THE SARCOPLASMIC RETICULUM OF A FAST-ACTING FISH MUSCLE

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INTRODUCTION

In the early years of this century Veratti (15) and a few other uncommonly observant cytologists (11) demonstrated in the sarcoplasm of striated muscle a delicate plexus of strands surrounding all of the myofibrils. The transverse elements of this network were located in a constant relation to the cross-banded pattern of the myofibrils. The majority of cytologists of that period seem not to have accepted the reality of this network, possibly because of their mistrust of the capricious metallic impregnation methods required for its demonstration. Therefore, it received little further study until some 50 years later when Bennett and Porter (3) and Andersson (1) directed attention to a vacuolar or tubular interfibrillar component of the sarcoplasm visible in electron micrographs of striated muscle. Extending these observations, Porter and Palade (9) later demonstrated that these membranous profiles are part of an elaborate reticular system of anastomosing tubules that surrounds all the myofibrils. The majority of the tubular elements of this *sarcoplasmic reticulum* run longitudinally, but there is, for each muscle, a characteristic repeating pattern of transverse channels occurring in register with particular bands in the cross-striations of the myofibrils. These transverse components, called the "triads" of the reticulum, consist of pairs of parallel tubes separated by an intermediate row of small vesicles. The continuous nature of the reticulum, its intimate relation to the myofibrils and to the cell surface have led to the suggestion that the membranes of this system may be involved in impulse conduction from the sarcolemma to the contractile elements in the interior of the muscle fiber (4, 7, 9). Comparative studies by Peachey and Porter (7) correlating cell size, speed of contraction, and degree of development of the reticulum have provided evidence tending to support this hypothesis. The investigation reported here extends these comparative observations on the sarcoplasmic reticulum to a particularly fast-acting fish muscle.

A number of teleost fish including the sea robins (*Prionotus*), the toadfish (*Opsanus*), the croakers (*Micropogon*) and drums (*Sciaenops*) are capable of making clearly audible sounds by a variety of mechanisms. The deep resonant sounds of the toadfish are made by rapid contraction of the intrinsic striated muscle in the taut wall of its gas-filled swim-bladder (14). In recent studies of the neuromuscular mechanism of sound production in this species, Skoglund (12) has shown that the muscle involved reaches its contraction peak and returns to complete relaxation very much more rapidly than do most vertebrate muscles. The present paper describes an elaborate development of the sarcoplasmic reticulum and other fine structural features of this muscle which may be related to its unusual physiological properties.

MATERIALS AND METHODS

The common Atlantic toadfish *Opsanus tau* and the closely related form *Opsanus beta* from the Gulf of Mexico were used in this study. The large heart-shaped gas bladder in these species is of the euphysoclist type. On its lateral surfaces are two prominent equatorial bands of striated muscle which are the subject of this electron microscopic investigation. The fibers are relatively short and run oblique to the long axis of the muscle mass.

Small strips of muscle were excised and fixed for electron microscopy by immersion in a 1 per cent solution of osmium tetroxide adjusted to pH 7.4–7.6 with veronal-acetate buffer. In the case of small fish the entire gas bladder was removed and submerged in the fixative. Small blocks were then cut from the blackened superficial layers of the muscle after 2 to 3 hours fixation. This procedure had the advantage of preserving the muscle *in situ* at resting length with minimal manipulation. The tissues were dehydrated

in a series of increasing concentrations of ethyl alcohol and embedded either in a mixture of 20 per cent methyl/80 per cent butyl methacrylate or in epon according to the procedure of Luft (6). Sections were cut with glass knives on a Servall microtome. Those displaying yellow interference colors were selected for examination. When indicated, the sections were stained with lead hydroxide as recommended by Watson (16) or with phosphotungstic acid. Electron micrographs were made with an RCA microscope, model EMU-3E, or with Siemens Elmiskop I or Elmiskop II at original magnifications of 4,000 to 14,000 diameters.

OBSERVATIONS

The fibers of this fish muscle differ from those in muscles of other vertebrates principally in the shape and disposition of the myofibrils. These differences can best be understood by examination of transverse sections (Figs. 1 and 2). The fibers are closely packed and round or polygonal in section. Each is surrounded by a delicate investment of fine collagenous fibrils which are clearly demonstrated in preparations stained with lead hydroxide (Fig. 1). The nuclei are located in a thin peripheral layer of sarcoplasm and the contractile elements occupy nearly all of the interior of the fiber. The myofibrils are, for the most part, flat ribbon-like structures with the long dimension of their cross-section oriented radially around a slender central core of sarcoplasm. They thus form a thick-walled hollow cylinder. Near the center of this *contractile cylinder* are variable numbers of smaller myofibrils which are not particularly elongated in a radial direction. Sometimes the axis of the fiber is filled with such myofibrils and no core of clear sarcoplasm is discernible. The ribbon-like myofibrils often branch and the distance between them thus remains remarkably constant from the center to the periphery of the contractile cylinder. The interfibrillar clefts are occupied almost exclusively by the sarcoplasmic reticulum. Mitochondria, which are lodged between the myofibrils in most types of muscle, are largely confined, in this muscle, to the core of the fiber or to the thin mantle of sarcoplasm immediately beneath the sarcolemma. Occasionally mitochondria are located among the smaller myofibrils near the center of the fiber, but they are almost never found between the broad flat myofibrils around the periphery.

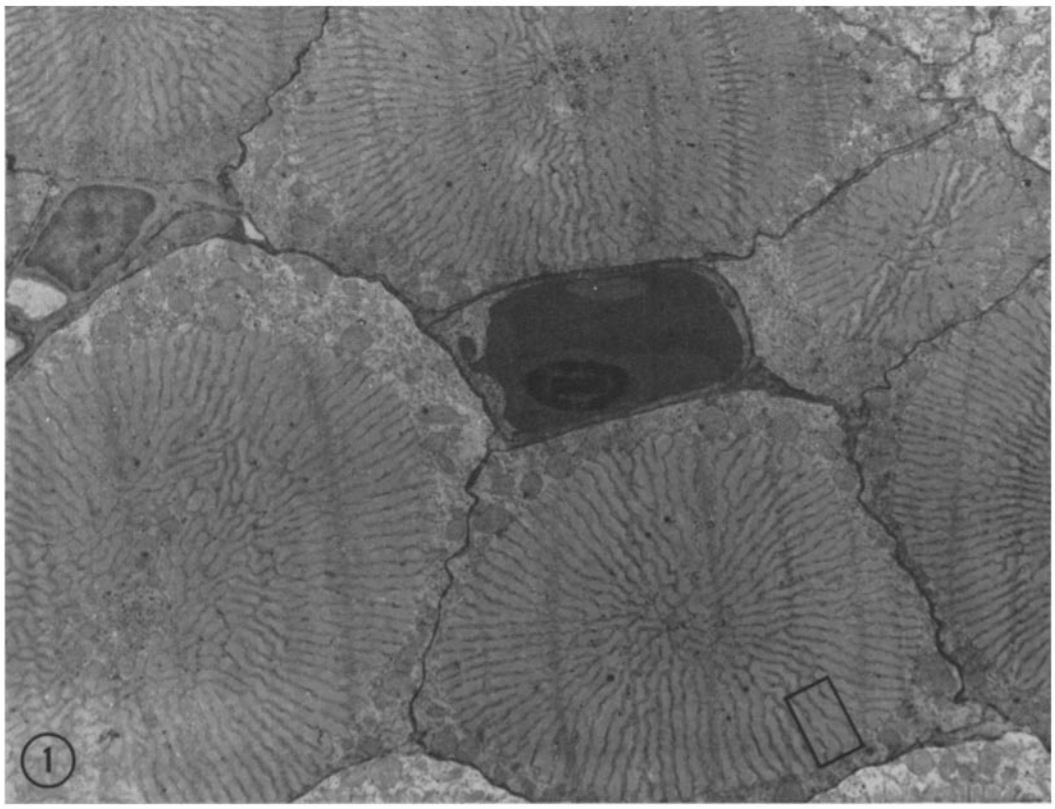
As a consequence of the peculiar internal geometry of these muscle fibers, the thickness of the myofibrils as seen in longitudinal sections varies depending upon the depth of the section in the contractile cylinder. In subtangential sections, the flat myofibrils present their narrowest dimension (300 to 400 mμ), whereas in sections along a diameter of the cylinder they present their broad dimension (5000 mμ or more). Sections coinciding with an interspace between the broad surfaces of

FIGURE 1

A low power electron micrograph of a transverse section of toadfish swim-bladder muscle. Included in the figure are parts of five muscle fibers and a capillary with its lumen occluded by erythrocytes. The lead hydroxide stain has blackened the collagen of the delicate reticulum which surrounds each fiber. Thin ribbon-like myofibrils are arranged radially around a central core of sarcoplasm to form a thick-walled contractile cylinder. In some fibers the sarcoplasmic core is lacking and the interior is occupied by smaller myofibrils of irregular shape. An area similar to that enclosed in the rectangle is shown at high magnification in Fig. 11. Epon section, stained with lead hydroxide. Magnification approximately 3,500.

FIGURE 2

A micrograph illustrating the polygonal outline of the closely packed fibers. The myofibrils take up most of the cross-section. The flattened nuclei are displaced to the outside of the contractile cylinder. Mitochondria generally do not occur between the myofibrils but are confined to the slender core of the fiber or the thin layer of sarcoplasm around its periphery. The cross-striations of the myofibrils are in register throughout the fiber, but when the orientation of the block is not perfect, cross-sections of the fibers shown parallel stripes where the plane of section intersects different band levels. The distance between these stripes varies with the obliquity of the section. Methacrylate section, unstained. Magnification approximately 3,000.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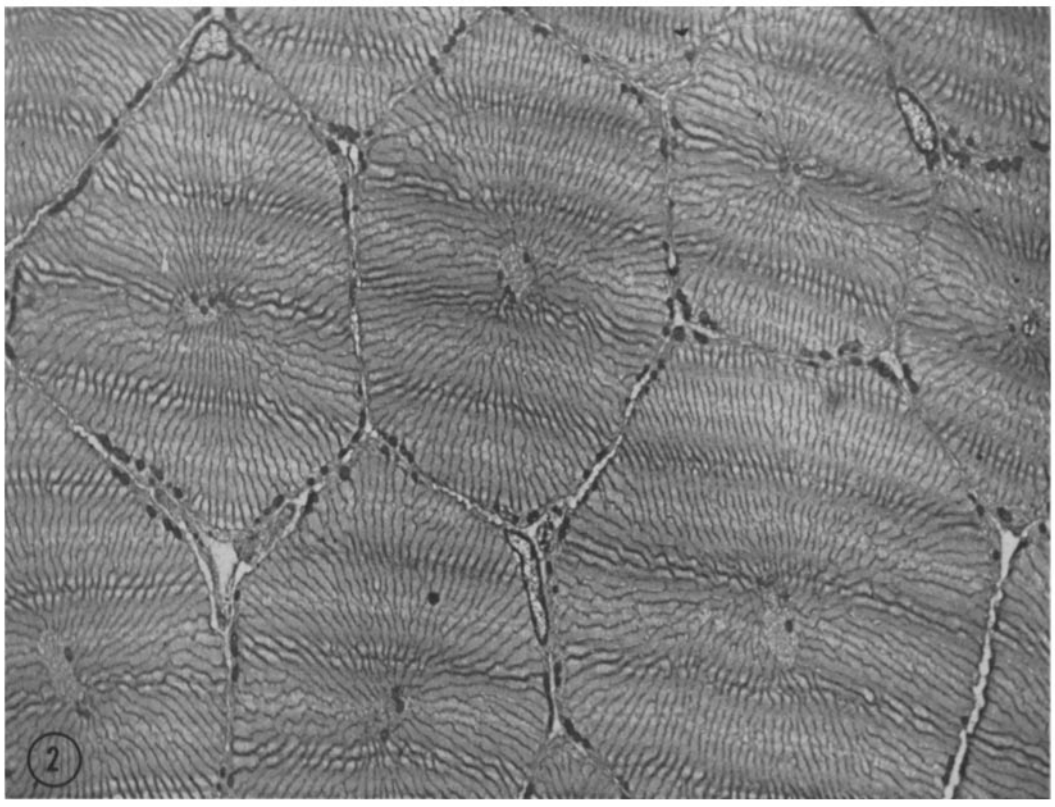
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adjacent myofibrils afford a far more extended view of the topography of the reticulum than can be obtained in any muscle having myofibrils of the usual shape.

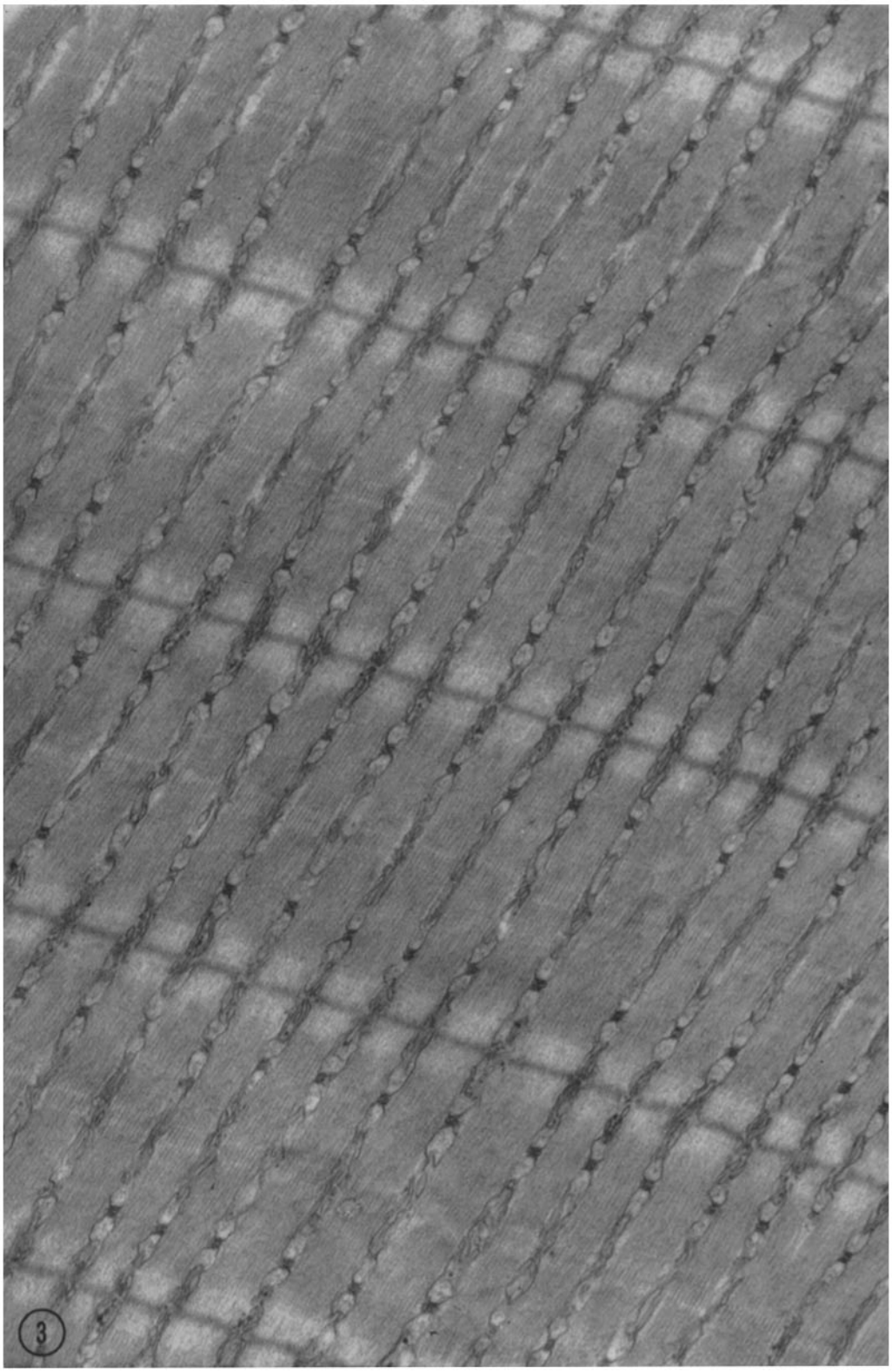
The most striking features of the muscle in longitudinal sections are the precise alignment of the cross-striations of all the myofibrils and the extensive development and impressive regularity of the sarcoplasmic reticulum. The several components of its repetitive pattern are invariably associated with exactly the same regions of each sarcomere (Fig. 3). The sarcomeres are approximately 2 μ long and show distinct A, H, I, and Z bands. The relatively long A band ($\backsim$ 1.5μ) takes up three-fourths of the sarcomere length. Two triads of the sarcoplasmic reticulum are found opposite the ends of the A band, very near the A-I junction. These run in the interfibrillar spaces, radially with respect to the axis of the contractile cylinder, crossing the broad surface of the myofibrils parallel to their striations. Thus, in subtangential longitudinal sections through the narrow dimension of the myofibrils, the triads are seen end on, and appear as pairs of elliptical profiles about 100 mμ in diameter on either side of a small circular profile 30 mμ in diameter (Fig. 3). Slender branches arising from the lateral elements of each triad run longitudinally toward the H and the Z bands respectively. These longitudinal components of the reticulum branch and anastomose freely to form networks between successive triads. In most types of striated muscle the plexus of tubules so constituted is in a single layer shared by adjacent myofibrils. In the muscle being described here, at least two distinct layers of branches take origin from each lateral member of the triad, and these form a double set of reticula in contact with the surfaces of both of the neighboring myofibrils (Figs. 4 and 5). The branches from the two triads associated with the same sarcomere are confluent in the region of the H band. The branches from triads of successive sarcomeres meet and often interdigitate at the Z band, but interruptions at this level are sufficiently common to suggest that the reticulum may not be continuous from one sarcomere to the next (see Fig. 4, at the arrows). It will be necessary to study muscle fixed in extension or to make reconstructions from serial sections to settle this point.

In sections that coincide for some distance with the plane of the reticulum, the triads are cut along their long axis and are seen in micrographs running parallel to each other and to the Z bands of the neighboring myofibrils (Figs. 6 and 7). The plexiform character of the reticulum between the triads is clear in some fields (Fig. 8) but may be obscured in others by crowding and superimposition of the layers of anastomosing tubes. Where the triads are cut midway between their layers of branches, each of the lateral elements appears to be bounded by a continuous membrane on both sides (Figs. 7 and 8). Sections at deeper or more superficial levels, however, pass through the sites of confluence of the longitudinal components of the reticulum with the triad. The outermost membranes of the triad then do not appear as straight lines but are continuous at many places along their length with the membranes of the longitudinally oriented tributaries. A section at this level is not illustrated here, but a few examples of continuity between the triads and longitudinal meshes of the reticulum are indicated by arrows in Fig. 6. Dense particles of glycogen 200 to 400 A in diameter are found in close association with the reticulum (Figs. 7 and 8). These occur singly or in small clusters between the meshes of the reticulum but not within its lumen.

The central component of the triads usually appears to be a row of discrete spherical or ellipsoidal vesicles 30 to 40 mμ in diameter (Fig. 6), but in some preparations it is a slender continuous tube (Fig. 9). Such tubes have been followed in

FIGURE 3

An area of a longitudinal section of a muscle fiber cut in a plane normal to a radius of the contractile cylinder. In this view the ribbonlike myofibrils present their narrow dimension. The most striking features of the muscle are the precise alignment of its cross-striations and the prominence and regularity of the sarcoplasmic reticulum in the interfibrillar clefts. The A band is long, the I band relatively short. There are two triads of the reticulum in each sarcomere located near the A-I junctions. Longitudinal branches extend in opposite directions from these triads, toward the H band and the nearest Z band. Lead hydroxide stained methacrylate section. Magnification approximately 30,000.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favorably oriented sections for 2 μ or more without interruption. In electron micrographs of muscle from *Opsanus tau*, continuity of the center element is frequent. In *Opsanus beta*, a continuous tubule can be found occasionally, but rows of vesicles occur more commonly. This variation is puzzling, but it seems possible that the middle member of the triad is normally a continuous tube and that the occurrence of vesicular fragments is due to inadequate preservation of its normal structure. The difference noted between the two groups of animals studied is probably not a true species difference but may merely indicate that good fixation was achieved more often in specimens of *Opsanus tau*. In cross-sections through the muscle at the level of the A–I junction the central component of the triad is often seen as a tubule studded with small outpocketings or diverticula. It is possible that the vesicular appearance of the central element of the triad is, in some instances, due to sectioning through these outpocketings, without cutting through the central component itself.

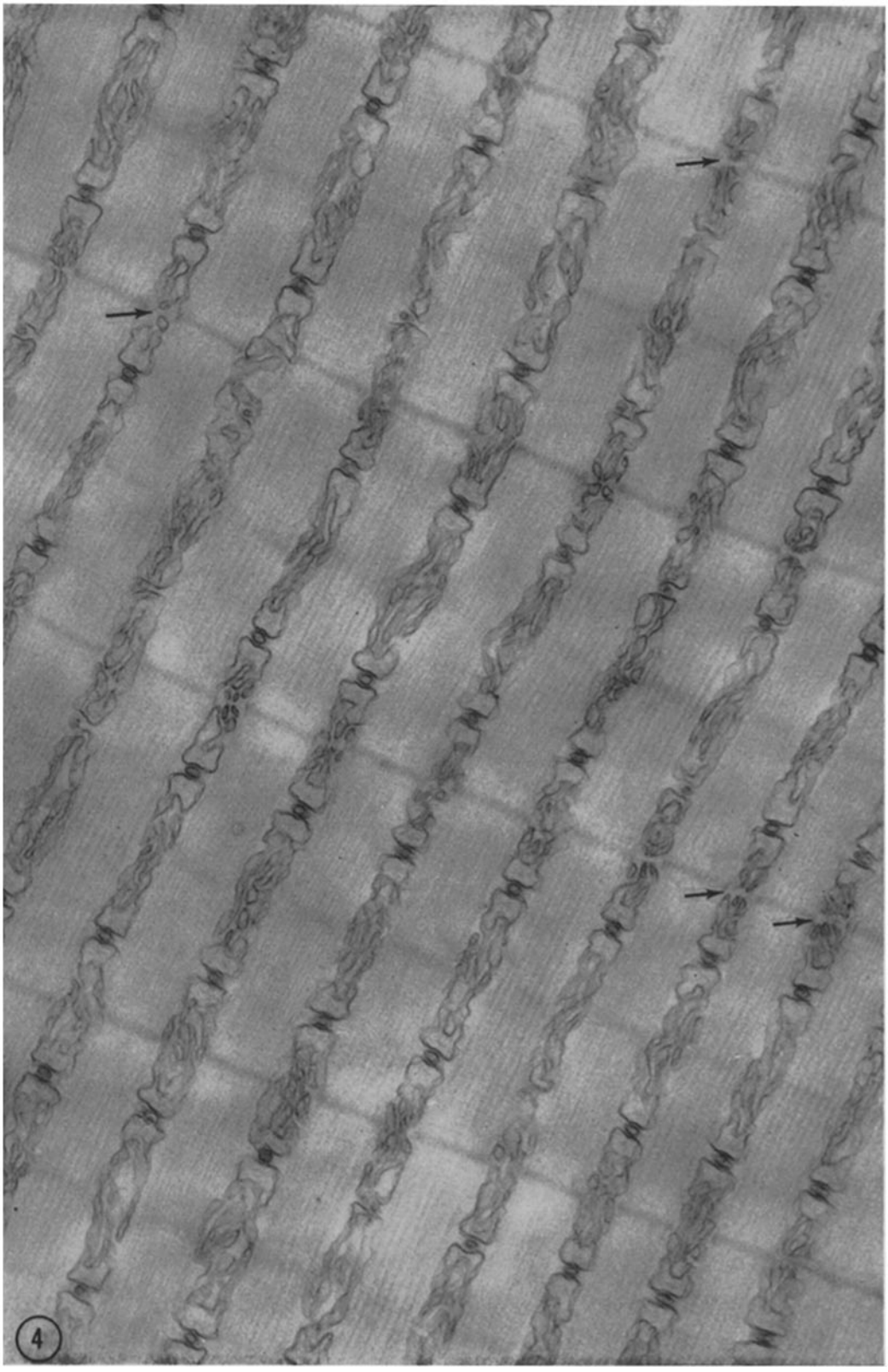
The longitudinal meshes of the reticulum appear somewhat darker than the triads (Figs. 7 and 8). It should be borne in mind, however, that these tubules are slender enough to be included in their entirety within the thickness of the section. Their greater density in electron micrographs is therefore attributable in part to the electron scattering of their limiting membranes, but they also seem to have an amorphous content of appreciable density. The lateral elements of the triads, being of larger diameter, are usually cut open in thin sections. Thus only one instead of two membranes is in the path of the electrons and these large open channels therefore appear lighter than the rest of the reticulum (Figs. 6 and 7). In addition to having an amorphous content of low density, they often show a fine dense stippling in a band of uniform width along the outer half of the channel (Figs. 7 and 8). This punctate material is presumed to be a fine-grained flocculent precipitate in the lumen, but it is difficult to explain its frequent localization along the side away from the middle element of the triad.

In previous descriptions of the sarcoplasmic reticulum, it has been reported that the membrane bounding the central member of the triad and the immediately adjacent membranes of the lateral members are thicker and more dense than membranes elsewhere in the reticulum. This was not obvious in the present material. Such density differences as are noted in the toadfish swimbladder muscle could be accounted for by differences in depth of the included membrane segments and their orientation with respect to the plane of section.

An aspect of the organization of the sarcoplasmic reticulum, which is of considerable importance to an understanding of its function is the relation of its components to the sarcolemma. This unfortunately is the region most difficult to study because the reticulum away from the myofibrils is tortuous and irregular in its course. The central component of the triads appears to terminate at the outer border of the myofibrils. The lateral channels continue and pursue a meandering course in the peripheral sarcoplasm. Owing to their sinuous course, they leave and re-enter the plane of section and therefore appear as discontinuous rows of elongated or oval profiles extending from the region of the triad toward the sarcolemma. A commonly observed pattern of peripheral continuation of the reticulum is illustrated in Fig. 10. The lateral elements of the triad undergo an abrupt reduction in the width of the lumen at the outer margin of the myofibrils. The narrowing of the lumen takes place in such a way that two slender tubes or flattened cisternae continue from that part of the lateral channels nearest the central

FIGURE 4

A small area of a muscle fiber cut longitudinally. The tissue in this case was embedded in epoxy resin. The cross-striations of the myofibrils and myofilaments are somewhat suppressed, but the sarcoplasmic reticulum stands out in good contrast. The "triads" consist of a slender intermediate tube $\sim$ 30 mμ in diameter, flanked by 2 larger lateral channels $\sim$100 mμ in diameter. These run across the broad face of the myofibrils in planes perpendicular to the page. The longitudinally oriented tributaries of the triads form tight networks parallel to the myofibrils. The branches of the two triads in the same sarcomere are continuous in the region of the H band but often appear to be discontinuous at the level of the Z band (see arrows). Magnification approximately 30,000.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component of the triad (see Fig. 10, at asterisks). Some of these slender tubes seem to run along the edge of the myofibrils connecting triads of successive sarcomeres, but others can be followed nearly to the sarcolemma (see rows of arrows, Fig. 10). Those shown in Fig. 10 come close to the surface, but in no instance has continuity between the two been demonstrated with certainty.

The examination of transverse sections of this muscle are not particularly helpful in analyzing the organization of the sarcoplasmic reticulum. The majority of sections in this plane simply show the interfibrillar clefts filled with irregularly shaped discontinuous profiles of the longitudinal tubules of the reticulum. They do illustrate, however, that where V-shaped branching of the myofibrils occurs (see Fig. 11, at A and B), the reticulum has a free edge between the arms of the V where it evidently cannot be continuous with the reticulum in adjacent interfibrillar clefts. Also, where a V-shaped myofibril is interposed between two unbranched myofibrils, as in the center of Fig. 11, the resulting Y-shaped interfibrillar space must necessitate a branching of the radially disposed triads. These and other consequences of the peculiar geometry of the myofibrils constitute marked differences in the organization of the reticulum in this muscle as compared to that of muscles with cylindrical myofibrils, but it is pointless to speculate upon their significance until more is known about the physiology of the reticulum.

Although not pertinent to the main topic of this paper, certain characteristics of the mitochondria and sarcoplasmic matrix are deserving of comment. The mitochondria tend to be oriented parallel to the long axis of the fiber. They are variable in size, but may be extraordinarily long. Profiles 5 μ in length are common and at least one has been observed to extend for 4 sarcomere lengths. Its length must therefore have been in excess of 8 μ (Figs. 12 and 13). Their matrix is moderately dense and the cristae very numerous but disordered in their arrangement. Dense granules are found similar to those described in the matrix of mitochondria in many other cell types. These, however, are numerous and very much larger than usual, with diameters of 900 to 1200 A. They are not homogeneous but have a porous or compartmented appearance with small areas of lower density uniformly distributed in the dense substance of the granule.

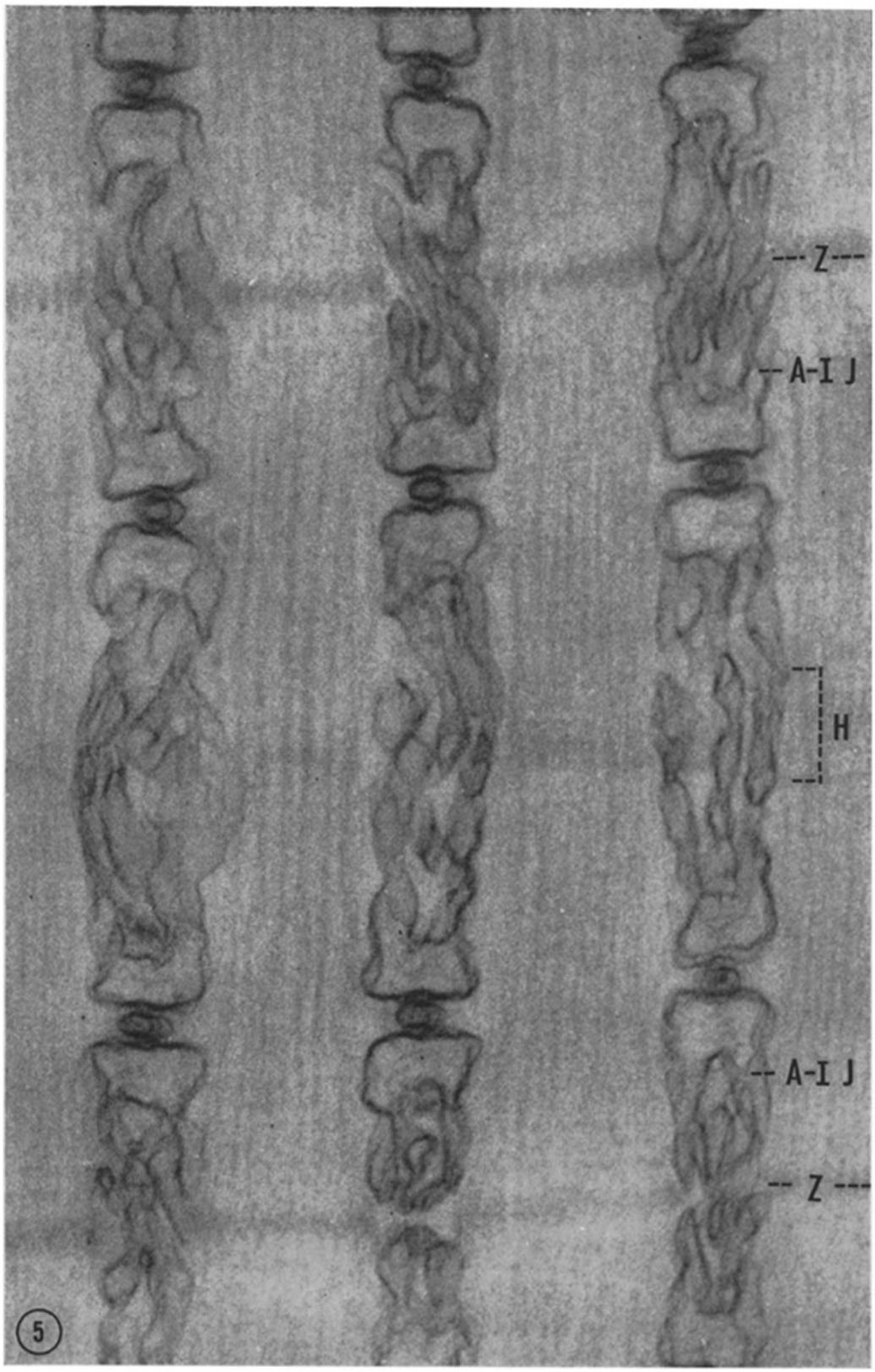
The presence of glycogen in small amounts in the interfibrillar sarcoplasm among the tubules of the reticulum has already been noted. It is more plentiful in the sarcoplasm at the periphery of the fiber (Figs. 8, 12) and is often particularly concentrated in the core of the contractile cylinder (Fig. 13). The granules are rather uniform in size (300 to 400 A) and have more sharply defined outer limits than the glycogen particles of most warm-blooded vertebrates (10). The granules have an appreciable density of their own but are brought out more clearly in sections stained with lead hydroxide. Ribonucleoprotein granules do not seem to be present in significant numbers in the sarcoplasm.

DISCUSSION

The physiological investigations of the swimbladder muscle of the toadfish have demonstrated that the lag between stimulation and response is very short. The earliest detectable movement occurs during the falling phase of the muscle action potential. The contraction peak is reached within 5 to 8 msec. and relaxation is complete in an additional 5 to 7 msec. (12). It requires a stimulation frequency as great as 300 cycles per second to tetanize (13). It is tempting to believe that these unusual physiological properties may be directly related to the extraordinary development of the sarcoplasmic reticulum revealed in the present electron microscopic study of this muscle. More specific discussion of the possible functional implications of this elaborate reticulum can best be undertaken after a brief review of the morphological and physiological observations which have

FIGURE 5

A higher magnification of units of the reticulum associated with a little more than one sarcomere length of four adjacent myofibrils. The relations of the components of the reticulum to the Z band, H band, and A-I junctions are indicated. It is evident that at least two layers of branches of the lateral members of the triads form networks in contact with each of the adjacent myofibrils. Magnification approximately 85,000.



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recently focused attention upon the possible involvement of this system in the contractile mechanism of striated muscle in general.

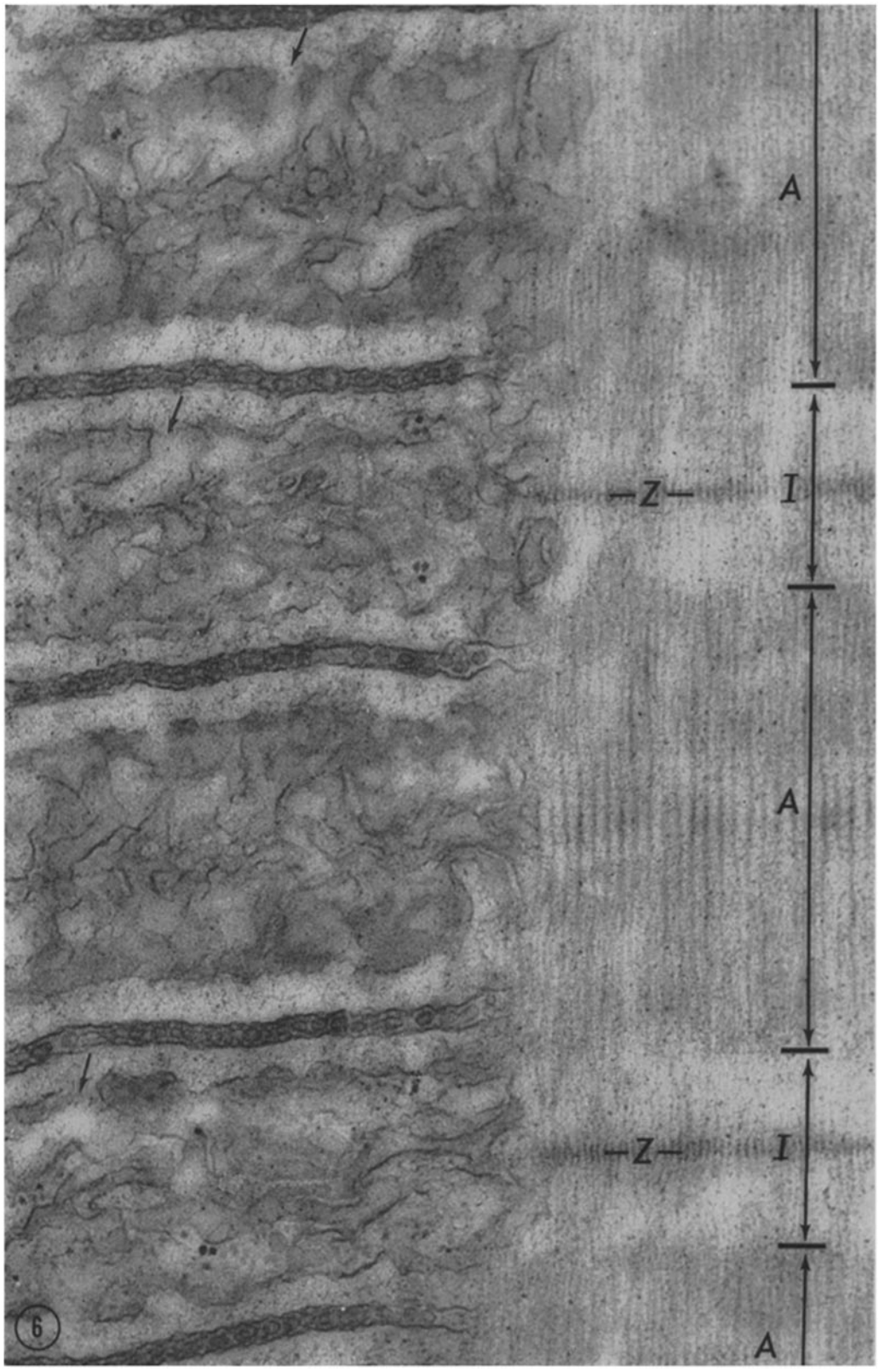
It has long been recognized that the time between depolarization of the surface membrane and contraction of the myofibrils in striated muscle fibers of large diameter is too short to be explained by intracellular diffusion of an activating substance. In investigating these paradoxical time distance relationships, Huxley (4) applied stimulating current with a microelectrode to different points on the surface of muscle fibers, and found that the muscle was unresponsive except at certain loci along the length of the fiber. In the frog semitendinosus, these sensitive points were opposite the isotropic band at the level of the Z line. Huxley and Taylor (5) therefore postulated the existence of a structural component at the level of the Z band capable of transmitting an impulse transversely toward the interior of the muscle. Their original thought, that this structure might be the Z band itself, was abandoned when later it was found in lizard muscle that a localized contractile response could not be elicited at the Z line, but only when the electrode was placed on the sarcolemma opposite the junction of the A and I bands. Electron microscopic studies later demonstrated that the transverse triads of the sarcoplasmic reticulum were associated with the Z band in the frog but were located at the A-I junction in the lizard. The close correspondence between the location of these elements and the responsive sites in Huxley's stimulation experiments strongly suggested that the triads of the reticulum were the structures responsible for inward spread of excitation. This hypothesis rests upon two assumptions: (*a*) that the membranes of the reticulum are polarized and capable of conducting an impulse, and (*b*) that they are continuous with the sarcolemma. If these assumptions are valid, then a wave of depolarization spreading over the cell surface might indeed be conducted inward at each sarcomere at the level of the triads.

The weakest points of evidence for this attractive hypothesis are concerned with the continuity of the triads across the fiber and their relation to the cell surface. In some of the slower vertebrate muscles they are not continuous for any considerable distance but are merely local expansions of the reticulum that do not even span the width of one myofibril (8). Even in the more rapidly acting muscles of warm-blooded vertebrates where the triads do form continuous bracelets around the myofibrils and communicate with triads of adjacent myofibrils, clear-cut examples of continuity between the membranes of the reticulum and the surface of the muscle fiber have rarely been seen in electron micrographs.

The sarcoplasmic reticulum of the toadfish muscle described here differs from that of many other vertebrate muscles in the large size of its transverse triads; their more precise localization at the A-I junctions of each sarcomere; their continuity over considerable distances; and the multiplicity of their branches, which form more than one layer of plexiform channels between adjacent myofibrils. Several of these features would seem to make the reticulum in this muscle more efficient than others as a system for intracellular impulse conduction. The same uncertainties exist, however, as to the relation of the reticulum to the sarcolemma. Prolongations of the lateral members of the triads into the subsarcolemmal sarcoplasm are described and some of these can be followed to points very near the surface, but actual continuity of the membranes has not been observed. The center component of the triad, which is considered by some workers to be the most likely candidate to conduct an impulse, usually appears in this, and in other muscles, as a row of discrete vesicles. However, the

FIGURE 6

A section parallel with the broad face of a myofibril. In the left half of the figure the plane of the section coincides with an interfibrillar cleft and hence shows an extensive view of the reticulum. In the right half it has cut through the reticulum to the underlying myofibril. The triads, now cut in their long axis, are seen running transversely across the myofibril parallel to each other and to the Z band. The intermediate member of the triad appears here as a row of small vesicles instead of a continuous tube. The longitudinal elements between triads are so crowded that their plexiform nature is not apparent. Some points of confluence of longitudinal tubules with the lateral members of the triad are indicated by arrows. Magnification approximately 55,000.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fact that it is sometimes found in the form of a tubule that is continuous over the broad face of the myofibrils for distances up to 2 or 3 μ suggests that the more common appearance of discontinuity could be due to inadequate preservation. The frequency of this occurrence in tissue which, by other criteria, seems excellently fixed, would then indicate that the membranes of this center component of the triad are exceptionally difficult to preserve. It is likely that there may be other differences in the physico-chemical properties of this membrane which set it apart from the other membranes of the reticulum. It is disappointing that even in those specimens where the tubular character of the center element has been successfully preserved, it has not been traced to the cell surface. Andersson-Cedergren (2) in a study of serial sections through the intercostal muscles of the mouse, has not been able to find evidence for the continuity of the center element with the sarcolemma. Huxley found that there are only a few sensitive points around the circumference of the fiber. Nevertheless, if communications with the sarcolemma occur at two levels in each sarcomere length, one would expect to encounter them with fair frequency even in thin sections. It may ultimately be found that this perifibrillar membrane system can be activated without its membranes actually being continuous with the surface.

In attempts to define the function of the sarcoplasmic reticulum, the emphasis to date has been upon the possibility that its membranes may have the capacity to conduct an impulse inward to activate the myofibrils, or that the lumen may serve as a pathway for rapid inward diffusion of an activating substance generated at the surface at the instant of depolarization of the plasma membrane. Our preoccupation with these possibilities may blind us to alternatives. One of these is suggested by the disposition of the mitochondria in this muscle. Their segregation in the core of the contractile cylinder and around its periphery is unusual and places myofilaments in the middle of the broad flat myofibrils a considerable distance from the nearest mitochondrion. In those fibers where the sarcoplasmic core is lacking and the central area of the contractile cylinder is occupied by smaller myofibrils of irregular shape, these may be 10 μ from the mitochondria at the periphery of the fiber. This degree of separation of the mitochondria from the myofibrils is surprising when one recalls the very close relation of these organelles to contractile elements elsewhere in nature. In most muscles, the mitochondria intermingle with the myofibrils and often encircle them at particular levels of the cross-banded pattern. In spermatozoa the mitochondria of the midpiece form a tight helix around the muscle-like fibers at the base of the tail. It has been assumed from such evidence that a close spatial relation between the mitochondria and the contractile elements was necessary for their enzymes to participate effectively in the transformation of chemical energy to mechanical work. The considerable distances between the mitochondria at the periphery of the toadfish muscle fiber and the interior of the myofibrils raise an interesting problem as to the source of energy for contraction deep in the fiber and foster the speculation that the elaborately developed reticulum may take part in the synthesis of energy-rich compounds or at least promote their uniform distribution throughout the contractile cylinder.

The current speculations concerning the role of the sarcoplasmic reticulum in the physiology of striated muscle will surely be tested in the next few years by chemical analysis of isolated membrane material and by direct experimental investigation of the electrical properties of these membranes *in situ*. The toadfish muscle described

FIGURE 7

A micrograph of a section through an interfibrillar cleft parallel to the broad face of a myofibril. The reticulum covers the entire figure except in small areas where the section has penetrated too deeply and reveals the underlying myofilaments and cross-striations. A planar view of the sarcoplasmic reticulum covering such a large area can be had only in species whose muscles have flat ribbon-like myofibrils. The middle element of the triads is discontinuous and the lateral members contain a fine flocculent or granular precipitate. The reticulum overlying the I band is more crowded and therefore darker than that over the A band. At both levels there are numerous granules of glycogen in the interstices between the anastomosing tubules of the reticulum. Magnification approximately 60,000.


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here may prove to be particularly suitable material for both kinds of investigation, because of its accessibility and the elaborate development of its sarcoplasmic reticulum.

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REFERENCES

1. ANDERSSON, E., The tubular system in the striated muscle cell, *in* Electron Microscopy, Proceedings of the Stockholm Conference, New York, Academic Press, Inc., 1956, 208.
2. ANDERSSON-CEDERGREN, E., Ultrastructure of motor endplate and sarcoplasmic components of mouse skeletal muscle fiber, *J. Ultrastruct. Research*, suppl. 1, 1959.
3. BENNETT, H. S., and PORTER, K. R., An electron microscope study of sectioned breast muscle of the domestic fowl, *Am. J. Anat.*, 1953, **93**, 61.
4. HUXLEY, A. F., Muscle structure and theories of contraction, *Progr. Biophysic. and Biophysic. Chem.*, 1957, **7**, 255.
5. HUXLEY, A. F., and TAYLOR, R. E., Activation of a single sarcomere, *J. Physiol. (London)*, 1955, **130**, 49.
6. LUFT, J., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
7. PEACHEY, L. D., and PORTER, K. R., Intracellular impulse conduction in muscle cells, *Science*, 1959, **129**, 721.
8. PORTER, K. R., The sarcoplasmic reticulum in muscle cells of *Amblystoma* larvae, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, suppl., 163.
9. PORTER, K. R., and PALADE, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
10. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
11. RETZIUS, G., Zur Kenntnis der quergestreiften Muskelfaser, *Biol. Untersuch.*, series I, 1881, **1**, 1.
12. SKOGLUND, C. R., Neuromuscular mechanism of sound production in *Opsanus tau*, *Biol. Bull.*, 1959, **117**, 438.
13. TAVOLGA W. N., 1960, personal communication.
14. TOWER, R. W., Production of sound in the chum fishes, the sea robin and the toadfishes, *Ann. New York Acad. Sc.*, 1908, **18**, 149.
15. VERATTI, E., Richerche sulla fine struttura della fibra muscolare striata, *Mem. reale ist. Lombardo di sc. e lettere*, 1902, **19**, 87.
16. WATSON, M., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

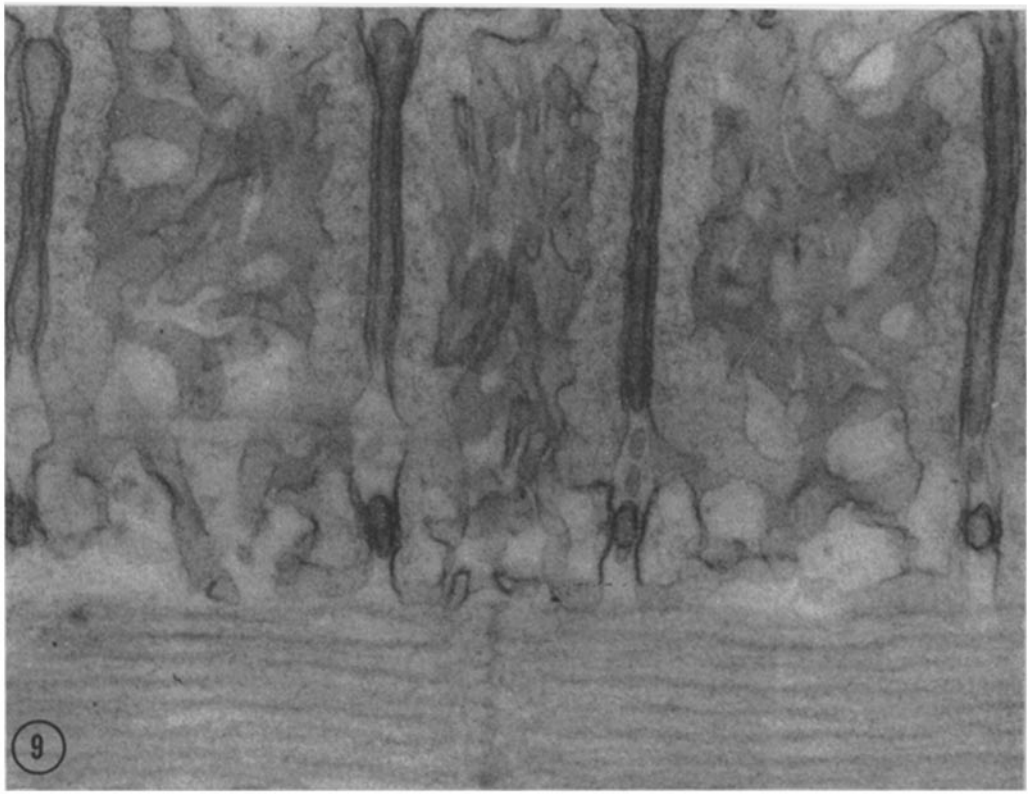
FIGURE 8

An oblique section through a myofibril and an area of reticulum at the outer margin of the contractile cylinder. The plexiform character of the reticulum is particularly clear here. The finely granular content of the lateral members of the triads forms two distinct parallel bands on the sides farthest from the intermediate element. Although the glycogen granules sometimes appear to be within the lumen of the reticulum, it is believed that they are actually in the intertubular spaces. Magnification approximately 60,000.

FIGURE 9

An example of reticulum from *Opsanus tau* in which the central element of the triad is not a row of vesicles but a continuous tube. The appearance of discontinuity toward the lower part of the figure is due to slight tortuosity of the tube which resulted in its leaving and re-entering the plane of the section. Magnification approximately 50,000.

8



9

FIGURE 10

An area including, from right to left, the sarcolemma (*Sl.*), the peripheral layer of sarcoplasm, the reticulum and two myofibrils cut obliquely. At the outer margin of the contractile cylinder, the lateral members of each triad undergo an abrupt reduction in diameter (at asterisks) and pursue a meandering course in the subsarcolemmal sarcoplasm. They extend to or nearly to the surface membrane but have not been observed to be continuous with it. If the intermediate element of the triad continues beyond the muscle's edge, it ceases to be morphologically distinguishable from other components of the reticulum. At present there is no evidence that it establishes contact with the cell surface. Magnification approximately 45,000.


SI
SI
10

FIGURE 11

A field of myofibrils like that enclosed in the box on Fig. 1, seen in cross-section at fairly high magnification. From the center to the periphery of the contractile cylinder, the flat ribbon-like myofibrils frequently branch. In this way the interfibrillar clefts remain of constant width. These are fully occupied by the membranes of the reticulum. The regular pattern of dots in the myofibrils are the primary filaments in the A band. The smaller, less dense dots around them are the thin secondary filaments. Magnification approximately 55,000.

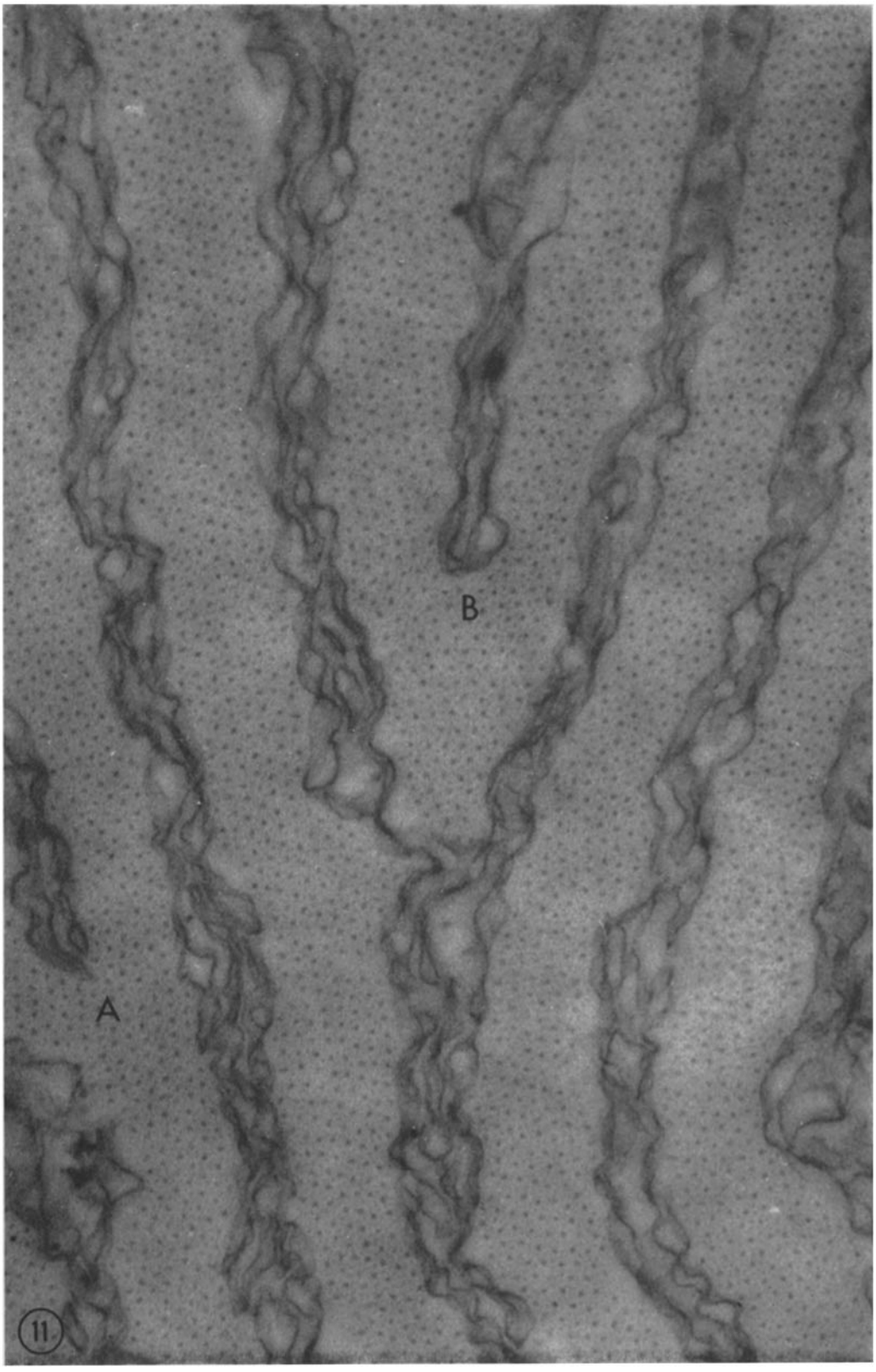

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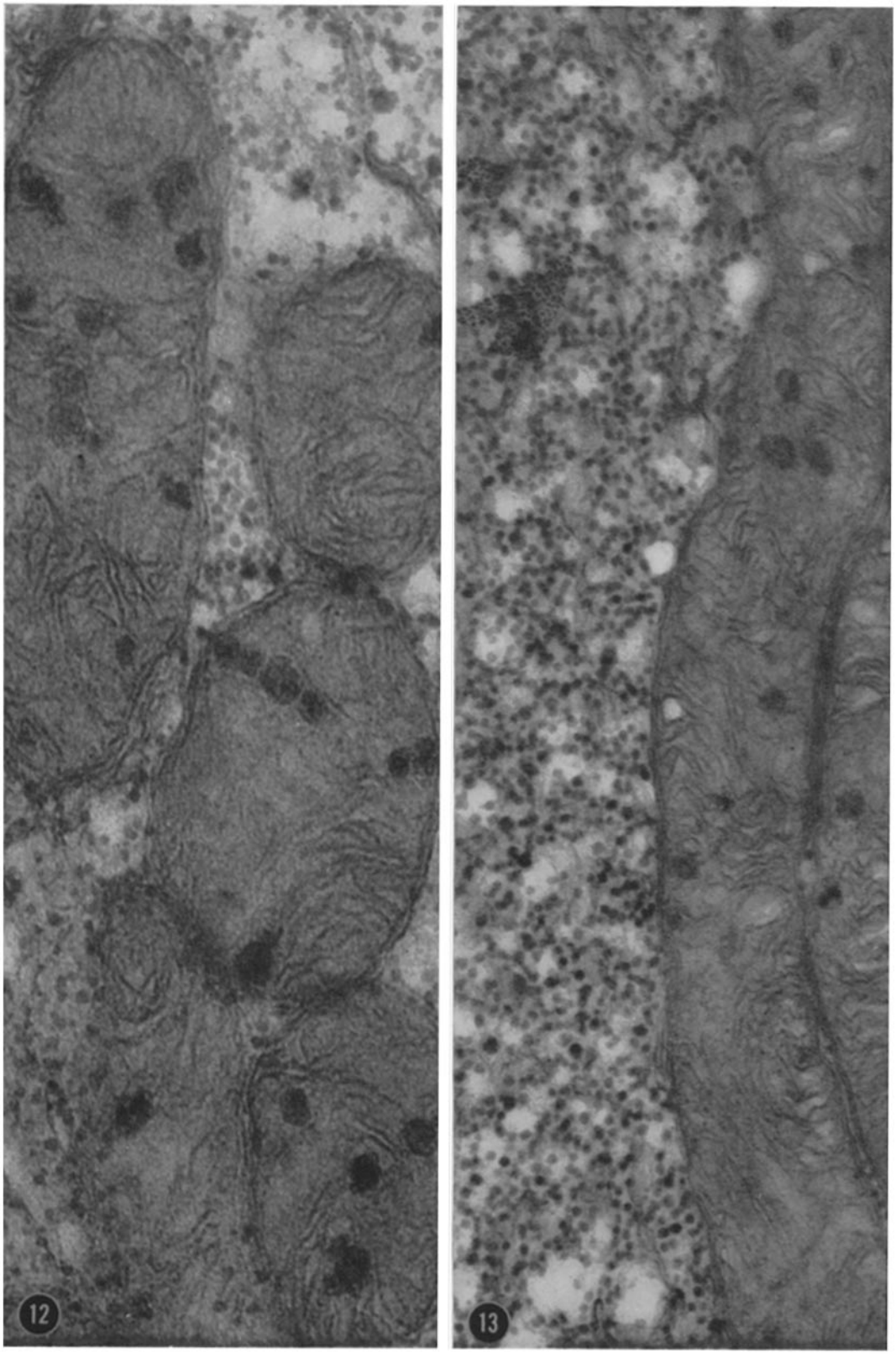
FIGURE 12

Mitochondria in the sarcoplasm immediately beneath the sarcolemma. The cristae are closely packed in a moderately dense matrix and are very numerous, but disorderly in their arrangement. The dense granules which have been described in the mitochondrial matrix of many cell types are particularly large in this muscle. They appear to have a compartmented fine structure with multiple minute spaces enclosed in the dense substance of the granule. Magnification approximately 80,000.

FIGURE 13

Mitochondria in the sarcoplasmic core of the fiber. The mitochondria are oriented longitudinally and may be very long. The one shown here in part remained in the plane of section for 4 sarcomere lengths and was therefore at least 8 μ long. Glycogen is unevenly distributed in the sarcoplasm but is often concentrated in the core of the fiber. The particles are quite uniform in size and unusually sharp in outline. Magnification approximately 120,000.


12
13