THE SARCOPLASMIC RETICULUM OF THE BAT CRICOTHYROID MUSCLE

J. P. REVEL, Ph.D.

From the Department of Anatomy, Harvard Medical School, Boston

ABSTRACT

The bat cricothyroid muscle is believed to participate in the production of the short bursts of frequency modulated ultrasound which these animals use as an echolocation device. The evidence seems to indicate that this muscle must be extremely fast acting. It possesses a very well developed sarcoplasmic reticulum, consisting of intercommunicating longitudinal and transverse tubular elements. The transverse elements, situated at the level of the junction between the A and the I bands, are tripartite complexes of tubules called triads, and these are sometimes replaced by more complex structures, the pentads. The intermediate element of the triad appears as a slender continuous tubule, which can be shown to come into close contact with the sarcolemma and also to share with it certain common staining properties. The longitudinal components of the reticulum consist of very numerous tubules which link successive triads to each other and anastomose to form multiple layers of close-meshed reticula in the interfibrillar sarcoplasm. Both the longitudinal and the transverse elements of the sarcoplasmic reticulum form a continuous network across the muscle fiber. It is suggested that the extraordinary development of the sarcoplasmic reticulum in the bat cricothyroid is related to the unusual physiological properties of this muscle.

INTRODUCTION

About 1793, de Jurine (1), a Swiss surgeon, suggested that bats use their ears to orient themselves. This view was received with great skepticism by naturalists of that period and it is only in the last twenty years that it has been demonstrated conclusively by Griffin and his collaborators (2) that bats of many species do indeed use echolocation to guide their flight. The species *Eptesicus fuscus* and *Myotis lucifugus*, on which we report here, emit short bursts of frequency-modulated sound which are apparently produced by the vibration of two thin laryngeal membranes. Examination of the anatomy of the larynx suggests that it is the extremely well developed cricothyroid muscles that control the tension on these vibratile membranes and are thus responsible for the modulation of frequency (3). Under laboratory conditions, each burst of sound lasts 2 to 3 milliseconds but, in the wild, the bat in pursuit of its insect prey may shorten the emissions of sound to as little as 1 millisecond and these may be repeated as rapidly as 200 times a second immediately before the capture. The frequency of the sound may drop from 100 to 30 kilocycles during a single 2- to 3-millisecond cry. To achieve this, the cricothyroid muscle must relax during sound emission. A maximum of 4 milliseconds is available for the contraction and 1 millisecond for the relaxation of the muscle. Inasmuch as evidence has been presented (3) that a motor nerve impulse precedes each burst of supersonic sound, it is not likely that any mechanism such as has been described for insect flight muscles (4) plays a role in sound production in the bat.

Because of the extraordinary performance of this muscle it was thought that an electron micro-

scopic study of its internal organization might disclose specializations of its fine structure for high speed action. Interest was centered especially on the sarcoplasmic reticulum (5, 6) which has recently been suspected of having an important role in the spread of the impulse from the sarcolemma to the contractile elements in the interior of the muscle fibers (7–9).

MATERIALS AND METHODS

The bats used in this study were of the species *Myotis lucifugus* and *Eptesicus fuscus*, collected in New Jersey and Massachusetts. They were killed by decapitation below the larynx. The complete larynx was then dissected out and fixed in ice-cold 1.3 per cent OsO_4 buffered with collidine at pH 7.4 to 7.6 (10). After about an hour the cricothyroid muscles were dissected in fixative, cut into blocks, and dehydrated rapidly in a graded series of increasing concentrations of cold ethanol. The tissues were allowed to come to room temperature in 100 per cent ethanol, transferred to fresh absolute ethanol at room temperature for 20 minutes to half an hour, and then embedded in Epon 812 mixtures as recommended by Luft (11). Light gold to gold sections were cut with glass knives on a Porter-Blum microtome and mounted on carbon-reinforced, celloidin-coated grids. The majority of the preparations were stained according to the method of Karnovsky (12) and then "sandwiched" with a thin layer of carbon before examination in the microscope. Some sections were stained instead with a 50 per cent ethanolic solution of uranyl acetate. An RCA EMU 3E and a Siemens Elmiskop I electron microscopes were used. Thicker sections were examined in phase contrast for orientation purposes.

RESULTS

The fine structure of the cricothyroid muscle in the two species studied was quite similar and the following description will apply to both except where minor differences are specifically pointed out. The muscle fibers have a diameter of 15 to 30 micra and their nuclei are located peripherally (Fig. 1). Numerous densely packed mitochondria often intervene between the myofibrils and the sarcolemma. The capillaries occupy grooves or shallow recesses in the surface of the muscle fiber (Fig. 2). Adjacent to the capillaries the conspicuous subsarcolemmal concentration of mitochondria is usually absent, leaving only a thin layer of sarcoplasm devoid of organelles between the myofibrils and the sarcolemma. In favorably oriented longitudinal sections, rows of long mitochondria are found in some of the sarcoplasmic clefts between the myofibrils (Fig. 1). The mitochondria, as in other muscles, have numerous cristae in close parallel array but display certain unusual features that will be the subject of a separate paper.

The myofibrils are more slender (0.2 to 0.5 μ) than in many mammalian muscles described heretofore. In specimens that appear to have been fixed in relaxation, the sarcomeres have a length of about 2.3 μ, of which the A band occupies 1.5 μ. In many of the muscles of ordinary speed described in the literature the sarcoplasmic reticulum is relatively inconspicuous, but in the bat cricothyroid it is evident, even in micrographs of low magnification, that each myofibril is surrounded by a close-meshed network of tubules (Figs. 1, 2). The reticulum actually seems in some instances to penetrate into clefts in the myofibrils, so that the latter may appear branched (Fig. 1, arrows). Small bundles of myofilaments, thus separated from a myofibril, may in turn fuse with another myofibril. In cross-sections the myofibrils vary in shape and size and may form a fairly irregular pattern.

In some specimens of *Myotis* sizeable bundles of filaments (100 to 200 A in diameter) having no distinguishable cross-striations are occasionally found in the subsarcolemmal sarcoplasm (Fig. 3). These filaments have a prevailing parallel orientation and may run either longitudinally or circumferentially. No sarcoplasmic reticulum seems to be associated with these structures. They have

FIGURE 1

A low power longitudinal section through two muscle fibers of a bat cricothyroid muscle. A peripherally placed nucleus is visible in one of the muscle cells. The mitochondria visible between the myofibrils are very long while those adjacent to the sarcolemma have a more rounded appearance. Much of the interfibrillar sarcoplasm is occupied by the sarcoplasmic reticulum. The arrows point to some branching myofibrils. × 9000.

no clear connection with the myofibrils and do not appear to have been described before.

The Triads of the Sarcoplasmic Reticulum

The extraordinarily well developed sarcoplasmic reticulum of the bat cricothyroid muscle consists of the same longitudinal and transverse elements described for other muscles (6, 13). The transverse elements, called triads by Porter and Palade, are found at each end of the A band, near the junction between the A and the I bands. Thus there are two triads to each sarcomere. In longitudinal sections of the muscle, the transverse elements of the sarcoplasmic reticulum are seen end on in the interfibrillar sarcoplasm (Fig. 4). In micrographs of such sections the triads appear as two vesicular profiles of equal size, the terminal cisternae, separated by a smaller intermediate element often somewhat compressed by the larger cisternae on either side. The surface of the two larger cisternal profiles that faces the intermediate element is often flattened or even indented by it. The narrow space between each terminal cisterna and the intermediate element is bridged by pairs of densities separated by a region of lower density (Fig. 4, arrows). This pattern of density variations may, in some instances, give the spurious appearance of minute vesicles interposed between the three principal elements of the triads.

In longitudinal sections tangential to the surface of the myofibrils one can readily observe the extent of the transverse triads and the longitudinal meshes of the reticulum that connect them. In Fig. 5 the plane of section passes through some myofibrils and tangential to others. In the latter case one sees a surface view of the reticulum. In favorable micrographs where the section chances to pass tangential to several neighboring myofibrils, a large area of sarcoplasmic reticulum is visible which is reminiscent of the extensive surface exposures of the reticulum obtainable in muscles having flat ribbon-like myofibrils, such as those of the toadfish swimbladder (14).

The cisternae which form the lateral elements of the triad are well shown in planar view in Figs. 5 and 6. They are 600 to 800 A high, along the longitudinal axis of the underlying myofibrils and 1000 A wide. The lumen of the cisternae is continuous with that of the longitudinal elements of the reticulum which connect the successive triads (Fig. 6, arrows). A fine meshwork interpreted as a precipitate is found in the lumen of each of the two cisternae of the triad, but does not extend into the longitudinal channels which are confluent with them (Fig. 6). The limiting membrane of the terminal cisterna which faces the intermediate element of the triad has a scalloped appearance and the outer surface of the membrane appears to be studded with uniformly spaced small projections, 100 A in diameter, of a density slightly lower than that of the membrane itself. It is not clear from the micrographs whether these structures are separate entities or whether they represent extensions of the limiting membrane of the terminal cisterna. These projections evidently correspond to the linear densities already described above as bridging the narrow space between the lateral and intermediate elements in cross-section of the triads (Fig. 4).

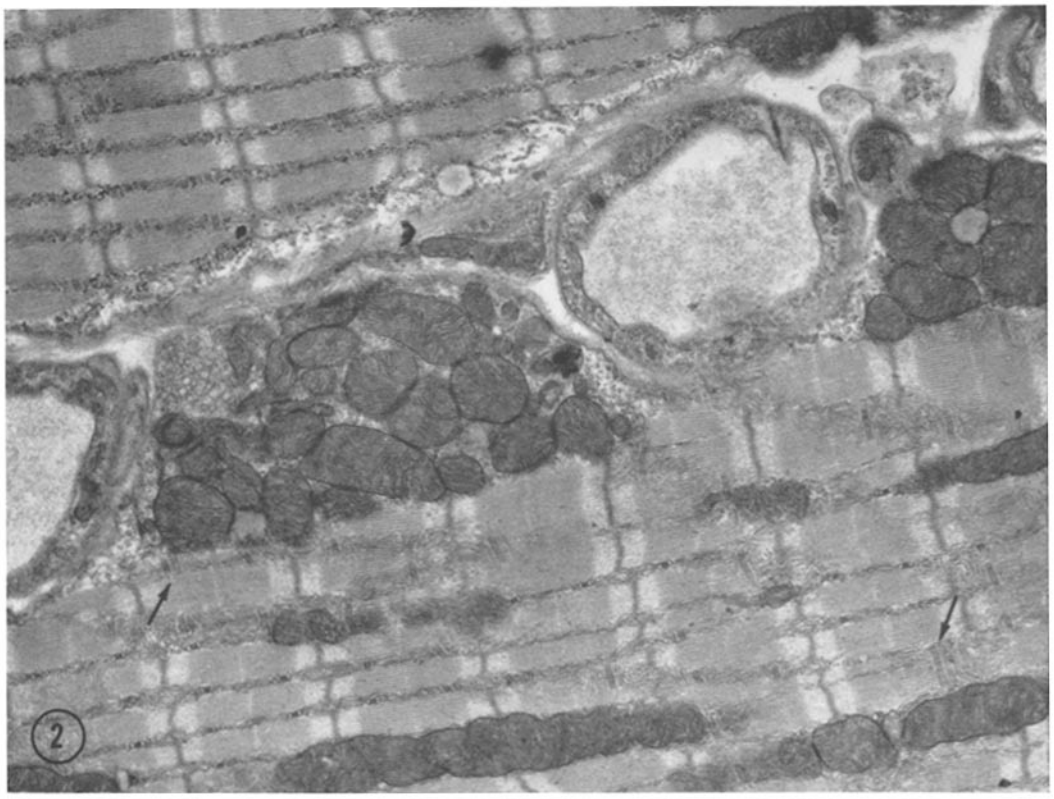
Although sometimes imaged as a row of round or elliptical vesicles, the intermediate element of the triad is usually a continuous tubule having an

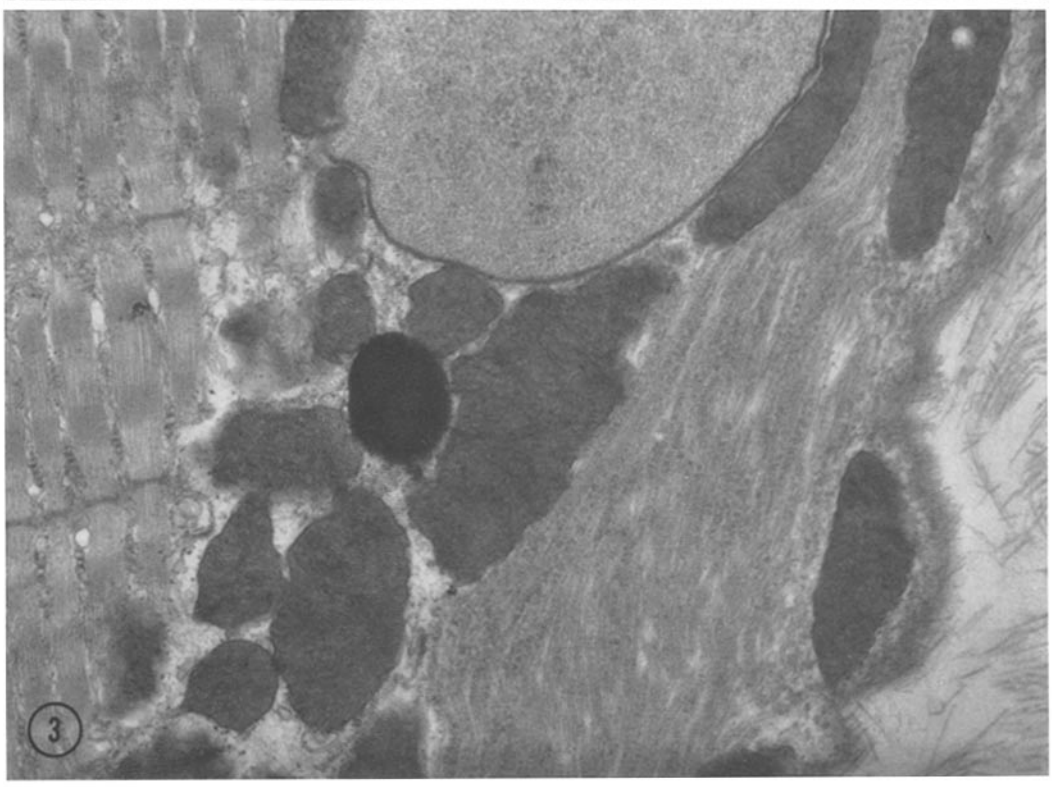
FIGURE 2

A longitudinal section through two cricothyroid muscle fibers of *Eptesicus*. The sarcoplasmic reticulum lies between the slender myofibrils. The transverse elements of the reticulum (triads) are found near the junction of the A and I bands. The arrows point out pentads, a more complex transverse element than triads (see text). Groups of mitochondria separate the myofibrils from the sarcolemma, and single rows of mitochondria are also found between the myofibrils. Two capillaries are seen resting in trough-like depressions of the sarcolemma. × 9000.

FIGURE 3

Low power electron micrograph of a longitudinal section of a cricothyroid muscle of *Myotis*. A large bundle of non-striated fibrils is found in the sarcoplasm underneath the sarcolemma. The myofibrils are somewhat stretched. A dense fat droplet is seen in the middle of the field. × 11,000.





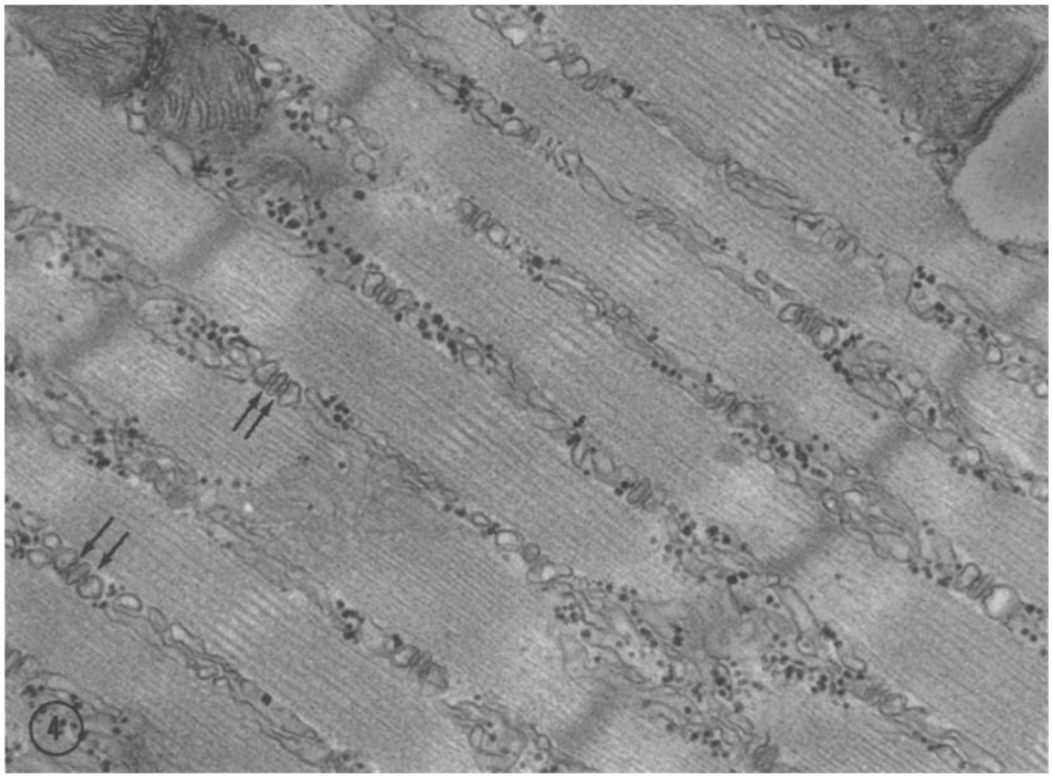


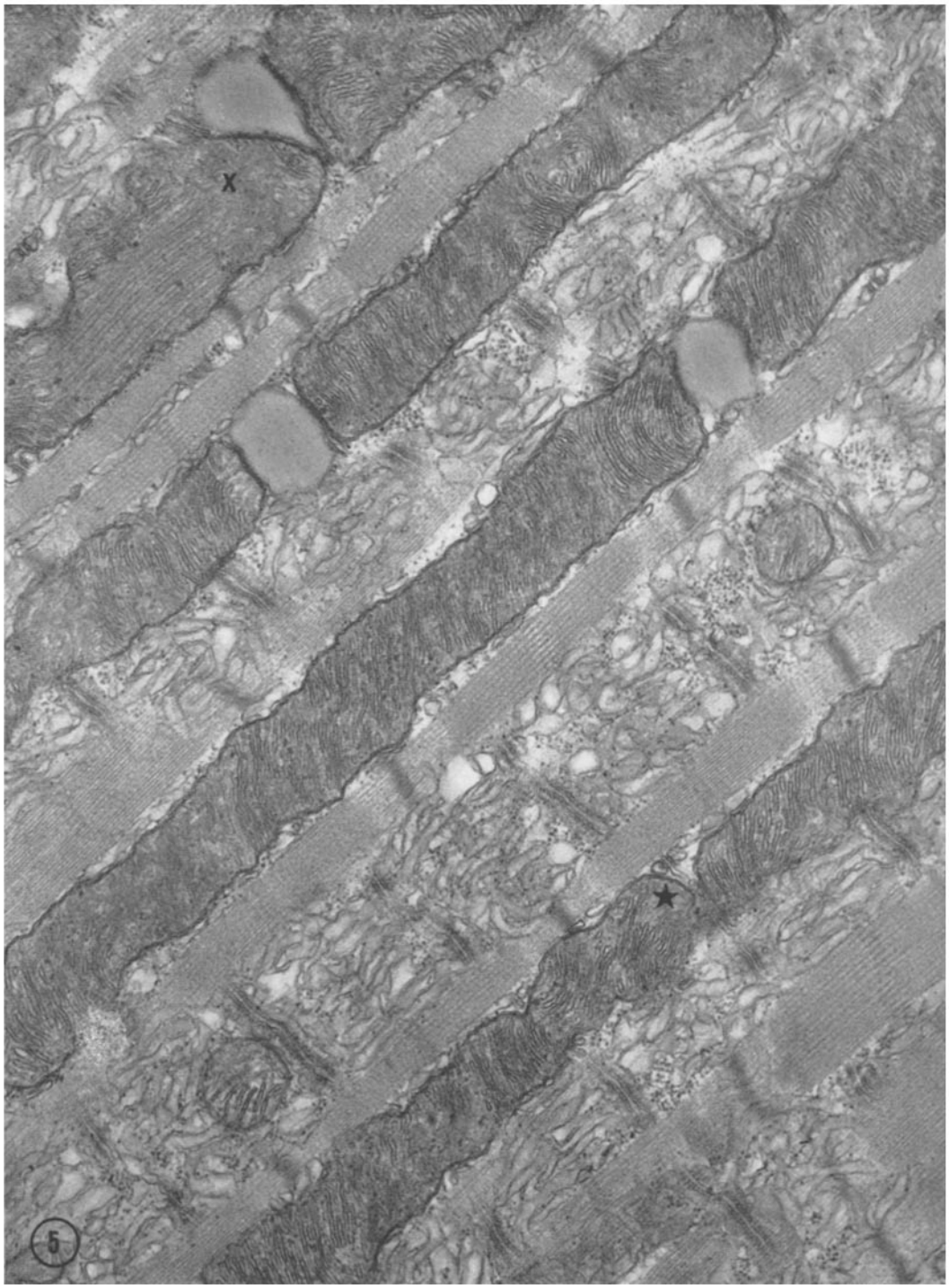
FIGURE 4

This field illustrates the appearance of the sarcoplasmic reticulum in the interfibrillar sarcoplasm. The triads, near the junction between the A and the I bands, are imaged as two terminal cisternae, flattened or indented, where they face a central elliptical vesicle, the intermediate element. The arrows point to the two pairs of linear densities found between the terminal cisternae and the intermediate element. Note the multilayered arrangement of the longitudinal elements of the reticulum. The heavily stained granules found in the interfibrillar sarcoplasm are glycogen particles. × 34,000.

elliptical cross-section (see Fig. 4) with its major axis 1,000 A and its minor axis 300 to 400 A. In favorable sections, it can be followed for several micra without interruption. The intermediate element appears to be continuous from the reticulum investing one myofibril to that surrounding the next. This is especially striking where the myofibrils are separated from each other by a row of mitochondria because, where this is the case, the intermediate element extends laterally beyond the

FIGURE 5

A surface view of the sarcoplasmic reticulum in *Eptesicus*. The reticulum completely invests the myofibril in the center of the field. The transverse elements (triads) can be seen near the junction between the A and the I bands. The longitudinal element link successive triads to each other. At the star (*) an intermediate tubule extends past the terminal cisternae of a triad, passes between two mitochondria, and becomes continuous with another triad on the opposite side of the obstruction. Note the closely packed cristae of the interfibrillar mitochondria and the pale fat droplets associated with them. The mitochondrion at *X* has a peculiar inner structure which is described in a separate publication. × 23,000.


X
5

termination of its associated cisternae. It passes then between the ends of adjacent mitochondria or through a groove on their surface to continue as the intermediate element of a triad in the reticulum enveloping the neighboring myofibril on the other side of the row of mitochondria (Figs. 5 and 6, stars).

The Pentads of the Sarcoplasmic Reticulum

In the bat *Eptesicus*, the triads are sometimes replaced by more complex structures which we designate as *pentads*. These consist of three large parallel transverse channels, corresponding to the terminal cisternae of a triad, separated by two small intermediate elements. Pentads (see Figs. 7 and 2) are found in the same location, with respect to the crossbanding of the myofibrils, as the triads. Favorable sections show that they are usually continuous with triads (Fig. 7). At the points of continuity the intermediate tubule of the triad bifurcates and an additional cisterna appears between the two branches. One can thus visualize the pentads as arising by an incomplete reduplication of a triad, in which one transverse cisterna is shared. Pentads thus far have been observed only in *Eptesicus* and not in *Myotis*.

The Longitudinal Elements of the Reticulum

The triads or pentads of the same or successive sarcomeres are connected by large numbers of longitudinal tubules that form an intricate network around the myofibrils (Figs. 4 to 6). Although their course is mainly longitudinal they exhibit considerable tortuosity, and short transverse connections between them are frequently observed. Such transverse connections are especially common at the level of the Z line (Fig. 9), where they may be aligned to form an irregular channel running transversely across the myofibril. These anastomotic connections between longitudinal meshes of the reticulum, together with the terminal cisternae of triads, form a continuous transverse canalicular system apparently extending across the whole muscle (Fig. 6).

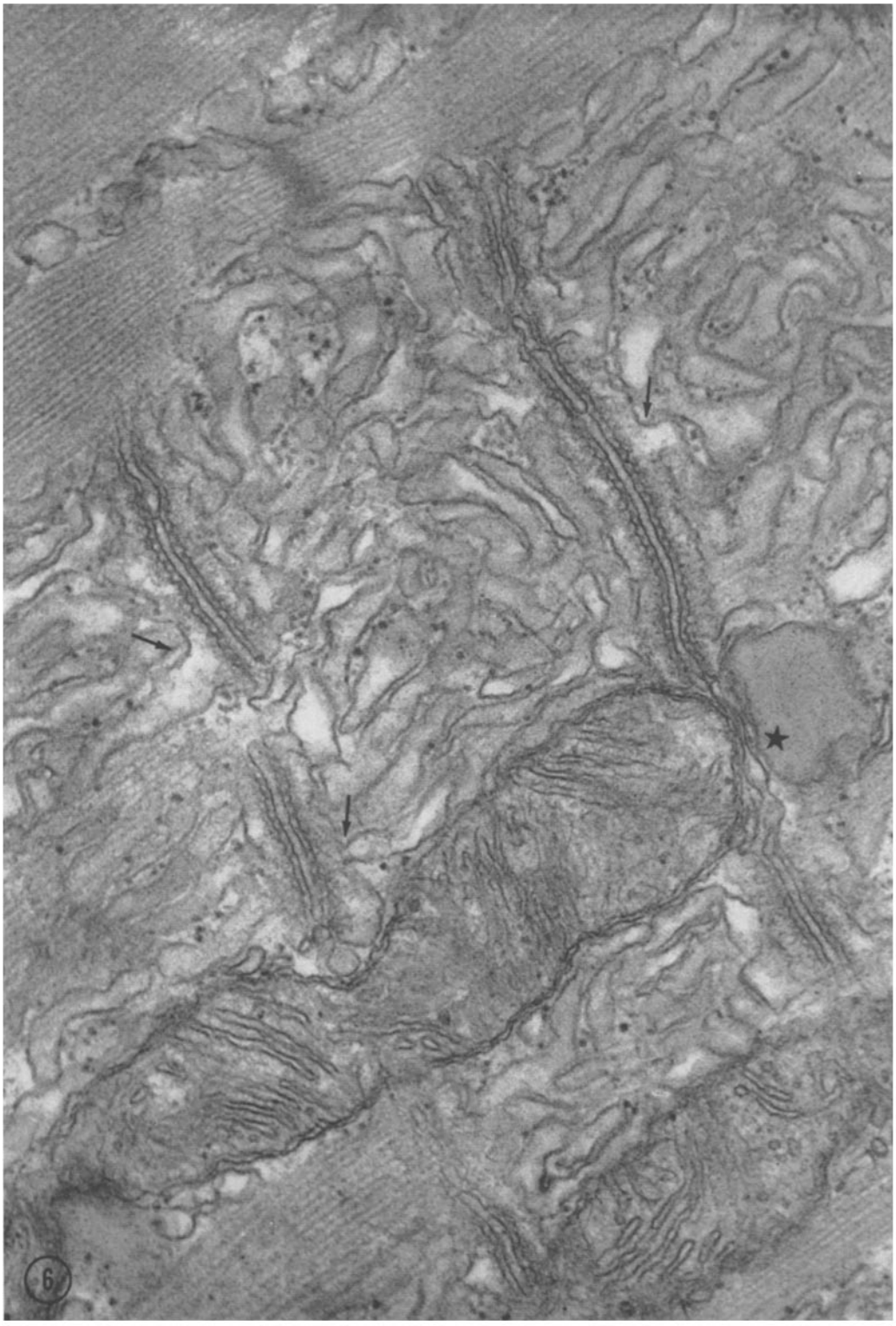
In longitudinal sections the sarcoplasmic reticulum in the interfibrillar clefts at the level of the A band usually consists of one or two layers of longitudinal tubules, whereas three or four layers are found at the level of the I bands (Fig. 4). It is not clear whether the longitudinal tubules arise from the terminal cisternae as a multilayered system, or whether the many layered appearance at the I band is due to partial contraction of the adjacent myofibrils and consequent crowding of the extremely tortuous elements. At their confluence with the cisternal elements of the triads the longitudinal tubules are often constricted, but elsewhere they are about 1,000 A wide in surface view and 200 to 400 A thick. The interstices between the strands of the reticulum often contain many glycogen particles.

Observations after Fixation in the Presence of $CaCl_2$

After fixation in the presence of 0.01 M $CaCl_2$ under certain conditions, a transverse element of the reticulum is strikingly outlined in electron micrographs of the muscle fibers by deposition of

FIGURE 6

A surface view of the reticulum of *Eptesicus* muscle seen at a higher power. The limiting membrane of the terminal elements facing the intermediate element has a scalloped appearance and is studded with uniformly spaced densities. Some material is precipitated in the lumen of the terminal cisternae. The intermediate element is a concontinuous tubule. We believe that the apparent interruptions of this continuity are due to minor changes in the orientation of the intermediate tubule, especially at the confluence of intermediate tubules between two myofibrils. At the star (*) the intermediate element is seen to extend past the terminal cisternae of a triad of one myofibril, passes between a mitochondrion and a fat droplet, and is in continuity with the intermediate element of the triad associated with a myofibril on the other side of the mitochondrion. The longitudinal elements form a closely meshed network of channels on the surface of the myofibrils. These channels are mainly oriented longitudinally but also show many transverse connections. The arrows indicate some points of confluence between the longitudinal elements and the transverse elements (terminal cisternae) of the reticulum. × 55,000.



dense particles on its surface (Fig. 8). Closer inspection reveals that the component selectively stained under these conditions is the intermediate element of the triads, recognizable by its small size and its position relative to the cross-banding of the myofibrils. No other element of the reticulum is stained so densely as the intermediate element. However, certain other membranous components of the muscle, including the mitochondria, are covered with a sparse, random stippling of similar electron-opaque granules. A few such particles are found scattered throughout the preparation. It must be emphasized that the conditions under which this "staining" reaction takes place have not been completely defined. The phenomenon is capricious and as yet not readily reproducible. Whenever there is staining, however, the pattern is as described above. It is not clear whether the staining is due to direct binding of Ca by the membranes or to some other factor associated with the presence of Ca in the fixative. Experiments are in progress that may lead to a better understanding of this phenomenon.

Despite our lack of understanding of the mechanism of this staining effect, the images obtained in this manner are useful in clarifying certain points in the organization of the reticulum of muscle cells which would otherwise be obscure. Fig. 8 includes several examples of pentads, recognizable here by the outlines of their paired intermediate tubules. The branching of the single intermediate element of a triad in the formation of a pentad is clearly visible (Fig. 8, *x* marks). Another point of interest which is difficult to detect without this staining is the occasional longitudinal connection between the intermediate tubules of successive triads. These connections span either the I band or the A band. The intermediate element abruptly changes its direction and runs longitudinally instead of transversally until it meets the next triad and becomes continuous with the intermediate element at that level. Electron micrographs of specimens fixed in the absence of Ca show (Fig. 10) that the whole triad complex does not participate in these connections, but only the intermediate element. It is humbling to compare these results with those of Veratti (15) in the early years of this century who illustrated, in an unspecified bat muscle impregnated with heavy metals, a reticulum in which transverse structures at each A-I junction were connected occasionally by bridging strands. The strands may have been similar to those seen in "calcium-impregnated" tissues described here. These were mainly connections within the sarcomere, but elements crossing the Z line were also depicted.

The Sarcolemma and its Relation to the Other Structures of the Muscle

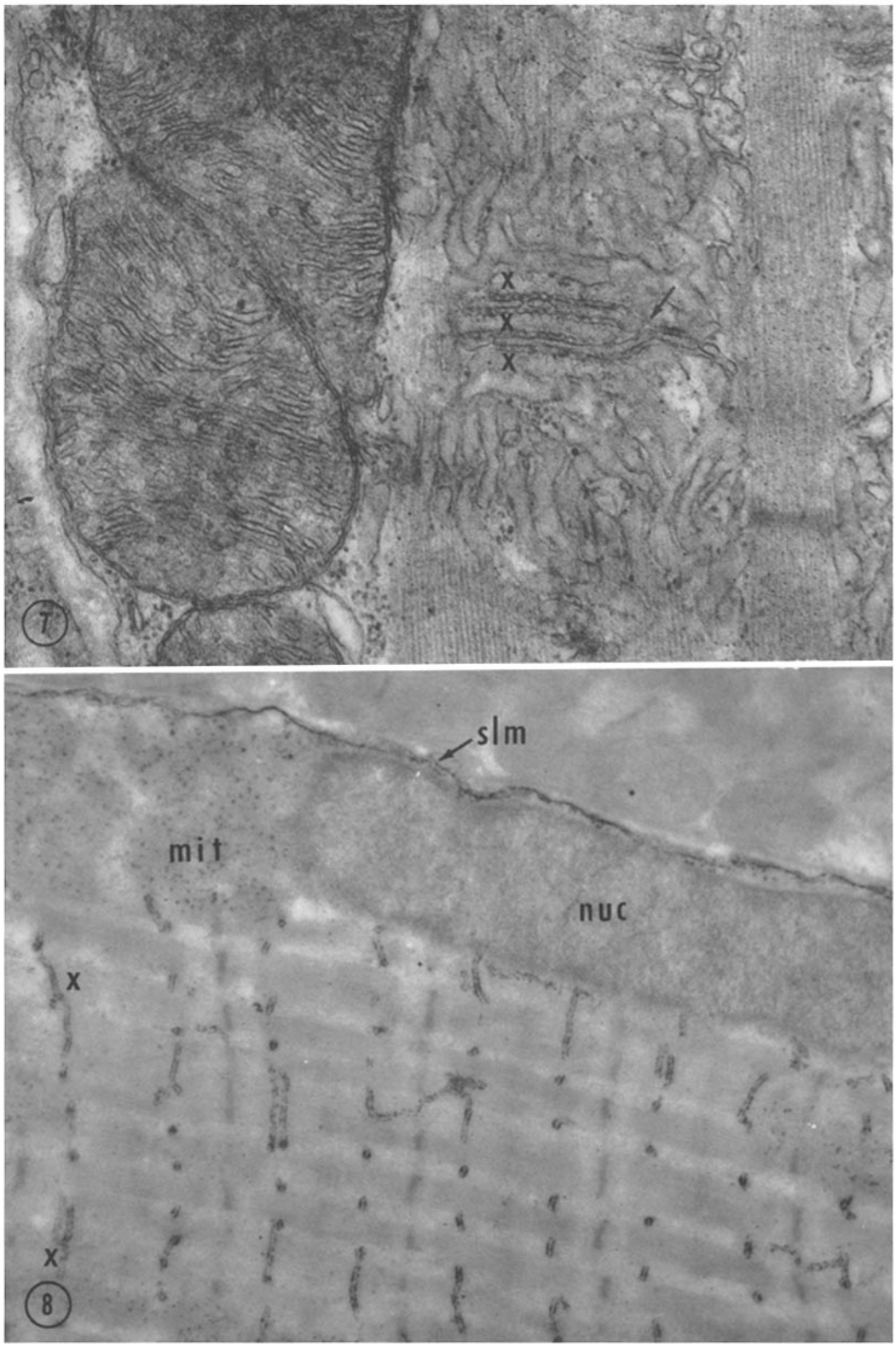
The sarcolemma of the cricothyroid muscle is similar in its fine structure to that found in other muscles (16, 17). It consists of a plasma membrane of the usual unit membrane thickness covered by a layer of basement membrane material 400 to 500 A thick which exhibits two zones: a dense outer zone separated from the plasmalemma by an inner zone of lower density. The sarcolemma sometimes forms cleft-like invaginations or incisures (Fig. 11) in the muscle fiber at the level of the Z lines. These are of variable depth. The basement membrane material

FIGURE 7

A pentad. Three transverse channels (crosses) corresponding to the terminal cisternae of the triads are seen, separated by two intermediate elements. At the arrow, the single intermediate cisterna of a triad is seen to branch, forming the double intermediate elements of the pentad. × 26,000.

FIGURE 8

A specimen "stained with calcium." With no further staining of the thin section the sarcolemma (*SLM*) and the intermediate cisterna of the transverse elements stand out in great contrast. Triads are represented by a single tubule, and pentads by two parallel tubules. The branching of the single tubule of the triad to form the paired tubules of the pentads is shown at the cross marks (*x*). Note also that the intermediate tubules can connect successive levels of transverse elements, either across the Z line (middle of the figure), or across the A band (close to the nucleus (*NUC*)). The mitochondria (*MIT*) are covered with a peppering of densities. × 16,000.


X
X
X
7
slm
mit
nuc
X
X
8

follows the sarcolemma and forms the lining of these invaginations. Aggregations of fine filaments about 100 A thick or of dense material, which may represent cross-sections through fine filaments, often extend from the Z line of the most superficial myofibrils toward the apex of these clefts (Fig. 11). Similar images may also be seen at the level of the H band, but less prominently. Very often, round or elongated membranous profiles are associated with the tip of the invaginations. These give the appearance of being sections through a tortuous tubule that may be continuous with the sarcoplasmic reticulum at the level of the Z line.

At the neuromuscular junction, the unmyelinated terminal arborizations of the axon lie in a trough, as is commonly found in other mammalian muscles (13, 18, 19), but this is often only a poorly marked gutter. The secondary clefts (19) are well developed (Fig. 13) and frequently converge toward the Z line of underlying myofibrils, even when, as commonly occurs, a layer of mitochondria separates the subneural apparatus from the myofibrils. The longitudinal elements of the reticulum can be observed to extend toward and to come into contact with the secondary clefts of the ending (Fig. 13). No direct continuity has been observed between the sarcoplasmic reticulum and the sarcolemma either at the end-plate or at other locations.

In specimens fixed in the presence of Ca, a close relationship can be demonstrated between the intermediate element of the triads and the sarcolemma (Fig. 12). The intermediate element can occassionally be followed right up to the sarcolemma, but it has never been observed to fuse with the latter. Darkly stained vesicles seen in this region probably represent cross- or oblique sections through the tortuous intermediate elements; but the possibility that these are pinocytosis vesicles cannot be excluded.

Distribution of Glycogen

The sarcoplasm between the meshes of the reticulum contains numerous glycogen granules, 200 to 300 A in diameter, which stain heavily with lead hydroxide (20). Their distribution is not always uniform. It is not uncommon to find glycogen granules concentrated between the longitudinal tubules of the reticulum at the level of the I band, while they are much less abundant in the A

FIGURE 9

This illustrates some of the well developed transverse tubules (arrows) at the level of the Z line. Part of a myofibril is seen on the extreme right of this electron micrograph, which otherwise shows a large expanse of sarcoplasmic reticulum in surface view. × 21,000.

FIGURE 10

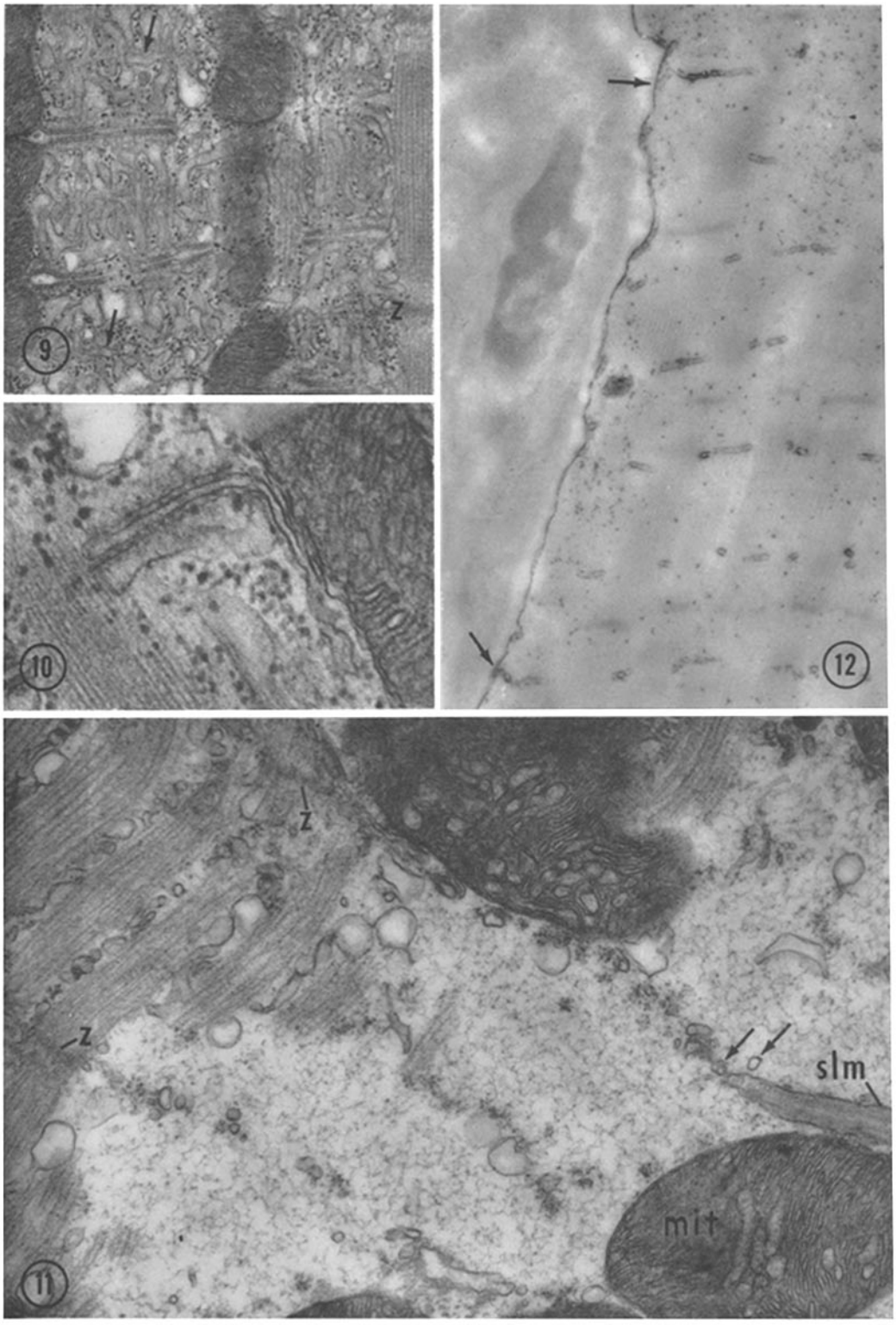
Shows the intermediate element of a triad running longitudinally to connect successive levels of triads. Compare this specimen with the one depicted in Fig. 8. × 55,000.

FIGURE 11

A section tangential to the surface of a muscle fiber cuts through the subsarcolemmal sarcoplasm and includes only the most peripheral myofibrils. At the level of the Z line, masses of dense punctate material in the sarcoplasm extend towards a cleft of the sarcolemma (*SLM*). This material represents cross-sections through fine filaments which can be seen in favorable planes of section. Vesicular profiles (probably sections through tubules of the sarcoplasmic reticulum) accompany this material and are found in close relationship to the sarcolemma (arrows). Similar observations can be made at the level of the H band. *MIT*, mitochondrion. × 27,000.

FIGURE 12

A "calcium-stained" specimen showing the close apposition of the intermediate tubules of the triads to the sarcolemma (arrows). No continuity between these structures has been conclusively demonstrated. × 16,000.


9
Z
10
12
Z
Z
slm
mit
11

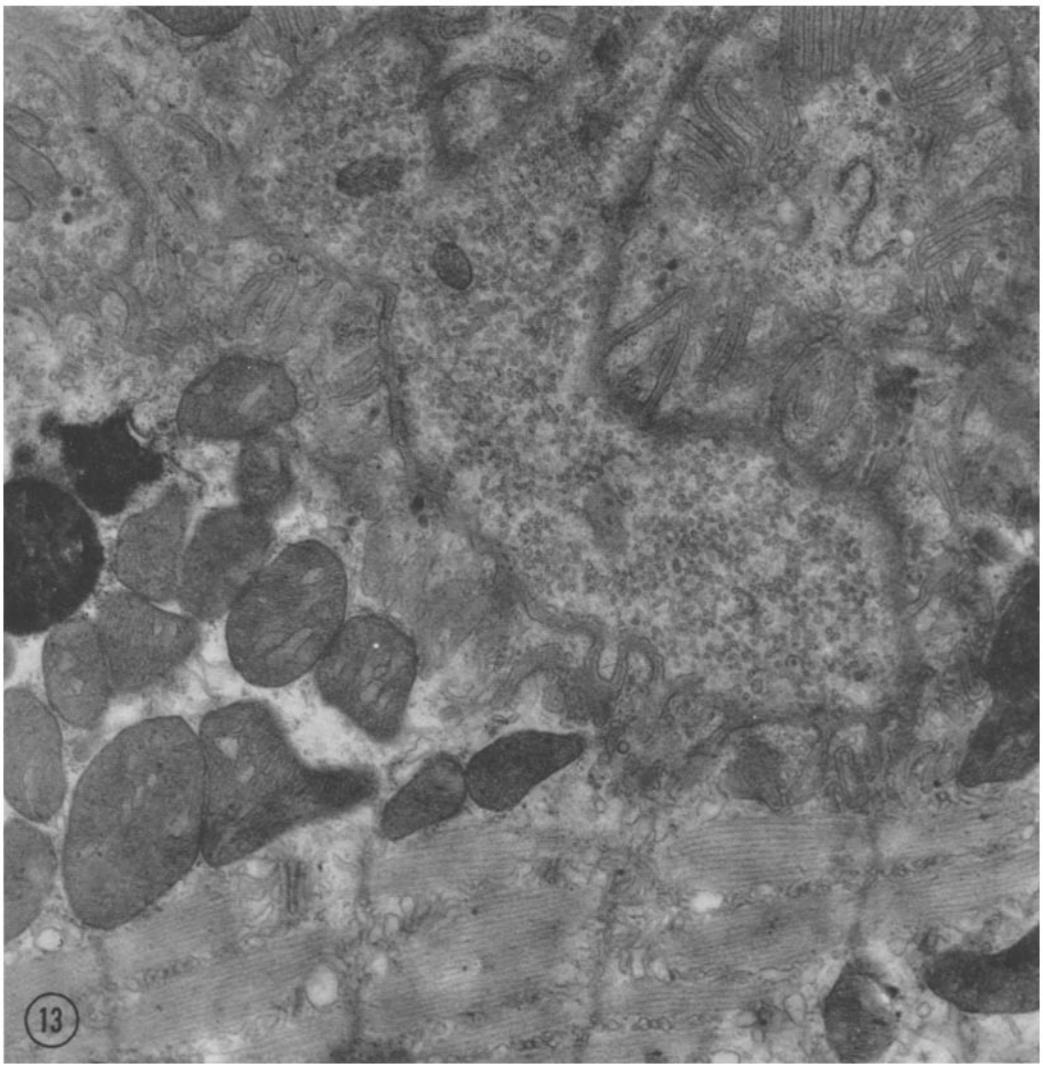


FIGURE 13

A tangential section through part of a nerve ending. The ending is filled with presynaptic vesicles and also contains mitochondria. It rests in a shallow trough on the surface of the muscle fiber, separated from the sarcolemma by a basement membrane. The sarcolemma is thrown into deep folds (secondary clefts) which penetrate deeply into the sarcoplasm. Basement membrane material is also found in the core of these clefts. Many ribonucleoprotein particles, either free or associated with membranous cisternae, are found in the sarcoplasm of the muscle cell in the vicinity of the ending. It may be possible to speculate that these have some function in the formation of acetylcholinesterase. The sarcoplasmic reticulum surrounding the muscle fibers extends towards and comes into close approximation with the secondary clefts. × 16,000.

band. In other areas, however, no such difference in distribution of glycogen between bands can be made out.

DISCUSSION

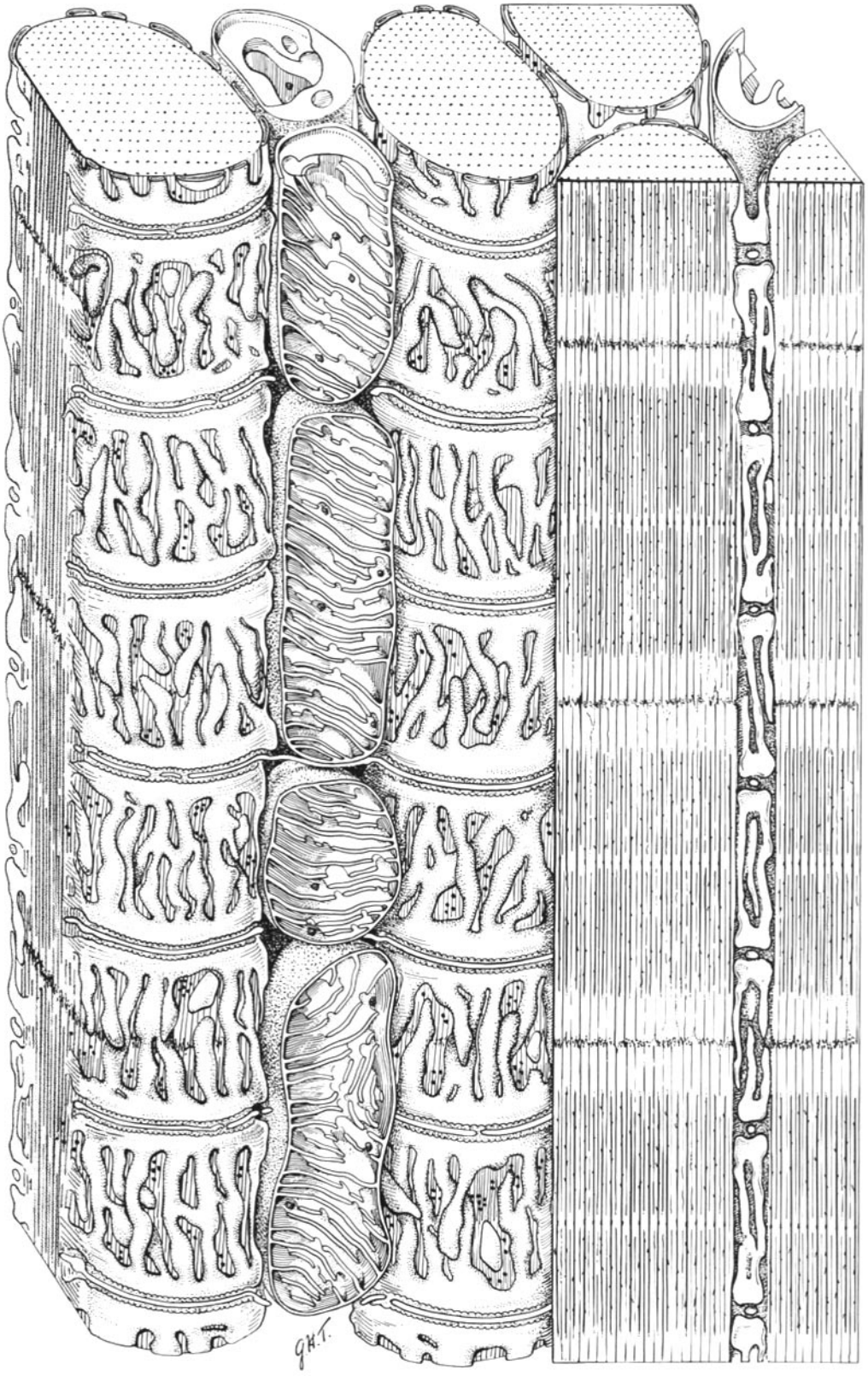
The circumstantial evidence reviewed in the Introduction indicates that the cricothyroid muscle of the bat is an extremely fast acting muscle. It evidently contracts within a few milliseconds after arrival of a nerve impulse, reaches maximal contraction in at most 4 milliseconds, and relaxes its tension in 1 millisecond. It is tempting to entertain the possibility that the degree of development and structural complexity of the sarcoplasmic

reticulum bears some relation to the speed of this muscle (21). The earlier observation that the fast acting swimbladder muscle of the toadfish is likewise provided with an extensively developed reticulum also favors this conclusion (14). This does not necessarily imply that all fast muscles will be found to have such an elaborate reticulum, for the problem of achieving very fast contraction may have been solved differently in various muscles of different animal species. In insect muscle, Edwards and Ruska felt that more reticulum was associated with slow muscles than with fast ones (22). It is true, in any case, that an unusually well developed reticulum is associated with rapid contraction in the bat cricothyroid muscle. Fig. 14, a diagram, summarizes some of the observations made. Very rough estimates based on our electron micrographs indicate that as much as 10 per cent of the volume of the muscle fibers may be occupied by sarcoplasmic reticulum. Because of the small diameter of the myofibrils, their innermost myofilaments can only be a few tenths of a micron from the reticulum. In most other muscles studied to date, the longitudinal elements of the reticulum form a sparse single-layered network in the interfibrillar sarcoplasm (6, 13). In the bat cricothyroid muscle, however, one finds a more complex network forming several layers of longitudinal tubules at the level of the I band. Although these channels are mainly oriented parallel to the long axis of the muscle fibers, they do, nevertheless, form a canalicular system that is continuous laterally throughout the muscle, either through numerous short side-to-side anastomoses or via more direct transverse channels which are sometimes found at the level of the Z lines.

The triads which are the principal transverse elements of the reticulum are well developed and form continuous bracelets around every myofibril. In addition to the triads, similar but more complex structures called pentads are found quite frequently. A new finding of special interest is the presence of regularly spaced specializations of that surface of the terminal cisternae that abuts on the slender intermediate element of the triads and pentads. Although such structures have been detected in toadfish swimbladder muscles (23), they have not previously been described in other muscles. The intermediate element may connect the reticula of adjacent myofibrils. It is a continuous tubule instead of a series of independent vesicles, as it is described in the literature. As we have previously pointed out (14), however, it is possible that the apparent vesicular nature of the intermediate element described in other muscles was attributable either to poor preservation of an extremely labile structure, or, as has been proposed by Andersson-Cedergren (13), to the fact that the intermediate element may be a sinuous tubule with out-pocketings which may not be wholly included in the plane of a single thin section. In the present study it has been found that the intermediate element, which normally runs transversely as a part of a triad, may leave its associated cisternae and run *longitudinally* forming shunts or direct connections between triads at different levels along the length of the muscle. Consistent with earlier observations (6, 13, 14, 24) is the finding that both the intermediate and the longitudinal elements of the reticulum come in close contact with the sarcolemma, but do not seem to be continuous with it.

One attractive hypothesis as to the role of the sarcoplasmic reticulum has grown out of a synthesis of physiological and ultrastructural observations. Experiments of Andrew Huxley and his collaborators (7, 25, 26) on local activation of skeletal muscle fibers showed that a subthreshold depolarization of certain points along a muscle fiber led to the contraction of the adjacent sarcomere or half sarcomere. The sensitive points at which this occurred were found only at the level where electron micrographs reveal the triads of the reticulum to be located. This seemed to indicate that the transverse elements of the reticulum may play a role in the inward spread of the impulse (6–8, 13).

It has been assumed that the mechanism of this inward spread is electrical in nature, perhaps a graded passive spread along a cable structure (7). One can imagine that such a structure is represented by the intermediate element of the triads which in the present case, and in the swimbladder muscle of *Opsanus tau* (14), has been shown to be a continuous tubule. Such an interpretation would be more difficult to defend if the discontinuous condition of the intermediate element described in the literature for other muscles proves to be valid. If, however, this appearance proves to have been an artifact of specimen preparation as suggested above, then it may be reasonable to consider the intermediate element of the triad as the structure chiefly responsible for the inward spread of the impulse. The very close apposition of the intermediate element to the sarcolemma which has been reported by others (6, 13) and also here is suggestive of such a role. As pointed out by Huxley (7), however, a direct continuity with the



sarcolemma would greatly simplify the hypothesis. The present work does present some evidence, though admittedly tenuous, which indicates that the intermediate element of the triad differs from the other tubular elements of the reticulum and that it may share some of the properties of the sarcolemma. Their similar calcium staining reaction suggests some chemical similarity between these two structures and thus tends to support the concept of inward conduction along the intermediate element. Upon stimulation the latter might conceivably release an "activating substance" which would initiate the sequence of reactions involved in the contraction of the myofibrils. In this view the occurrence of pentads would be interpreted as an elaboration of the usual triad pattern which might lead to the release of more "activator." Such an interpretation requires only the presence of an intermediate element and does not explain the presence of the other components which make up the reticulum. It is possible that these other elements play some role in the contractile mechanism other than impulse conduction. The possibility also remains that the longitudinal elements do play a role in conduction while the triads act as a "trigger" of muscular contraction (6). Indeed, both the longitudinal elements and the intermediate element form a continuous channel system across the muscle and closely approach the sarcolemma. If this interpretation be entertained, an alternate explanation for Huxley's experiments must be found, since one would not expect to find the spots sensitive to local stimulation to be situated at the level of the triads but rather where the longitudinal elements come into close contact with the sarcolemma. A possible explanation may be that the subthreshold stimuli used by Huxley were strong enough to fire the triads, releasing the trigger substance, but not sufficient to stimulate the conducting system.

It is of course also possible that either or both of these functional interpretations of morphological data are completely or partially invalid. There certainly are other ways to think about the role of the reticulum. Others have already pointed out that the sarcoplasmic reticulum of muscle may play a role in the general metabolism of the muscle cell (6, 13, 14) rather than being directly involved in the excitation contraction coupling. The latter view, however, is very tempting to consider, especially in the light of recent evidence (for review see 27, 28) on the existence in microsome fractions (presumably originating in the sarcoplasmic reticulum of itnact cells) of factors which can affect the physiological state of the myofibrils.

This work was supported by grant number G-12916 of the National Science Foundation.

The author would like to express his appreciation for the constant help and advice he has received from Dr. D. W. Fawcett.

Received for publication, August 14, 1961.

BIBLIOGRAPHY

1. DE JURINE, L., *J. physique*, 1798, **46**, 145 (quoted in 2).
2. GRIFFIN, D. R., Listening in the Dark, New Haven, Yale University Press, 1958.
3. GRIFFIN, D. R., Listening in the Dark, New Haven, Yale University Press, 1958, 111–119.
4. PRINGLE, J. W. S., Insect Flight, Cambridge University Press, 1957.
5. BENNETT, M. S., and PORTER, K. R., *Am. J. Anat.*, 1953, **93**, 61.
6. PORTER, K. R. and PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.

FIGURE 14

This composite diagram summarizes observations made on the toadfish swimbladder muscle and on the bat cricothyroid muscle. Two myofibrils in surface view and two myofibrils in longitudinal section are represented. The triads of the reticulum are at the level of the junction between the A and the I bands. The intermediate element of each triad is depicted as a continuous tubule. Approximately in the middle of the diagram an intermediate tubule is seen extending across the interfibrillar space which is occupied by a row of mitochondria. Successive triads are connected by several layers of tubules which form the longitudinal elements of the reticulum. Glycogen particles are shown between the meshes of the reticulum. Both the triads and the longitudinal elements form networks which are continuous laterally throughout the muscle.

7. HUXLEY, A. F., *Proc. New York Acad. Sc.*, 1959, **81,** 446.
8. BENNETT, H. S., *Am. J. Phys. Med.*, 1955, **34,** 46.
9. BENNETT, H. S. *in* Structure and Function of Muscle, (G. H. Bourne, editor), New York, Academic Press, Inc. 1960, **1,** chapter 6.
10. BENNETT, H. S., and LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 113.
11. LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
12. KARNOVSKY, M. J., *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 729.
13. ANDERSSON-CEDERGREN, E., *J. Ultrastruct. Research*, Suppl. 1.
14. FAWCETT, D. W., and REVEL, J. P., *J. Biophysic. and Biochem. Cytol.*, 1961, **10,** No. 4, suppl., 89.
15. VERATTI, E., *Mem. reale Inst. Lombardo*, 1902, **19,** 87.
16. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 374.
17. FAWCETT, D. W., and SELBY, C. C., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 63.
18. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 381.
19. DE HARVEN, E., and COËRS, C., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 7.
20. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 575.
21. PEACHEY, L. D., and PORTER, K. R., *Science*, 1959, **129,** 721.
22. EDWARDS, G. A., and RUSKA, H., *Quart. J. Micr. Sc.*, 1955, **56,** 151.
23. FAWCETT, D. W., and REVEL, J. P., unpublished observations.
24. SIMPSON, F. O., and OERTELIS, S. J., *Nature*, 1961, **189,** 758.
25. HUXLEY, A. F., and TAYLOR, R. E., *J. Physiol. (London)*, 1955, **130,** 49.
26. HUXLEY, A. F., and STRAUB, R. W., *J. Physiol. (London)*, 1958, **143,** 40.
27. NEEDHAM, D. M., *in* Structure and Function of Muscle, (G. H. Bourne, editor), New York, Academic Press, Inc., 1960, **2,** chapter 2.
28. PORTER, K. R., *J. Biophysic. and Biochem. Cytol.*, 1961, **10,** No. 4, suppl., 219.