

OBSERVATIONS ON THE FINE STRUCTURE OF PHARYNGEAL MUSCLE IN THE PLANARIAN *DUGESIA TIGRINA*

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ABSTRACT

Pharyngeal muscle of the planarian *Dugesia tigrina* was studied by electron microscopy after osmium tetroxide fixation. The muscle cell was observed to contain one myofibril or bundle of myofilaments parallel to its longitudinal axis. The myofilaments were of two types, different in size and distribution. No Z lines or myofilament organization into cross or helical striations were seen. Dense bodies were seen as projections from an invagination of the plasma membrane and as dense lines parallel to the myofilaments. The muscle cells are surrounded by a plasma membrane which is structurally associated with dense body projections, with vesicles and cisternae of sarcoplasmic reticulum, and with synaptic nerve endings. The cell has sarcoplasmic projections perpendicular to its long axis; these projections are seen to contain the nucleus or mitochondria and granules. Mitochondria and granules are also seen in a sarcoplasm rim around the fibril. The dense bodies may serve as attachment for thin myofilaments and function in transmission of stimuli from plasma membrane to the interior of the fibril.

INTRODUCTION

The structure of cross-striated muscle has been extensively studied in both invertebrate and vertebrate animals, and the contraction of cross-striated muscle has been correlated with the striations and the arrangement of protein filaments within the fibril (7, 8, 12). The sliding filament mechanism of contraction postulated for cross-striated muscles may hold for some smooth muscles as well (10). However, muscles classified as smooth exhibit much variability in structure. Muscles previously classified as helical smooth muscle in several invertebrates have been shown to possess striations, though these are obliquely arranged along the fibril length (9, 10, 13). Thus, Hanson and Lowy (10) proposed that the presence or absence of segregated arrays of filaments could serve as the main criterion for distinguishing striated from smooth muscle, although additional features such as the absence of the Z line appear

also to be characteristic of the latter. There are other invertebrate muscles which cannot be classified as striated on the basis of segregated arrays of myofilaments, but which, like helical "smooth" muscle, do have two types of myofilaments. They thus differ from the vertebrate smooth muscles studied, which are definitely non-striated and have only one type of myofilament (2, 4, 15, 19, 20).

The sliding filament mechanism of contraction depends on certain structural features, namely, cross-linked myofilaments of an actin-myosin type as found in striated muscle. Further examination of the ultrastructure of smooth muscle of a variety of invertebrates and vertebrates seems desirable to establish in what manner muscles now classified as smooth may be similar to cross-striated muscle and in what manner they may be entirely different. Such information is necessary for a

correlation of structure with function and in particular for reexamination of the classification of types of smooth muscle in terms of possible contractile mechanisms.

The present investigation on the electron microscopy of the pharyngeal muscles of the planarian *Dugesia tigrina* presents data on the fine structure of muscle cells that may be tentatively classified as smooth, but have two types of filaments. Dense bodies similar to those described in several vertebrate smooth muscles (2, 19) and in some invertebrate muscles (10) were observed. They seem to originate as projections from the plasma membrane at membrane invaginations. Their possible role in attachment of thin filament and in transmission of stimuli is discussed.

The muscle cell is described in relation to its sarcoplasm, nucleus, mitochondria, sarcoplasmic reticulum vesicles, and the surrounding structures.

MATERIAL AND METHODS

The observations were made on sections of muscles of the pharynx of the fresh water planarian *Dugesia tigrina*. Animals kept in spring water and fed beef liver and egg yolk twice weekly were maintained in a healthy condition in the laboratory for several months. Animals were fasted for 1 week prior to fixation. The pharynxes were removed from the animals with fine forceps, and placed immediately in ice cold 1 per cent osmium tetroxide buffered at pH 7.6 with veronal-acetate buffer (16) and fixed for 1 hour. No attempt to stretch the muscle or to extract it in water-glycerol prior to fixation was made for this part of the investigation. The muscles were partially contracted when fixed. After fixation, the specimens were dehydrated through a graded series of ethyl alcohol from 25 to 100 per cent and two changes of propylene oxide. Embedding was done in epoxy resin anhydride mixture (Epon 812) which was polymerized for 24 hours at 62°C (14). Sections were cut with glass knives on a Porter-Blum microtome, and

placed on 200-mesh copper grids. To increase contrast, sections were stained in saturated uranyl acetate for 1 hour at room temperature. Sections were viewed on an RCA EMU-3D microscope, and photographed on Kodak contrast lantern slides with development in Dektol. Initial magnifications of micrographs were from 5000 to 33,000; photographic enlargements were made at 4 or 8 times the initial magnification.

Some specimens were fixed in 95 per cent EtOH, dehydrated, cleared, and embedded in paraffin in a routine way in order to observe sections under the light microscope.

OBSERVATIONS

General Organization

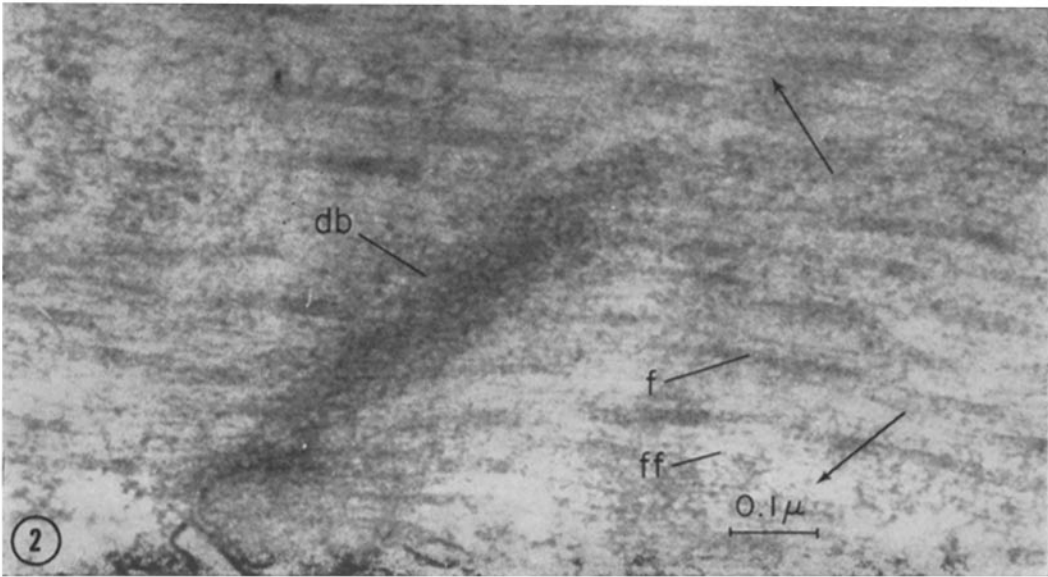
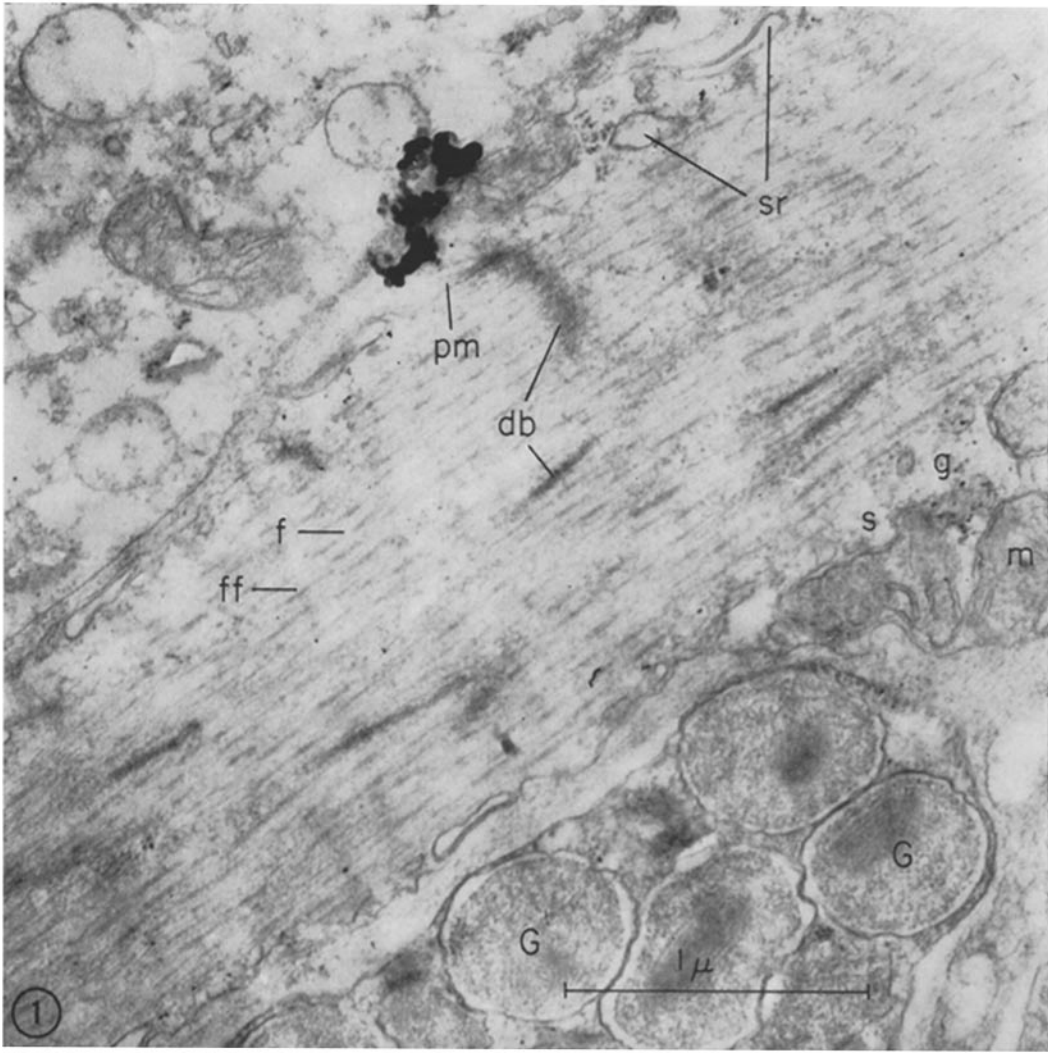
The muscles in the pharynx of the planarian as observed by the light and electron microscope are arranged into a muscularis interna and a muscularis externa. The muscularis interna consists of an outer layer of longitudinally oriented muscle fibers and an inner layer of circularly oriented muscle fibers; the muscularis externa consists of an outer layer of longitudinally oriented muscle fibers and an inner layer of circularly oriented muscle fibers. In the circular and longitudinal layers of both the muscularis interna and the muscularis externa there are individual muscle fibers running radially and obliquely through the layers. Between the two sets of muscles are numerous gland cells filled with granules. Individual muscle fibers running radially are observed between gland cells.

Muscle Fiber

Each muscle cell or fiber consists of parallel longitudinally arranged myofilaments (Figs. 1 and 2). The myofilaments are not grouped into many bundles, such as the myofibrils of striated

FIGURE 1 Electron micrograph of a longitudinal section of a radially oriented muscle in the pharynx of the planarian *Dugesia tigrina*. The micrograph shows the two types of myofilaments, thick filaments (*f*) and thin filaments (*ff*). Dense bodies (*db*) are seen parallel to the myofilaments, and one dense body is seen as a dense projection from the plasma membrane (*pm*). The profiles of vesicles and cisternae of the sarcoplasmic reticulum (*sr*) are seen beneath the plasma membrane. An area of sarcoplasm (*s*) containing mitochondria (*m*) and small granules (*g*) is also seen. Large granules (*G*) are seen near the muscle cell but are not part of the muscle. The extremely dense mass is an artifact. $\times 40,000$.

FIGURE 2 Enlarged micrograph of a longitudinal section of muscle. Two types of myofilaments, thick (*f*) and thin (*ff*), are evident. A dense body (*db*) is seen as a projection at an acute angle from plasma membrane. Note filamentous structure of dense body. At arrows are possible cross-links between thick and thin filaments. $\times 120,000$.



muscle. The myofilaments occupy most of the muscle cell; thus, the muscle cell can be considered to consist of one myofibril. The muscle fibers are round or ovoid in cross-section (Figs. 3 and 4). The fibers measure from 1.3 to 3.5 μ in their greatest diameter; their total length could not be measured, but has been estimated to be 100 to 300 μ from light microscope observations.

Plasma Membrane and Sarcoplasm

Each muscle cell is distinct and surrounded by a plasma membrane, which is approximately 75 A thick. The plasma membrane exhibits infoldings and invaginations from the surface; some of the invaginations are associated with dense projections into the interior of the fibril. Other parts of the plasma membrane are associated with flattened cisternae and vesicles immediately beneath the plasma membrane. The plasma membrane also surrounds outpocketings of sarcoplasm; some of these outpocketings are observed to be associated with synaptic nerve endings. The sarcoplasm occupies a narrow zone just beneath the plasma membrane and around the fibril (Figs. 1, 3, and 4). It is also found as small outpocketings or as larger projections perpendicular to the longitudinal axis of the fibril (Fig. 3). Some of these projections may be seen to contain material of low density and unattached granules. Clusters of dense granules with a diameter of about 150 A are thought to be ribosomes (17). The ribosomes may be seen in clusters of 4, 5, or more in the sarcoplasm (Fig. 5). Granules attached to membranes as in endoplasmic reticulum were not observed in the cells. Other unattached and less dense granules such as are seen among myofilaments (Fig. 1) may be glycogen.

Nucleus

The nucleus is not often seen in sections. It occupies a position in a projection peripheral to the longitudinal axis of the fibril. Sarcoplasmic projections from the fibril have been observed as cell processes in turbellarian subepidermal muscle cells after silver preparation by Gelei (5). The nucleus, which has a diameter of 2 to 3 μ , is separated from the fibril by a strand of sarcoplasm (about 1 μ wide) which appears continuous around the nucleus. Granules and vesicles can be seen in the sarcoplasm adjacent to the nucleus. The nucleus has a double membrane and a granular interior, and contains aggregates of presumably chromatin material (Fig. 4).

Mitochondria

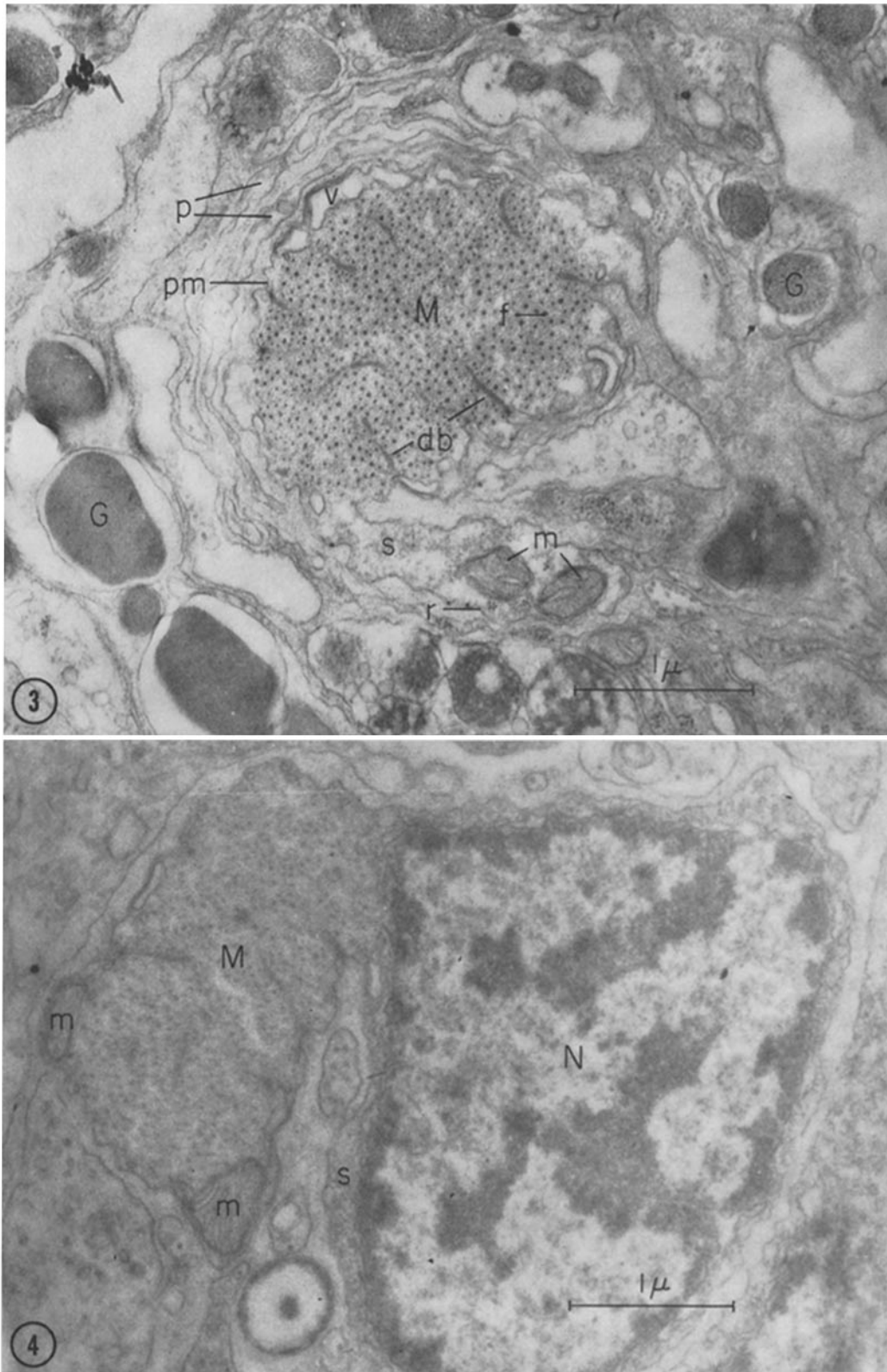
The mitochondria, which are round, oval, or rod-shaped, average 350 by 200 $m\mu$ in size. They are found beneath the plasma membrane in the sarcoplasm along the length of the fibril. They also appear in the sarcoplasmic projections (Fig. 3); as many as 16 have been observed in a section of one projection. Their internal structure consists of cristae or ridges varying in number from 1 to 6 in the plane of section. The double membrane characteristic of mitochondria can be observed (Fig. 5).

Sarcoplasmic Reticulum

Immediately beneath the plasma membrane there are observed many ovoid vesicles and flattened cisternae. The vesicles have a diameter of about 800 A, and the flattened cisternae average 300 $m\mu$ in length and 500 A in width. The cisternae often appear more collapsed in the center of their cross-sections (Figs. 1, 3, and 6). These agranular structures appear by their form and

FIGURE 3 Electron micrograph of a transverse section of an individual muscle fiber (*M*) among a large number of gland cells, whose large granules (*G*) and protoplasmic extensions (*p*) are seen around the muscle cell. The plasma membrane (*pm*) is seen around the muscle cell; it is in close proximity to vesicles (*v*) of the sarcoplasmic reticulum. A projection of sarcoplasm (*s*) is shown containing two mitochondria (*m*) and granules. Some granules are seen in a cluster and resemble ribosomes (*r*). Dense bodies (*db*) are seen among the myofilaments; thick filaments (*f*) are more readily observed at this magnification. $\times 27,000$.

FIGURE 4 Electron micrograph of a cross-section of a muscle cell (*M*) seen with its nucleus (*N*) in a sarcoplasmic projection perpendicular to the long axis of the fiber. A thin rim of sarcoplasm (*s*) surrounding the nucleus contains granules. The double membrane and aggregations of chromatin are present. Two mitochondria (*m*) are seen just beneath the plasma membrane. $\times 25,000$.



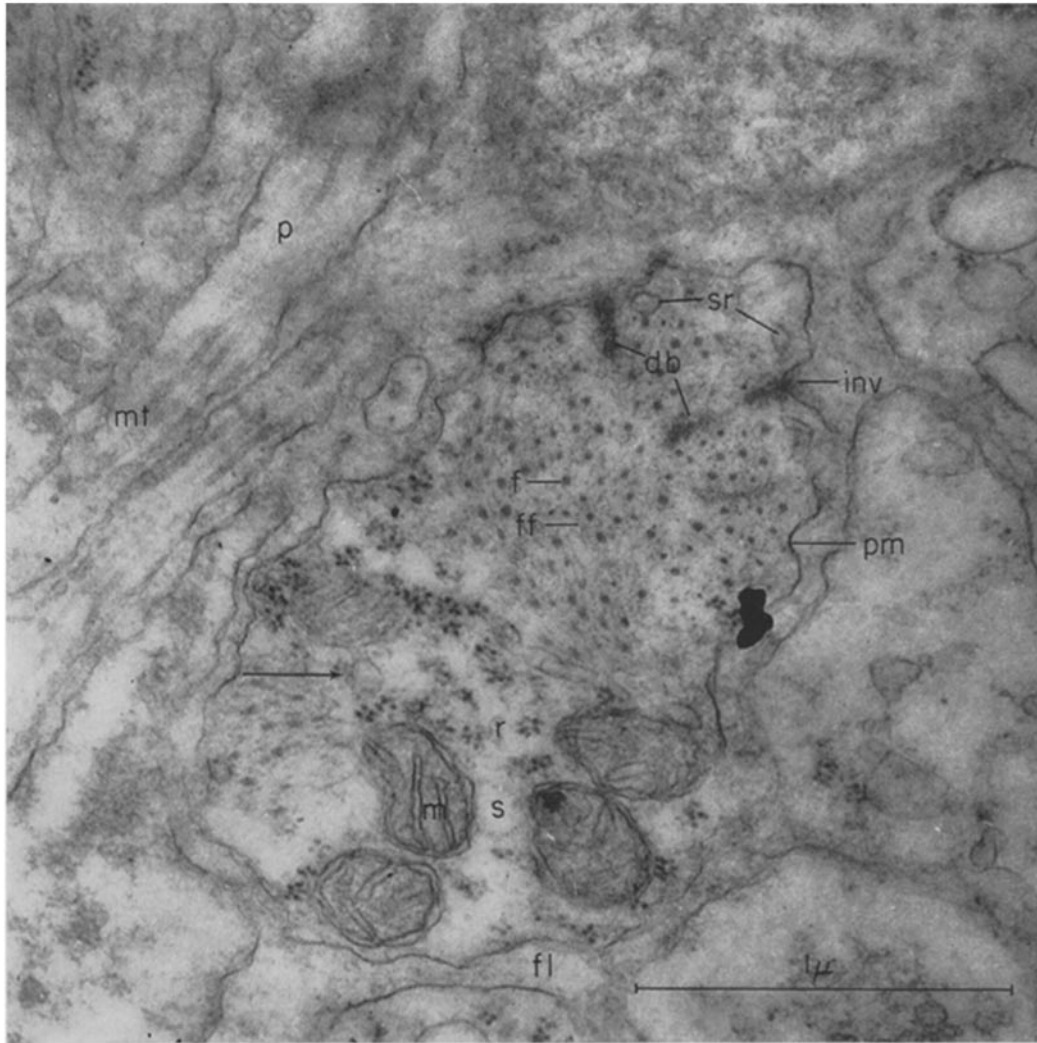


FIGURE 5 Electron micrograph of a cross-section of an individual muscle cell. The myofilaments (*f* and *ff*) are seen to occupy two different areas of the cross-section. Some filaments are cut in a slightly oblique plane. The plasma membrane (*pm*) is seen surrounding the fiber. Two dense bodies (*db*) are seen as projections from the plasma membrane. They appear from denser areas at invaginations of the plasma membrane (*inv*). Circular profiles of the sarcoplasmic reticulum (*sr*) are seen beneath the plasma membrane. The cross-section also contains five mitochondria (*m*) and numerous clusters of granules, presumably ribosomes (*r*), in sarcoplasm (*s*). A bulbous projection of the outer membrane of one mitochondrion is seen (arrow). The muscle fiber is surrounded by an intercellular space containing a finely dispersed and fibrillar material (*fl*). Microtubules (*mt*) cut longitudinally are seen within protoplasmic extensions (*p*) of gland cells in the surrounding area. $\times 50,000$.

location to be the sarcoplasmic reticulum. There is no evidence that the vesicles are organized in any longitudinal pattern. The vesicles and cisternae often appear closely associated with the

plasma membrane; the outer membrane of the cisternae appears denser where it is directly adjacent to the overlying plasma membrane (Figs. 3 and 6).

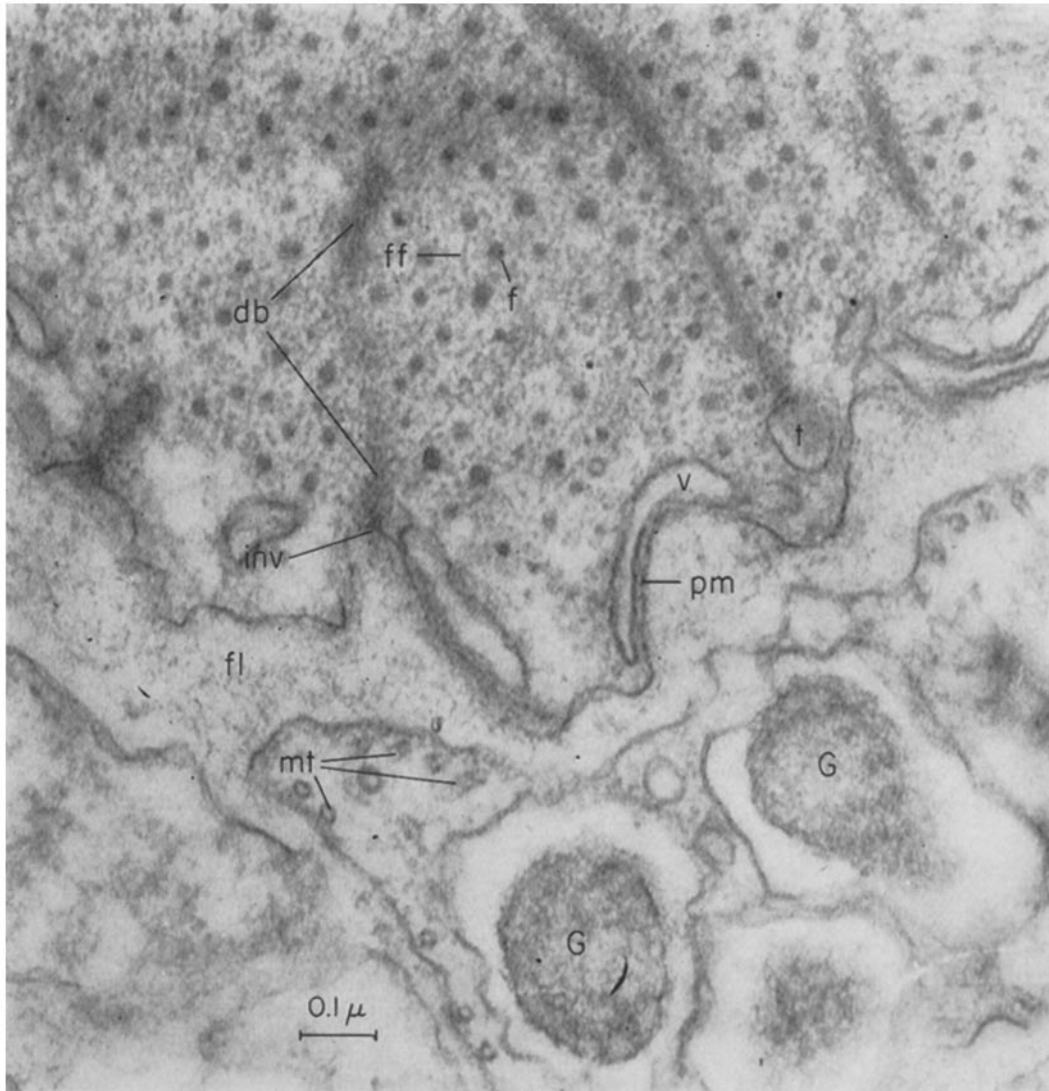


FIGURE 6 High magnification of a part of a cross-section of a muscle fiber. Several modifications of the plasma membrane are seen: an increased density of the plasma membrane (*pm*) and of the outer membrane of the flattened vesicle (*v*) at the point of their contact; the plasma membrane invagination (*inv*) at the apparent origin of the dense body projection into the fibril with an increase in the density of the projection at the point of origin; invagination of the plasma membrane cut almost in cross-section, showing a tubular structure (*t*). The material of increased density at the point where the dense body projection begins at the plasma membrane is not limited by the membrane, but seems to have a continuity with the fibrillar (*fl*) material of the intercellular space. The filamentous nature of the dense body (*db*) can be observed. Note the different sizes and variable density of the thick filament (*f*) cross-sections. Thick filaments may appear granular, and a few appear with a much less dense center. Part of a gland cell is also seen containing membrane-enclosed granules (*G*) and microtubules (*mt*) cut in cross and oblique section near the gland cell membrane. *ff*, thin filaments. $\times 110,000$.

Myofilaments

The myofibril is composed of two types of parallel myofilaments oriented longitudinally in the fibril (Figs. 1 and 2). The thick filaments vary from 100 to 220 Å in diameter, and the thin filaments are about 50 Å wide. The distance between two thick filaments varies from 400 to 800 Å.

The myofilaments normally occupy practically all of the cell cross-sectional area, but occasionally only about a third of the area (Fig. 5). The packing of the myofilaments within the fibril is not always regular, since areas with no filaments can sometimes be observed in cross-sections (Fig. 5). Radial lines suggesting cross-linkage between the

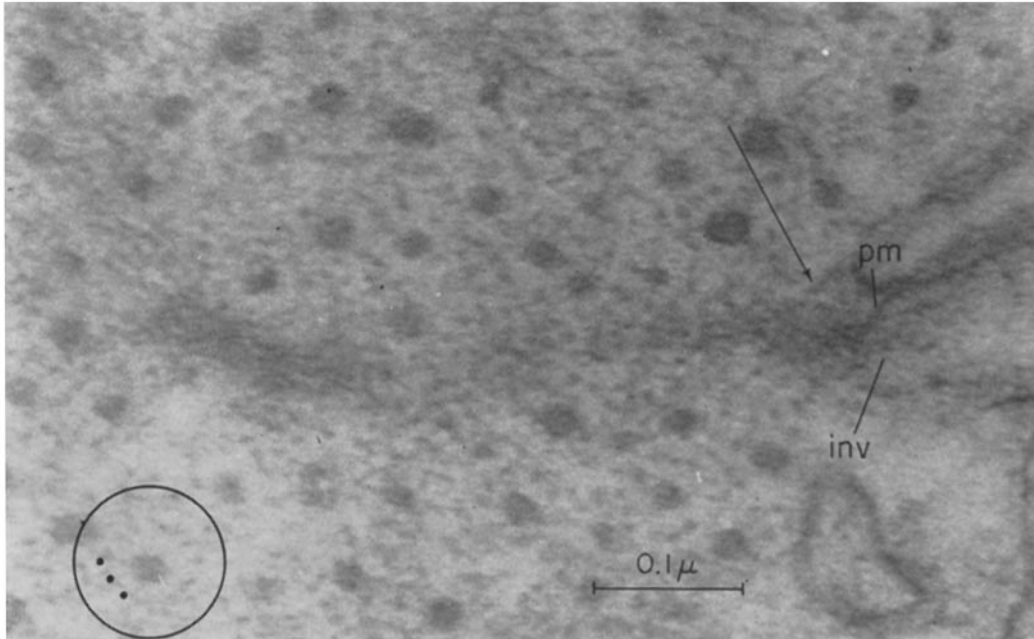


FIGURE 7 Higher magnification micrograph of a dense body projection (*db*) of Fig. 6. Note its origin at the plasma membrane (*pm*). At the invagination (*inv*) of the membrane note the fibrillar material from the intercellular space which appears continuous with the dense body projection. At arrow, note filaments forming the dense body. Radial lines appearing as cross-links between central thick filament and three thin filaments in its orbit are shown at the three dots within the circle. $\times 200,000$.

The thick filaments are not homogeneously dense in cross-section. Some filaments appear granular with areas of varying density; other filaments appear to have a less dense center (Fig. 6). The thin filaments are seen between the longitudinal thick filaments. In cross-sections the thin filaments can more readily be observed individually, scattered rather randomly among the thick filaments. An occasional orbital arrangement of 10 to 15 thin filaments has been observed around one thick filament (Figs. 6 and 7).

The individual filaments can be followed for some distance, but their leaving the plane of section prevents tracing them for their complete length.

thick filament and some of the thin filaments in the surrounding orbit can be seen in cross-sections (Figs. 6 and 7). Possible cross-links are occasionally discernible between thick filaments and adjacent thin filaments in longitudinal sections of the fibril (Fig. 2, arrows).

Organization of the myofilaments into cross-structures corresponding to the A and I bands of striated muscle could not be observed (Figs. 1 and 2). There is no clear evidence of a Z band such as is seen in striated muscle. The myofilaments seem to maintain a parallel orientation throughout the length of the fiber and to span the entire length of the cell. However, the same fila-

ments could not be traced from one end to the other of the cell.

Dense Bodies

Observations of both longitudinal and cross-sections of muscle fibers suggest that there are ribbonlike dense body structures which project from their apparent attachment to the plasma membrane toward and into the interior of the fibril, without intersecting the thick filaments. Projecting structures of this sort would give rise in cross-section (Figs. 3 to 7) to the observed dense lines separating the dots corresponding to the thick myofilaments, and in longitudinal sections (Figs. 1 and 2) to the observed dense line segments parallel to the filaments. These dense line projections are thought to be ribbonlike (Fig. 2). They measure 300 to 400 $m\mu$ in width and 300 to 800 \AA in thickness; though they vary in length, projections extending as far as 1 μ into the fibril have been measured. Often projections appear to penetrate into the fibril at an acute rather than a right angle (Fig. 2). The plasma membrane is observed to invaginate into the sarcoplasm surrounding the fibril at the point of origin or attachment of the dense body projection (Figs. 3, 5, 6, and 7). The plasma membrane invagination may extend into the fibril, since in some cross-sections the dense line projection is seen to originate from a large isolated tubular structure in the fibril (Fig. 6). This tubular structure is thought to be the invagination of the plasma membrane. At the plasma membrane invagination where the dense body begins there is often observed a small area of greater density (Figs. 5 to 7). This denser material does not seem to be limited to the interior of the cell by a plasma membrane, but seems continuous with intercellular fibrillar material (Figs. 6 and 7). The dense bodies or projections are not homogeneous but seem to contain small filaments (Fig. 7). In the interior of the fibril in some preparations two projections appear to meet or to branch. In longitudinal sections, it can be observed that the area extending beyond the ends of the dense line seems to contain few or no thick filaments, usually only thin filaments (Figs. 1 and 2). The presence of only thin filaments in the area of dense bodies is more conspicuous in the electron micrographs presented by Hanson and Lowy (10) of the dense bodies in longitudinal sections of stretched adductor muscle of the oyster.

Surrounding Structures

The muscle fibers are surrounded by an intercellular space which contains material appearing as thin fibrillae (Figs. 5 to 7). This intercellular material may appear more electron opaque than the cytoplasm of the surrounding cells. Individual muscle fibers, not associated with external or internal layers of muscle fibers, are observed to have protoplasmic processes of surrounding gland cells partially encircling the muscle cell. The protoplasmic processes contain long microtubules which are arranged parallel with the length of the processes (Fig. 5). Their length can be considerable; 1.5 μ has been the greatest uninterrupted length measured. The microtubules are seen as hollow tubules in cross-section, usually evenly spaced and close to the plasma membrane of the processes or the main body of the gland cell (Fig. 6). The diameter of the microtubules is 200 \AA . The processes and microtubules belong to cells which have an expanded main cell body containing rather conspicuous granules each surrounded by a membrane and containing an electron-opaque matrix (Fig. 6). The granules measure 500 to 800 $m\mu$ and are presumably secretory products.

Neuromuscular Junction

Nerve plexuses are often observed in the vicinity of the muscle fibers. Synaptic nerve endings can be observed in close contact with sarcoplasmic outpocketings from the fibril. At the point of membrane contact there is an increased osmiophilia in the membranes of both muscle and nerve cells (Fig. 8). The synaptic nerve endings contain vesicles of several types, small ovoid or round clear vesicles which measure in diameter 200 to 400 \AA , and larger osmiophilic granule-containing vesicles ranging in diameter size from 400 to about 800 \AA (Fig. 8). Some vesicles, particularly the larger ones, have less dense granules. A few vesicles could not be classified as being granule-containing or not, since the granular substance was exceedingly pale. In the sarcoplasmic projection which is in contact with the nerve ending, there are at least three invaginations of the plasma membrane with dense body projections (Fig. 8, arrows).

DISCUSSION AND SUMMARY

The observations presented suggest that the pharyngeal muscles of the planarian *Dugesia ti-*

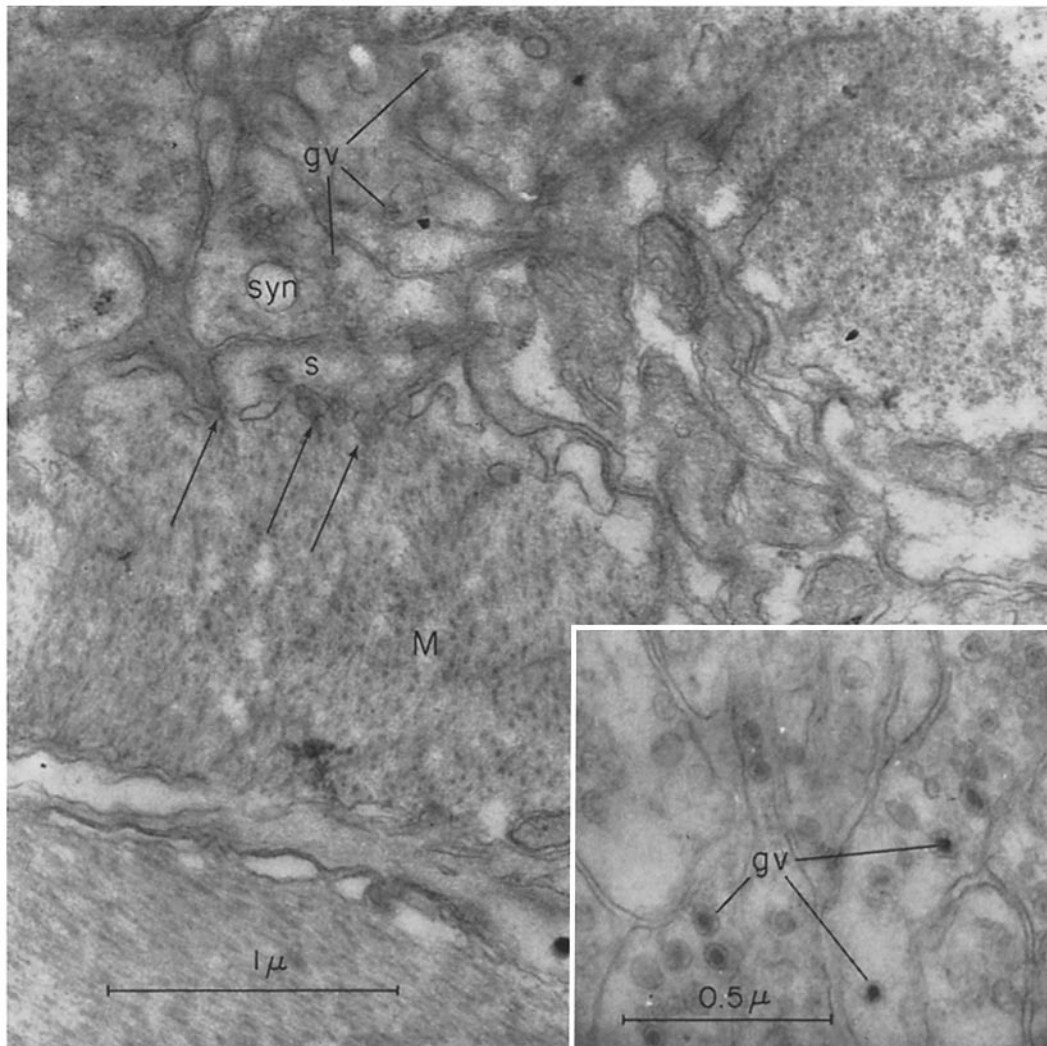


FIGURE 8 Electron micrograph of part of a muscle fiber (*M*) with a sarcoplasmic projection (*s*) in contact with a synaptic nerve ending containing numerous synaptic vesicles (*syn*). The vesicles are of two main types: ovoid or circular pale vesicles and osmiophilic granule-containing vesicles (*gv*). At the point of contact of the intact sarcoplasmic membrane and axolemma there is increased osmiophilia. Note three invaginations of plasma membrane (arrows) and corresponding dense body attachments in the area of the synaptic nerve ending. $\times 38,000$.

Insert shows greater detail of different vesicles of synaptic nerve endings from a neighboring nerve plexus. Ovoid or round pale vesicles are seen together with granular vesicles (*gv*) or "catechol-amine bodies." $\times 56,000$.

grina lack cross-striations and Z lines. They have two distinct types of myofilaments, different in diameter and distribution within the fibril. The filaments are not segregated into arrays corresponding to A and I bands. The filaments are

described as thick or thin on the basis of their diameter measurements.

On these morphological grounds, these muscles cannot be classified as striated. Though two types of myofilaments are present, they differ from

striated muscle, both vertebrate and invertebrate, in lack of striations as well as in absence of Z line or disc. They differ from invertebrate helical muscle in absence of a helical arrangement of myofilaments, but they are similar in having two types of myofilaments and in absence of Z line. They differ from vertebrate smooth muscle in having two types of filaments rather than one type as described for several vertebrate smooth muscles (2, 4, 19, 20). The position of the nucleus in a sarcoplasmic projection peripheral to the fibril in the planarian muscles observed also differs from that of the nucleus of vertebrate smooth muscle, which occupies a position in the center of the cell. There is one similarity to vertebrate smooth muscle (2, 19), namely the presence of the conspicuous dense body or dense line seen among the myofilaments of the planarian muscle. Dense bodies have also been observed in other invertebrate smooth muscles (10). In the earthworm body wall muscle, Hanson (6) reported a regularly spaced series of transverse strips of two kinds, alternating along the fiber length. Hanson suggested that they may be comparable to the Z and M lines of cross-striated muscle fibers. Although the dense body projections have an arrangement which is not regular in the planarian muscle, the strips may have some functional similarity. The apparent origin of the dense lines or bodies at the plasma membrane may indicate that they serve to transmit stimuli intracellularly from the membrane. It is interesting to note the presence of plasma membrane invaginations with dense body projections in the sarcoplasm in contact with a synaptic nerve ending (Fig. 8).

The osmiophilic granule-containing vesicles in the synaptic nerve endings have been considered to be "catechol-amine bodies" and usually associated with adrenergic nerve endings (1, 3, 18). The simultaneous presence of the granular vesicles and the usual type of clear synaptic vesicles at the nerve endings has been seen by other investigators in other organisms (18). It has been suggested (18) that more than one transmitter could be liberated at a single synapse.

The dense bodies may act as Z lines to allow for some spatial arrangement of the thin filaments with respect to the thick filaments. This suggestion is based on observations of the general spatial disposition of these three components of the fibril. It cannot be established from these data whether the thick filaments are continuous or not, since

they seem to pass out of the plane of section. Thin filaments give an impression of continuity along the long axis of the fibril. Although they may have a discontinuity, such as the H band in cross-striated muscle, it was not readily observable in these micrographs of contracted muscle. Less dense irregularly spaced areas seen in cross-section may correspond to the discontinuous segments of thin filaments. In a longitudinal section, the thin filaments are seen in association with the ends of the dense bodies; the ends of the dense bodies are relatively devoid of thick filaments. When thick filaments appear in the area, it may be due to a somewhat oblique plane of section. If these observations are correct, then the dense lines or bodies may serve as a Z line for the attachment of thin filaments.

There is evidence that cross-links between the thick and thin filaments may exist. Whether the cross-links are indeed of the same type as those observed between the thick and thin filaments of cross-striated muscle (12) cannot be concluded, since the proteins composing the filaments can only be assumed at this time to be similar to myosin and actin from data on their general appearance, size, and relative distribution. The diameter of the planarian thick filaments varies from 100 to 200 A, and thus they are somewhat larger and more variable than the thick filaments of myosin of striated muscle, reported as 100 A in diameter (8). The planarian thin filaments are of approximately the same diameter (50A) as the thin filaments of actin in striated muscle (8). The variation in size among the thick filaments may be due to their being somewhat spindle-shaped and thicker in the middle.

On the basis of morphological similarities, it would seem that these planarian pharyngeal muscles with two types of myofilaments and cross-links do have the structural requisites for contraction by the postulated sliding filament mechanism, although this fact does not entirely exclude a different mechanism of contraction for these or other smooth muscles. Hanson and Lowy (10) have stated that the sliding mechanism would explain contraction in other invertebrate smooth muscle with two kinds of filaments. More recently, their work on isolated filaments has demonstrated that an identical structure for actin is found in a large variety of both striated and smooth muscles (11), adding support to the hypothesis that the sliding filament mechanism will eventually be

found in all contractile systems, regardless of their original classification. Nevertheless, changes in the fine structure of these smooth muscles will have to be studied in relation to physiological performance before their contraction can be definitely stated to be due to a sliding filament or a folding filament mechanism.

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