THE SARCOPLASMIC RETICULUM, THE T SYSTEM, AND THE MOTOR TERMINALS OF SLOW AND TWITCH MUSCLE FIBERS IN THE GARTER SNAKE

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ABSTRACT

Twitch and slow muscle fibers, identified morphologically in the garter snake, have been examined in the electron microscope. The transverse tubular system and the sarcoplasmic reticulum are separate entities distinct from each other. In twitch fibers, the tubular system and the dilated sacs of the sarcoplasmic reticulum form triads at the level of junction of A and I bands. In the slow fibers, the sarcoplasmic reticulum is severely depleted in amount and the transverse tubular system is completely absent. The junctional folds of the postsynaptic membrane of the muscle fiber under an "en grappe" ending of a slow fiber are not so frequent or regular in occurrence or so wide or so long as under the "en plaque" ending of a twitch fiber. Some physiological implications of these differences in fine structure of twitch and slow fibers are discussed. The absence of the transverse tubular system and reduction in amount of sarcoplasmic reticulum, along with the consequent disposition of the fibrils, the occurrence of multiple nerve terminals, and the degree of complexity of the post junctional folds of the sarcolemma appear to be the morphological basis for the physiological reaction of slow muscle fibers.

Extrafusal muscle fibers may be classified both morphologically and physiologically as twitch or slow fibers. The twitch fiber shows propagated impulse activity and non-graded contractile activation (1–3) and has a fibrillar pattern in which the fibrils are regular, distinct, and punctate (*Fibrillenstruktur*), and it receives a single nerve ending of the end plate or "en plaque" type (3–7). The slow fiber usually does not display impulse activity and undergoes a graded contraction or contracture (1–3) and has larger, irregular, poorly defined fibrils (*Felderstruktur*) and receives multiple motor endings of the "en grappe" type (3–7). That the physiologically slow fiber and the fiber with the more irregular distribution of fibrils and multiple motor terminals are the same can be illustrated by referring to the anterior part of the latissimus dorsi of the chick in which all the fibers are physiologically slow (2) and all have the irregular distribution of fibrils and multiple motor endings (5). This same correspondence between morphological and physiological characteristics in a type of muscle fiber has also been found in the frog (7). In addition, Hess and Pilar (3) conclude that "correlation of the morphological and physiological data (in cat extraocular muscles) provides further evidence that the fibres with diffuse, poorly delineated fibrils incompletely surrounded by sarcoplasmic reticulum and with multiply-distributed nerve endings are physiologically of the slow-fibre type." Hence, since there is a strong correlation between the physiological reaction and the morphological appearance and innervation of the muscle fiber, it is apparent that extrafusal muscle fibers can be classified, using either morphological or physiological standards, as twitch type or slow type.

Recently, these two types of fibers have been identified morphologically in the garter snake (8). It is the purpose of the present investigation to study in detail with the electron microscope the structure of the sarcoplasmic reticulum, the transverse tubular system, and the nerve endings of these two different types of muscle fiber in the garter snake.

MATERIAL AND METHODS

The segmental muscle originating on the ribs and inserting into the skin of the garter snake (*Thamnophis sirtalis*) was used. This flat muscle has approximately 100 muscle fibers, about 25 of which are of the slow type. The muscle fibers run uninterrupted from the origin to the insertion of the muscle. The muscle was fixed outstretched in Millonig's fixative (9) for 1 hour or in 5 per cent glutaraldehyde (10) in phosphate buffer for 90 minutes. The glutaraldehyde-fixed muscle was postfixed in 1 per cent osmium tetroxide in water for an additional hour. The tissues were then dehydrated, some were divided longitudinally into two pieces, and embedded in Epon 812 (11) or Maraglas (12, 13). Thin sections, cut on a Porter-Blum MT-1 microtome, were collected on Formvar-coated copper grids, stained with lead (14), and examined in an RCA-EMU 3F microscope. Stained thick epoxy resin sections were examined in the light microscope to determine the location of nerve bundles and nerve terminals in the block of tissue.

RESULTS

The Muscle Fiber

The elements of the sarcoplasmic reticulum and the T system, called the triad, have been considered to be of great importance in muscle fibers (15). The triad consists of longitudinally oriented elements of the sarcoplasmic reticulum which form dilated cisterns or terminal sacs and between which occurs the transverse tubular or intermediate element. This latter structure is oriented at right angles to the muscle fibrils. Because of its different orientation and possible physiological significance, the intermediate element has become known as the transverse tubule or T system of the muscle fiber. In vertebrates, the triad occurs at the level of junction of A and I bands or at the level of the Z line.

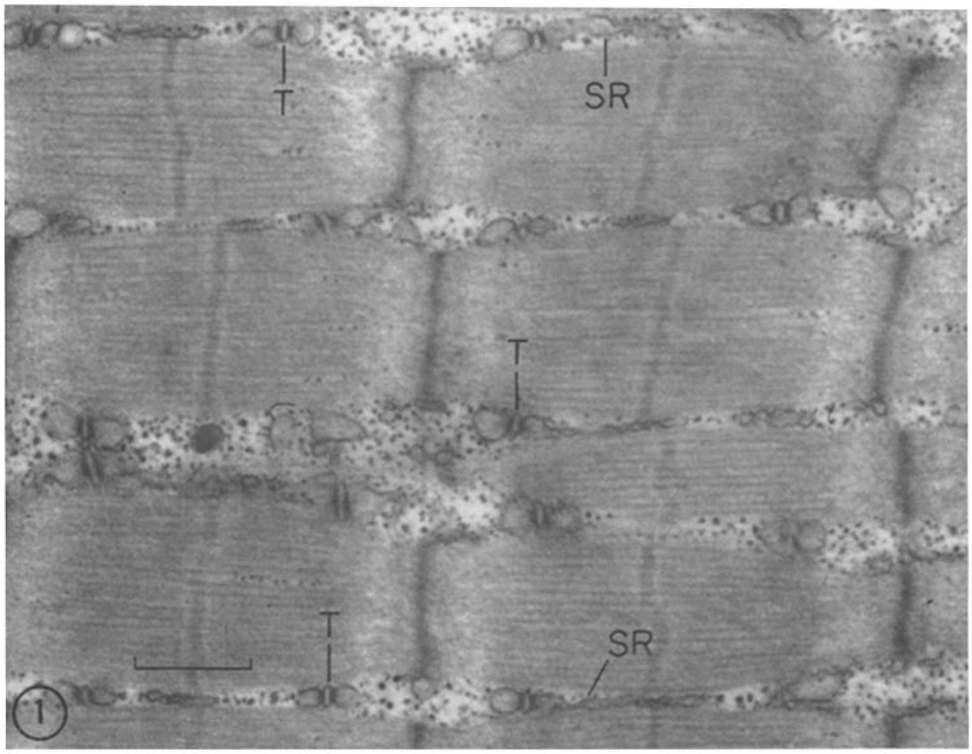
A muscle fiber of the snake can be identified as being morphologically of the twitch or slow type, in cross- or longitudinal sections viewed in the electron microscope, by the distribution of its fibrils (8). The fibrils of a twitch fiber are regularly separated from each other by sarcoplasmic reticulum and are rather regular in size and shape (*Fibrillenstruktur*) (Figs. 1, 6). The fibrils of slow muscle fibers are only irregularly separated from each other, if at all; hence, the fibrils are irregular in size, shape, and distribution (*Felderstruktur*) (Figs. 2, 7). Frequently, but not always, the Z line of the slow fiber has a zigzag appearance in longitudinal section (Fig. 2), whereas the Z line of the twitch fiber runs straight across the fibril (Fig. 1).

It has recently and convincingly been pointed out that the T system, seen best after glutaraldehyde fixation, is an entity distinct from the sarcoplasmic reticulum in the muscles of fish (16). This can also be seen clearly in snake twitch muscle fibers. The sarcoplasmic reticulum is

All illustrations are electron micrographs of snake muscle fibers. The lines on the photographs indicate 0.5 μ.

FIGURE 1 Twitch muscle fiber, longitudinal section, glutaraldehyde fixation. *T* indicates the triad at the level of junction of A and I bands and consisting of the transverse tubule between two dilated sacs of sarcoplasmic reticulum (*SR*). Densities occur between the membranes of the transverse tubule and the dilated sacs of sarcoplasmic reticulum. The muscle fibrils are regularly separated from each other by sarcoplasmic reticulum (*Fibrillenstruktur*). Compare with Fig. 2. $\times$ 32,000.

FIGURE 2 Slow muscle fiber, longitudinal section, glutaraldehyde fixation. The triads and the transverse tubules are absent. The sarcoplasmic reticulum (*SR*) is severely depleted in amount. Adjacent muscle fibrils are irregularly separated from each other (*Felderstruktur*). In this fiber, the Z line runs in a zigzag manner across the fibril compared to the relatively straight Z line seen in the twitch fiber of Fig. 1. Compare with Fig. 1. $\times$ 32,000.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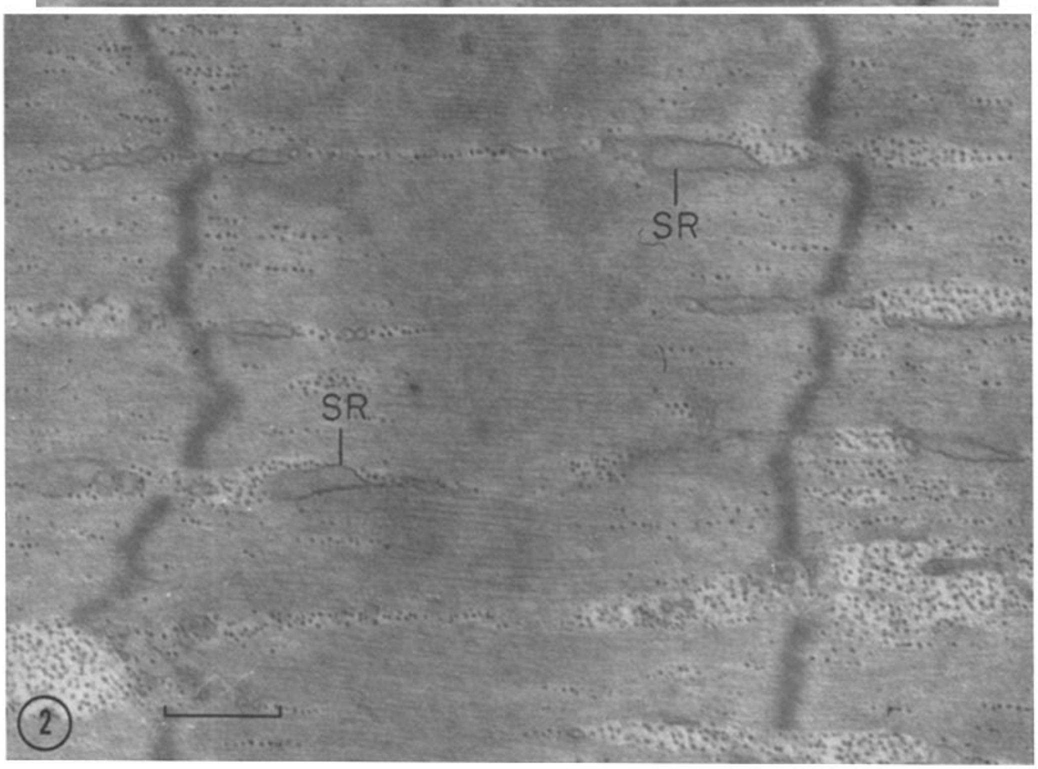
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disposed in the same way as that of the muscle of other vertebrates. The elements of the sarcoplasmic reticulum are oriented longitudinally in the spaces between the fibrils. Near the junction of A and I bands, the longitudinal elements of the sarcoplasmic reticulum dilate and form terminal cisterns or sacs. Between the two terminal sacs, a small vesicle, representing the T system of the fiber, occurs. The two cisterns and this intermediate element form the triad at the level of junction of A and I bands (Fig. 1). A surface cut shows the elements of the sarcoplasmic reticulum anastomosing with each other and forming a canalicular system surrounding the muscle fibril (Fig. 3).

In longitudinal sections of the muscle, the T system can be seen running across the muscle fiber (Fig. 3). The transverse tubules apparently run without interruption across the muscle fiber. However, they are seen usually in only relatively short stretches since they pass in and out of the plane of section. The T system of the snake muscle appears like a tubule; there is nothing remarkable about its contents. In fish, the T system is often occupied by strands of glycogen-rich cytoplasm limited by a membrane and belonging to an adjacent fiber (16). The space between the membrane of the T system tubule and the membrane around the terminal sacs of the sarcoplasmic reticulum is usually dense in the electron microscope, so the transverse tubules have very dark borders. In cross-sections, it is difficult to identify the T system since the dense borders of the transverse tubule cannot be seen in transverse sections passing through its lumen. However, in slightly oblique sections passing through A and I bands, the T system can be seen as a dense tubule occurring between the A band with its thick and thin filaments and the I band with only thin filaments (Fig. 5).

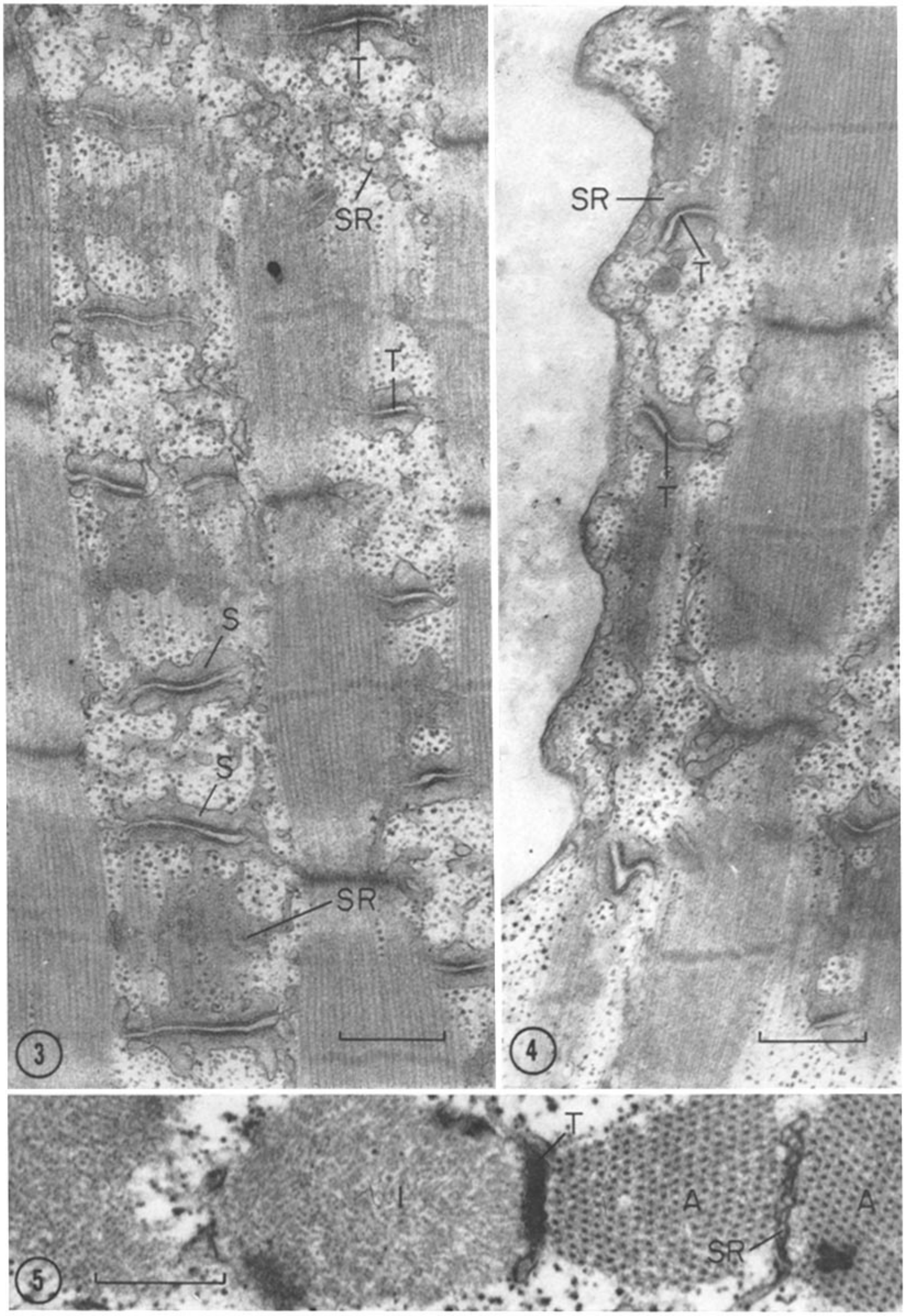
A direct connection between the T system and the sarcolemma or limiting membrane of the muscle fiber has not been found in the snake. The transverse tubules come close, in some sections, to the sarcolemma (Fig. 4), but an invagination of the latter to form the T system has never been seen. Perhaps a direct continuity of invaginating sarcolemma and transverse tubule has been missed in section. If so, the places in which sarcolemma invaginates to form the T system must be very infrequent. An invagination of sarcolemma to form the T system has been seen in fish (16), but not in frog (17) muscle. A scalloping of the membranes of the T system and the sarcoplasmic reticulum, similar to that seen in the fish (16) and frog (17), also occurs in the snake (Figs. 3, 4). Elements of the sarcoplasmic reticulum can also approach closely to the sarcolemma; however, continuity between elements of the sarcoplasmic reticulum and the sarcolemma was never seen (Fig. 4).

The slow fiber differs radically from the twitch fiber in its content of sarcoplasmic reticulum and transverse tubules. The sarcoplasmic reticulum is severely depleted in the slow muscle fiber (Fig. 2). A few irregular individual strands occur, but they do not regularly separate adjacent muscle fibrils from each other. Elements of the sarcoplasmic reticulum are of most frequent occurrence at the level of the I band; only very few strands,

FIGURE 3 Twitch muscle fiber, longitudinal section, glutaraldehyde fixation. The transverse tubules (*T*) are seen running across the muscle fiber at the level of the junction of A and I bands. On either side of the transverse tubules, dilated sacs (*S*) of the sarcoplasmic reticulum are seen. Densities occur between the membranes of the transverse tubule and the dilated sacs of the sarcoplasmic reticulum. The canalicular system on the surface of the muscle fibril formed by the sarcoplasmic reticulum (*SR*) is seen. × 32,000.

FIGURE 4 Twitch muscle fiber, longitudinal section, glutaraldehyde fixation. The transverse tubules (*T*) come close to, but do not join, the limiting membrane of the muscle fiber. Elements of the sarcoplasmic reticulum (*SR*) can be seen near the limiting membrane of the muscle fiber. × 32,000.

FIGURE 5 Twitch muscle fiber, cross- (oblique) section, glutaraldehyde fixation. The dense transverse tubule (*T*) occurs between the A band (*A*) with its thick and thin filaments and the I band (*I*) with only thin filaments. Elements of the sarcoplasmic reticulum (*SR*) occur between two A bands. × 40,000.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if any, can be seen between the fibrils around the A band. The anastomosing channels seen in surface view can be present, but are of irregular occurrence. Apparent terminal sacs at the level of junction of A and I bands have been seen, but do not occur at all regularly. The T system is completely absent (Fig. 2). A search for it in longitudinal sections near the sarcolemma or between the fibrils yielded negative results. In some cross-sections, structures resembling triads can be seen; however, closer examination reveals these so called triads to be composed of three elements of the sarcoplasmic reticulum alone and not to include a transverse tubule. Absence of triads in frog slow muscle fibers has already been noted (7).

The presence or absence of the T system in longitudinal section and the distribution of the fibrils in cross- or longitudinal section can be used as criteria to characterize a muscle fiber as twitch or slow.

The Nerve Endings

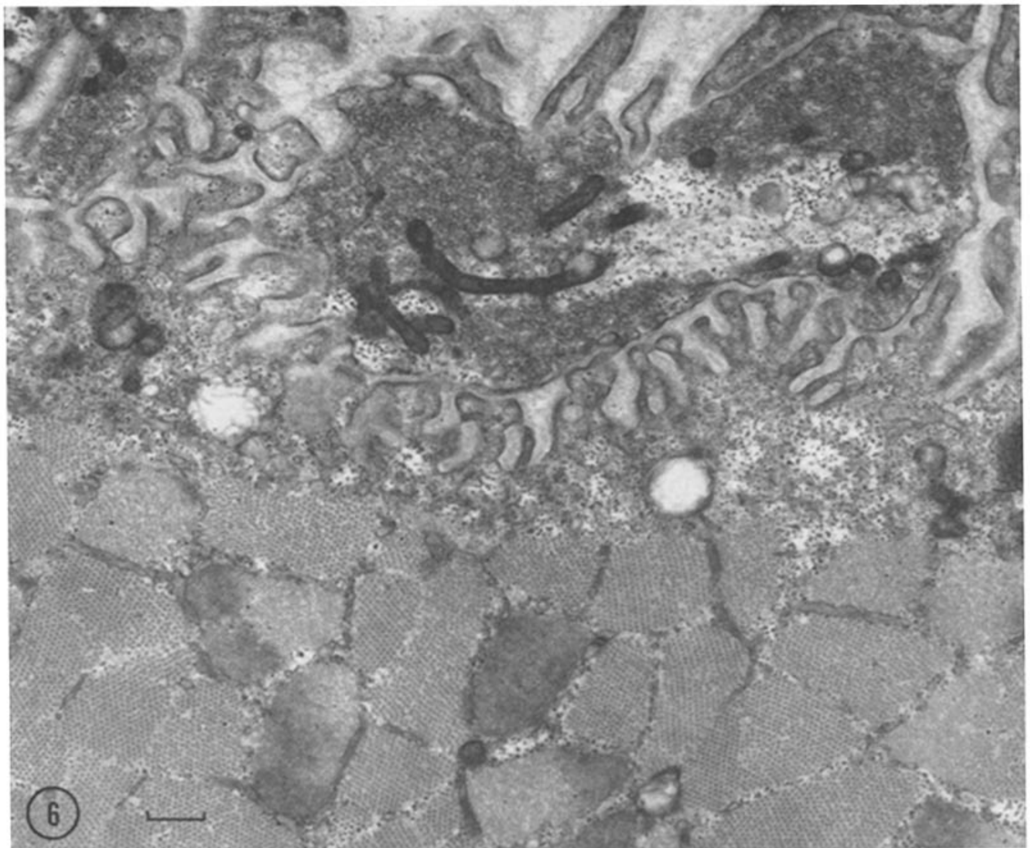
The twitch muscle fibers of the snake have a single, robust, compact nerve ending ("*en plaque*"), whereas the slow fiber has more delicate filamentous terminals, several of which occur on an individual fiber ("*en grappe*") (8). The postjunctional folds of the sarcolemma under motor end plates have been described many times in the literature; "en grappe" terminals have yet to be seen in the electron microscope.

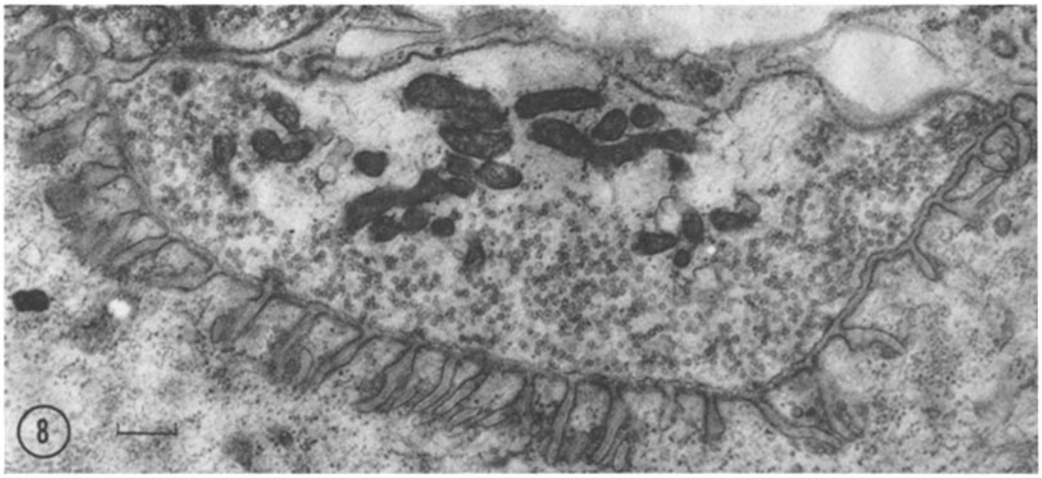
In muscle fibers identified as twitch fibers using the standards designated above, the nerve terminals are seen as rather large endings with several units, producing a conspicuous elevation on the muscle fiber (Figs. 6, 8). Each unit of the ending contains an accumulation of synaptic vesicles. The junctional membrane of the muscle fiber is thrown into many folds under the ending. These folds are demonstrated in cross- (Fig. 6) or longitudinal (Fig. 8) section. The folds are elongate and push into the muscle fiber for a considerable distance. The folds also occur rather regularly spaced from each other. Some of the indented folds of the sarcolemma can branch and appear Y-shaped. The folds frequently appear slightly dilated toward the muscle side. Basement membrane material, identified by its density, occurs between the nerve terminal and muscle fiber and passes into the space between the junctional folds.

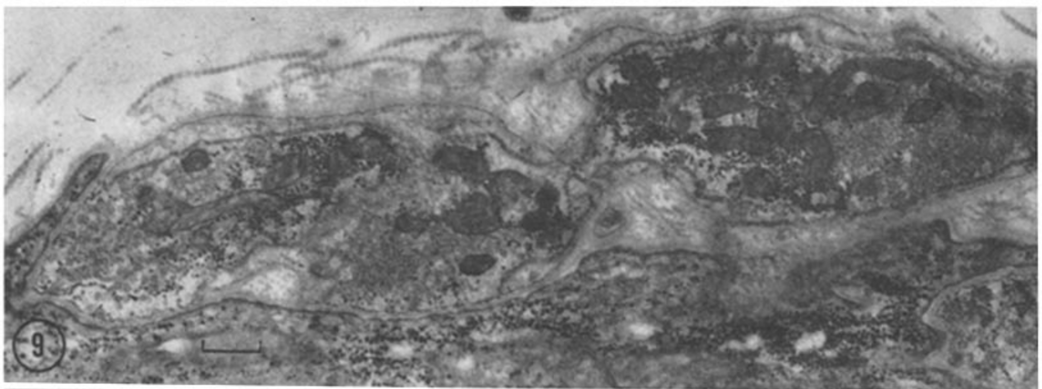
The slow muscle fibers can be identified by the distribution of sarcoplasmic reticulum and fibrils, as described above, and by the absence of the T system. In addition, multiple nerve terminals, separated by variable distances from each other, can be seen on the same slow muscle fiber. The nerve terminals in the longitudinal sections of slow muscle fibers in the illustrations (Figs. 9, 10) are one of at least two terminals found on the same muscle fiber. The endings on the slow muscle fiber also can be seen as an elevation on the muscle fiber (Figs. 7, 9, 10). However, the elevations do not appear as conspicuous as on the twitch fiber. The nerve endings also appear as several units, each of which is filled with vesicles entirely similar in appearance to those seen in terminals on twitch fibers. The postjunctional folds of the sarcolemma under *en grappe* terminals are rather scarce in cross- (Fig. 7) or longitudinal (Figs. 9, 10) section. At many levels, they do not occur. In the most extensive folding seen on a slow fiber, the folds do not occur with regularity, are narrow, and are not so elongated as in the twitch fiber. The specialized area of sole plate sarcoplasm is not so extensive in the slow muscle fiber as in the twitch fiber and hence the folds are not so elongate

Figure 6 Motor end plate, twitch muscle fiber, cross-section. The vesicle-filled units of the nerve terminal form an elevation on the muscle fiber. Under the terminals, the sarcolemma is thrown into many rather regularly occurring postjunctional folds. The muscle fibrils are regularly separated from each other (*Fibrillenstruktur*). Compare with Fig. 7. × 16,000.

Figure 7 *"En grappe"* terminal, slow muscle fiber, cross-section. The vesicle-filled units of the nerve terminal form a slight elevation on the muscle fiber. The postjunctional folds of the sarcolemma under the terminals are essentially absent. The muscle fibrils are only irregularly separated from each other (*Felderstruktur*). Compare with Fig. 6. × 16,000.







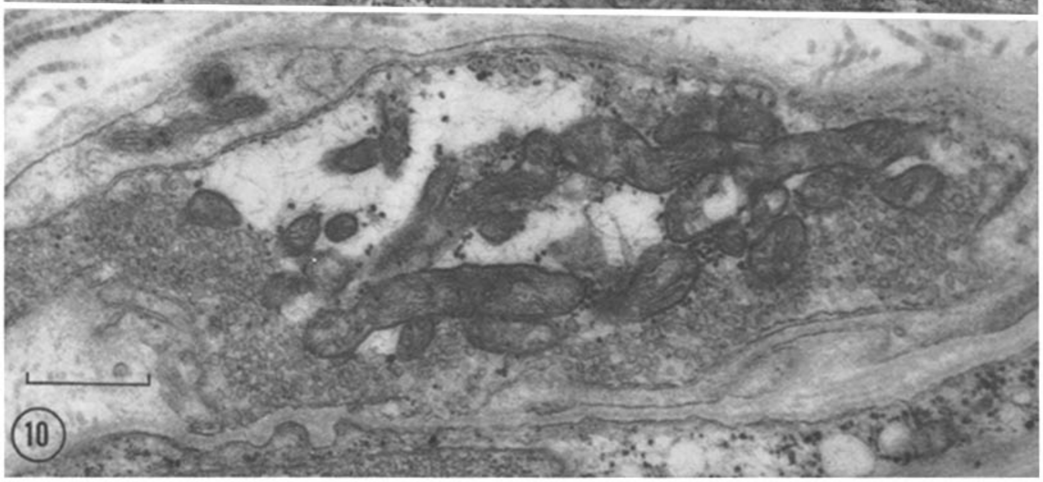


FIGURE 8 Motor end plate, twitch muscle fiber, longitudinal section. Vesicle-filled terminal units of the end plate are seen. Under the terminals, the sarcolemma is thrown into regularly occurring folds. Compare with Figs. 9 and 10. × 16,000.

FIGURES 9 and 10 *En grappe* terminals, slow fibers, longitudinal sections. The terminals shown are one of at least two nerve endings which were seen on the same muscle fiber. The vesicle-containing nerve terminals are shown. Under the terminals, the postjunctional folds of the sarcolemma are very few in number, very short in extent, and irregularly occurring. Compare with Fig. 8. Fig. 9, × 16,000; Fig. 10, × 32,000.

and appear in some sections to run in a longitudinal direction, rather than to push into the muscle fiber. It is almost as if the folds start to invaginate, but soon come near the fibrils of the slow muscle fiber due to relatively sparse amounts of sole plate sarcoplasm, and are forced to turn and change direction. Nuclei tend to accumulate near the terminal in both types of muscle fiber, but are not so numerous or so large in slow fibers as in twitch fibers. As in twitch muscle fibers, basement membrane material separates the nerve terminals from the muscle fiber and enters into the postjunctional infoldings.

DISCUSSION

There is, as yet, no evidence for the snake that the morphologically slow type fibers with multiple nerve terminations are physiologically slow fibers. However, thus far, wherever such muscle fibers have been found unequivocally, using morphological criteria (3–5, 7), they have been found to be physiologically slow and to undergo a contracture, rather than a contraction or twitch, response to a nerve stimulus (1–3).

The structure and the biochemical and physiological implications of the sarcoplasmic reticulum, the T system, and the triad have been well reviewed (15). The T system of the muscle fiber has been considered as the unit responsible for conducting the impulse from the limiting membrane of the muscle fiber to the fibrils in the interior of the fiber. The T system of the snake twitch fiber, even though it is not a direct continuation of an invaginated sarcolemma, comes close enough to the sarcolemma at the level of junction of A and I bands that it might still be able to allow for the transverse transmission of excitation into the interior of the muscle fiber. If this is so and the T system really performs this function, the absence of such an element in slow fibers might well be the basis for the slow contracture undergone by these fibers in contrast to the relatively rapid twitch of ordinary fibers. Similarly, a relaxing factor, said to be present in the sarcoplasmic reticulum or triads, might well be reduced in amount or absent in slow fibers and hence could account for the prolonged contracture of these fibers. The absence of the T system and reduction in amount of sarcoplasmic reticulum, along with the consequent disposition of the fibrils, and the occurrence of multiple nerve terminals appear to be the morphological basis for the physiological reaction of slow muscle fibers.

In addition, then, to the above differences between twitch and slow muscle fibers, the degree of complexity of the junctional folds must also be added. It is possible that the shape and amount of the folding of the postsynaptic membrane of the muscle fiber might influence significantly the passage of the nerve impulse from the nerve terminal to muscle fiber. This might perhaps be especially so if the synaptic vesicle in the nerve terminal as seen in the electron microscope is indeed equivalent to the quantum of acetylcholine which supposedly passes from nerve terminal to postsynaptic membrane (18). If so, then the kind and amount of folding of the postsynaptic membrane conceivably could influence the amount of quanta available to the muscle fiber and the time necessary for these to pass from nerve to muscle. At any rate, the differences in frequency and regularity of occurrence and in width and length of folding of the postsynaptic membrane under the nerve terminal on a twitch or slow fiber exist. The physiological significance of these differences remains to be determined.

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