STRUCTURE OF THE LONGITUDINAL BODY MUSCLES OF AMPHIOXUS

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ABSTRACT

The structure of the longitudinal body muscles of *Branchiostoma caribaeum* has been studied by light and electron microscopy. These muscles are shown to be composed of fibers in the form of flat lamellae about 0.8 μ in thickness, more than 100 μ wide, and reaching in length from one intermuscular septum to the next, a distance of about 0.6 mm. Each flat fiber is covered by a plasma membrane and contains a single myofibril consisting of myofilaments packed in the interdigitating hexagonal array characteristic of vertebrate striated muscle. Little or no sarcoplasmic reticulum is present. Mitochondria are found infrequently and have a tubular internal structure. These morphological observations are discussed in relation to a proposed hypothesis of excitation-contraction coupling. It is pointed out that the maximum distance from surface to myofilament in these muscles is about 0.5 μ and that diffusion of an "activating" substance over this distance would essentially be complete in less than 0.5 msec. after its release from the plasma membrane. It is concluded that the flat form of amphioxus muscle substitutes for the specialized mechanisms of excitation-contraction coupling thought possibly to involve the sarcoplasmic reticulum in higher vertebrate muscles.

INTRODUCTION

As part of an experimental investigation of the role of the sarcoplasmic reticulum in muscle function, a comparative study has been made of the structure of several types of muscle cells with widely different morphological and physiological properties.[1] The objective of this study has been a correlation of functional variation with structural variation, with particular reference to the sarcoplasmic reticulum and its morphological and possible physiological relationships to the cell surface and to the contractile elements of the cell. The present paper is a report on one phase of this study. The light and electron microscopic structure of the longitudinal body musculature of amphioxus (*Branchiostoma caribaeum*) is described and the results are discussed in terms of a proposed function of intracellular membranes (sarcoplasmic reticulum) in the coupling of surface excitation to contraction. These results have been described briefly in preliminary form (1).

Historical Background

Grenacher, in 1867, described fine lamellae in cross-sections of the musculature of amphioxus (2). This was in conflict with earlier reports on the histology of amphioxus muscle that either reported nothing special in the way of histology (3, 4) or reported the musculature to be composed of a collection of myofibrils that were not organized into fibers but packed directly together

[1] The author's doctoral dissertation, entitled "Morphological Pathways for Impulse Conduction in Muscle Cells," The Rockefeller Institute, New York, May, 1959.

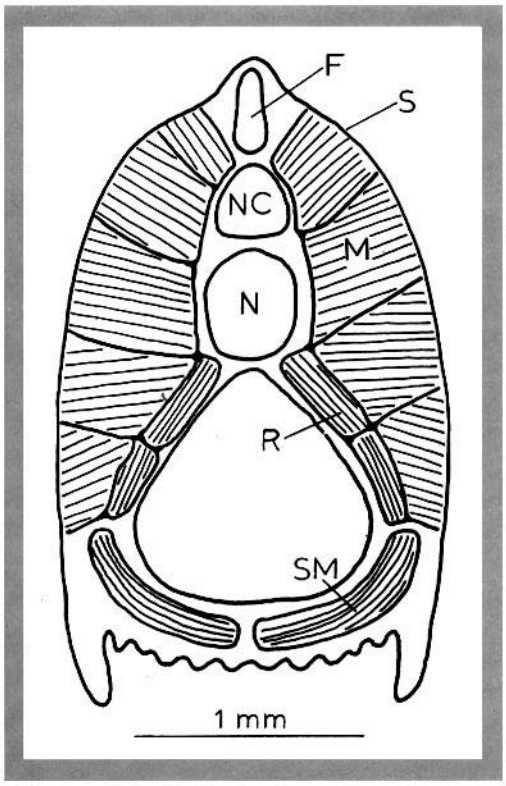


FIGURE 1

Diagram after Willey (14) showing the location of the body musculature (*M*) in a transverse section of amphioxus. Other muscles shown are the rectus abdominis (*R*) and the subatrial muscles (*SM*). Also indicated are the notochord (*N*), the nerve chord (*NC*), the dorsal fin ray (*F*), and the skin (*S*). × approx. 50.

to form the muscle mass (5). Grenacher found that the whole muscle mass of amphioxus was composed of very thin lamellae that ran outward from the internal organs to the skin. He placed the thickness of these lamellae between 0.8 and 1.0 μ and their width at about 12 μ, and reported that they contained few nuclei and had no sheath. This description, as will be shown here, is quite accurate at the light microscope level with respect to the form and thickness of the lamellae and the paucity of nuclei. The lack of a sheath, however, must be reinterpreted in light of the evidence presented here and new knowledge based on electron microscopy of the muscle cell sheath or sarcolemma.

Fig. 1 shows diagrammatically the arrangement of the muscles of amphioxus in transverse sections. The body musculature (*M*) extends outward from the nerve chord, the notochord, and the rectus abdominis to the skin, and is divided by the intermuscular septa into a number of roughly rectangular areas. Other muscles indicated in Fig. 1 are the rectus abdominis and the subatrial muscles. These latter muscles will not be described in this paper.

MATERIALS AND METHODS

Specimens

Branchiostoma caribaeum 3 to 6 cm. in length were collected from the Gulf of Mexico along the Florida coast and flown to New York in oxygenated sea water.[2] They were easily kept alive for several weeks in a circulating, filtered marine aquarium and used as needed.

Preparation of Tissues for Microscopy

Specimens for light microscopy were fixed in Bouin's fixative, dehydrated in a series of ethyl alcohol and water mixtures, and embedded in paraffin.[3] Sections were cut at about 4 μ and stained with iron alum and iron hematoxylin.

Specimens for electron microscopy and phase light microscopy were immersed in fixative until dead, and then transected into pieces 1 to 2 mm. long that were further cut longitudinally into pieces representing about one-quarter of the cross-section. Fixation was continued for a total of ½ to 1 hour. The fixing agent was 1 per cent osmium tetroxide in acetate-veronal buffer (6), at pH 7.4, with 7.2 per cent sucrose and 0.001 M calcium chloride added. Better fixation was obtained using this fixative than when the sucrose was omitted. Dehydration was carried out in a series of ethyl alcohol and water mixtures, and embedding was done at 60°C. in *n*-butyl methacrylate plus 2 per cent of 50 per cent 2,4-dichlorobenzoyl peroxide in dibutyl phthalate (Luperco CDB) as initiator and plasticizer. Thick sections for phase microscopy and thin sections (about 60 mμ) for electron microscopy were cut on a Porter-Blum microtome (7) using glass knives. All thin sections were mounted on carbon coated specimen grids. Unless otherwise specified in the figure legend, all sections shown were "stained" with lead

[2] The author is indebted to Dr. E. Lowe Pierce of the University of Florida, Gainesville, Florida, for collecting and shipping the animals.

[3] The author wishes to thank Mr. Peter Satir of The Rockefeller Institute for supplying the paraffin-embedded specimens.

hydroxide to increase contrast (8, 9) and covered with a thin film of collodion to reduce beam damage (10).

Microscopy

Light micrographs of stained paraffin sections were made using Zeiss Planachromat objectives. Methacrylate sections 1 to 2 μ thick were mounted in 1.46 refractive index immersion oil for phase microscopy using the Zeiss Neofluor series of objectives. Electron micrographs were made in a modified RCA EMU 2B and a Siemens Elmiskop I. Details are given in the figure legends.

RESULTS

Myotomes

The longitudinal body musculature of amphioxus is divided into myotomes by transverse connective tissue septa in a manner similar to but not identical with that found in the fishes. The characteristic V shape of the intermuscular septa forming the longitudinal boundaries of the myotomes is shown in the sagittal section in Fig. 2. The species studied here, *Branchiostoma caribaeum*, has an average of 58 myotomes on each side of the body (11) and a longitudinal spacing of about 0.6 mm. between septa.

The arrangement of septa in amphioxus is simpler than the arrangement in fishes, in which the septa bend obliquely backward on the sides of the body so that each myotome partly covers the one behind it. In amphioxus, the intermuscular septa run straight out from the axis of the body and the myotomes do not overlap. This can be seen in the frontal section in Fig. 3. At higher magnification, as in Fig. 4, the longitudinally oriented muscle fibers can be seen to be striated and to insert at their ends on the intermuscular septum. The striation spacing is about 2 μ.

Lamellar Structure

The lamellar form of amphioxus muscle can clearly be seen in images of transverse sections, as in the phase micrographs shown in Figs. 5 and 6. Fig. 5 shows a transverse methacrylate section of

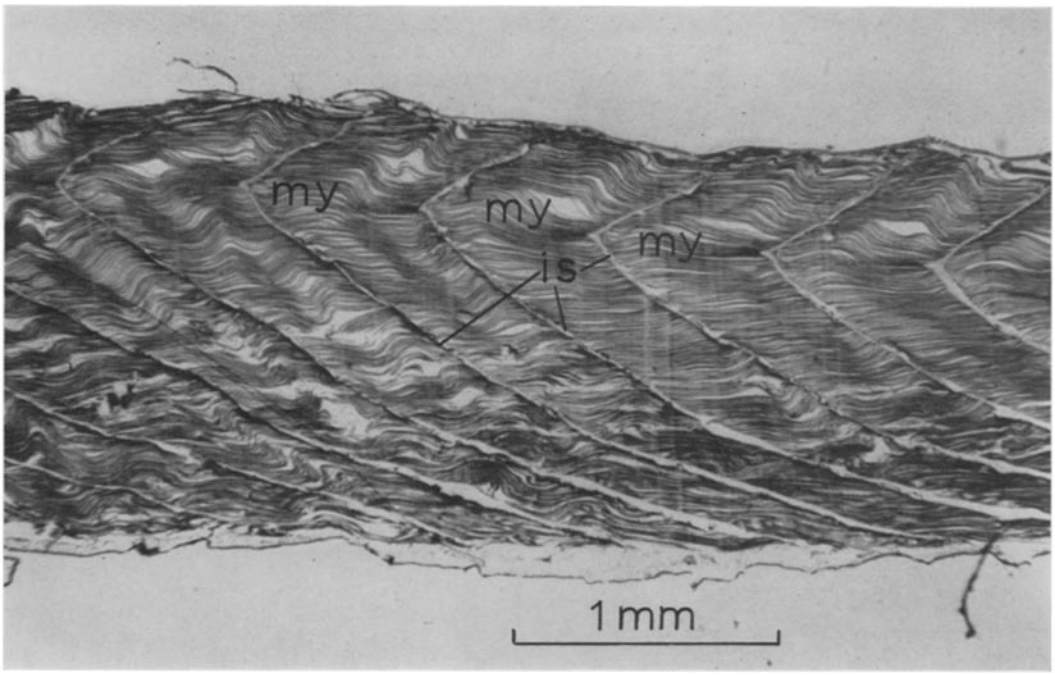


FIGURE 2

Light micrograph of a sagittal paraffin section of amphioxus, anterior end to the left. The body musculature is divided into myotomes (*my*) by the V-shaped intermuscular septa (*is*), between which the very thin, longitudinally oriented muscle fibers can be seen. Zeiss 2.5/0.08 Planachromat objective. × 35.

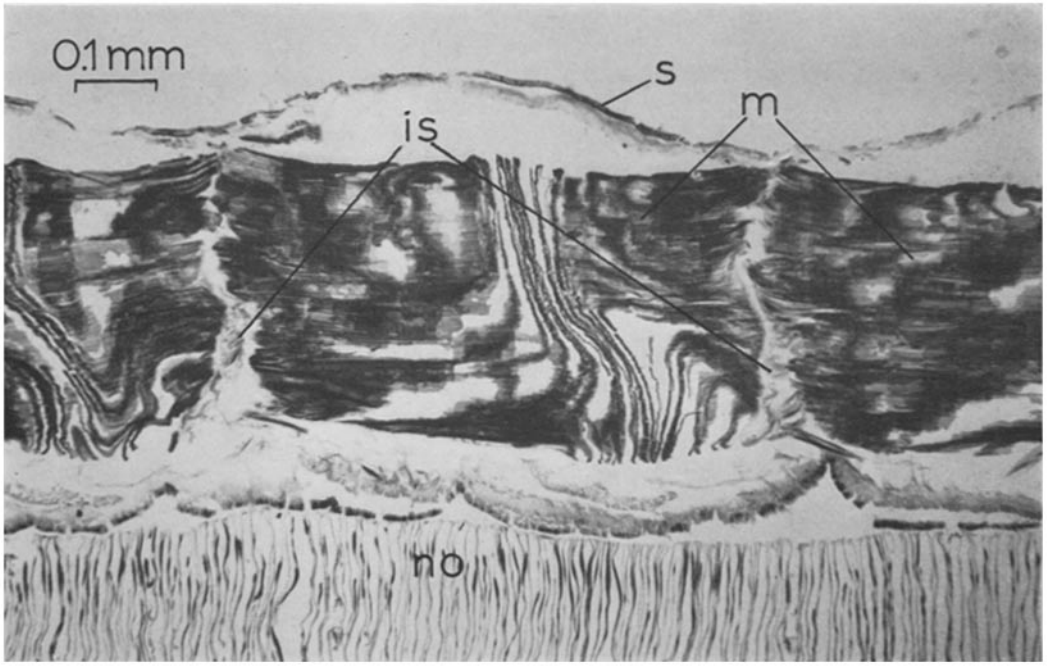


FIGURE 3

Light micrograph of a frontal section showing the orientation of the intermuscular septa (*is*) perpendicular to the axis of the animal. The muscle lamellae (*m*) are in the plane of the section near the ends of the myotome shown, and are cut more transversely near the center of the figure. The notochord (*no*) is at the bottom of the figure and the skin (*s*) is at the top. Zeiss 10/0.22 Planachromat objective. × 110.

the entire complement of lamellae between two intermuscular septa. The lamellae are rather folded, but arranged in a roughly radial pattern. Fig. 6 shows, at higher magnification, the attachment of the lamellae, from two adjacent myotomes, to their common intermuscular septum. This is an oblique view of the junction, since the septum, being inclined to the axis of the animal, runs obliquely through transverse sections.

The relationship of these lamellae to the fibers and myofibrils of "ordinary" striated muscle (*e.g.*, frog twitch muscle fibers) cannot be determined from light microscope images. The thickness of the lamellae, which is a little less than 1 μ, corresponds to the usual diameter of myofibrils, whereas the width of the cross-sectioned profiles is considerably greater and corresponds approximately to the usual fiber diameters. Proper clarification of this point requires determination of how many lamellae compose one fiber, that is, are contained within one plasma membrane. As will be shown in the description of the electron micrographs, the lamellae represent individual myofibrils, each of which is also an entire fiber completely surrounded by its own plasma membrane.

Fibers and Myofibrils

The muscle lamellae are seen in longitudinal section in Fig. 7. The banding of the myofibrils is the same as that commonly observed in vertebrate muscles. This area is contracted, so that little or no I band is seen at the Z line, but even in uncontracted areas the I bands of these muscles are short in relation to the A bands (see Fig. 14 for a less contracted area). A light zone, the H zone, with a central dark M band is found at the center of each A band. The lamellae are completely filled with myofilaments, and the membranous nature of the boundaries of the lamellae can be seen.

Figs. 8 to 10 show the fine structure of the lamellae in transverse sections. The low magnification micrograph in Fig. 8 shows the myofilament

masses which completely fill the lamellae, and the membranes on their surfaces. It appears, from this figure, that these membranes are not part of a network or system of tubules, such as the sarcoplasmic reticulum of many vertebrate striated muscles, but rather that there is a distinct membrane covering the large, flat surface of each lamella. In many preparations, the membranes bounding the lamellae appear more folded, and isolated profiles of membranes (arrows, Fig. 9) appear within the lamellae. It is not immediately clear, however, whether these represent intracellular membranes (sarcoplasmic reticulum) or are profiles of folds and extensions of the membranes between the lamellae. When the plane of section is favorable, as in Fig. 10, considerable folding of these membranes (*pm*) can be observed. Furthermore, the thickness (about 75 A) and appearance of the isolated membranes are identical with those of the clearly surface membranes. Thus it seems most probable that membranes in amphioxus muscle are largely limited to the surfaces

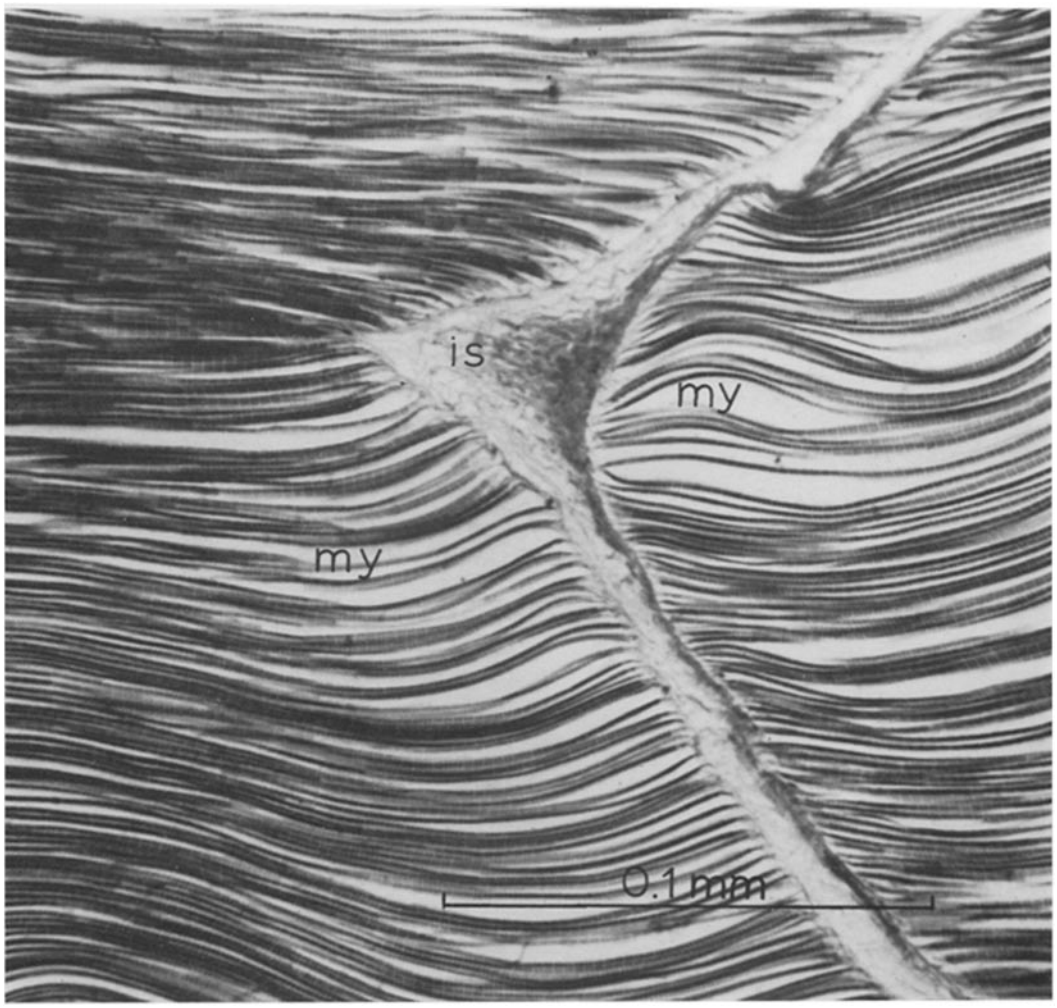


FIGURE 4

Higher magnification light micrograph of a section similar to the one in Fig 2. The insertion of muscle fibers from two myotomes (*my*) on their common intermuscular septum (*is*) near its apex is shown. The striation spacing measures about 2 μ, of which more than half is A band with a prominent H band. The I bands are short and no Z line is seen. Zeiss 40/0.63 Planachromat objective. × 650.

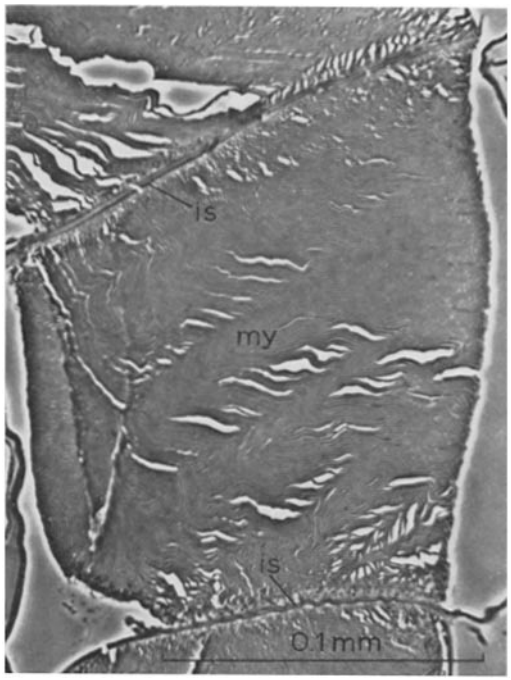


FIGURE 5

Phase micrograph of a transverse section showing the lamellar form of the body musculature. The lamellae of one myotome (*my*) are shown bounded by two intermuscular septa (*is*). 2 μ methacrylate section, unstained. Zeiss 25/0.60 Neofluor phase objective. × 440.

of the lamellae; intralamellar membranes are certainly not common.

An important question is whether these membranes bounding the lamellae are plasma membranes separating sarcoplasm from extracellular space or whether they are a new form of the sarcoplasmic reticulum lying between the lamellae. We have answered this question by examining the lateral edges of the lamellae and the ends of the muscle lamellae where they insert on the intermuscular septa, that is, the myotendonal junction. The form of the myotendonal junction in amphioxus is the usual form observed in vertebrates and consists of tubular penetrations of extracellular connective tissue, bounded by plasma membrane, into the ends of the muscle fibers. This is illustrated in transverse section in Fig. 11 and in longitudinal section in Fig. 12. In these figures, the plasma membranes that separate connective tissue components on the extracellular side from myofilaments on the intracellular side, at the ends of the fibers, can be seen (*pm*). The continuity of these membranes with the membranes between the flat surfaces of the muscle lamellae (*pm'*, Fig. 12) settles the question of the nature of the latter membranes by identifying them as plasma membranes. Furthermore, at the edges of the lamellae, *e.g.* at the sheath of the notochord or at the skin, continuity of the plasma membrane around the edges of the fibers can clearly be seen (Fig. 13). No evidence of branching between lamellae has been found either in longitudinal or in transverse sections. Thus, each of the muscle lamellae of amphioxus is a continuous flat plate bounded on all surfaces by plasma membrane. It is an entire fiber, in that it is the entire cell bounded by a particular plasma membrane, and contains a single mass of myofilaments, that is, a single myofibril.

When the plane of the section coincides with the plane of one of the flat muscle fibers, rather large areas of myofibril can be seen, as in the section in Fig. 14. The large width of the myofibrillar mass and the continuity of the band structure across the flat fiber can be seen, further illustrating the point that each fiber contains only a single myofibril. This area is less contracted than the area shown in Fig. 7, and the short I bands can more clearly be seen. The average width of the fibers, as measured in electron micrographs, is greater than the 12 μ reported by Grenacher (2) for *Branchiostoma lanceolatus*, a species which is of about the same size as the species studied here. In many cases a single fiber could be traced in transverse sections across the entire width of a hole in the electron microscope specimen screen, a distance of about 200 μ, and clearly defined edges of fibers were never found except at the lateral boundaries of the myotomes. The average width of the fibers therefore must be much larger than 12 μ and is probably at least 200 μ, and it seems quite possible that most or all of the fibers extend across the width of the myotome.

Myofilaments

The myofilaments are shown at higher magnification in Fig. 15. They are arranged in a double hexagonal array of two sets of filaments, with the smaller secondary filaments placed at the trigonal

points of the primary filaments. This section passes through the region of the A band where both sets of filaments are found. In certain other parts of the A band, more specifically the H zone and its central M band, only the primary filaments are seen. Only secondary filaments are found in the I bands. This arrangement of filaments in amphioxus muscle is entirely consistent with the arrangement found in several other vertebrate muscles (12).

Sarcoplasmic Reticulum

As pointed out above, only a small complement of membranes that might be intracellular can be found in the muscle fibers of amphioxus, and it seems highly probable that these are the result of folding of the plasma membranes. Therefore, it can be concluded that little or no sarcoplasmic reticulum is present.

Mitochondria

Irregularly shaped bodies about 0.5 to 1.0 μ in size are found infrequently in amphioxus muscle fibers and are identified on structural grounds as mitochondria. One such body is shown in Fig. 9, and another in Fig. 16. These bodies are bounded by double membranes and have a tubular internal structure. A possible significance of the scarcity of mitochondria in amphioxus muscle will be discussed.

Nuclei

Nuclei are found infrequently in sections of amphioxus muscle and thus far no more than one

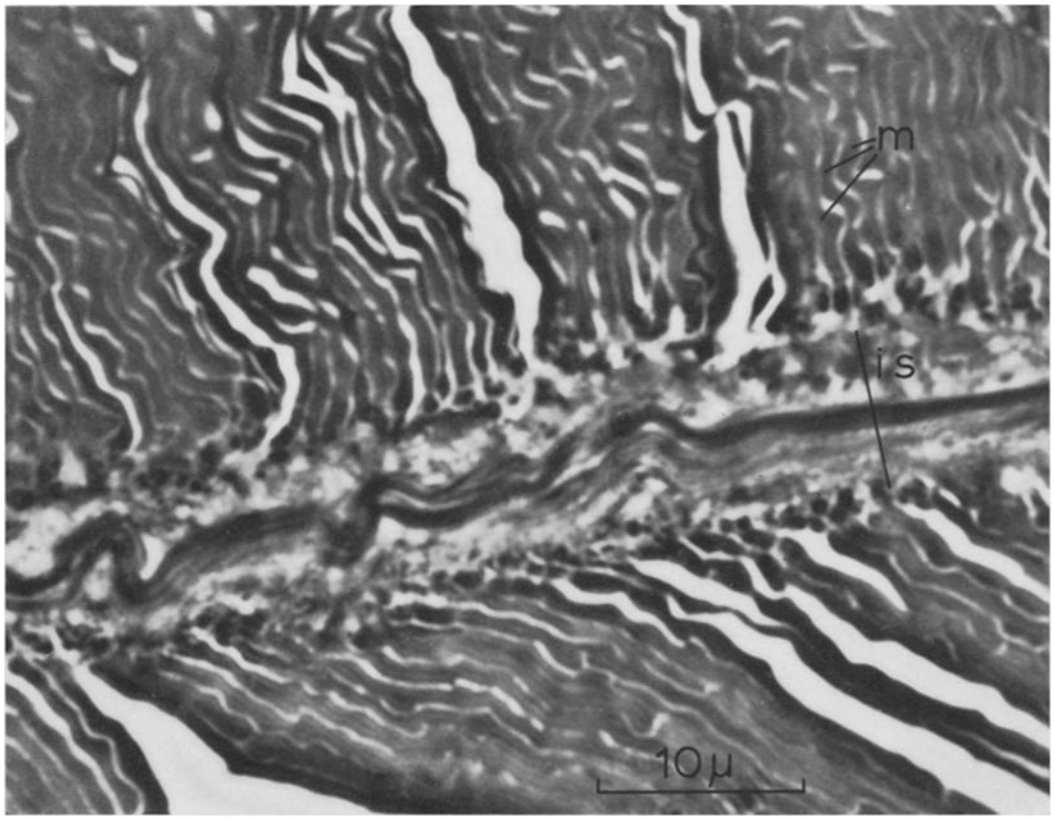


FIGURE 6

Higher magnification phase micrograph of a section similar to the one in Fig. 5, showing the muscle fibers (*m*) and an intermuscular septum (*is*) in greater detail. The average thickness of the fibers measures about 0.8 μ in this section. Zeiss 100/1.30 Neofluor oil immersion phase objective. × 2700.

nucleus has been found in a single muscle fiber. This is in agreement with Hatschek (13), who describes each lamella as developing from a single mononucleated mesodermal cell, with the single nucleus retaining a position in a bulge at the outer edge of the developing plate. The appearance of the nuclei of amphioxus muscle is similar to that of nuclei in many vertebrate muscles. Fig. 17 shows a typical nucleus, in amphioxus muscle, with a large dense nucleolus and a granular or filamentous nucleoplasm. The muscle fiber is thickened where the nucleus is present, and the single myofibril passes to one side of the nucleus.

Sarcolemma

The plasma membrane appears, in amphioxus muscle, not to be associated with any extracellular fibrils, and it has only a minimum of ground substance or basement membrane such as is found in the sarcolemma of vertebrate muscle fibers. This is probably related to the lack of connective tissue components between the fibers and supports the view that the outer layer of the vertebrate sarcolemma is of connective tissue origin and not derived from the muscle fiber itself.

Three-Dimensional Structure

The structure of the muscle fibers of amphioxus is summarized in the three-dimensional reconstruction shown in Fig. 18. Parts of five fibers are depicted projecting forward and cut transversely a short distance from their insertions on an intermuscular septum. The spacing between the lamellae is somewhat exaggerated in this drawing in order to show each fiber clearly. The arrangement of lines and dots representing

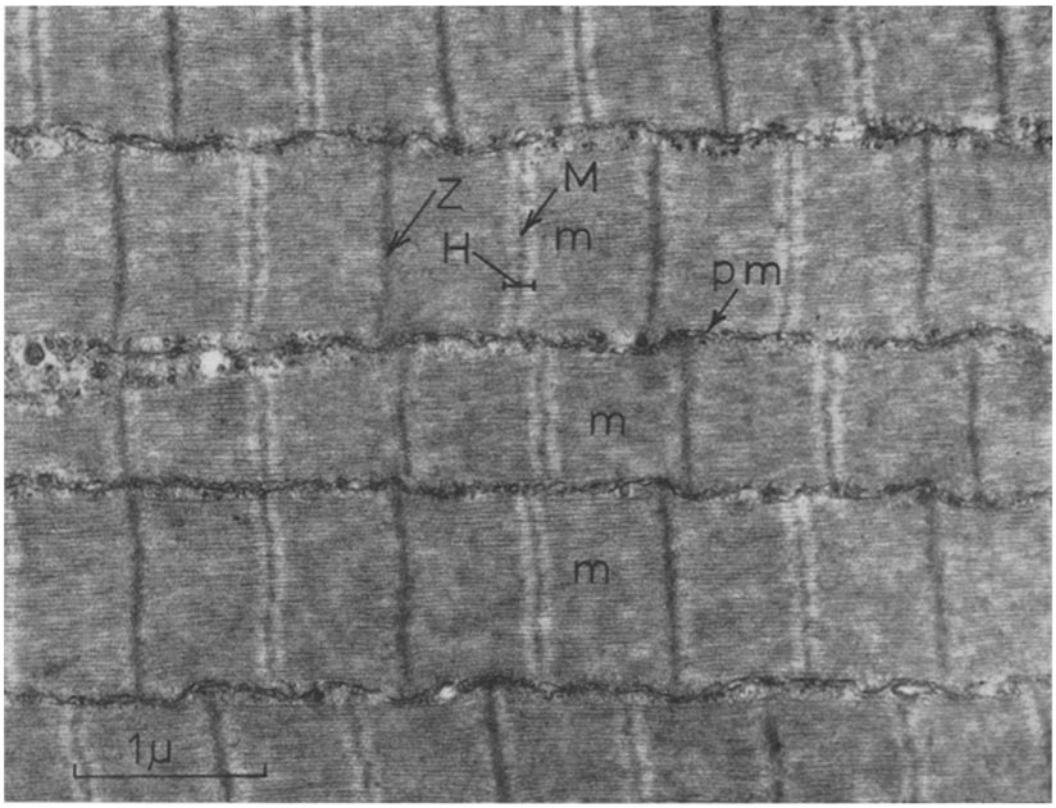


FIGURE 7

Electron micrograph of a sagittal section showing several muscle fibers (*m*) separated by membranes (*pm*). The Z line, H band, and M line of one sarcomere are labeled. RCA. × 25,000.

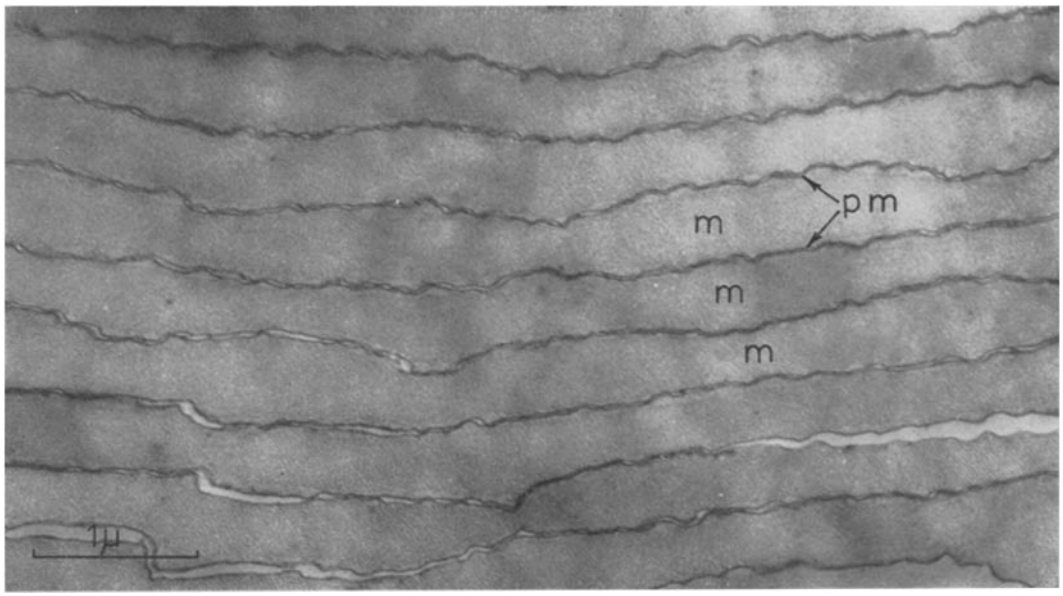


FIGURE 8

Low magnification electron micrograph of a transverse section showing the membranes (*pm*) covering top and bottom of each flat muscle fiber (*m*). RCA. × 22,000.

myofilaments in this figure is intended only for purposes of shading and is not meant to imply structural organization of the myofilaments, which, as mentioned, are arranged in an interdigitating hexagonal array.

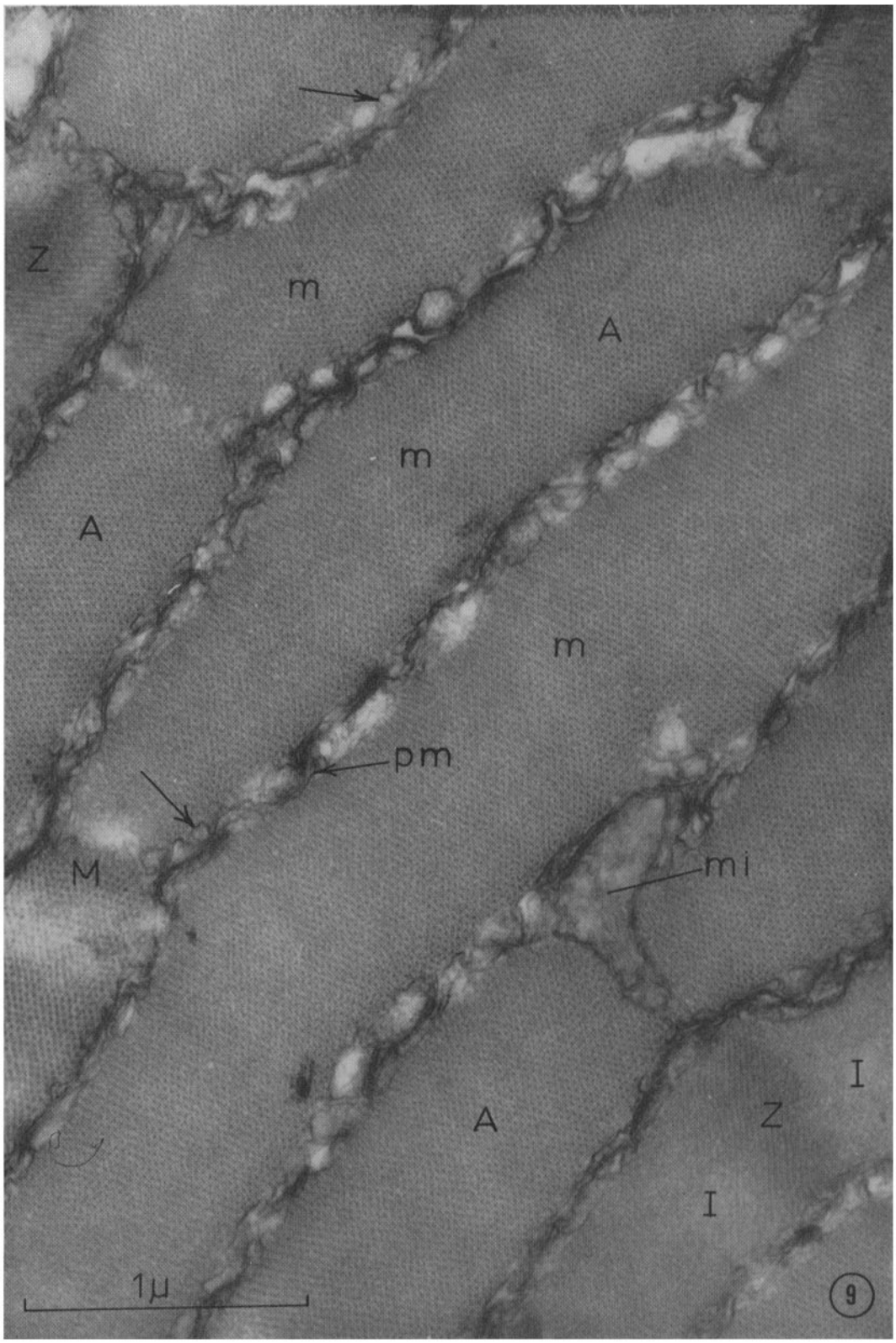
DISCUSSION

Muscle Structure

The fine structure of amphioxus muscle as described here is unique among all muscles of which the fine structure is known. However, there are some striking similarities between the fine structure of amphioxus muscle and that of vertebrate skeletal muscle. These similarities make it seem appropriate to describe amphioxus muscle as having a "modified" or, considering the probable significance of amphioxus in vertebrate evolution as a descendant of a primitive chordate ancestor of the vertebrates (14, 15), a "primitive" vertebrate form at the fine structural level. Prominent among these similarities is the arrangement of myofilaments in amphioxus, as in the vertebrates, in an interdigitating hexagonal array of primary and secondary filaments, with the smaller secondary filaments at the trigonal points of the seminumerary primary filaments. This is identical with the arrangement in mammalian (rabbit) and amphibian (frog) skeletal muscle (12), and can be contrasted with the arrangement in insect indirect flight muscle, where the smaller secondary filaments are situated between two primary filaments rather than being trigonally located (16).

Plasma membranes and nuclei can be listed as additional structures in amphioxus that show no striking variation from the usual vertebrate form. The plasma membrane is certainly different in gross form but probably not in structure. Thus it seems as if the contractile machinery of amphioxus muscle, at the level of the plasma membrane and also of the myofilament, is identical with that demonstrated for vertebrates. Any difference that makes a comparison of muscle of amphioxus with that of the vertebrates interesting, therefore, will most likely be found in phases of the contractile cycle that lie between the activity of the plasma membrane and the actual contraction of the myofilaments, or, structurally, in components other than the plasma membrane and the myofilaments themselves.

The most striking difference between amphioxus



muscle and the usual vertebrate muscle is to be found in the shape of the myofibrils and fibers. Compared in cross-section with the roughly cylindrical and 0.5 to 1 μ diameter vertebrate myofibril, the myofibrils of amphioxus are considerably larger in one dimension and roughly the same size in the perpendicular direction. Furthermore, each fiber of amphioxus muscle contains only one myofibril, in contrast to the usual vertebrate fiber, which may be composed of several thousand myofibrils. The importance of these differences will be considered below in relation to problems of excitation-contraction coupling.

Origin of the Sarcoplasmic Reticulum of Vertebrate Muscles

The presence of plasma membranes between the myofibrils of amphioxus muscle, in the position occupied in vertebrate striated muscles by the sarcoplasmic reticulum (17), must be taken as highly suggestive of the origin of at least certain parts of the sarcoplasmic reticulum from the plasma membrane in vertebrate evolution. The production of intracellular membranes from infolding of the plasma membrane is not an uncommon phenomenon in cytology. It occurs in phagocytosis and pinocytosis in many types of cells. Furthermore, it has been shown by Smith (18) that in an insect muscle (fibrillar flight muscle of *Tenebrio molitor*) the plasma membrane infolds and forms a very close association with some of the myofibrils. A very extensive system of infoldings of the sarcolemma has also been found in crab striated muscle (extensor carpopodite of *Carcinus maenas*) by Peachey[1] (19). It thus seems quite possible that such infoldings may detach and become completely intracellular, so that a portion of the sarcoplasmic reticulum might arise evolutionarily and/or morphogenetically from the plasma membrane. In any case, this idea may be a valuable one to keep in mind in future studies on muscle cells.

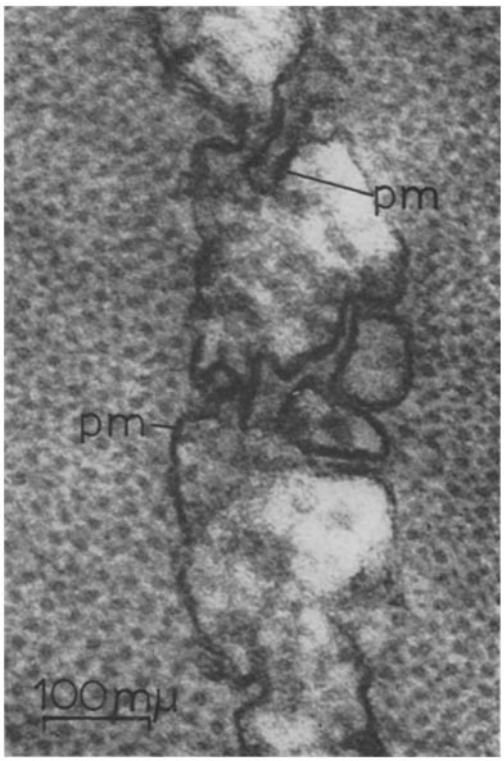


FIGURE 10

Transverse section of two adjacent fibers showing infolding of the plasma membrane (*pm*). RCA. × 140,000.

Mitochondria

The paucity of mitochondria in amphioxus body muscles, as compared with most other striated muscles, is interesting in its correlation with the apparent high degree of fatigability of amphioxus. Couch observed that when live animals (*Branchiostoma lubricum* Costa) were disturbed three or four times, they swam violently for a few seconds

FIGURE 9

Higher magnification micrograph of a transverse section demonstrating the arrangement of myofilaments in a hexagonal array identical with that found in vertebrate striated muscles. Various bands can be identified from the local arrangement of filaments and are marked with their usual designations (A, I, M, and Z). The mitochrondrion (*mi*) in one of the fibers near the lower right corner of the micrograph shows profiles of what appears to be a tubular internal structure. Arrows indicate profiles of infoldings of the plasma membranes (see text). RCA. × 44,000.

and then suddenly became quiescent and sank to the bottom of the tank (20). They were then inexcitable for some time. This was also observed by the present author for the species studied here, where the quiescent period seemed to last for one-half minute or more. This suggests that the body muscles have a rather low reserve of the immediate energy source for contraction and a low rate of oxidative recovery, in correlation with the observed small mitochondrial population.

Excitation-Contraction Coupling

It is generally believed that the significant event, at the surface of a muscle fiber, leading to contraction of the fiber is membrane depolariza-

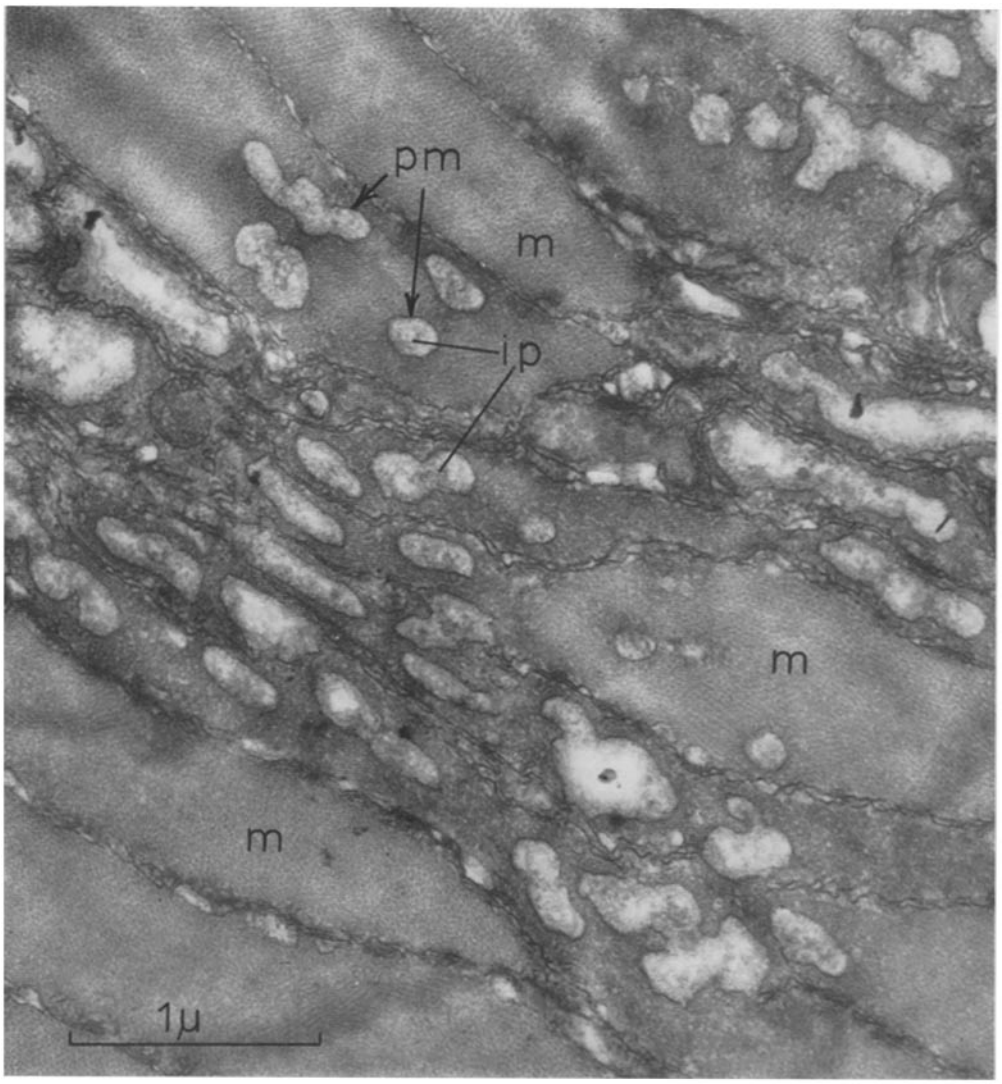


FIGURE 11

Transverse section showing the insertion of several muscle fibers on an intermuscular septum. Finger-like projections of the septum (*ip*) bounded by plasma membranes (*pm*) are seen projecting into the muscle fibers (*m*). RCA. × 33,000.

tion. Other theories, such as the longitudinal current theory, have been proposed, but none has had extensive experimental support or been widely accepted (for a recent rejection of the longitudinal current theory, see Frank, 21). It is well known that Hill (22, 23) demonstrated that diffusion of an "activator" from the surface of a muscle fiber 50 μ in diameter, as commonly found in many vertebrate striated muscles, is not fast enough to account for the observed rapid transition from rest to activity of the entire contractile content of the fiber. Some process more rapid than bulk diffusion must act to convey the effects of surface depolarization transversely inward to the interior of the fiber. This unknown process has become known as *excitation-contraction coupling*.

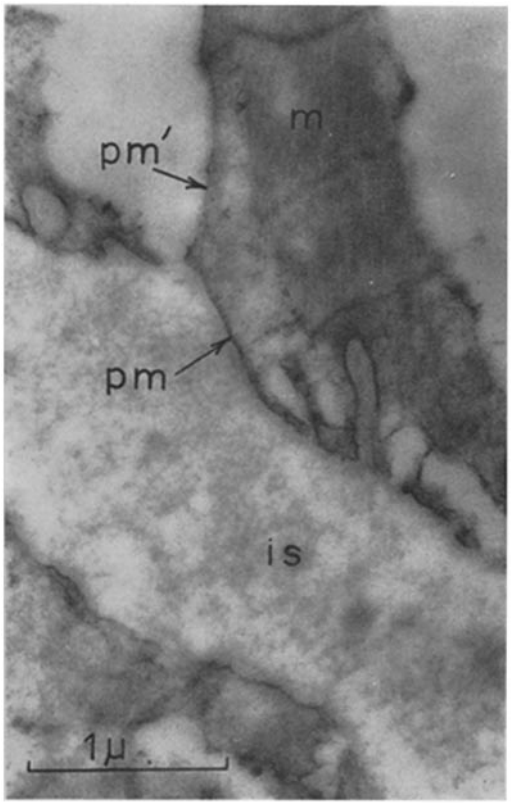


FIGURE 12

Longitudinal section of the region of the myotendonal junction, demonstrating the continuity of the plasma membranes (*pm*) facing on the intermuscular septum (*is*) with those (*pm'*) covering the flat surfaces of the lamellar muscle fibers (*m*). Unstained, uncovered section. Elmiskop I. × 26,000.

In the striated muscles of higher vertebrates, it is suspected that the effects of excitation are carried in from the surface of the fiber along pathways formed by intracellular membranes (1, 24, 25), although there is little or no direct evidence to support this view. In the cases in which the localization of such pathways has been determined by local stimulation (24), it appears as if a portion of the triad of the sarcoplasmic reticulum, as described by Porter and Palade (17), is involved in this function. Considering that the element termed the "intermediary vesicle" by Porter and Palade has been shown by Andersson-Cedergren (26) to be the only part of the triad that forms a transversely continuous pathway, this element is, at present, the most likely candidate as the coupling pathway. Although attempts to show continuity, in mature muscle fibers, between the intermediary vesicles and the plasma membrane have failed, there is evidence of close association and structural similarity between the two, suggesting that the intermediary vesicles may be derived from the plasma membrane. We must still consider the possibility that it will yet be shown that the two are permanently or transiently connected in the mature muscle fiber. Should this be shown to be the case, the content of the intermediary vesicles would be similar to extracellular fluid and the name "xenoplasm" which has been proposed for this content[1] would seem appropriate.

The muscle fibers of amphioxus have been shown to be planar with a thickness of about 1 μ. The analysis of Hill, which considers only cylindrical fibers, cannot be applied to amphioxus muscle. Judging from the rapid swimming motion of amphioxus, its muscles have roughly the same speed as vertebrate striated twitch muscles. This crude judgment of speed is clearly not good enough to allow more than a rough comparison of amphioxus muscle with vertebrate striated muscles. A more accurate comparison will have to await a physiological determination of the speed of amphioxus muscle. However, if activation is truly transferred from the surfaces of the fibers to the contractile proteins in these simple muscles by a diffusing activator, regardless of whether this activator enters through the membrane or is released from its inner surface following depolari-

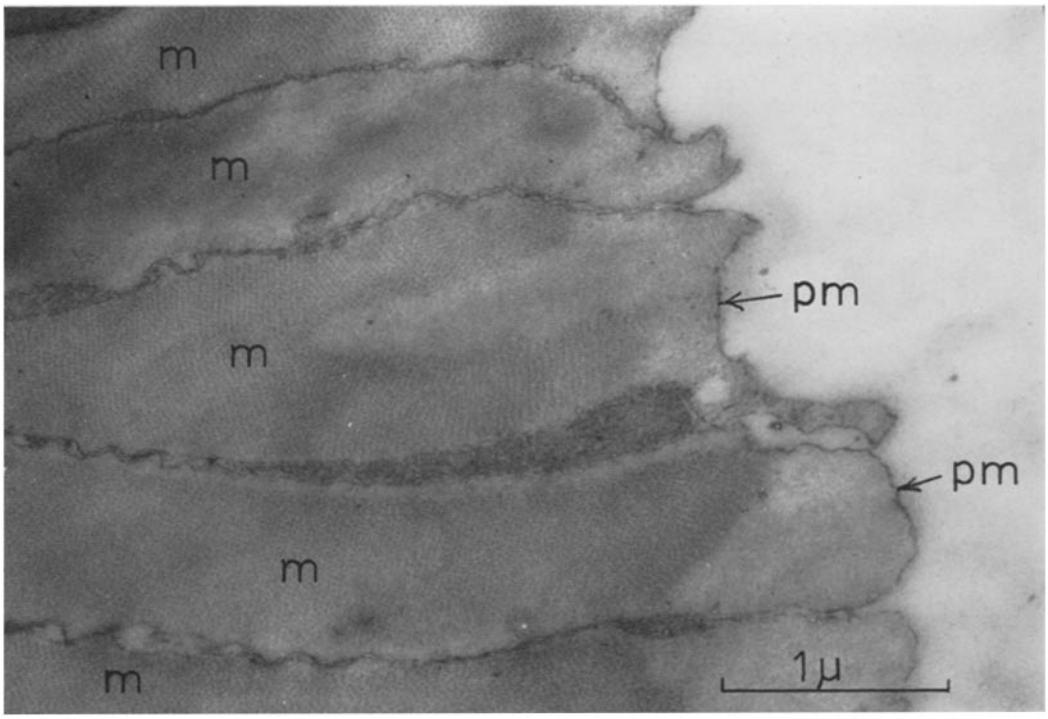


FIGURE 13

Transverse section at the lateral edge of a myotome. Continuity of the plasma membrane (*pm*) around the edge of each muscle fiber (*m*) is seen. RCA. × 30,000.

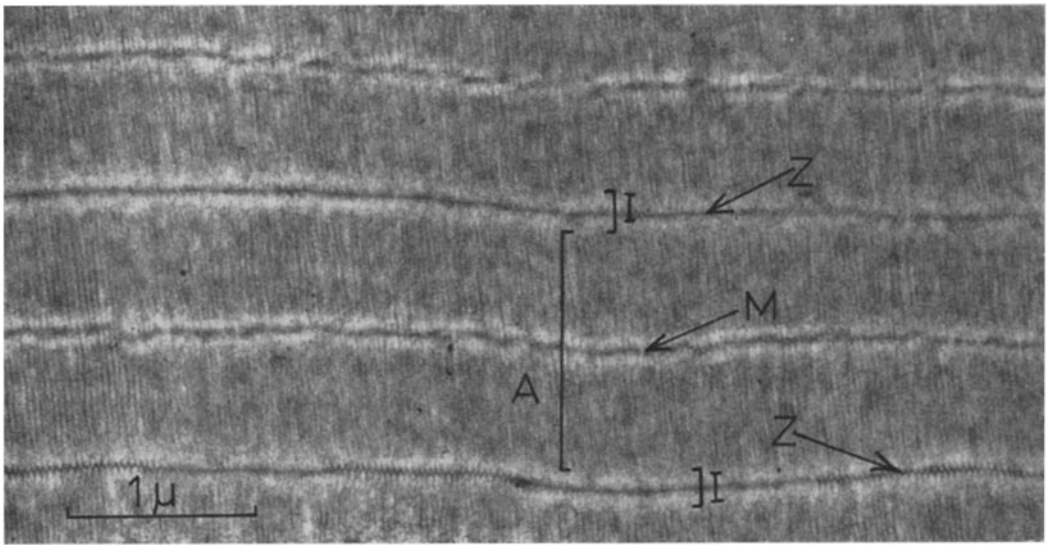


FIGURE 14

Section cut parallel to the plane of a muscle fiber, illustrating the large width of the myofilament mass and the lack of a division of this mass into myofibrils. A, I, M, and Z bands are indicated. The lower Z line shows a zigzag appearance at three places, as has been observed in a variety of other striated muscles. RCA. × 25,000.

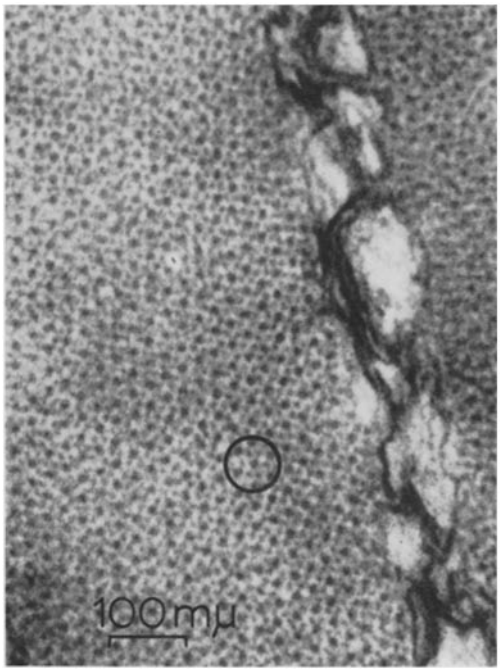


FIGURE 15

High magnification electron micrograph of a transverse section showing in greater detail the hexagonal arrangement of myofilaments. Each primary filament is surrounded by six smaller secondary filaments, each of which is placed equidistant from three primary filaments. This is seen especially clearly in the area marked by the circle. RCA. × 100,000.

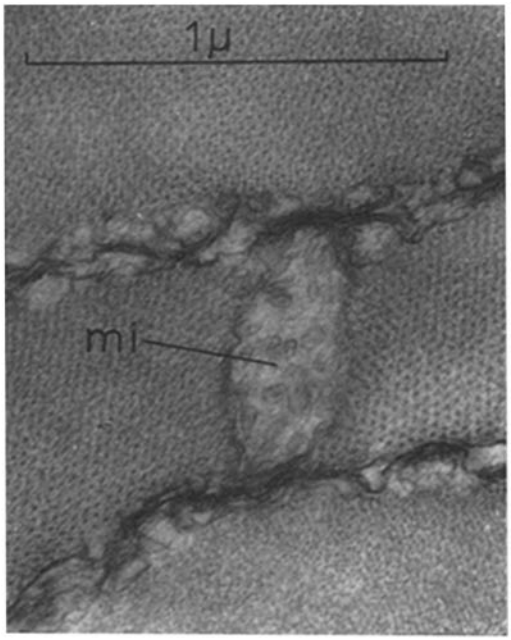


FIGURE 16

Transverse section of a muscle fiber containing a body identified as a mitochondrion (*mi*). The appearance of tubular internal structure is evident in this micrograph. RCA. × 56,000.

zation, the distance over which diffusion must act is 0.5 μ or less. Preliminary results from a numerical computer method of diffusion analysis (27) indicate that diffusion of a substance, such as calcium chloride, into lamellae 1 μ thick is essentially complete (reaches equilibrium) in less than 0.5 msec. after diffusion has begun (0°C., diffusion constant = 0.6 × 10^{-5} cm.²/sec.). It thus is clear that diffusion alone could account for a rapid link between surface and myofilaments in amphioxus muscle.

It is interesting to note that no sarcoplasmic reticulum is present in amphioxus muscle; nor is one needed for purposes of excitation-contraction coupling. Admittedly, the same arguments could be applied with equal success to other possible functions of the sarcoplasmic reticulum, as, for example, metabolite transfer, relaxation, etc. It seems reasonable, however, to conclude, on the basis of the morphological results presented here and the diffusion analysis mentioned, that the problem of excitation-contraction coupling is solved in amphioxus muscle by a unique arrangement of flat fibers so thin that no part of the contractile machinery is more than about 0.5 μ from the plasma membrane and extracellular space, a distance over which diffusion can quickly act.

Note Added by the Author: After the completion of this manuscript and a brief presentation of some of the results to the European Regional Conference on Electron Microscopy in Delft in August, 1960, two papers by K. Zapf and Abdel Aziz Ali Mohamed on the fine structure of amphioxus muscle were kindly brought to my attention by Dr. Zapf (*Acta Biol. et Med. Germ.*, 1959, **2**, 331, 508). These authors arrive at several conclusions on the structure of the muscle fibers which are not in agreement with the results presented here, and discuss a mechanism of contraction, consistent with their results, in which the length of the I band remains constant during con-

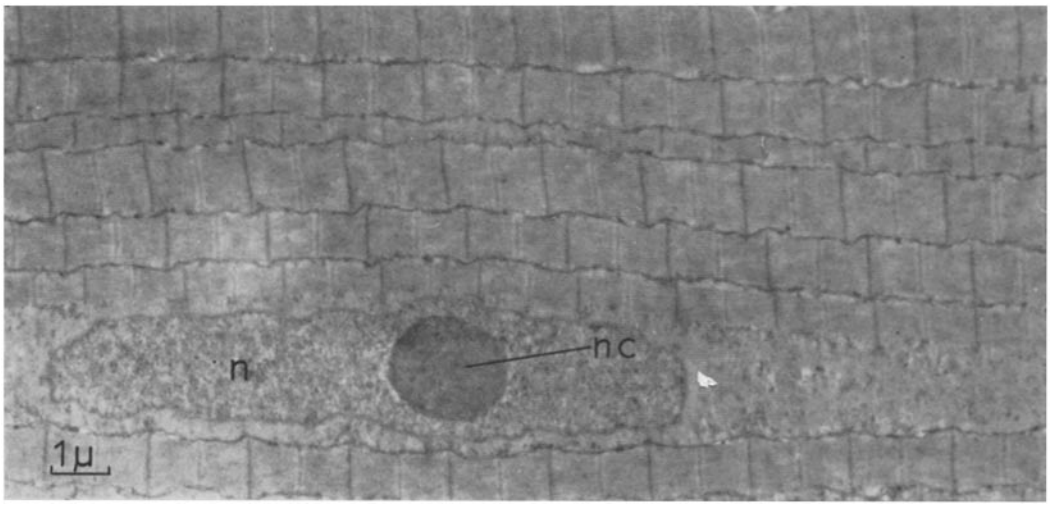


FIGURE 17

Longitudinal section through a nucleus (*n*) with a large nucleolus (*nc*) in a muscle fiber. Unstained, uncovered section. Elmiskop I. × 8,000.

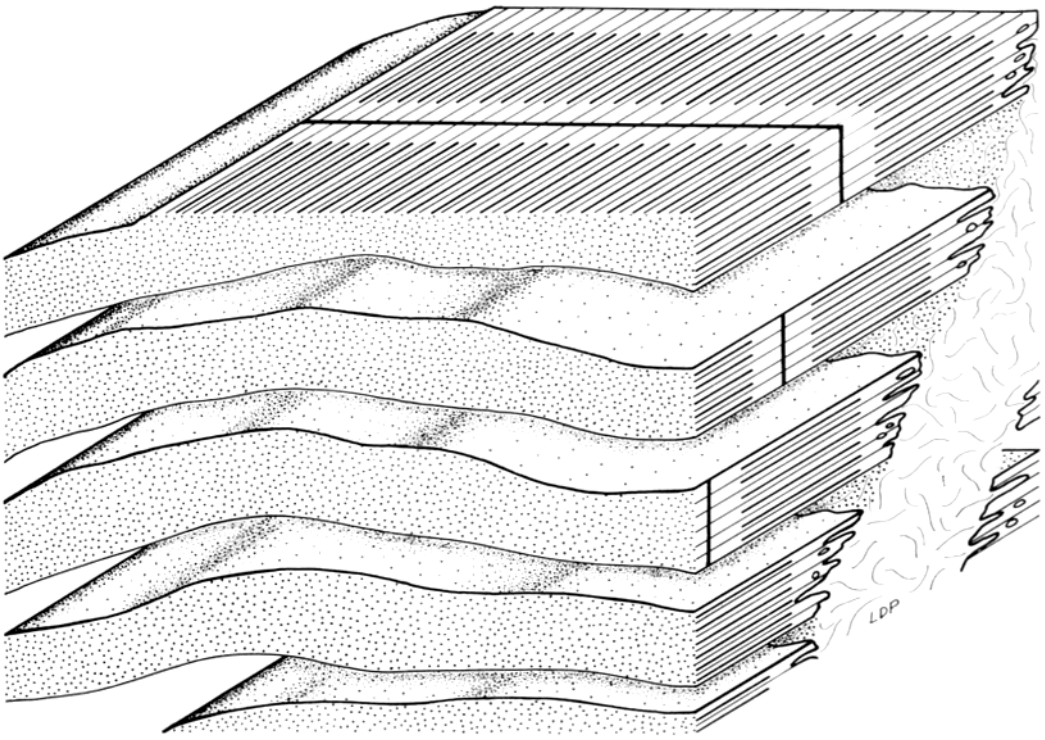


FIGURE 18

Three-dimensional reconstruction of the form of the body musculature of amphioxus. Parts of five muscle fibers are depicted projecting forward from their insertion on an intermuscular septum that runs diagonally from the rear to the bottom surface of the block of tissue shown in the drawing. The space between the fibers is exaggerated in order to show clearly their lamellar form, and the arrangement of lines and dots representing myofilaments is not intended to depict their form accurately. × approx. 25,000.

traction, while the H zone shortens. Comparison of their Fig. 8 (second paper), however, with Figs. 7 and 14 of the present paper indicates that Zapf and Mohamed have misidentified H zones as I bands and *vice versa*. These two regions of the sarcomere have approximately the same length in relaxed amphioxus muscle but can be distinguished by the fact that the H zone is slightly less dense than the I band. Identification of the I bands is made positive in Fig. 14 of the present paper by the zipper-like or zigzag appearance of the Z line at several places in the lower I band. This appearance of the Z line has been observed in a variety of striated muscles, some of which have I bands that are considerably longer than the H zones, being almost as long as the whole A band of which the H zone is only a part, making the I bands easily distinguishable from the H zones. This being the case, the length changes observed by Zapf and Mohamed would then occur in the I bands, in agreement with the figures shown in the present paper and with the sliding filament model of muscle contraction. The H zone, however, would then remain constant in length, a result not in agreement with this model. More work needs to be done on this problem before clear conclusions on the mechanism of contraction of amphioxus striated muscle can be drawn.

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