 μ.

FIGURE 5

A phase contrast micrograph of a transversely sectioned group of fibers of *Aeshna* flight muscle. The radial orientation of their component structures is striking: the sarcosomes appear dark and the myofibrils light. The central core of nuclei in each fiber is one of the characteristics of "tubular" muscle. This figure is of a 1.5 μ section of Epon-embedded material. × 1000.

FIGURE 6

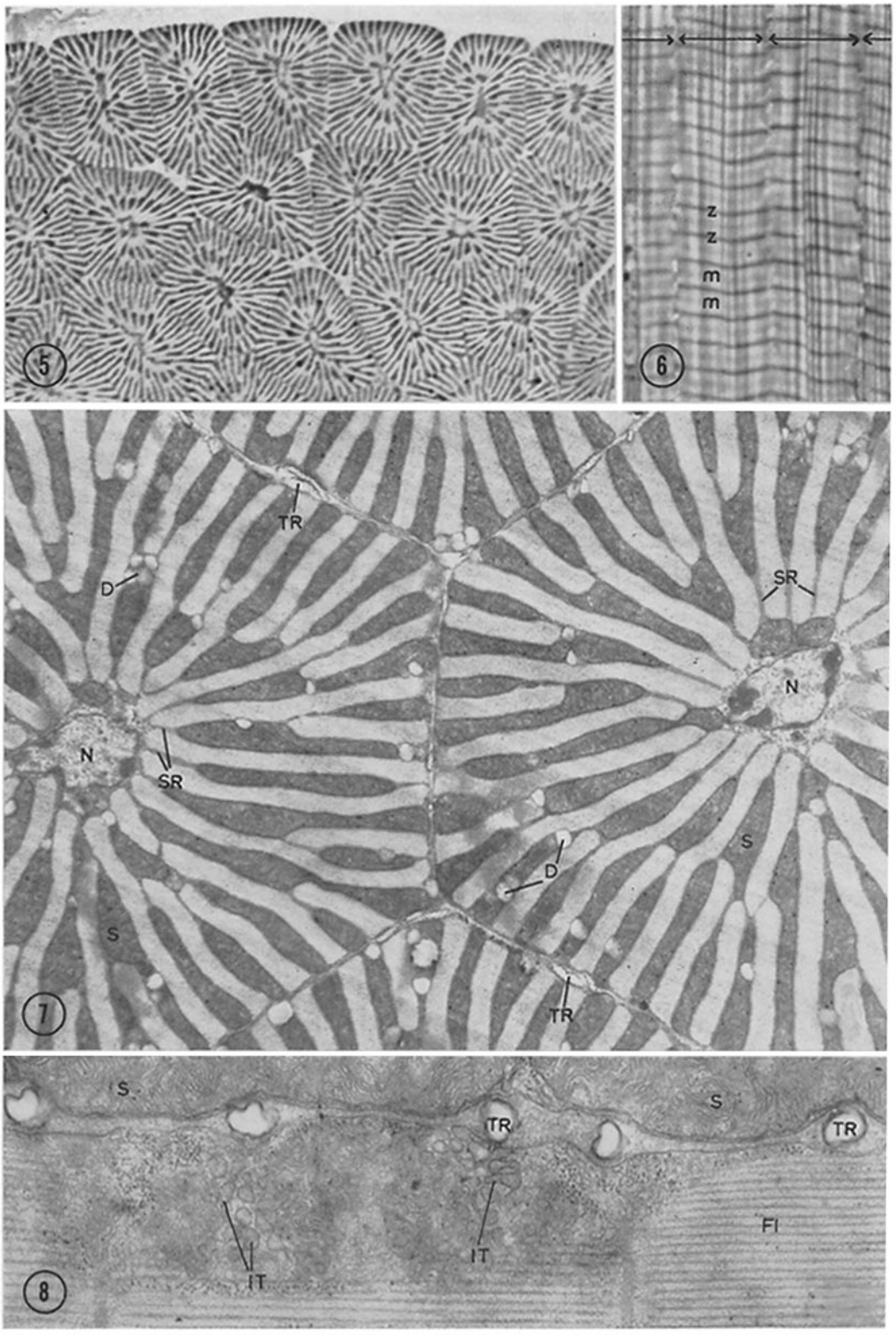
A longitudinal section of portions of four fibers (arrows). The sarcomere period of 3.2 μ is demarcated by the Z bands, between which lie the fainter M bands. The I bands of this muscle are very short (a feature that may be correlated with the relatively isometric conditions under which it operates) and are not visible here. On the left of the figure the fibrils are sectioned more or less frontally, and on the extreme right perpendicularly to their long axis. In the latter instance the dark sarcosomes are seen lying between the narrow fibril profiles. An Epon-embedded 1.5 μ section viewed in phase contrast. × 1250.

FIGURE 7

A survey electron micrograph of a portion of four transversely sectioned fibers. Note the radially arranged fibrils and sarcosomes (*S*), surrounding the sarcoplasmic reticulum (*SR*) and the central nuclei (*N*). The tracheoles (*TR*) lie exclusively between the fibers and do not indent their surface. The irregular bodies (*D*) situated between the sarcosomes and fibrils have not been identified with certainty, but they may contain lipid and carbohydrate materials. × 7000.

FIGURE 8

A longitudinal section through peripheral areas of two adjacent fibers. In the upper of these the plane of section passes through sarcosomes (*S*) and in the lower through a fibril (*FI*). The insertion of the tracheoles (*TR*) between the fibers is the most intimate relation between respiratory and contractile portions of this type of muscle. This section passes just above the surface of a fibril in the central region of the figure; the irregular profiles (*IT*) lying opposite each side of the Z bands are believed to represent sections through the highly convoluted plasma membrane invaginations (intermediary tubules) seen to better advantage in Figs. 17 through 19. × 25,000.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8

THE FIBRILS

The radial arrangement of the components of *Aeshna* flight muscle around a central core of nuclei is clearly seen in the phase contrast micrograph reproduced in Fig. 5, which includes transverse sections of several fibers each 20 to 30 μ in diameter; this diameter is only one-tenth of that of *Tenebrio* flight muscle fibers, but comparable to that of many muscles in vertebrates. Even at this low magnification, the great development of the sarcosomes (noted by Marcus, 1921) is evident; these appear as dark wedges inserted between the light fibrils. The identity of these components may be established by comparing this figure with the survey electron micrograph shown in Fig. 7. The strap-like fibrils, each 0.5 to 0.8 μ wide, extend radially across part or most of the distance from the nucleus to the periphery; thus in many instances a diametric transect of a fiber passes through two fibrils only. Each fibril is closely applied to the outer margin of the fiber and ends a short distance from the nucleus. At this magnification almost the entire remaining portion of the fiber appears to be occupied by profiles of sarcosomes, but in the narrow spaces intervening between fibrils and sarcosomes lie the membranes of the sarcoplasmic reticulum, which will be considered in detail in due course. In methacrylate-embedded material the double array of primary and secondary myofilaments, described by Huxley and Hanson (1960) in insect and vertebrate muscles, has been identified here, but in the Epon-embedded material illustrated here only the wider primary array of filaments is clearly visible.

From the appearance of these fibers in transverse section, it is evident that those in the longitudinal plane will present much variation in the aspect of fibrils and interposed sarcosomes, depending on whether the section is perpendicular or tangential with respect to the fibril face. An instance of the former is shown in Fig. 9, which includes several sarcomeres about 3.2 μ in length. The M band is indistinct, but the central region of each sarcomere is demarcated by a localized scattering of small granules of unknown nature, which have also been observed in *Tenebrio* muscle. In the phase contrast micrograph (Fig. 6) both Z and M bands are clearly visible. A more tangential

FIGURES 9 AND 10

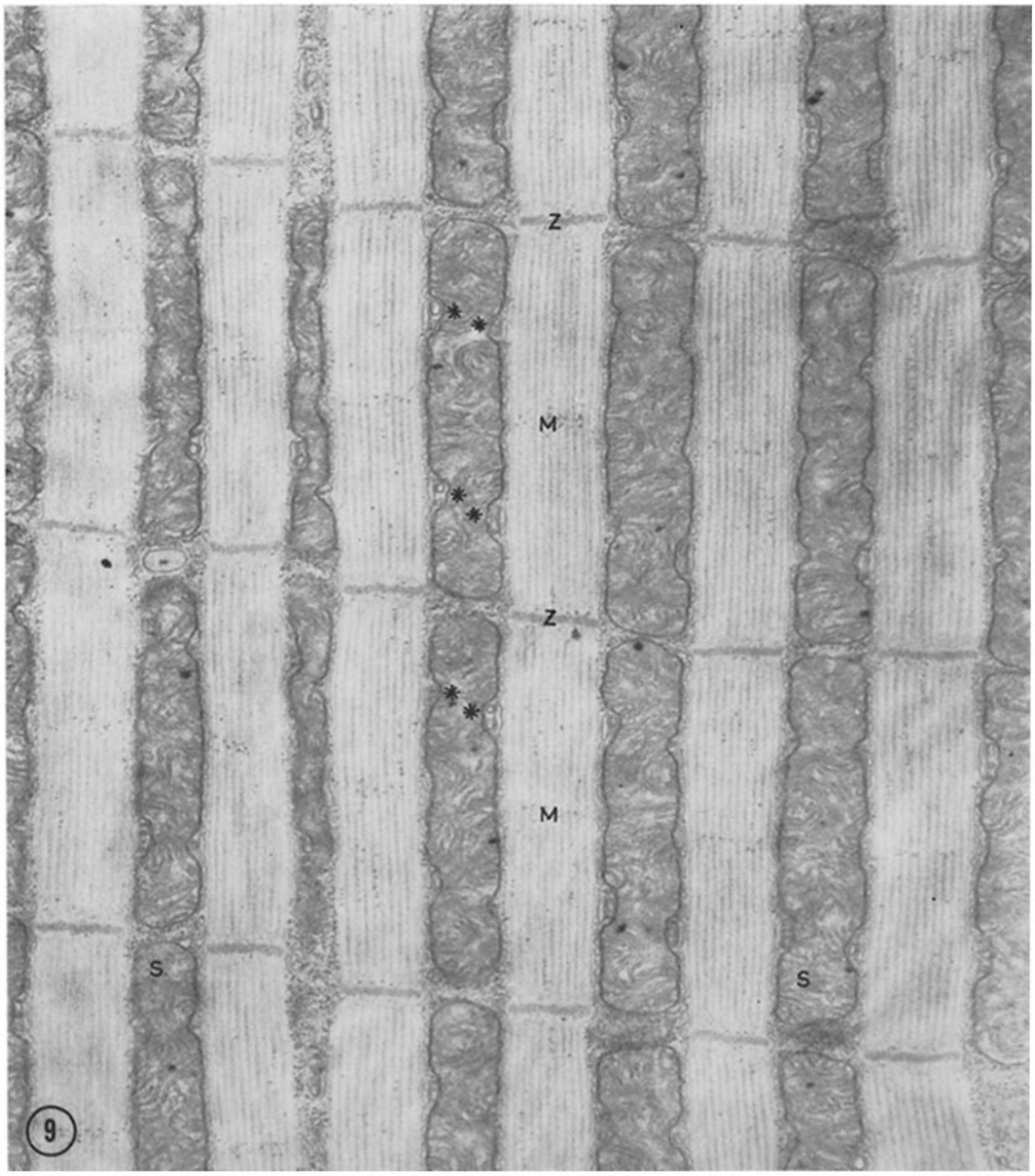
Electron micrographs of longitudinal sections of *Aeshna* flight muscle cut in a plane perpendicular to the long axis of the fiber.

FIGURE 9

In this micrograph two entire sarcomeres and portions of adjacent ones are included, over a width of six fibrils. The sarcomeres, 3.2 μ in length, are demarcated by the Z bands, while in the mid-sarcomere region the level of the M band contains numbers of small granules of unknown nature. The correspondence between the positioning of sarcosomes and of sarcomeres is very precise. The sarcosomes (*S*), *ca.* 3 μ high and 0.4 to 0.6 μ wide, are arranged radially within the fiber, sometimes reaching a length of almost 10 μ. Note that each sarcosome is indented (*) at a point midway between the Z and M levels of the fibrils. The detailed organization of these indentations is seen more clearly at higher magnification in Fig. 10. × 16,000.

FIGURE 10

A higher power electron micrograph of one sarcomere (in the same plane of section as in Fig. 9), together with portions of the two accompanying sarcosomes (*S*). Within the indentations along the sides of the latter (*) are seen isolated membrane-limited profiles. Between the sarcosome surfaces and the fibrils, and extending across the entire sarcomere, lies a membrane system (*SR*) seen in surface section in Fig. 17, interpreted here as a perforated curtain-like cisterna of the sarcoplasmic reticulum. This passes across the mouth of each sarcosomal indentation or notch (arrow), which contains profiles of the intermediary tubules (*IT*). The three-dimensional reconstruction showing the relationship between these membrane components of the fiber and the fibrils and sarcosomes, based on electron micrographic evidence, is shown in Figs. 23 and 24. × 41,000.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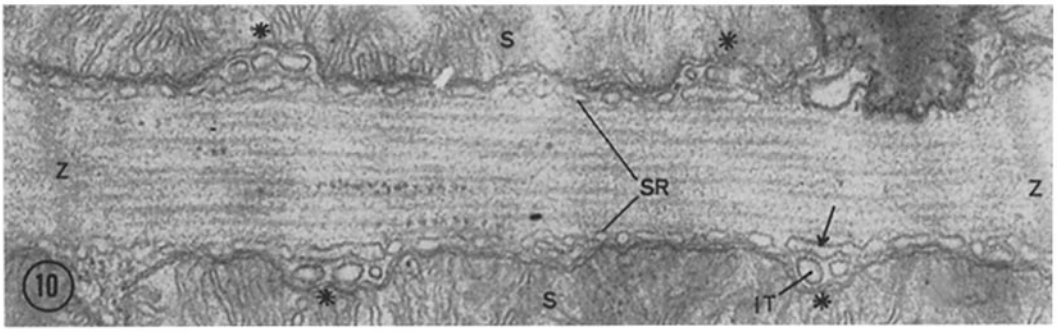
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section of a fiber is seen in Fig. 20, and here the Z band is similarly evident and the I bands very narrow, presumably reflecting, as in fibrillar muscle, the relatively short distance by which these muscles contract during the flight cycle.

The possibility of branching of the fibrils, suggested by the variable length of their profiles seen in transverse sections, appears to be ruled out by observations made in the longitudinal plane; each fibril is a strap-like structure up to 10 μ in breadth, about 0.6 μ wide, and, in this species, several mm in length, extending as an independent unit from ventral to dorsal thoracic insertions.

THE SARCOSOMES

The sarcosomes of this dragonfly flight muscle are larger than those of any other cell so far examined in the electron microscope. Despite their great size, their internal organization is similar to that of other mitochondria and, as seen in Figs. 18, 20, 21, etc., they contain large numbers of cristae, arranged in whorled and subparallel arrays. In transverse sections of the fiber, the sarcosomes are seen to lie between the fibrils in a similarly radial disposition. Their length is variable but may approach the extent of the fiber radius (10 μ), and their width varies from 0.6 to 1.0 μ. Orientation and distribution of sarcosomes with respect to the cross-striation of the fibrils has been described in several types of muscle in both light and electron microscopic studies, and this phenomenon is most striking in *Aeshna* flight muscle, for, as may be seen in longitudinal sections (Fig. 9), the sarcosomes are arrayed alongside each fibril precisely opposite the sarcomeres defined by successive Z bands.

The slab-like form of these sarcosomes and of the fibrils ensures a very large area of contact or close apposition between them, a feature presumably reflecting the high metabolic activity of this tissue. Measurements of longitudinal and transverse sections carried out with a map measurer suggest that a *minimum* of 70 per cent of the surface area of each fibril is in contact with sarcosomal limiting membranes, or, rather, separated from them by a very narrow space in which lies the sarcoplasmic reticulum (Fig. 22). A similar value obtains in the case of *Tenebrio* flight muscle, in which the cylindrical fibrils are packed in with sarcosomes distributed in a fashion unrelated to the striation (Smith, 1961). In addition it was estimated that in *Aeshna* flight muscle the sarcosomes account for no less than 40 per cent of the fiber volume.

THE NUCLEI AND SURROUNDING SARCOPLASM

A row of nuclei occupies the central core of each fiber in *Aeshna* flight muscle, the arrangement characteristic of insect "tubular" muscle. Each nucleus is about 2 μ in diameter and several microns in length. Whereas in *Tenebrio* flight muscle, nuclear pores were found to be of rare

FIGURES 11 AND 12

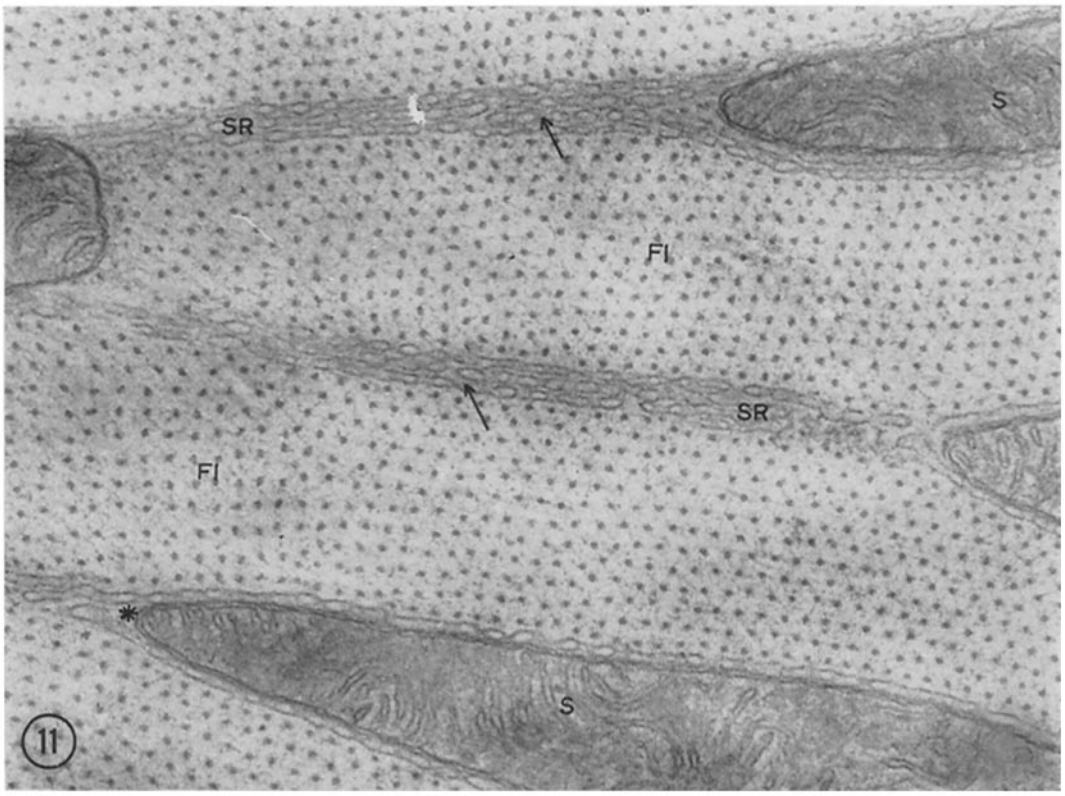
Transverse sections of *Aeshna* flight muscle in a plane lying between the sarcosomal indentations (see Fig. 9), close to either the Z or the M region of the fiber.

FIGURE 11

Portions of four fibrils are included (*FI*) together with sarcosomes (*S*) and profiles of the cisternae of the sarcoplasmic reticulum (*SR*). Two to four such cisternae are present where no sarcosome intervenes (arrows), while only one or two occur, as in the lower part of the field (*), separating the mitochondrial surface from that of the fibrils. These cisternae are perforated and curtain-like (see Fig. 17), hence the "beaded" appearance afforded by sections in this plane. × 50,000.

FIGURE 12

A more extensive aspect of a central region of a fiber sectioned in a plane similar to that shown in Fig. 11. The cisternae (*SR*) frequently loop around the inner extremity (*) of the fibrils (*FI*) and pass close to the nucleus (*N*) when no sarcosome stands in their way. × 28,000.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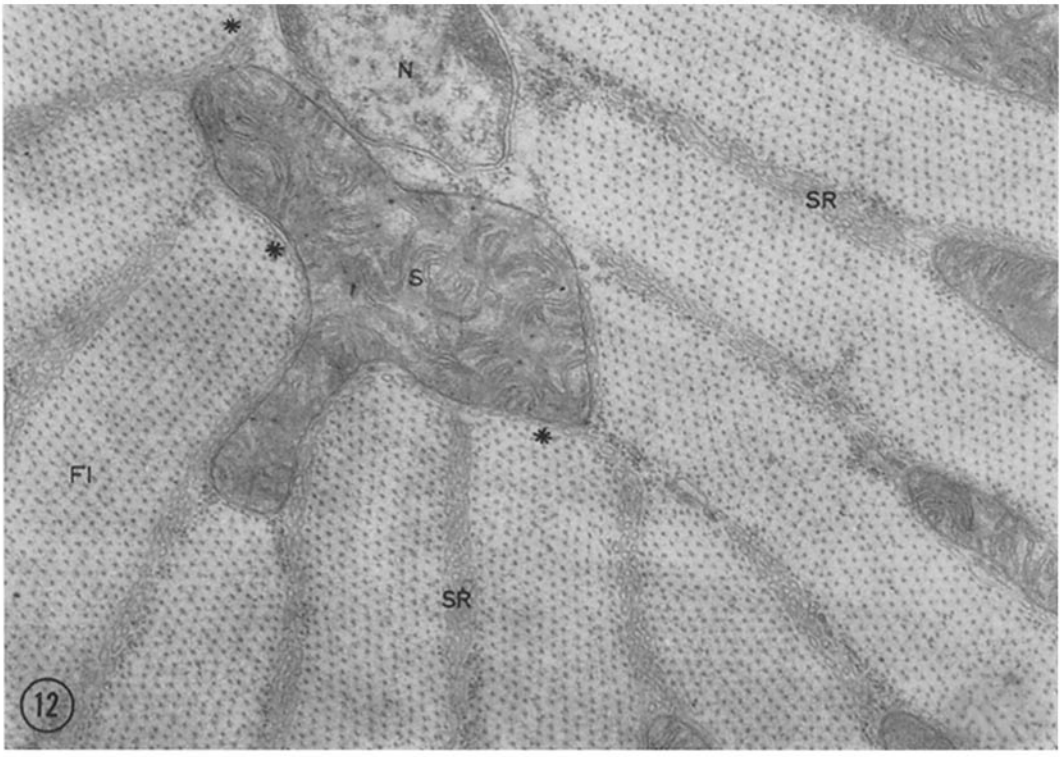
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occurrence, in *Aeshna* such pores (*ca.* 50 to 60 mμ in diameter) are very frequent (Fig. 16). The fibrils and sarcosomes terminate a short distance from the nuclear envelope; the narrow region of sarcoplasm that intervenes contains groups of granules, 150 to 250 A in diameter, and these extend into the narrow interstices between the fibrils and the sheet-like cisternae of the sarcoplasmic reticulum. In addition, the outer membrane of the nuclear envelope bears small numbers of particles, 150 A in diameter, presumably of ribonucleoprotein, differing from the sarcoplasmic granules in their rather smaller size. The latter are especially abundant in the spaces between adjacent sarcosomes at each Z band, but also extend across the face of each fibril (Fig. 17). Fawcett and Selby (1958) issued a timely warning against the practice of indiscriminate labeling of cytoplasmic granules and particles as RNP, a warning that is apposite here. Thick (3 μ) longitudinal sections of methacrylate-embedded fibers of *Aeshna* muscle give a marked diffuse positive PAS reaction throughout the sarcomere, while the Z bands are more intensely stained though less so than the area aroung the nucleus. This PAS-positive material is not digested by saliva or by a solution of crystalline amylase. However, glycogen (with which these sarcoplasmic granules

FIGURE 13

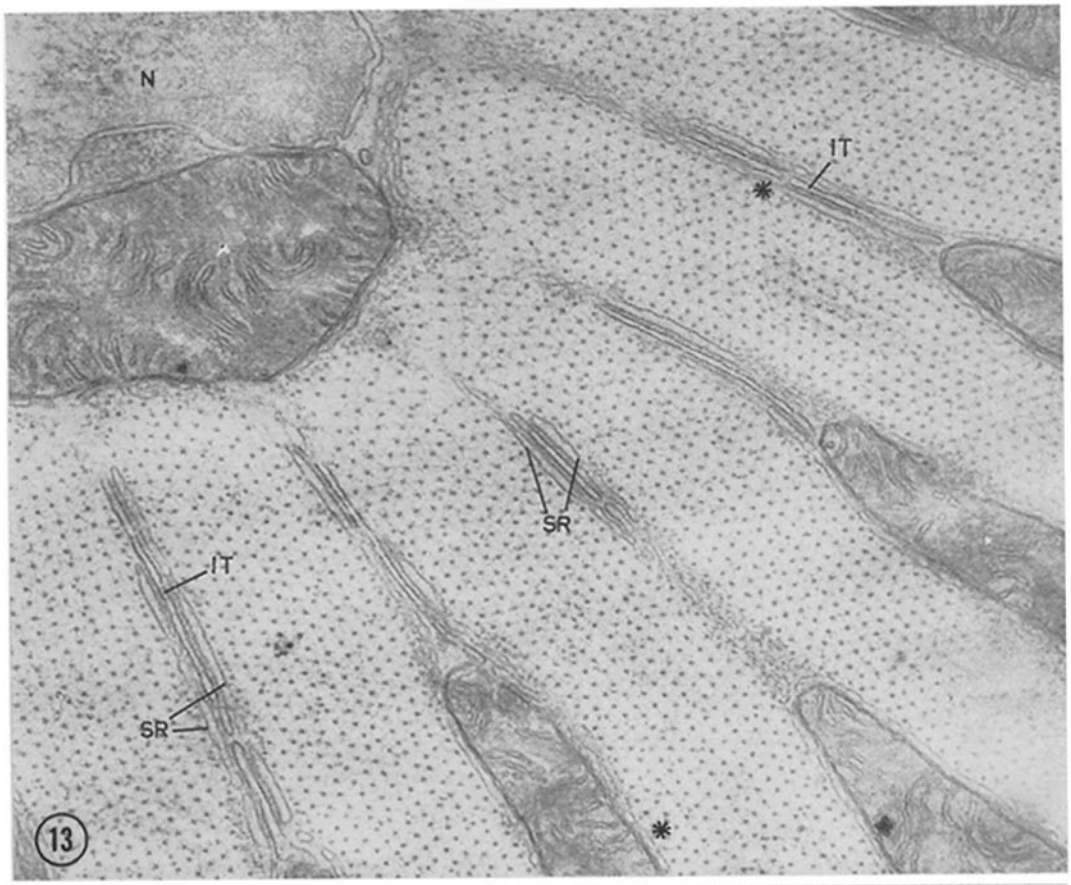
A transverse section passing through the level of sarcosomal indentations, shown in a plane perpendicular to that in Fig. 9. In the present micrograph, elongated profiles take the place of the cisternae indicated in Figs. 11 and 12. It is suggested that these represent two different components of the sarcoplasmic reticulum: first, sections through tubules lying in the indentations of the sarcosomes (*IT*) (here seen more or less in longitudinal section), and second, flanking these, profiles of those portions of the cisternal curtains adjacent to them (*SR*). The latter, as may be seen in Fig. 10, appear to have few perforations opposite the indentations, hence relatively uninterrupted profiles are to be expected here. Regions of transition to the perforated portion of the cisternae are indicated by asterisks. Further evidence on the nature of these two components of the sarcoplasmic reticulum is presented in the next figure. *N* denotes the nucleus. × 37,000.

FIGURE 14

The plane of section of this micrograph is identical with that of the last, and represents an area at the periphery of the fiber. The plasma membrane (*PM*) is clearly invaginated (between arrows) alongside a sarcosome (*S*), and this invagination is flanked by elongated profiles (*SR*) adjoining the fibril, having no connection with the plasma membrane. It is suggested that the profiles occupying the notches in the sarcosomes (Figs. 9 and 10) represent tubules derived from the plasma membrane which, as may be inferred from frontal or surface sections (Fig. 17), are highly convoluted. This convolution results in the apparent interruption of the tubules met with in transverse sections of the fiber (Fig. 13). The tubules appear to be selectively impregnated by the "black reaction" (Figs. 1 through 4) where they are seen to extend from the periphery of the fiber to the central axis. × 19,000.

FIGURE 15

An oblique transverse section, to be compared with Figs. 9, 12, and 13. The Z bands of successive fibrils are included in the upper part of the micrograph. Some distance from the Z bands the accompanying sarcosomes are indented (*) (as in the longitudinal section, Fig. 9), and within each indentation lies a membrane profile, the elongated appearance of which, as well as the increased distance from the Z bands (as compared with Fig. 9), is attributable to the obliquity of the section. The cisternal curtains (*SR*) between the fibrils and sarcosomes are perforated over most of their extent (*cf.* Fig. 11) except opposite the indentations (arrows), where they thus afford more unbroken profiles. × 19,000.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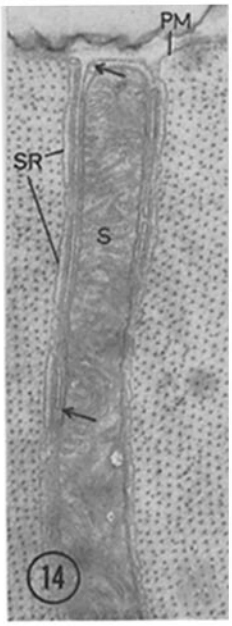
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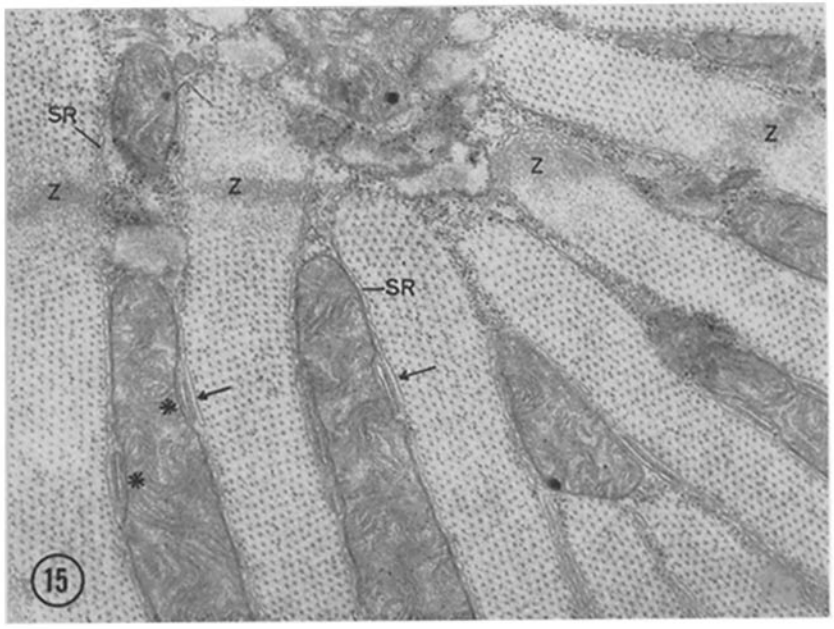
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are probably to be identified) often cannot be removed enzymatically from osmium-fixed material. Fawcett and Selby (1958) found "enormous numbers of moderately dense granules 100–250 A in diameter" in skeletal and cardiac muscle of the frog and turtle, and these they identified as particulate glycogen. This form of glycogen may be compared with the rosettes of granules described in liver (Carasso, 1960) and, like these, are intensely "stained" with solutions of lead hydroxide.

The PAS reaction also demonstrates another sarcoplasmic component: the large (0.4 to 0.6 μ) bodies located, for the most part, opposite the Z bands and inserted between the sarcosomes (Figs. 17 and 20). The stainability of these structures is similarly not destroyed by amylase or saliva treatment in methacrylate-embedded sections. They have a rather osmiophilic amorphous content suggestive of lipidic material; if they are indeed fat droplets perhaps associated with polysaccharide, then from their osmiophilia and their failure to stain with Oil Red O may be inferred their unsaturated nature. It is interesting to note in this connection that George and his coworkers (1958) found unusually large amounts of lipase in dragonfly flight muscle, though unfortunately values of the Q_{10} of this tissue (which may contain substantial quantities of carbohydrate and lipid) do not seem to be available. In other insects it is known that the immediate energy source may be either lipid (*e.g.* locusts, Krogh and Weis-Fogh, 1951, butterflies, Zebe, 1954) or carbohydrate (*e.g.* fruit flies, Wigglesworth, 1949; bees, Beutler, 1937). It would be by no means surprising should both prove to be present together, as may be the case here.

THE TRACHEAL SUPPLY TO THE FIBERS

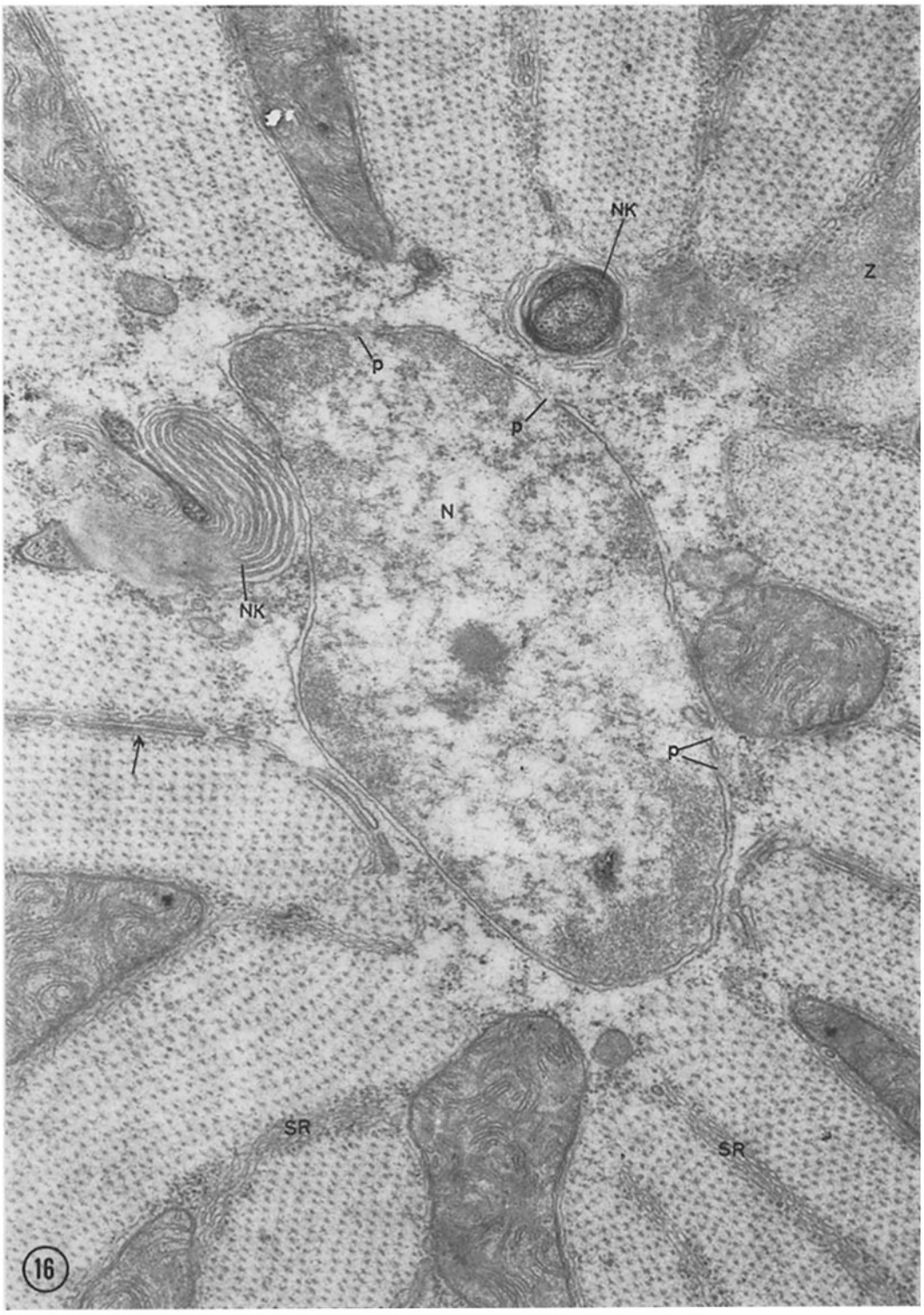
It will be remembered that invasion of the fiber by the fine tracheolar branches is a feature associated with the flight muscles of the "fibrillar" type, and to some extent with close-packed fibers as in the active Orthoptera (Vogell *et al.*, 1959). The flight muscles of dragonflies, in common with insect leg and trunk muscles, possess only a surface tracheation, the tracheoles passing between the fibers and never indenting them. These tracheolar branches, which arise from tracheal end-cells likewise inserted between the fibers, may be as little as 0.1 to 0.2 μ in diameter. Figs. 7 and 8 illustrate their characteristic disposition. In *Tenebrio* the tracheoles were found to draw with them into the fiber concentric sheaths of muscle plasma membrane (Smith, 1961), and it is evident that in *Aeshna* the situation is quite different in this respect. This will be considered in the next section, when the probable pathway of impulse conduction within the fiber is discussed.

THE PLASMA MEMBRANE AND THE SARCOPLASMIC RETICULUM

At the time when the techniques of electron microscopy were first applied to considerations of muscle structure, the early descriptions (notably those of Veratti, 1902) of a filamentous reticular system distinct from the sarcosomes and lying in the interfibrillar sarcoplasm were virtually forgotten. The first electron microscopic description of a membrane-bound system of this nature, the "sarcoplasmic reticulum" of Bennett and Porter (1953), was followed by many others, and it became evident that a reticulum of this nature is present in one form or another in every striated muscle so far examined, whether from vertebrates or from other animals. Porter and Palade (1957) recognized that in the skeletal and cardiac fibers they examined, the reticulum is arranged in series of units that are repeated in register with the striation of the myofibrils. Between the "terminal cisternae" of adjacent segments of the reticulum they observed small discrete "intermediary vesicles" which with the cisternae

FIGURE 16

An electron micrograph of the central region of a fiber. Note the nucleus (*N*) and the double nuclear envelope with numerous wide pores (*p*). The nebenkern-like structures of whorled membranes (*NK*) occur quite frequently in this type of muscle, but their significance is not known. This section passes through a Z band in the upper right of the field, and is presumably slightly oblique. Whereas in most regions the plane of section passes solely through the perforated cisternae of the sarcoplasmic reticulum (*SR*), in places the more continuous profiles characteristic of the level of the sarcosomal indentations are included (arrow) because of slight obliquity in the section or through some degree of lack of register in the alignment of neighboring myofibrils. × 33,000.


NK
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NK
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SR
16

flanking them constituted the "triads." Specializations of this general nature have subsequently been found in other instances, and many muscles may be placed in one of two groups, depending on whether the triads or corresponding structures lie opposite the Z bands or, alternatively, at or near the level of the A-I junction. However, the position of most insect and other invertebrate muscles in this respect is not yet clear, though in the case of insect fibers available evidence suggests that specialization of the sarcoplasmic reticulum typically occurs at two levels alongside each sarcomere, as in *Periplaneta* flight muscle (Edwards *et al.*, 1956), *Blatta* abdominal muscle (Edwards, 1959), and tymbal muscle of the cicada *Tibicen* (Edwards *et al.*, 1958). The fibrillar flight muscle of *Tenebrio* exhibits a highly modified disposition of the reticulum (Smith, 1961) in which "dyads" consisting of profiles of isolated vesicles associated with tubules derived from the plasma membrane of the fiber are abundant, though not oriented or distributed in register with the striation. In *Aeshna* flight muscle, the situation is strikingly different from this last example.

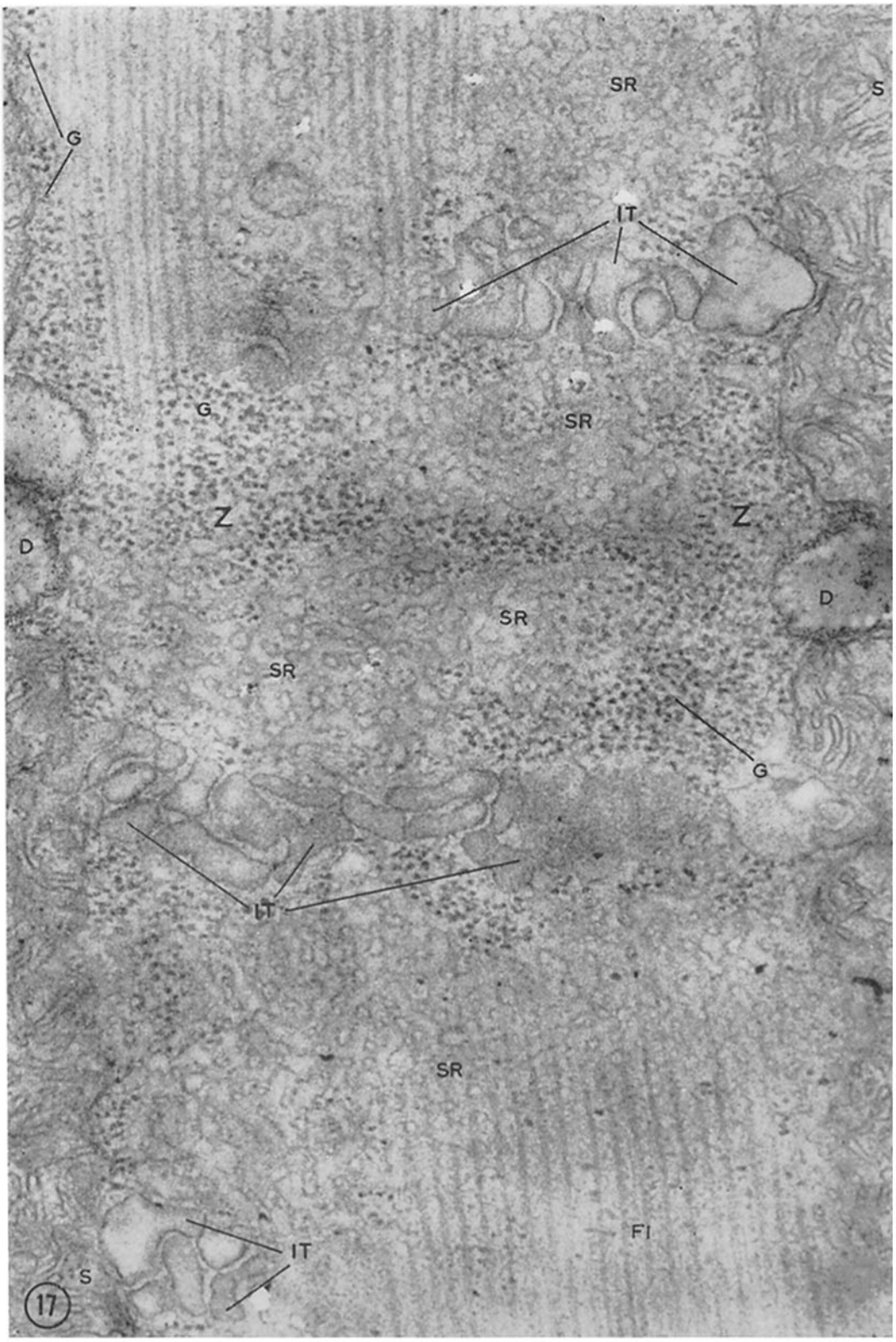
In the low power longitudinal section shown in Fig. 9 it may be seen that the sarcosomes are indented on each side in two places, at levels almost midway between the Z bands and the region corresponding to the M band, which is marked by a series of sparsely distributed granules. Within each of these indentations lie from one to three circular or ovoid membrane profiles. From the universality of this appearance, it may be inferred that two tubular systems (continuous or otherwise) run alongside the face of each sarcomere, perpendicular to the plane of section shown in Figs. 9 and 10. In addition, a second membrane system is present, running between each fibril and the adjacent sarcosomes without interruption at the Z band level or elsewhere, and thus traversing the sarcosomal indentations (Figs. 9, 10, and 15). At first sight the latter system appears, in longitudinal sections, to consist of a single or, especially in the mid-sarcomere region, of a double series of vesicles. However, close examination shows that each such series represents a profile of a membranous curtain-like cisterna which contains frequent small fenestrations and extends over the entire surface of each fibril. The fenestrations are more or less absent only opposite the indentations in the sarcosomes and their enclosed membrane profiles (Fig. 10).

Consideration of a complex arrangement of this nature cannot, of course, be based on sections in one plane. It is clear that the appearance of the reticulum elements will vary, depending on the level along the sarcomere included in transverse sections. In addition, both oblique transverse sections and especially sections passing almost parallel to the surface of a fibril will be informative, as the latter, for example, should include an extensive surface view of the components of the reticulum. As an additional guide to the inter-

FIGURE 17

An electron micrograph of a section of *Aeshna* flight muscle which passes just above the surface of a fibril (*FI*). A Z band crosses the field above the center, and between the ends of the sarcosomes (*S*) at this level (*cf.* Fig. 9) are inserted irregular bodies (*D*) which may contain lipid and polysaccharide materials.

On each side of the Z band and equidistant from it are seen series of irregular membrane-limited profiles (*IT*) which are held to represent sections of convoluted tubular invaginations of the plasma membrane. At the lower left, a further portion of this system lies within the plane of section, adjoining the adjacent Z band lying immediately below this field. Traversing the level of the Z band and extending across much of the fibril surface is visible, in surface aspect, a perforated curtain-like cisterna (*SR*) seen in other planes of section in Figs. 10 and 13 through 16. The fenestrations are irregularly distributed; each is 200 to 300 A in diameter, and together they represent about 15 to 25 per cent of the total area of the cisterna. The deeply "stained" granules (*G*) concentrated above the Z band and between the fibril and adjacent sarcosomes may be particulate glycogen. Further information on the disposition and nature of the membrane profiles straddling the Z band is offered in Figs. 18 to 20. Note that the level of the Z band is indicated (*Z Z*) as situated opposite the ends of the sarcosomes, although the fibril surface lies just beneath the plane of section. × 46,000.


SR
S
G
IT
G
SR
Z
Z
D
D
SR
SR
G
IT
SR
FI
IT
S
17

pretation of electron micrographs illustrating these planes of section, the reader may find the reconstruction diagrams (Figs. 23, 24) useful at this point. Fig. 15 shows an oblique transverse section passing through a series of Z bands of adjacent fibrils. Here, as in the longitudinal section (Fig. 9), the narrow continuous system of membranes lying between the fibrils and sarcosomes is seen, and likewise, at some distance from each Z band, the sarcosomal indentations containing in this case oblique profiles which are therefore somewhat longer than those noted in more precisely longitudinal sections. In this figure the terminations of the sarcosomes on either side of the Z bands may also be pointed out.

In the transverse sections shown in Figs. 11 and 12 the reticulum is represented by profiles corresponding to those lying alongside the length of the fibrils in longitudinal sections (Figs. 9 and 10), and it appears that the plane of section in the present instance lies between the levels of sarcosomal indentation, either just on one side of the Z region or between the indentations in the mid-sarcomere region. The continuous nature of the reticulum is more clearly seen at higher magnification (Fig. 11); this micrograph also demonstrates that where the reticulum borders a sarcosome one or two cisternae are found, whereas three or four are closely applied to one another between adjacent fibrils in those places where no sarcosome is interposed. Thus far, then, the similarity in the appearance of the cisternae in these longitudinal and transverse sections supports the suggestion that this portion of the reticulum consists of perforated curtain-like cisternae, the fenestrations of which result in the "chained" appearance presented by the reticulum in the planes of section so far considered.

A very different aspect of the reticulum is displayed in Fig. 13, where, in a transverse section, elongated membrane profiles predominate between the myofibrils. This field represents part of an area adjoining a nucleus which, as may be seen at low magnification, is relatively free of sarcosomes (Fig. 7). The plane of section in Fig. 13 passes through a series of fibrils at the level of sarcosomal indentation, though in this field the sarcosomes do not extend fully between the fibrils. The elongated membrane profiles represent more or less longitudinal aspects of the tubular struc-

FIGURE 18

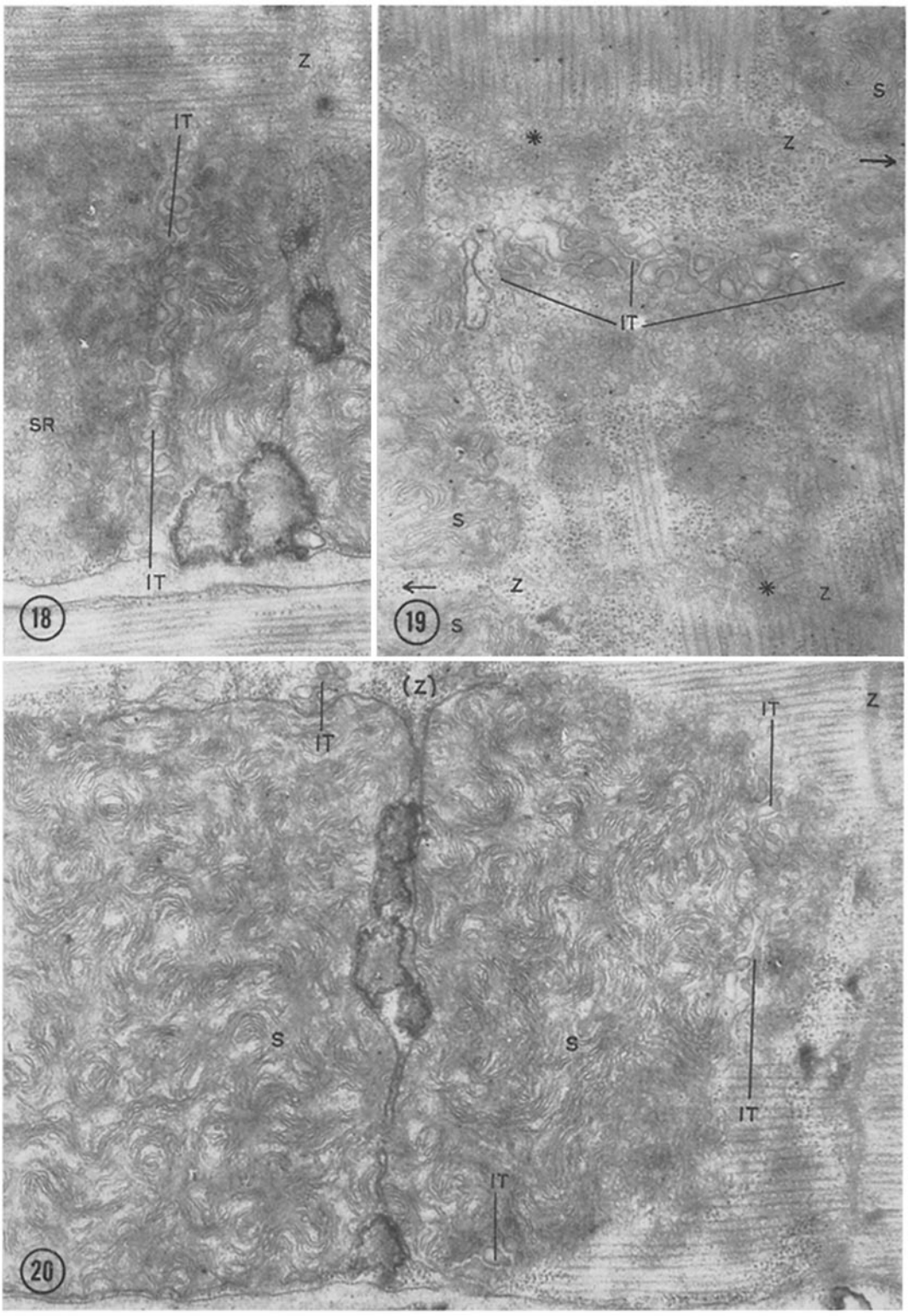
A field sectioned similarly to that shown in Fig. 17, but lying at the periphery of a fiber. The plane of section passes directly through a sarcosomal indentation, but the profiles of the convoluted plasma membrane tubules (*IT*) are not included in the section when the adjoining fibril is reached. In Fig. 8 portions of a pair of these series of tubule profiles are to be seen; each series lies midway between adjacent Z bands and the center of the sarcomere, the position (as seen in Fig. 9) occupied by the sarcosomal indentations. *SR* indicates a portion of the cisternal sheet of the sarcoplasmic reticulum, in surface view. × 20,000.

FIGURE 19

A somewhat oblique section passing just above a sarcomere. The position of the Z bands has been indicated—opposite the ends of the sarcosomes (*S*) (arrows)—though the fibril surface lies just beneath the plane of section. Only one extensive series of profiles of the convoluted plasma membrane intermediary tubule (*IT*) is included. The perforated cisterna of the sarcoplasmic reticulum is continuous over the Z bands (*), as may be seen also in Fig. 10. × 23,000.

FIGURE 20

Another field at the periphery of a fiber in which portions of two sarcosomes (*S*) are included, sectioned parallel to their largest surfaces, and bordering a fibril. Note the complex whorls of cristae within the sarcosomes, and the fragmentary profiles of the intermediary tubules (*IT*) lying on either side of the Z bands. The Z band on the right is clearly seen, and the position of its neighbor in the center of the field, opposite the junction of the two sarcosomes, is indicated by the bracketed *Z*. × 23,000.


Z
IT
SR
IT
18
S
Z
IT
S
Z
Z
19
S
(Z)
IT
IT
Z
S
S
IT
IT
20

tures lying within the indentations, together with the accompanying cisternal sheets. The relatively uninterrupted profiles of the latter are consistent with the observation in longitudinal sections (Fig. 10) that opposite the indentations fenestrations are infrequent. Both these series of profiles end before the nucleus is reached, and in the upper part of Fig. 13 a cisterna follows the curved termination of the fibril, while Fig. 12 suggests that these cisternae may sometimes loop around the fibril from one surface to the other. The section shown in Fig. 16 is presumably slightly oblique, a Z band being included at the upper right, and the transition of the fenestrated cisternae to a more uninterrupted system at the level of sarcosome indentation may in places be seen.

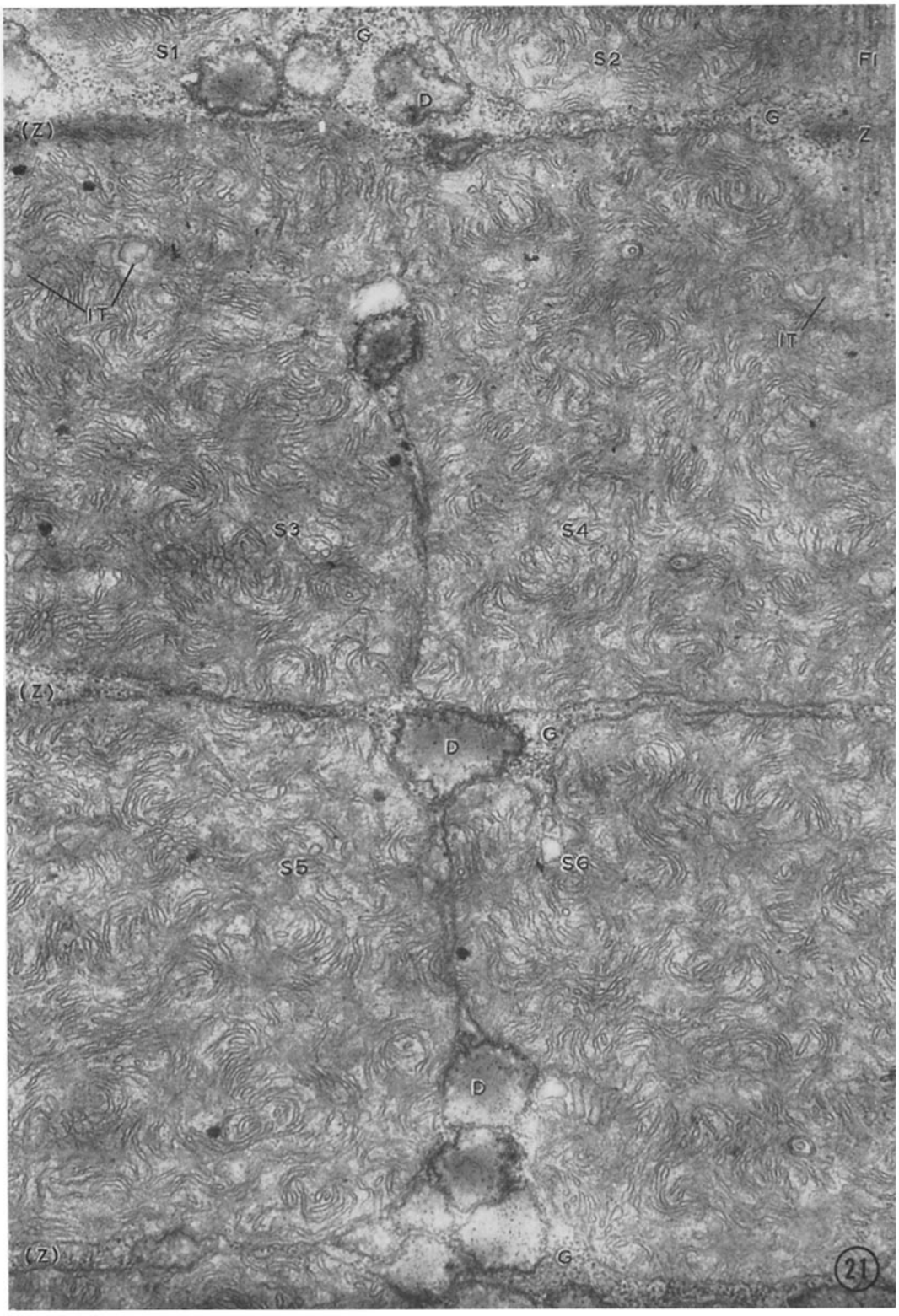
The question of the total uninterrupted length of the tubules lying alongside the fibrils in the indentations of the sarcosomes remains. In Fig. 13 the greatest such extent is over 1 μ, and in a survey of similar fields continuous profiles up to 3 μ have been noted, representing 20 to 30 per cent of the fiber radius. An important additional piece of information concerning the nature of these profiles derives from examination of the disposition of the plasma membrane at the surface of the fiber. This membrane lies immediately beneath a delicate and seemingly amorphous basement membrane, confluent with that of the tracheal cells. Although the plasma membrane in most places is seen (in transverse sections) to run a simple circumferential course, it is sometimes (Fig. 14) invaginated radially into the fiber to varying depths. Where this occurs the plasma membrane tubule lies alongside a sarcosome and is separated from the adjoining fibril by elongated profiles having no connection with the plasma membrane and evidently representing the fenestration-free region of one of the cisternal "curtains" in an aspect comparable with those seen lying close to the center of the fiber in Fig. 13.

If it is admitted that these plasma membrane invaginations are identical with the tubules or membranous structures lying in the sarcosomal indentations and thus traversing each sarcomere on either side of the Z band, then three possibilities remain to account for the apparent local discontinuities in the radially disposed profiles. It might be supposed (*a*) that the invaginations are simple tubules visible in their entirety only when sectioned perfectly longitudinally, (*b*) that they are indeed broken into segments either permanently or momentarily in the living fiber, or (*c*) that the apparent discontinuity seen in transverse sections of the fiber results from sectioning a convoluted or sinuous tubular structure. An indication that the last of these alternatives is to be preferred is afforded by sections passing parallel to and just above the surface of a fibril, hence either through or slightly above or below the sarcosomal indentations as in Figs. 17 through 20.

In Fig. 17 the plane of section passes over the surface of a fibril and just below the level of notching of the flanking sarcosome. The Z band is more or less obscured by large numbers of granules, probably of glycogen, and inserted between the sarcosomes opposite the Z band are seen irregular ovoid bodies which may contain

FIGURE 21

An electron micrograph illustrating the size and complexity of the sarcosomes in a section perpendicular to that shown in Fig. 10. At the top of this figure, small portions of two sarcosomes are seen (*S1*, *S2*), and immediately beneath is situated the Z band of a fibril (*FI*) visible on the right and lying beneath the plane of section on the left (upper bracketed *Z*). Between this and the next two Z bands, the levels of which are indicated by bracketed *Z* left center and lower left, lie larger portions of four sarcosomes (*S3*, *S4*, *S5*, *S6*) containing large numbers of whorled or irregularly arranged cristae. Note that only portions of these sarcosomes are seen here. They are each about 3 μ high (*Z-Z* level) and reach 10 μ in length along the radius of the fiber, and thus appear to be the most massive mitochondria recorded. Dense bodies (*D*), possibly containing lipid and carbohydrate materials, lie in most cases at the level of the Z bands, between the sarcosomes. In addition, small granules (*G*), probably of glycogen, are inserted between the sarcosome surfaces and also (Fig. 17) extend over the fibrils. The section passes for a short distance through sarcosomal indentations where fragments of the intermediary tubules (*IT*) of the sarcoplasmic reticulum are visible. × 29,000.


S1
G
S2
F1
D
(Z)
G
Z
IT
IT
S3
S4
(Z)
D
G
S5
S6
D
(Z)
G
21

lipid and polysaccharide materials. A short distance from the Z band on each side of it lies a series of irregular membrane profiles traversing the fibril surface but passing out of the plane of section at the edge of the sarcosomes. Fragmentary profiles of the corresponding system relating to the other half of each sarcomere are also visible. In addition to these profiles, a surface view is obtained of the fenestrated cisternal component of the sarcoplasmic reticulum, perforated by holes 200 to 300 A in diameter. A similar field is shown in Fig. 19, but in this case the plane of section is more oblique with respect to the fibril surface, and above the single sarcomere shown only one of the transverse series of irregular profiles is included. Although many of these profiles are apparently isolated, some degree of continuity is evident between them. The plane of section in Fig. 18 has passed longitudinally through one indentation of a peripherally placed sarcosome, and the chain of irregular profiles, again lying some distance from a Z band, is flanked by mitochondrial cristae before passing out of the section. In Fig. 8, portions of a pair of such profile chains are seen, each situated midway between adjacent Z bands and the center of the sarcomere, at which position, in longitudinal sections such as in Fig. 9, the sarcosomes are seen to be indented to accommodate the transversely oriented tubules, the longitudinal and surface aspects of which have just been described.

Thus far it has been established that the tubule profiles lying in the indentations seen in longitudinal sections of fibers perpendicular to the long axes of fibrils and sarcosomes are represented, in sections passing close to the fibril surface, not by simple tubules, but by irregular profiles which may be connected over short distances. These structures, on the other hand, in transverse sections of the fiber, appear as elongated profiles lying within the plane of section over distances of up to 30 per cent of the fiber radius. Two possibilities remain by which these aspects may be coordinated or reconciled. Firstly, the twin systems traversing the face of each sarcomere may be convoluted tubules affording more or less isolated profiles in surface section, but more continuous profiles in the plane perpendicular to this. Secondly, it may be that this system is not a static one, being momentarily either more or less continuous. It is not possible categorically to decide at the moment between these possibilities, but it is suggested that in the flight muscles of the dragonfly *the plasma membrane is invaginated to form convoluted tubes at two places along each sarcomere face at least for an appreciable distance, and quite probably along the whole radius of the fiber.*

At this point it should be remembered that Porter and Palade (1957), Andersson-Cedergren (1959) and others found not a direct invagination of the plasma membrane at the triad level (whether opposite the Z or the A-I junction level), but at least a very close association between the intermediary vesicles (the T system of Andersson-Cedergren) and the plasma membrane, at the periphery of the fiber. It is suggested here that *Aeshna* flight muscle, despite its peculiarities, may be placed in the category of muscles which have two triads or similar specializations per sarcomere, and that the counterpart of the intermediary vesicles surrounding fibrils in vertebrate muscle may be the plasma membrane tubes extending across the surface of the slab-like fibrils in *Aeshna* muscle; to stress this suggested homology, these tubes will here be referred to as the *intermediary tubules.*

In the original descriptions of the sarcoplasmic reticulum, the intermediary vesicles were considered to be one component of the system; indeed, the terms "triad" and "dyad" of Porter and Palade described the relation or association between these vesicles and the cisternal tubework of the rest of the reticulum. The term "sarcoplasmic reticulum" itself (in the connotation established on electron microscopic evidence) was employed to underline the suggested homology between it and the "endoplasmic reticulum" of other cells which, although possibly having momentary connection with the plasma membrane in certain cells, is in general an entirely distinct formation. If, as now seems likely, the intermediary vesicles of the triad have the special function of channeling nervous excitation within the fiber by virtue of some relationship with the plasma membrane, the question arises whether "sarcoplasmic reticulum" should properly be retained as a term connoting two separate components of the muscle cell, or whether it should relate only to the system of membrane-limited cisternae extending between the intermediary vesicles.

In *Tenebrio* muscle (Smith, 1961) the name "dyad" was given to the dual association between invaginated plasma membrane tubules and isolated vesicles, the former possibly corre-

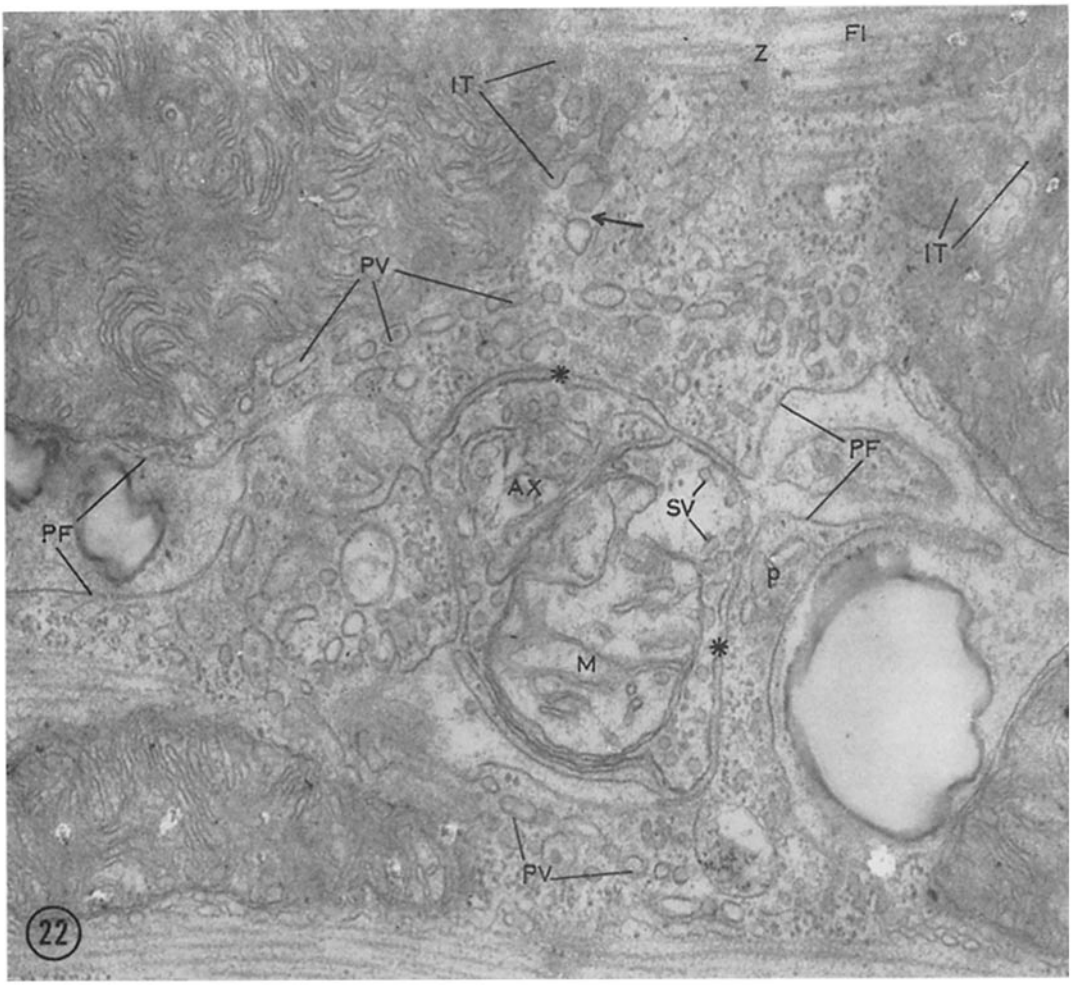


FIGURE 22

A myoneural junction in the flight muscle of the dragonfly *Aeshna*. In this tissue such endings usually appear to involve a single axon, as in this instance. At the synaptic region the axon (*AX*) is without the lemnoblast with which it is ensheathed throughout its peripheral course, and it is closely applied to the plasma membrane of the fiber (*PF*). The postsynaptic sarcoplasm is often produced into elongated fiber-like processes (*p*) which presumably increase the area of synaptic apposition. The axon shown here contains a large mitochondrion (*M*) and a few synaptic vesicles (*SV*), though a much greater concentration of these is frequently found. The postsynaptic sarcoplasm contains a number of vesicles of variable size (*PV*), fewer in number but similar in appearance to those described in *Tenebrio* flight muscle (Smith, 1960). This section passes through a series of profiles of an intermediary tubule (*IT*) or plasma membrane invagination, lying to one side of the Z band of the fibril (*FI*), and these profiles are in intimate relation with those of the postsynaptic vesicles in the region indicated by an arrow.

In this micrograph the axon appears to effect synapse with two adjacent fibers simultaneously (*), an unusual occurrence, the significance of which is not known. × 33,000.

sponding to the intermediary vesicles in terms of their suggested role in internal conduction of excitation. In *Aeshna* muscle a highly ordered though differently oriented system of plasma membrane invaginations is present, lying in close relationship with continuous sheet-like cisternae. In the case of vertebrate muscle, although the intermediary vesicles may well have a functional

relationship with the plasma membrane, they have never been shown to form a continuous system throughout the fiber; that is, it seems they cannot be regarded as plasma membrane invaginations in the same (morphological) sense. However, in view of the present incompleteness of our knowledge concerning the role played by these membranes, and particularly those of the extensive cisternae, it is the opinion of this author that the term "sarcoplasmic reticulum" should for the present be retained in its original sense *provided it is clearly understood that it probably includes two distinct components, only one of which is directly or indirectly connected with the plasma membrane.* The patently plasma membrane nature of the oriented tubular system in *Aeshna* flight muscle would thus place this tissue as a special case in a more general framework.

Between the intermediary vesicles of many vertebrate fibers lies an irregular latticework of wide cisternae which appear to correspond, except for the small size and greater number of the perforations in the present example, to the membranous sheets covering the fibrils of *Aeshna* muscle. The possible functional parallel between corresponding components in vertebrate and *Aeshna* muscle will be discussed later. The interpretation of the three-dimensional fine structure of the dragonfly muscle described here has been incorporated into the drawings shown in Figs. 23 and 24.

One further aspect of the structure of *Aeshna* flight muscle should be mentioned: the organization of the myoneural junctions. Edwards (1960) found that in longitudinal thoracic muscle of a dragonfly (not a muscle of flight) multiaxonal, multijunctional synapses occur, and in particular that the final junction between nerve and muscle involves pairs of axons, one axon of a pair being smaller than the other, supposedly representing the "slow" and "fast" axons characteristic of insect skeletal muscle other than certain flight muscles. During the present study, many of the junctions have been found to contain only a single axon as in *Tenebrio* flight muscle (Smith, 1960), and where more than one is involved such distinctive size difference is absent. As in other myoneural junctions, the synapsing axons contain large numbers of vesicles from 300 to 450 A in diameter, and, as in *Tenebrio* but unlike other insect and vertebrate myoneural junctions, the lemnoblast which surrounds the presynaptic axon is shed before synapse is effected. As may be seen in Fig 22, the sarcoplasm of the immediately postsynaptic region of the fiber contains moderate numbers of vesicle profiles *ca.* 500 to 1500 A in diameter having more or less dense contents and resembling, except for their sparser distribution, the postsynaptic vesicles found in *Tenebrio.* In Fig. 22 the plane of section passes close to the surface of a peripherally located myofibril and it is evident that the vesicles of the postsynaptic area are in close spatial relationship with the profiles of the intermediary tubule lying on one side of the Z band.

APPLICATION OF THE "BLACK REACTION" TO *AESHNA* MUSCLE

The extensive comparative work of Veratti (1902) on filamentous reticula lying within the sarcoplasm provides, if not the first, certainly the definitive light microscopic account of what proved to be the sarcoplasmic reticulum of electron microscopy. The method employed by Veratti—a modification of Golgi's "black reaction"—was carried out on *Aeshna* flight muscle fibers during this study. In longitudinal sections (Fig. 1) where the staining has succeeded, twin chains of black granules are deposited on either side of the Z bands of the fibrils, and these appear to correspond to the profiles of the intermediary tubules seen in a similarly oriented electron micrograph in Fig. 10. In transverse sections of this muscle (Figs. 2, 3, and 4), series of intensely impregnated filaments are seen, extending radially from the periphery toward the central axis of the fiber and having precisely the disposition of the intermediary tubules inferred from the electron micrographic evidence. It is interesting to note that the cisternal "curtains" of the sarcoplasmic reticulum appear not to be stained by this procedure, at least in these preparations, pointing to a possible qualitative difference between the two membrane systems which together compose the sarcoplasmic reticulum of these fibers.

DISCUSSION

The need for a conduction system by which membrane excitation initiated by the motor nerve impulse is channeled into the striated muscle fiber (Peachey and Porter, 1959) is now generally agreed upon. Hill (1948, 1949) showed, on theoretical grounds, that simple diffusion from the periphery of the fiber could not result in the

adequate transfer throughout the fiber of any substance or ionic disturbance liberated or initiated at the depolarized fiber membrane, in the observed time elapsing between the arrival of the impulse and the start of contraction. In vertebrate muscle the microdepolarization experiments of Huxley, Taylor, and Straub (see references in Huxley, 1959) pointed to the level of the triad or similar structure as that at which internal conduction takes place. This suggestion derives morphological support from the observed close association between the plasma membrane and one component of the triads—the intermediary vesicles.

Fibrillar flight muscle of insects is typically composed of very large fibers, and it has been shown in the mealworm beetle *Tenebrio* (Smith, 1961) that the maximum distance between plasma membrane and fibril is reduced from a distance equal to the radius of the fiber (100 to 150 μ) to a few microns, by virtue of a rich system of plasma membrane tubules drawn into the fiber along with the invading tracheoles. In *Aeshna* muscle, as indeed in all insect muscles other than those of the fibrillar type and the corresponding flight muscles of a few other actively flying forms such as the higher Orthoptera, no such internalized tracheolar system is present, and these fibers therefore might be expected to be more directly comparable with those of vertebrates and other non-tracheated animals as regards the relationship between the plasma membrane and the sarcoplasmic reticulum.

Evidence has been offered here that the reticulum of *Aeshna* fibers is morphologically comparable with that of striated muscle of vertebrates, and, in particular, with that exhibiting a repeating pattern based on alignment of triads opposite the A-I junction or between the A-I junction and the H zone. That in *Aeshna* muscle the intermediary tubule is a direct invagination of the plasma membrane, whereas the intermediary vesicles of other muscles are probably not, may perhaps be attributed to the fact that in *Aeshna* the fiber radius characteristically includes only one fibril, whereas the vertebrate fiber is composed of a large number of cylindrical or irregular close-packed fibrils.

It has a number of times been suggested that the sarcoplasmic reticulum may represent or include the pathway of internal conduction of excitation (Bennett and Porter, 1953; Ruska *et al.*, 1958; Peachey and Porter, 1959). On morphological grounds it now appears that the intermediary vesicles of the triad (or in the present case the corresponding tubular invaginations) may be the *primary* conduction channels reaching across the fibrils at or between the Z bands of every sarcomere. If this is the case, then the question of the role played by the intervening cisternae remains. That these too may be intimately concerned in muscle contraction, perhaps through regulation of the cycle of contraction and relaxation of the fibers initiated by the arrival of the impulse, is suggested, for example, by the work of Muscatello and co-workers (1961), who showed that the "sarcotubular" (cisternal) fraction of homogenized frog skeletal muscle has high Mg^{++}-stimulated, Ca^{++}-inhibited ATPase activity, while Revel (unpublished observations) has demonstrated that calcium appears to be present in local high concentrations at the level of the intermediary vesicles. In *Aeshna* muscle the cisternae lie physically between the plasma membrane tubules and the contractile material, a fact which supports the suggestion that they act in some way as intermediaries between the fibrils and the plasma membrane.

In vertebrate "twitch" fibers a motor impulse arriving at the single end-plate initiates a propagated wave of membrane depolarization, whereas in arthropod muscle several myoneural junctions are typically present on a single fiber. Presumably local regions of depolarization are set up as the impulse reaches the surface of the fiber along the axon branches. In fibers of *Tenebrio* flight muscle the membrane-fibril distance is reduced to about 2 μ by an irregular though profuse system of tubular invaginations. In *Amphioxus* muscle the fibers are sheet-like with a width of only about 1 μ (Peachey, 1961), and in *Carcinus* the branching plasma membrane invaginations analogously reduce the distance across which membrane depolarization must act upon the fibrils (Peachey, unpublished observations). In each of these instances the maximum distance over which diffusion must operate is similar to the maximum distance from intermediary vesicles to fibrillar material in the triads of vertebrate muscle. In *Aeshna* flight muscle the distance between the intermediary tubules and any point in the fibrils is less than 1 μ. Despite structural variation between different muscles, a similar proximity between myofibrils and an impulse conduction system seems always to be established.

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FIGURE 23

A semidiagrammatic reconstruction of the three-dimensional structure of *Aeshna* flight muscle as interpreted from the electron micrographs shown here. The front face of the "block" is sectioned longitudinally across the narrowest dimension of the fibrils and sarcosomes (*cf.* Fig. 9). The upper plane of section is obliquely transverse, passing on the left above, in the middle through, and on the right below successive Z band levels. The precise orientation of the sarcoplasmic reticulum and of the slab-shaped sarcosomes is indicated.

The notches or indentations in the sarcosomes, situated on each side of the Z bands, contain the convoluted tubules representing invaginations of the plasma membrane. When these lie within the plane of section, elongated profiles accompanying the sarcosomes result (Figs. 13 through 15) together with relatively uninterrupted profiles of the cisternal curtains bordering the indentations. On the extreme right of this figure, a portion of a perforated cisterna has been exposed, and elsewhere profiles of this component of the sarcoplasmic reticulum are visible, inserted between the radially oriented surfaces of the fibrils and sarcosomes, both in longitudinal and in transverse sections of the fiber.

Only a portion of each sarcosome is shown in this drawing. Low magnification transverse sections of the fiber (Fig. 8) show that these may extend radially from the periphery to the center of the fiber, ending close to the nucleus. The double envelope of the nucleus is indicated, and close to this are included a number of profiles of the smooth-membraned vesicles of the well developed Golgi region.

Note that while the Z and M bands of each sarcomere have been indicated in this drawing, no attempt has been made to incorporate into it the detailed organization of the myofilaments. These, in fact, are arranged in the double hexagonal arrays of primary and secondary filaments described by Huxley and Hanson (1960) in both vertebrate and insect striated muscle fibers.

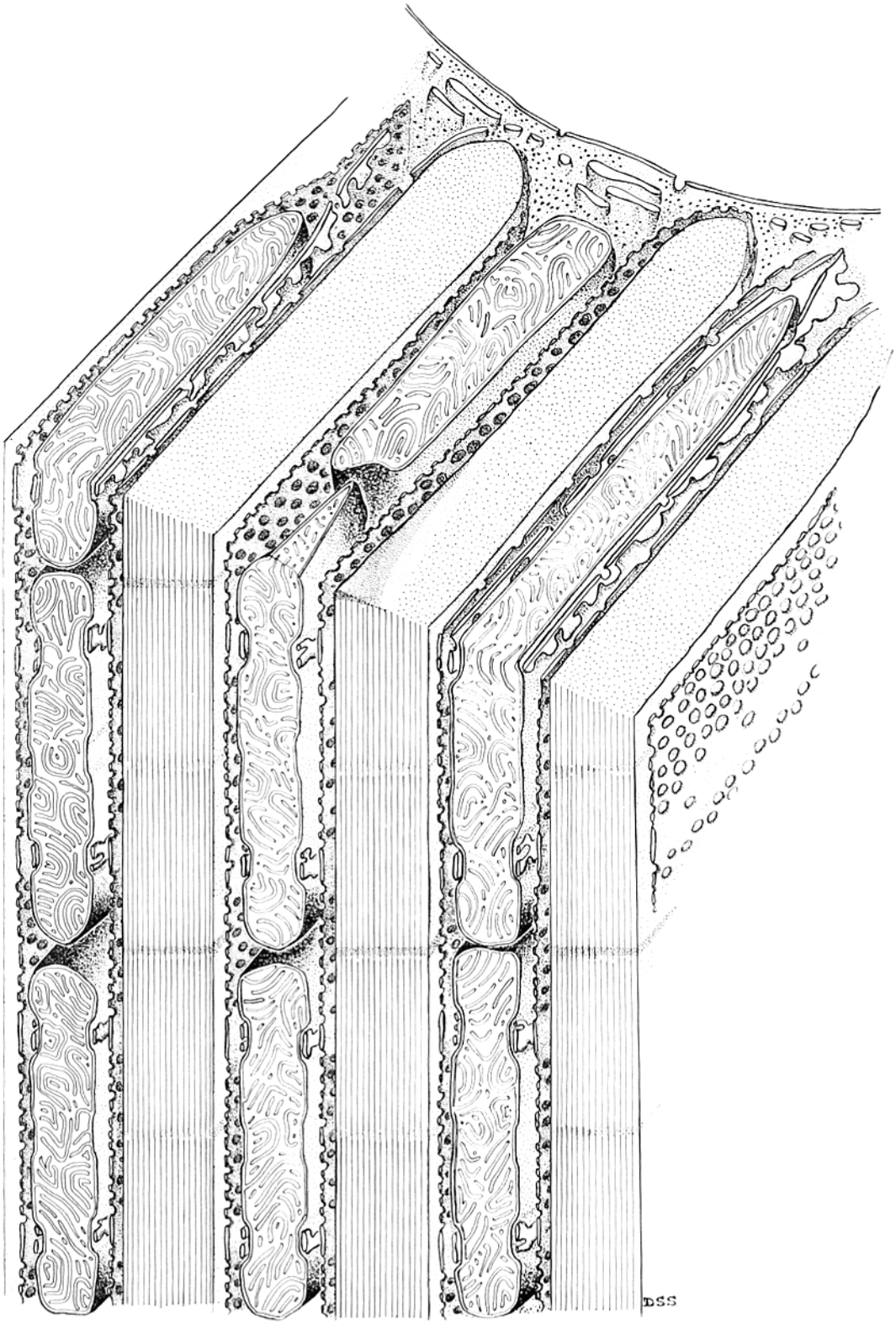
DSS

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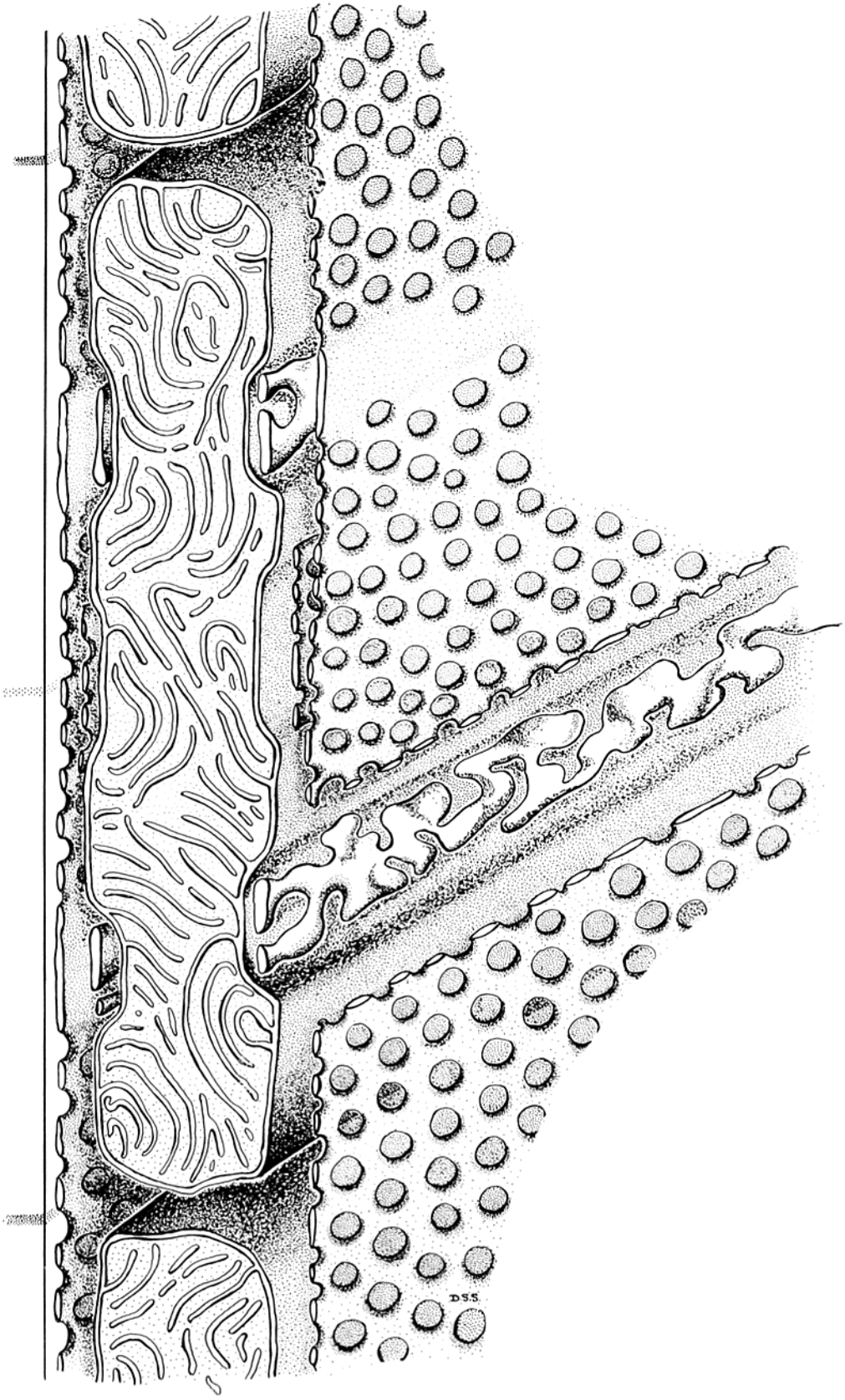
FIGURE 24

A semidiagrammatic interpretation of the organization of the sarcoplasmic reticulum associated with a single sarcomere of *Aeshna* flight muscle. The edge of one fibril is indicated on the left (with the M band in the center of the figure), while the neighboring fibril has been removed to display, in surface aspect, the continuous perforated cisternal curtain, which is frequently doubled in the mid-sarcomere region. This cisterna is represented as being cut away over a convoluted intermediary tubule (lower right). Transversely sectioned profiles of the three other such tubules associated with this sarcosome are also shown.

Note that in this and the preceding figure, the distance between the fibrils, the sarcosomes, and the reticulum cisternae has been exaggerated for purposes of clarity, as also has the relative size of the fenestrations in this last component of the reticulum. The extremely intimate relationship that actually exists between these is clearly evident in the accompanying electron micrographs.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