FINE STRUCTURE OF RAT INTRAFUSAL MUSCLE FIBERS

The Polar Region

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ABSTRACT

An ultrastructural comparison of the two types of intrafusal muscle fibers in muscle spindles of the rat was undertaken. Discrete myofibrils with abundant interfibrillar sarcoplasm and organelles characterize the nuclear chain muscle fiber, while a continuous myofibril-like bundle with sparse interfibrillar sarcoplasm distinguishes the nuclear bag muscle fiber. Nuclear chain fibers possess well-defined and typical M bands in the center of each sarcomere, while nuclear bag fibers contain ill-defined M bands composed of two parallel thin densities in the center of the pseudo–H zone of each sarcomere. Mitochondria of nuclear chain fibers are larger and more numerous than they are in nuclear bag fibers. Mitochondria of chain fibers, in addition, often contain conspicuous dense granules, and they are frequently intimately related to elements of the sarcoplasmic reticulum (SR). Striking differences are noted in the organization and degree of development of the sarcotubular system. Nuclear bag fibers contain a poorly developed SR and T system with only occasional junctional couplings (dyads and triads). Nuclear chain fibers, in contrast, possess an unusually well-developed SR and T system and a variety of multiple junctional couplings (dyads, triads, quatrads, pentads, septads). Greatly dilated SR cisternae are common features of nuclear chain fibers, often forming intimate associations with T tubules, mitochondria, and the sarcolemma. Such dilatations of the SR were not encountered in nuclear bag fibers. The functional significance of these structural findings is discussed.

INTRODUCTION

It is now well known that mammalian muscle spindles contain at least two structural types of intrafusal muscle fibers. On the basis of diameter, length, and nuclear arrangement, Boyd (3), in the cat, classified intrafusal muscle fibers into a larger diameter and longer "nuclear bag" muscle fiber and a smaller diameter and shorter "nuclear chain" muscle fiber. Histochemical evidence to support this structural dichotomy of mammalian intrafusal fiber types has subsequently been presented by several workers employing a variety of staining techniques. That the small-diameter nuclear chain fibers display greater mitochondrial and/or oxidative enzyme activity than their nuclear bag fiber counterparts has been independently verified. This was done with the demonstration of high succinic dehydrogenase activity (37, 39, 71, 72) and high mitochondrial adenosine triphosphatase (ATPase) activity (27) in nuclear chain fibers, and with Sudan black B staining (35).

Higher myofibrillar (actomyosin) ATPase activity in the nuclear chain fiber than in its nuclear bag counterpart has been demonstrated (37, 38, 65, 66), and it has been shown that nuclear chain fibers are myoglobin-poor whereas nuclear bag fibers are myoglobin-rich (30).

That the two structural types of mammalian intrafusal muscle fibers differ functionally was first shown by Smith (63) in the rat, and later by Boyd (4, 5) and Diete-Spiff (11) in the cat. In response to direct electrical stimulation, Smith showed that the small-diameter (presumably nuclear chain) fiber responded with fast and vigorous contractions while the large-diameter (presumably nuclear bag) fiber elicited slower, prolonged contractions. Similar conclusions were drawn by Boyd, who observed response to indirect stimulation via the fusimotor nerves, and by Diete-Spiff, who recorded tension changes in the intrafusal fibers after direct stimulation.

Ultrastructural differences between nuclear bag and nuclear chain fibers in the mammal were first noted by Landon (33) in the rat, and later by Corvaja et al. (8) in the cat, and Corvaja and Pompeiano (9) in the rabbit. On the basis of myofibrillar density, mitochondrial content, and presence or absence of an M band, Landon concluded that nuclear chain fibers more closely resembled frog "twitch" extrafusal fibers while nuclear bag fibers were more like frog "slow" extrafusal fibers (see Peachey and Huxley [46] and Page [41]).

It has been suggested that a direct correlation exists in other skeletal muscles between the amount of sarcotubular system (SR and T system) in a given muscle cell and its speed of contraction (45). Some workers have noted that both nuclear bag and nuclear chain fibers in the cat contain only "aberrant" T-system elements (28) while others have more recently stated that triads are more numerous in nuclear chain fibers than in nuclear bag fibers (8). In view of the known functional differences which distinguish the two intrafusal fiber types (4, 5, 11, 63), it was thought that a more extended study of the internal organization of mammalian intrafusal fibers with emphasis on the organization and degree of development of the sarcotubular system would be worthwhile. Accordingly, the present investigation was undertaken. A preliminary report of a portion of this study has been published elsewhere (40).

MATERIALS AND METHODS

Adult male Sprague-Dawley rats, ranging in body weight from 200 to 300 g each, were used. The hindpaw IV lumbrical muscle, from one or both sides of the animal, was chosen because of its small size and suitability for electron microscopic preparative procedures (33) and because of its comparatively high muscle spindle content (70). Under pentobarbital anesthesia, a cannula was inserted into the abdominal aorta and each animal was subsequently perfused with cold, isotonic, buffered fixative (1.2% glutaraldehyde in 0.067 M sodium phosphate buffer, pH 7.4, at 4°C). The inferior vena cava above its point of bifurcation was cut for proper drainage. After a period of 30 min, in which time 150–175 cc of fixative had perfused through the lower extremities, the IV lumbrical muscle was quickly dissected out *in toto*. After the whole muscle was placed on moderate stretch by firmly fixing its proximal and distal ends in a stainless steel clamp, it was immersed in a beaker containing fresh buffered fixative for an additional 3–6 hr. The muscle was then removed from the clamp, cut into small pieces (1 × 2 × 3 mm), and reimmersed in fresh fixative for an additional hour.

The minced tissue pieces were washed in repeated changes of 0.36 M sucrose buffer for 2 hr. Postfixation with cold 1% osmium tetroxide in Millonig's (36) phosphate buffer at pH 7.4 was subsequently undertaken for 2 hr. Rapid dehydration in a graded series of ethanols (30, 50, 70, 80, 95, and 100%) and propylene oxide was followed by infiltration and embedding in Epon 812 (34). In addition, several muscles were infiltrated and embedded with Epon *in vacuo* overnight for better penetration of the embedding medium into the intracapsular spaces of the muscle spindles.

Precise orientation for flat embedding in both transverse and longitudinal planes was undertaken with the aid of a dissecting microscope. Serial transverse and longitudinal semithin sections, 1 μ thick, were cut with glass knives on an LKB ultramicrotome (Ultrotome III), placed on glass microscope slides, and stained with a 0.1% solution of Azure II in 1.0% aqueous borax on a hotplate at 100°C (52). This method was employed for each new block examined, for the purpose of identifying and properly orienting the muscle spindles embedded in the muscle. Transverse and longitudinal thin sections through various regions of several muscle spindles encountered by this method were subsequently cut with glass knives on the LKB Ultrotome III (LKB Produkter, Stockholm, Sweden), picked up and mounted on naked copper grids and stained with uranyl acetate (69) and lead citrate (51). Grids were examined in an RCA EMU 4 electron microscope operated at an accelerating voltage of 50 kv with a 25 μ objective aperture.

Identification of the Intrafusal Muscle Fibers

Semithin Epon sections of muscle spindles stained with Azure II (as described above) were initially used for distinguishing the intrafusal fibers as nuclear bag or nuclear chain. Light microscope identification was made on the basis of cross-sectional diameter and myofibrillar density of the muscle fibers in polar regions. In such regions, nuclear bag fibers exhibit a greater over-all diameter than nuclear chain fibers. Estimation of the cross-sectional diameters of a total of 232 fibers, for example, resulted in a bimodal distribution of fiber diameters. The mean diameter of nuclear chain fibers (n = 124) was 6.85 μ, while that for nuclear bag fibers (n = 108) was 10.28 μ. Measurements were not corrected for shrinkage. Moreover, the large diameter (nuclear bag) fiber usually exhibited an "areal" or "afibrillar" pattern in transverse section, while the small diameter (nuclear chain) fiber exhibited a more punctate "fibrillar" pattern. Several individual fibers were, in addition, traced serially to equatorial regions where distinct differences in the disposition of nuclei in the two intrafusal fiber types were readily apparent.

RESULTS

Myofibrillar Organization

The polar regions of intrafusal muscle fibers are almost completely occupied by contractile myofilaments. When such regions are examined in transverse section, striking differences in the distribution and pattern of myofibrillar organization in the nuclear bag[1] and the nuclear chain[2] muscle fiber are readily apparent.The polar region of a bag fiber is characterized by ill-defined myofibril-like units which appear tightly packed and fused together, forming a somewhat continuous bundle or mass of myofilaments occupying most of the fiber (Fig. 1). Small isolated areas of interfibrillar sarcoplasm are occasionally interspersed among the predominating myofilaments. In contrast, a corresponding profile of a chain fiber usually exhibits myofilaments which are clearly demarcated into discrete myofibril units (Fig. 2). Moreover, the interfibrillar sarcoplasm of the chain fiber appears more abundant and the presence of various sarcoplasmic organelles, such as glycogen, mitochondria, and elements of the sarcotubular system, is more apparent in the chain fiber than in the bag fiber.

[1] Hereafter designated "bag fiber."

[2] Hereafter designated "chain fiber."

Mitochondria

A feature which clearly distinguishes the two intrafusal fiber types is the presence of larger and more numerous mitochondria in the chain fiber (Figs. 2, 3). Those in the bag fiber are considerably smaller and less numerous (Figs. 1, 4). Mitochondria of both muscle fibers tend to be unbranched and are usually oriented parallel to the longitudinal axis. Furthermore, mitochondria of the bag fiber usually extend within the limits of one sarcomere, while those of the chain fiber frequently span two or more sarcomeres. In addition, dense intramitochondrial granules, 200–1000 A in diameter, are a prominent feature of mitochondria of chain fibers (Fig. 3), while such granules are smaller and less frequently encountered in mitochondria of bag fibers (Fig. 4).

M Band

An important criterion which serves to distinguish the two intrafusal fiber types in longitudinal section is M-band structure. A prominent M band, 800–900 A wide, is present in the chain fiber (Fig. 3). It is situated in the center of a well-defined pseudo–H zone and appears similar in structure to the M band of neighboring extrafusal muscle fibers. In contrast, the presence of an ill-defined M band, located in the center of an often poorly demarcated pseudo–H zone, is encountered in longitudinal sections of the bag fiber (Fig. 4). At low magnifications, the M band of the bag fiber is inconspicuous and frequently appears to be absent or barely visible (Fig. 4). At higher magnifications, however, a distinct substructure is evident. It is characterized by two parallel thin densities which traverse the entire width of the center of a sarcomere. Each density, 250–275 A in width, is, in turn, separated by a slightly lighter interspace of 150–175 A. Hence the total width of an entire M band, from the outer limit of one dense line to the other, is about 725 A. This is appreciably narrower than the M band of the chain fiber, whose mean width is 850 A.

In transverse sections, on the other hand, typical-appearing M bands, exhibiting triangular cross-sectional profiles with M bridges interconnecting the A filaments, are present in the bag fiber (Fig. 1) as well as in the chain fiber (Fig. 2).

Sarcotubular System

SARCOPLASMIC RETICULUM: One of the most striking differences between bag and chain

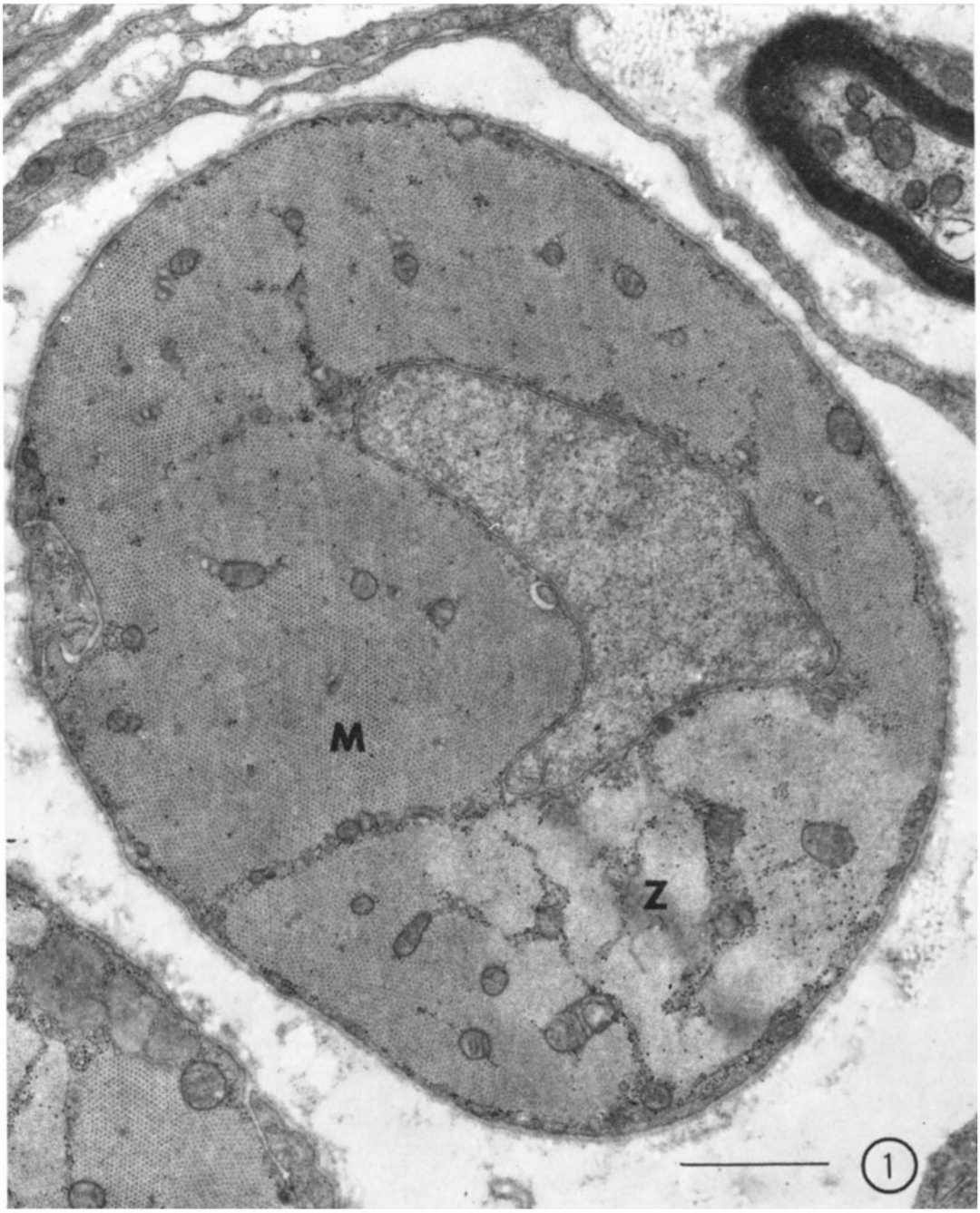


FIGURE 1 Transverse section of a bag fiber. Myofilaments appear tightly packed and aggregated into a continuous bundle. The interfibrillar sarcoplasm is sparse, mitochondria are small, and glycogen particles are sporadic. M band, *M*; Z band, *Z*. × 20,800.

muscle fibers is the distribution and degree of development of the sarcoplasmic reticulum (SR). This is particularly evident in transverse sections where the SR appears considerably more abundant in the chain fiber (Fig. 2) than in its bag fiber counterpart (Fig. 1). This is especially apparent at I- and Z-band levels of the chain fiber where myofibrils are almost completely encircled by SR

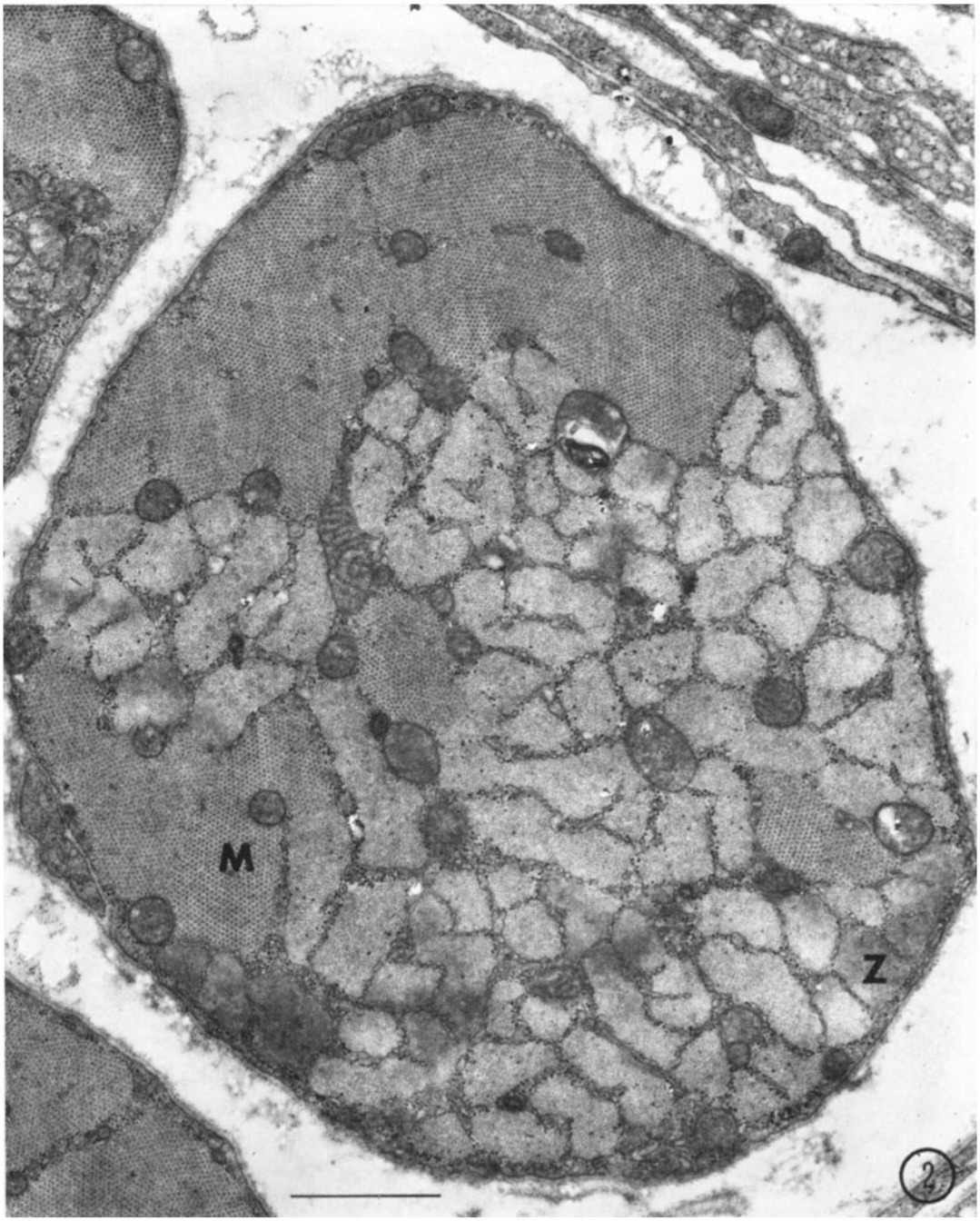


FIGURE 2 Transverse section of a chain fiber. The majority of myofilaments are delineated into distinct myofibril units. Glycogen particles and the interfibrillar sarcoplasm are abundant. Mitochondria are large and numerous. M band, *M*; Z band, *Z*. × 20,800.

elements (Fig. 2). Very little SR, however, occurs at A-band levels. In contrast, the extreme paucity of the SR at all sarcomere levels in the bag fiber results in poorly defined and tightly anastamosing myofibril-like units. Moreover, only sporadic elements of the SR are encountered at I- and Z-band levels (Fig. 1).

T SYSTEM AND TRIADS: Transverse (T) tu-

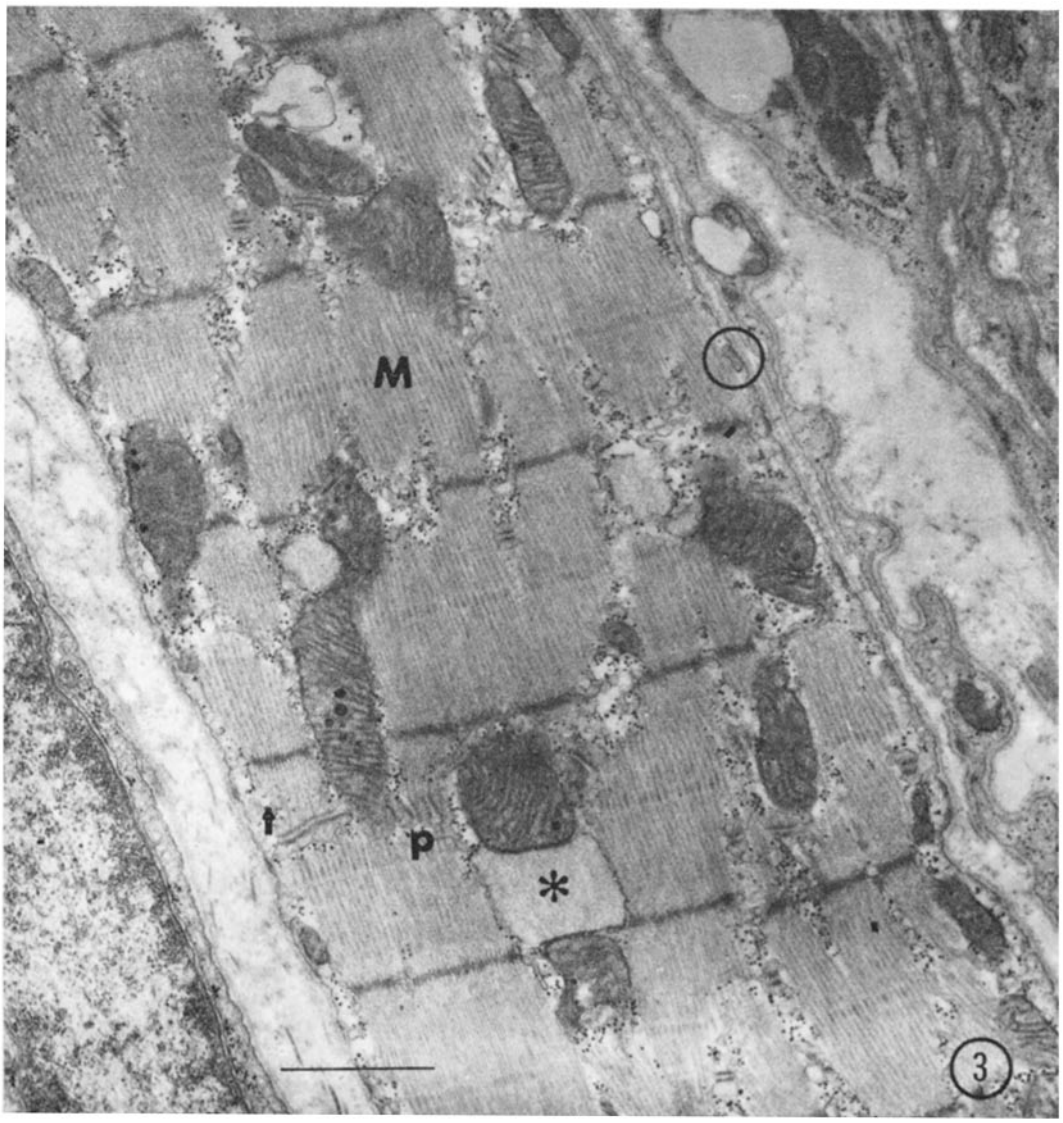


FIGURE 3 Longitudinal section of a chain fiber. Distinct M bands (*M*) are situated in the center of each sarcomere. Mitochondria are large and numerous, often containing conspicuous dense granules. Triadic (*t*), pentadic (*p*), and peripheral (circle) couplings are present. Note the terminal dilated SR cisterna (asterisk) intimately associated with two mitochondria. × 20,200.

bules are present in both intrafusal fiber types. They are oriented longitudinally as well as transversely and they are considerably more prevalent in the chain fiber than in the bag fiber (Figs. 3, 4). Such a consistent difference in the number of T tubules is further emphasized by the rare occurrence of junctional couplings (between T tubules and SR cisternae) in the bag fiber (Fig. 4) and a relative abundance of such contacts in the chain fiber (Figs. 5, 6). In both fiber types, profiles of T tubules usually appear somewhat flattened with a short diameter which varies between 400 and 800 A.

Triads are present in both intrafusal fiber types and appear similar in structure to those seen in neighboring extrafusal muscle fibers. In most cases, dense serrated bridges or "dimples" (31) traverse the interspace at regular intervals be-

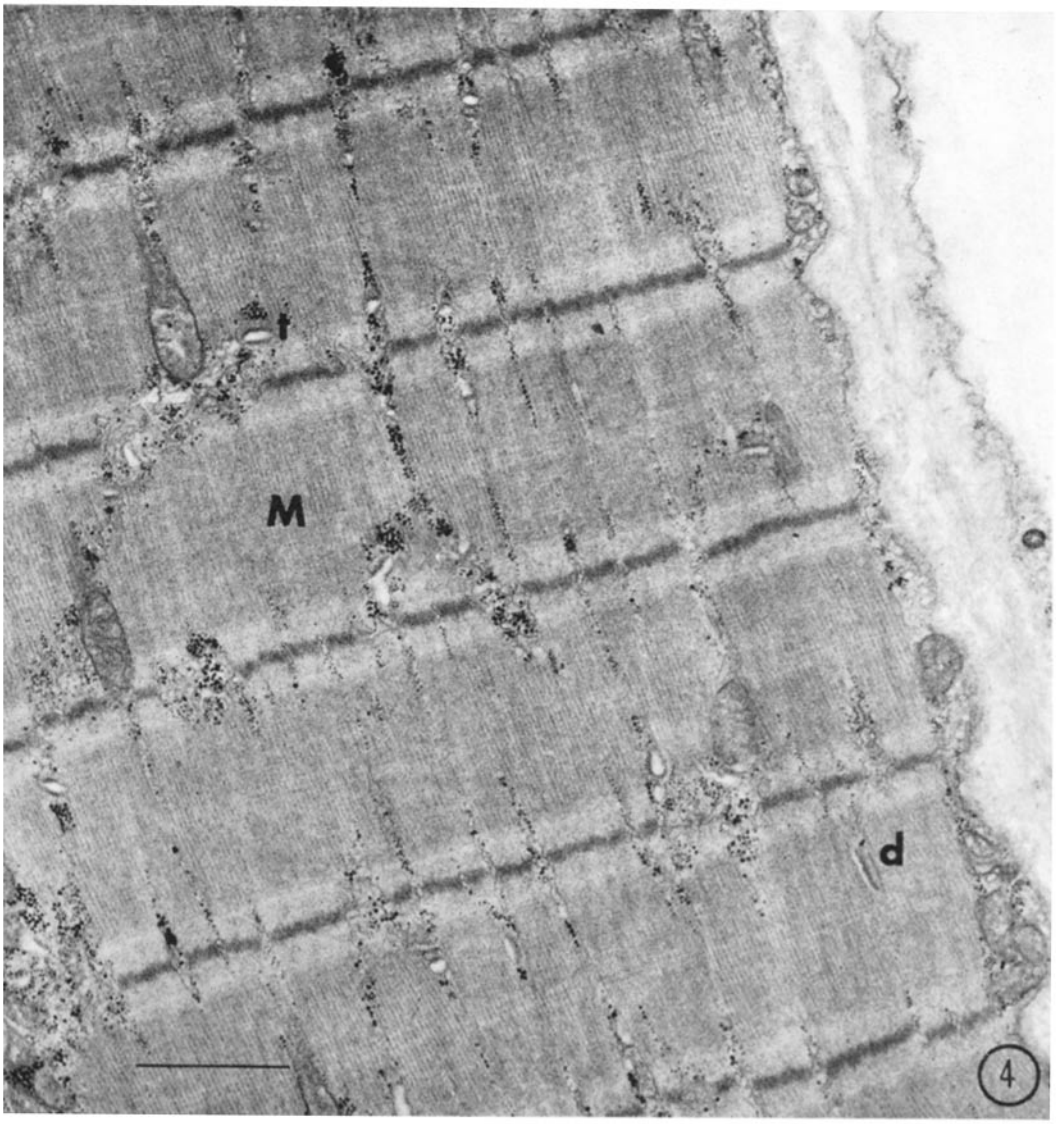


FIGURE 4 Longitudinal section of a bag fiber from the polar region of the same muscle spindle as in Fig. 3. M bands (*M*) are ill defined and appear as a double density. Mitochondria are small and scarce. Occasional, irregularly oriented dyads (*d*) and triads (*t*) are present. × 20,200.

tween the apposed membranes (Fig. 5). In some instances, a uniform and narrow dense line, intermediate and parallel to the apposing membranes, is encountered within the interspace (Fig. 7). Although most intrafusal triads are located at, or near, the A-I junctions (Figs. 5, 6), triads are frequently encountered well within the A band in the center of the sarcomere (Fig. 7). Triads in chain fibers, in addition, are often situated directly underneath the sarcolemma of the cell (Figs. 3, 6, 11).

Chain fibers usually contain one triad, and often two, for each sarcomere (Fig. 5). In some instances, some sarcomeres completely lack them. On the other hand, bag fibers commonly exhibit only one triad for every two, three, or four sarcomeres (Fig. 4), and two triads per sarcomere are rarely present.

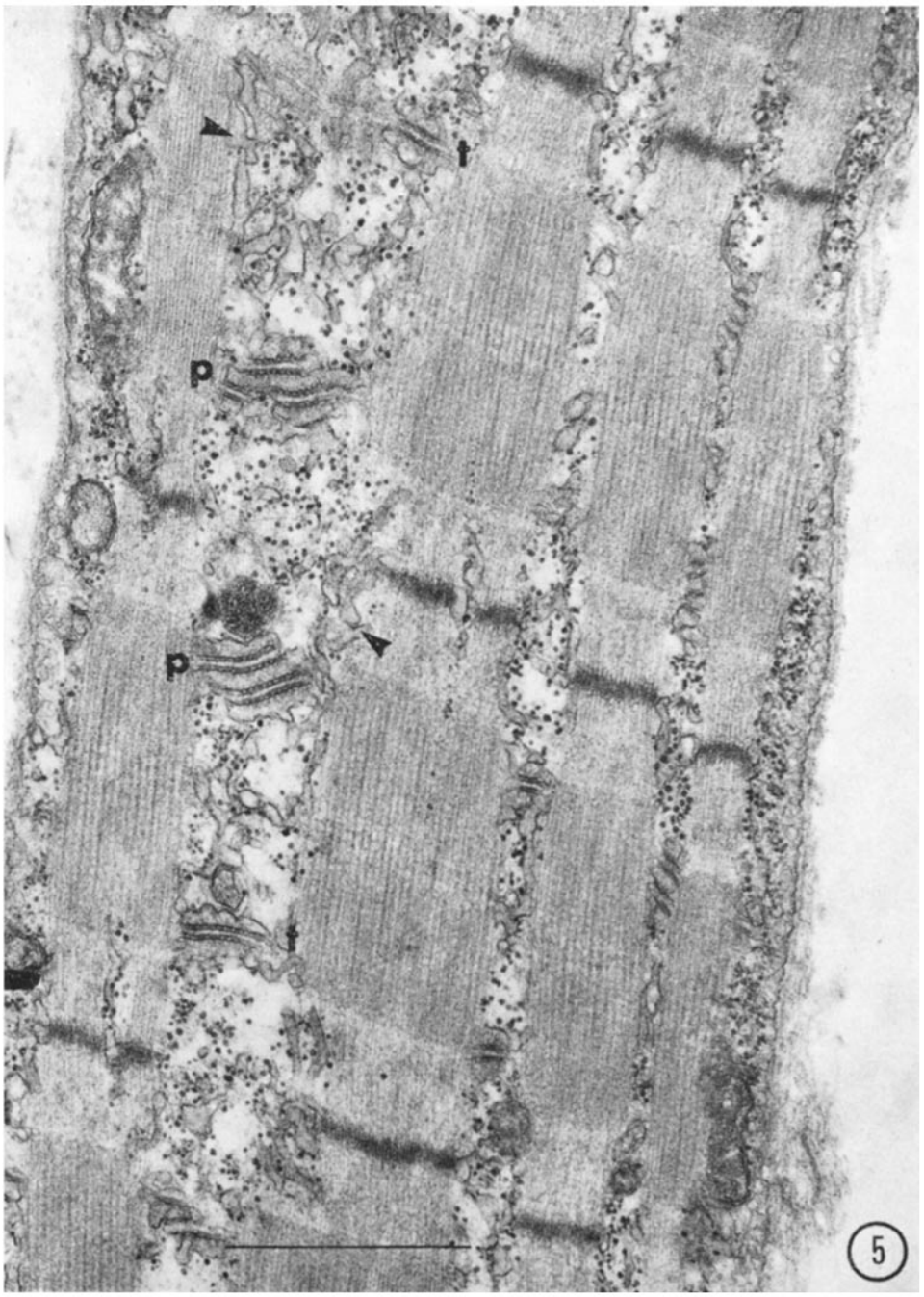


FIGURE 5 Longitudinal section of a chain fiber. Note the relative abundance of junctional couplings at the A-I junctions of each sarcomere. Two triads (*t*) and two pentads (*p*) are conspicuous. The SR (arrowheads) consists of an elaborate network of interconnected cisternae. Note the branching T tubule in the upper pentad (*p*). Micropinocytotic vesicles appear directly under the sarcolemma. × 33,600.

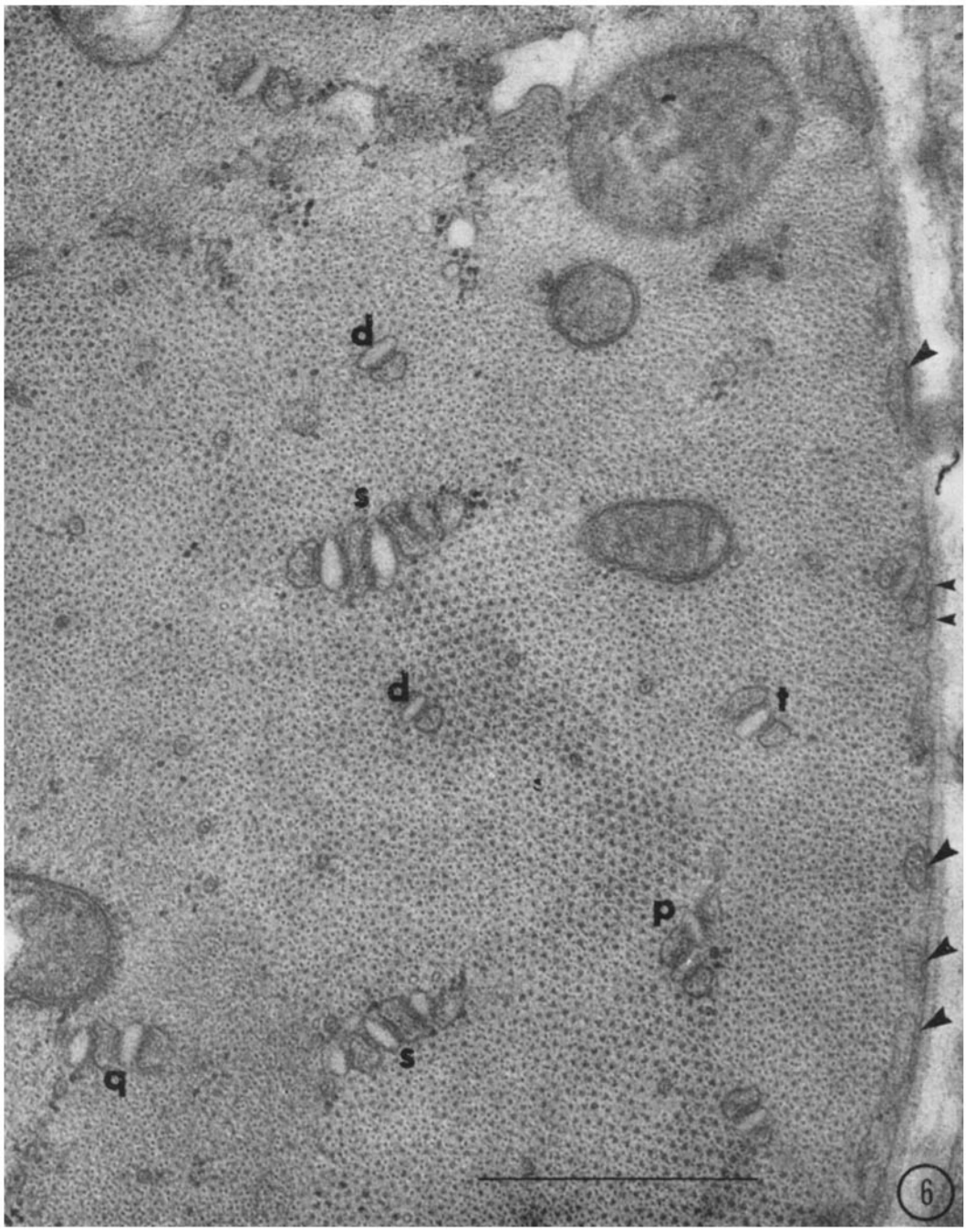


FIGURE 6 Transverse section of a chain fiber. Several kinds of longitudinally oriented junctional couplings are present. Note the dyads (*d*), quatrad (*q*), pentad (*p*), and septads (*s*). Several subsarcolemmal SR cisternae (large arrowheads) and a lateral SR element of a triad (small arrowheads) appear coupled to the sarcolemma. × 40,000.

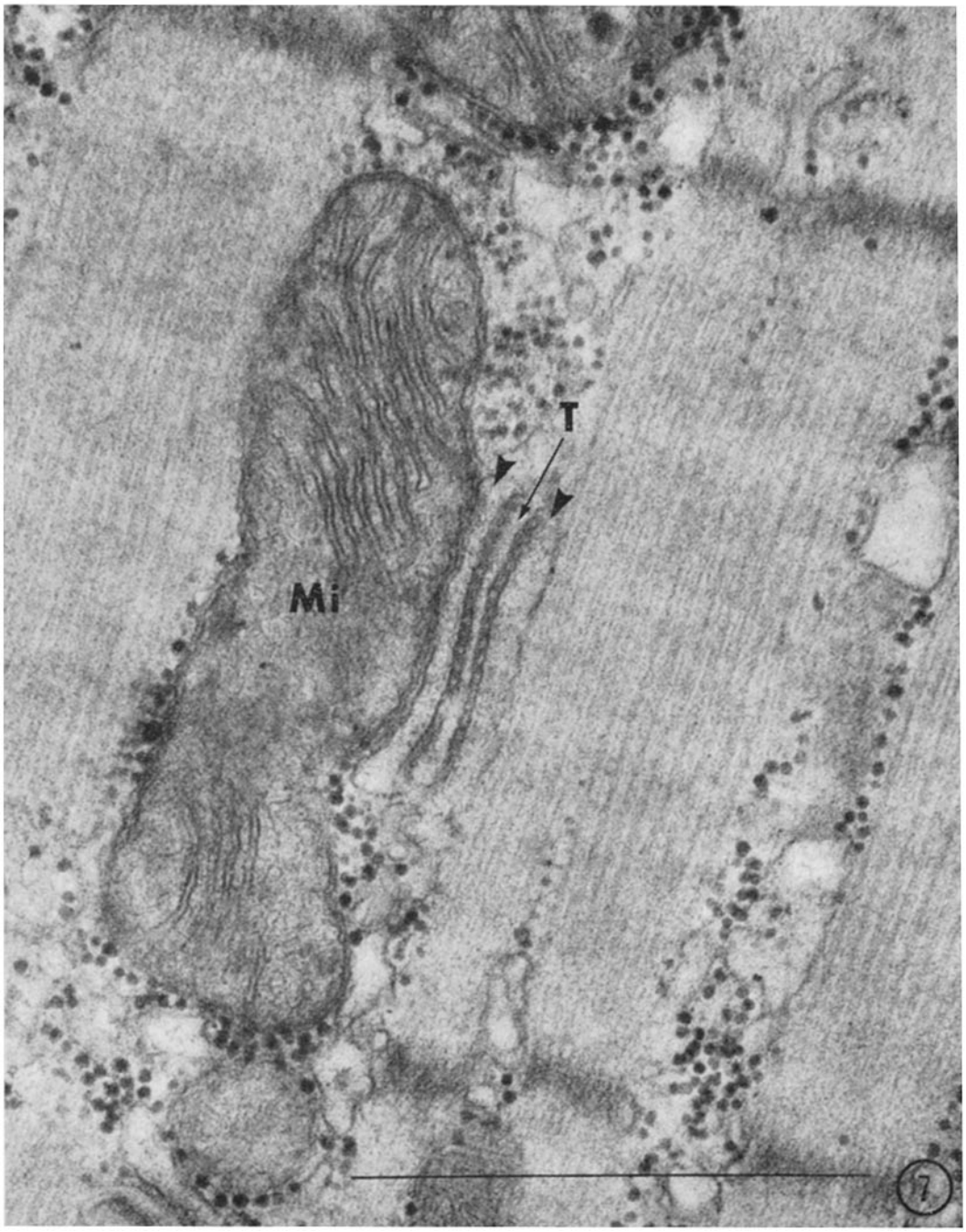


FIGURE 7 Longitudinal section of a chain fiber. A longitudinally oriented triad is situated in the center of a sarcomere. A central T tubule (*T*) is coupled to two terminal SR cisternae (arrowheads). Note the narrow intermediate dense line between the apposing SR and T-tubular membranes. One of the terminal cisternae is intimately associated with the outer membrane of a large mitochondrion (*Mi*). × 75,000.

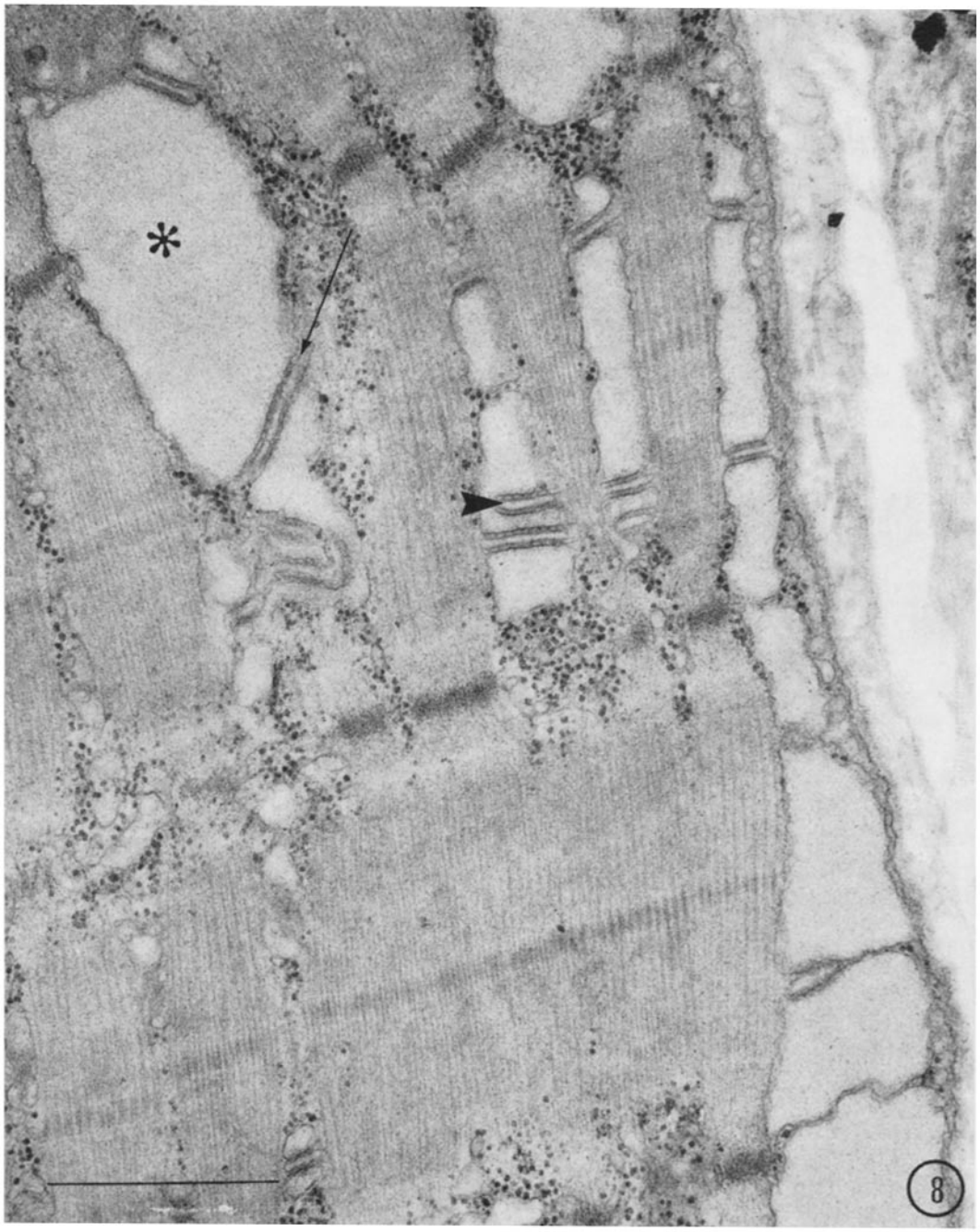


FIGURE 8 Several dilated SR cisternae are present in a chain fiber. One cisterna (asterisk) extends directly across a Z band; others are located directly under the sarcolemma on the right. Many cisternae are coupled to nondilated T tubules. One such T tubule (arrowhead) protrudes into the center of an SR cisterna, forming a pentad. Another T tubule (thin arrow) is coupled to two dilated SR cisternae in a labyrinthine fashion. Note the close association of numerous glycogen particles with elements of the SR. × 33,600.

DYADS: Dyads are junctional couplings composed of a single T tubule and a single intimately apposed SR cisterna (61). Although they are not commonly encountered in most adult vertebrate skeletal muscle fibers, such couplings are prominent features of both intrafusal fiber types (Fig. 4, 6). Their location within the sarcomere is variable. Usually located at the A-I junctions, dyads may also be situated well within the A band in the center of the sarcomere (Fig. 4). Moreover, they are usually oriented parallel to the longitudinal axis of the muscle cell (Figs. 4, 6). One member of the pair of vesicles in the dyad, identified as the SR cisterna, is usually filled with a dense granular material. Whereas the other component of the dyad, identified as the T tubule, is distinguished by a clear empty lumen.

PERIPHERAL COUPLINGS OR SUBSARCOLEMMAL CISTERNAE: The chain fiber is further distinguished by the frequent occurrence of SR cisternae which appear directly coupled to the inner aspect of the sarcolemma at the periphery of the cell (Figs. 3, 6). Most subsarcolemmal cisternae intimately approximate the inner aspect of the sarcolemma for variable distances, and may be separated from it by a 100–150 A interspace (Fig. 6). On occasion, periodic or serrated densities connecting the two apposing membranes are encountered in the interspace. The occurrence of such peripheral couplings (64) in bag fibers is extremely rare, and no such couplings were encountered in the surrounding extrafusal muscle fibers.

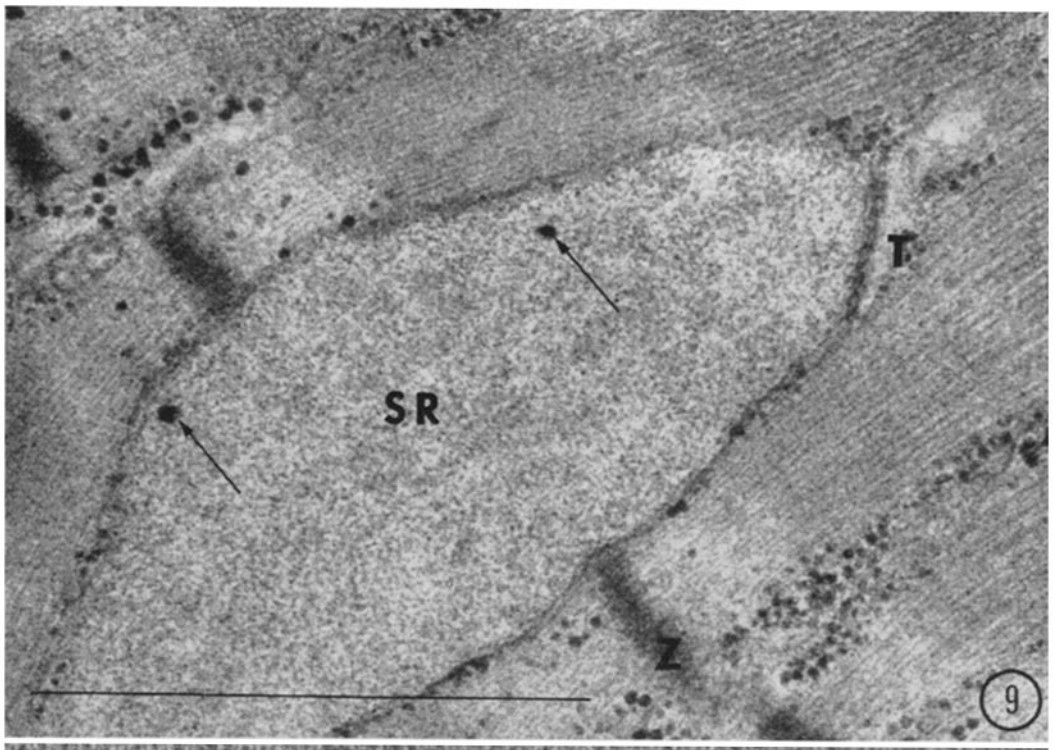
COMPLEX JUNCTIONAL COUPLINGS: In addition to the existence of dyads, triads, and peripheral couplings, there is the occurrence of even more complex multiple junctional couplings between T tubules and terminal SR cisternae in the chain fiber. It is noteworthy that such multiple couplings were not encountered in the bag fiber or in any of the surrounding extrafusal muscle fibers.

T tubules in the chain fiber, for example, are frequently seen to undergo bifurcation within a sarcomere (Fig. 5). Such duplication of T-tubular elements often creates a quatrad configuration consisting of two T-tubular profiles closely interdigitating with two SR cisternae (Fig. 6). More extensive branching of both T tubules and SR cisternae, or multiple numbers of T tubules and cisternae, often form more complex configurations such as pentads and septads. Pentads usually consist of two T-tubular profiles closely alternating with three terminal SR cisternae (Figs. 3, 5). Septads are composed of three T tubules intimately associated with four terminal SR cisternae (Fig. 6). These multiple couplings are usually located at, or near, the A-I junctions, and, in some cases, as many as two are encountered in one sarcomere. Their orientation is both longitudinal (Fig. 6) as well as transverse (Figs. 3, 5). On close inspection of these multiple couplings, a terminal SR cisterna is distinguished by the presence of dense osmiophilic material within its lumen, while a T-tubular element is characterized by a considerably less dense or clear lumen.

DILATED CISTERNAE OF THE SARCOPLASMIC RETICULUM: Extreme focal dilatations of the SR are common and unique features of chain fibers (Figs. 3, 8). No such dilatations were present in the T system, and no marked enlargements of the SR were encountered in bag fibers or in any of the surrounding extrafusal fibers. Moderately dilated cisternae are usually seen located adjacent to, and intimately contacting, a transverse tubule at the A-I junction of a sarcomere (Figs. 8–10). Because of the characteristic dyadic, triadic, or pentadic configurations formed by these structures, such dilated vesicles were identified as terminal cisternae of the SR. Extensively dilated cisternae vary greatly in size, shape, and location within the cell (Fig. 8). They frequently measure up to 3 μ in size and usually exhibit elongated or flattened profiles in longitudinal section. They often span

FIGURE 9 Longitudinal section of a chain fiber. A dilated SR cisterna (*SR*) extends across a Z band (*Z*). Such a cisterna forms a dyadic coupling with a T tubule (*T*). A fine granular material and two isolated electron-opaque particles (arrows) are present within the lumen of the SR cisterna × 75,000.

FIGURE 10 High-magnification transverse section of a chain fiber. A small triad (*t*) is situated on one side of a small mitochondrion. Another triad, composed of a central T tubule (*T*) and two terminal SR cisternae (*SR*), is seen on the left. One cisterna (thin arrow) appears nondilated, while the other cisterna (thick arrow) is markedly dilated. × 86,000.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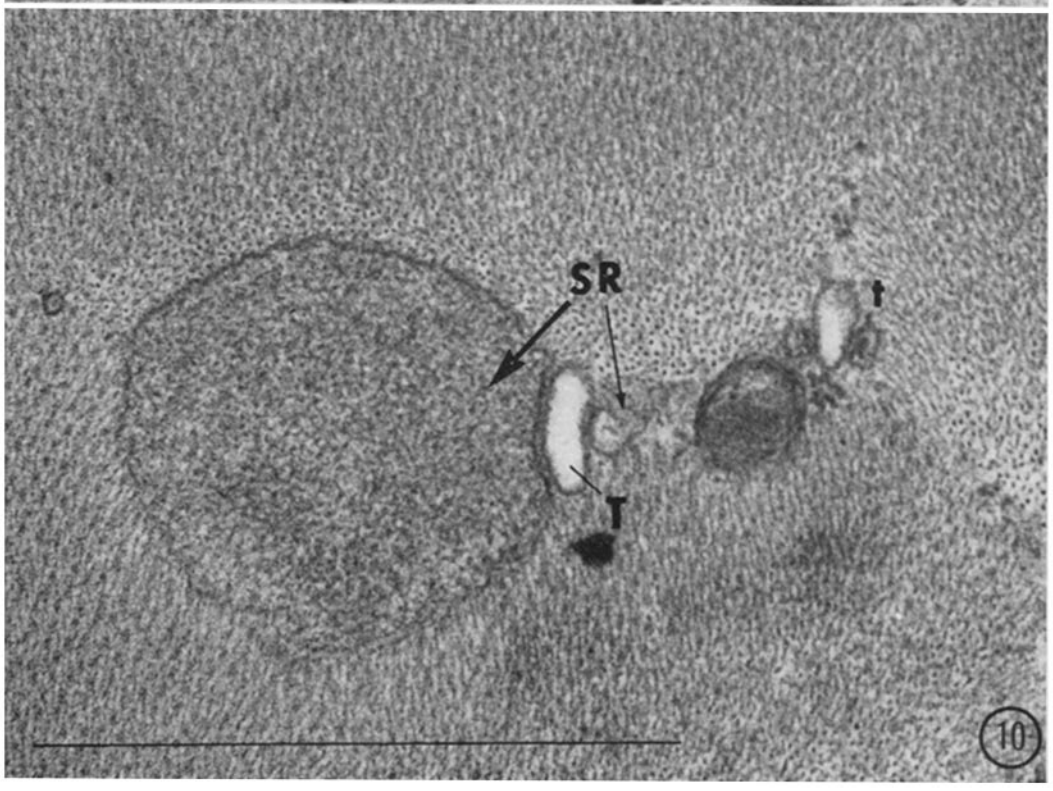
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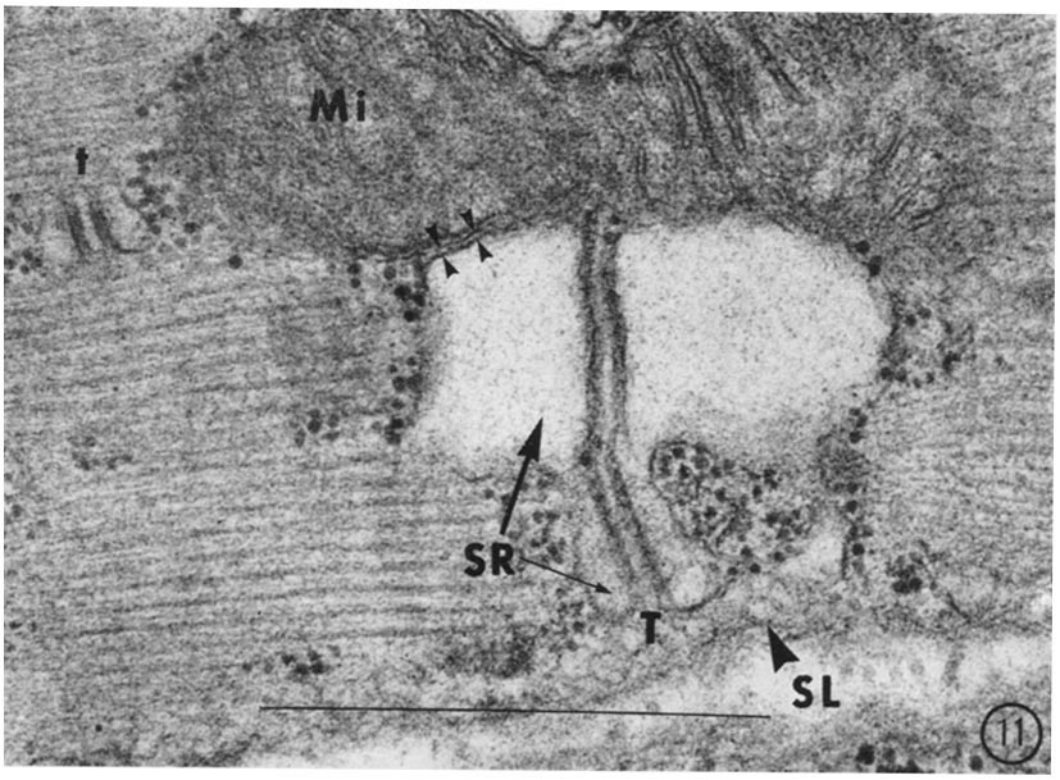


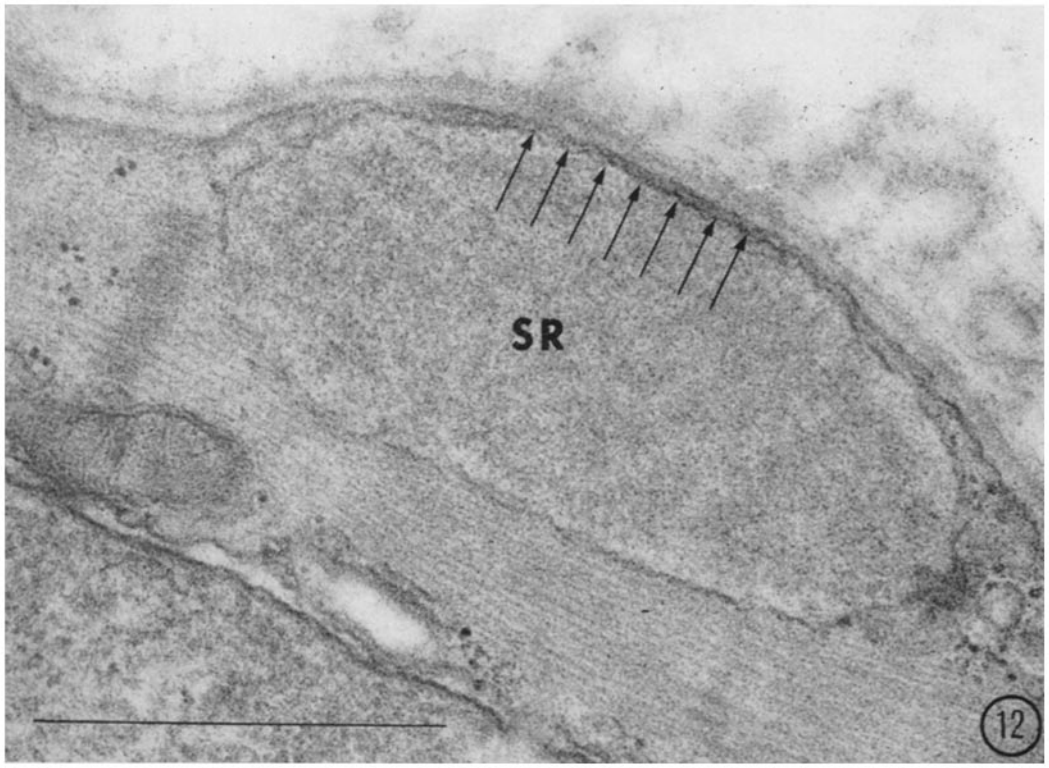
FIGURE 11 Longitudinal section of the periphery of a chain fiber. A T tubule (*T*) extends from the vicinity of the sarcolemma (*SL*) and is coupled to two terminal SR cisternae, forming a triad. A portion of each SR element appears nondilated (thin arrow), while another portion appears dilated (thick arrow). The two portions are continuous. The outer membrane of a mitochondrion (*Mi*) is intimately associated with membranes of the SR cisternae (see small arrowheads). Triad (*t*). × 67,500.

two successive sarcomeres, usually extending directly across a Z band from one sarcomere to the adjacent one (Figs. 8, 9). Lumina of most dilated cisternae are commonly filled with a dense, homogeneous, finely granular material similar to that found in the majority of nondilated cisternae. Occasionally, however, greatly enlarged cisternae are found to contain a few, large, isolated osmiophilic particles, 50–375 A in diameter, within their lumina (Fig. 9).

Points of direct continuity between nondilated lateral elements of the SR and larger dilated cisternae were noted in the same section (Fig. 11). Moreover, triads in which one lateral SR element appeared nondilated while the other lateral element appeared markedly dilated were also encountered (Fig. 10). Dilated cisternae may also be located in the periphery of the cell in close association with the sarcolemma (Fig. 12). In such cases, an intervening space between the two apposing

FIGURE 12 Longitudinal section of the periphery of a chain fiber. A dilated SR cisterna (*SR*) is intimately associated with the sarcolemma (see arrows). × 55,200.

FIGURE 13 High-magnification transverse section of a chain fiber. Note the two dilated SR cisternae (*SR*), each filled with fine granular material. The cisterna in the upper right contacts two T tubules (thin arrows). The cisterna in the lower left is closely associated with two small mitochondria (arrowheads) and a T tubule (thin arrow). × 82,600.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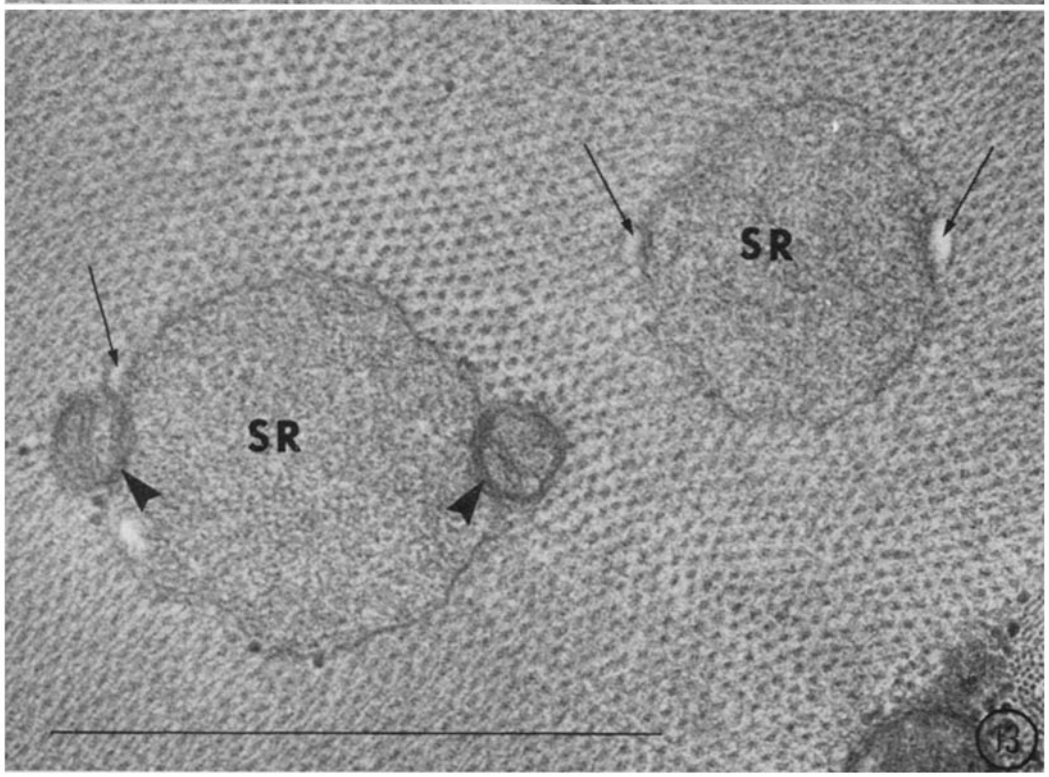
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membranes, ranging in width from 100 to 300 A is encountered.

UNUSUAL SARCOTUBULAR ARRANGEMENTS: In contrast to bag fibers, chain fibers frequently contain unusual "triadic" configurations composed of a single dilated SR cisterna and two laterally placed nondilated T tubules (Fig. 13). SR cisternae of these "atypical triads" are easily distinguished by the presence of dense granular material within their lumina.

Close associations between mitochondria and terminal SR cisternae are frequently present in the chain fiber (Figs. 3, 7, 8, 11, 13). It should be noted that no such close associations between mitochondria and the SR were encountered in the bag fiber or in any of the surrounding extrafusal fibers. In Fig. 7, a lateral SR element of a triad in a chain fiber is intimately associated with the outer membrane of a mitochondrion for a considerable distance. A fairly constant interspace of 75–100 A between the two closely apposed membranes is evident. Although such a constant interspace was encountered in the majority of cases, no periodic or serrated densities were ever found to bridge the gap. In addition, dilated SR cisternae were frequently seen to be intimately related to mitochondria in such a manner that a centrally located cisterna was closely apposed to two laterally placed mitochondria (Figs. 3, 13).

Micropinocytotic Vesicles

A common feature of both intrafusal fiber types is the presence of moderate-to-dense accumulations of micropinocytotic vesicles immediately beneath the sarcolemma (Figs. 3, 5). Such vesicles vary in size from 350 to 950 A and usually exhibit clear lumina. They are often situated in close proximity to dilated subsarcolemmal SR cisternae or are interposed between a triad and the sarcolemma of the cell (Fig. 11). No consistent differences in the number of micropinocytotic vesicles between bag and chain fibers were noted.

Glycogen

The sarcoplasm of both intrafusal fiber types contains varying amounts of glycogen appearing in the form of distinct electron-opaque particles, 180–260 A in diameter. Such particles are usually more abundant in the chain fiber (Figs. 2, 5) than in the bag fiber (Fig. 1). Most glycogen particles are located in the interfibrillar sarcoplasmic spaces of the muscle cell and are commonly situated in close proximity to cisternae of the SR (Figs. 3, 8, 9, 11). Some glycogen is also situated within the myofibrils. In such cases, glycogen particles are usually concentrated at the I-band levels of the sarcomere. Many may also be seen lined up in rows between the actin (thin) filaments (Fig. 3), or on both sides of the Z band (Fig. 8).

DISCUSSION

It was shown that bag fibers commonly exhibit a single, tightly packed myofilament bundle in contrast to the discrete myofibrillar arrangement found in chain fibers. This is in agreement with the original findings of Landon (33) in the rat, and more recently with the work of Corvaja and coworkers in the cat (8) and rabbit (9). The present study has shown that the myofilament pattern of bag fibers closely resembles that of other skeletal muscle fibers of the slow "felderstruktur" type, while that of chain fibers closely resembles the myofilament pattern of fast "fibrillenstruktur" fibers (41). Such a difference in the geometrical arrangement of the contractile myofilaments may account for differences in the contractile activity of the two intrafusal fiber types (see 4, 5, 63).

The subdivision of chain-fiber myofilaments into separate and discrete myofibril units was shown to be accounted for in a large part by a very extensive SR surface area and a well developed T system in contrast to that seen in bag fibers. Furthermore, the distribution and complexity of SR-T junctional couplings were shown to be considerably greater in chain fibers than in bag fibers. The roles of the T system and the SR in excitation-contraction-relaxation coupling in muscle cells have recently been the subject of intensive study (44, 56, 57, 62). Continuity of the T system with the sarcolemma (21, 29) has implicated the T tubules in the inward spread of excitation (surface depolarization) from the sarcolemma to the interior of the cell. Other studies (10, 54) have shown that the terminal SR cisternae, forming the lateral elements of each triad, are principally involved in the storage of calcium and its subsequent release into the sarcoplasm, resulting in activation of the myofibrils for contraction. Relaxation occurs by the uptake and storage of the released calcium by the terminal cisternae (25). In view of this, it seems reasonable to assume that sarcotubular content and number of junctional couplings might be closely related to the speed of contraction and the duration of the

contractile time in a given muscle cell. The striking differences noted above, therefore, concerning the extent of junctional surface area between T tubules and terminal SR cisternae in the two intrafusal fiber types may partially explain the vigorous twitchlike response of chain fibers and the slow, prolonged contractile response of bag fibers to direct (63) or indirect (4, 5) electrical stimulation.

Relevant to this discussion is the peculiar overdevelopment and extensive branching of the T system in chain fibers, resulting in a variety of elaborate multiple couplings with the SR. Pentads, for example, were previously reported to occur in the fast-contracting cricothyroid muscle of the bat (50). More recently, Corvaja and coworkers (8) described an "overdeveloped" and "labyrinthine" network of "confluent tubules" at the A-I junctions of chain fibers in the cat; however, they were unable to ascertain its continuity with the T system. Overdeveloped T-tubular profiles, similar to those reported in the present study, have also been described in other muscles, i.e. in response to experimental denervation of rat extrafusal muscle fibers (47), during castration atrophy of rat dorsal bulbocavernosus muscle (23), during early stages of development of rat skeletal muscle (58), and in human rhabdomyoma myopathy (7).

Although many T tubules and triadic couplings were frequently encountered directly under the sarcolemma, especially in chain fibers, it should be noted that no direct continuity of T tubules with the sarcolemma was observed in the present study. A consistent feature, however, was the presence of numerous micropinocytotic vesicles interposed between such triads and the sarcolemma (see Fig. 11). In examining surface features of mammalian skeletal muscle fibers, Rayns et al. (49) demonstrated that T tubules were directly continuous with the extracellular space via continuity of such vesicles with T tubules. If such a situation also exists in the intrafusal fibers, it is possible that an appreciable increase in volume and surface area of the T system, relative to the surface, would occur in these fibers. Moreover, since membrane capacitance is known to be related to the extent of T system within a given muscle cell (15), it is possible that micropinocytotic vesicles directly continuous with T tubules may play a significant role in contributing to the total membrane capacitance. Subsarcolemmal micropinocytotic vesicles are common features of mammalian myocardial cells, and Forssmann and Girardier (20) maintain that the number of such vesicles in a given cell is inversely proportional to the number of T tubules present. Although such a relationship was not readily apparent in the intrafusal fibers, future attention must be directed to this problem.

The SR of chain fibers is, furthermore, extraordinary in that the terminal dilated cisternae serve to increase its surface area and volume many times over that seen in the SR of bag fibers. Such dilatations of the SR are peculiar to chain fibers and have only been reported previously in cases of periodic paralysis of skeletal muscle (13, 60) and after denervation of developing skeletal muscle (59). The functional significance of such dilatations is not known at present. However, since it has been suggested that the speeds of contraction and relaxation of skeletal muscle are, in part, determined by the calcium-transporting activity of the SR (17, 56), it is possible that such an increase in SR surface area and volume in the terminal dilatations may significantly increase rates of calcium release and uptake, and, in turn, of contraction and relaxation in chain fibers.

The presence of dense granular material and conspicuous electron-opaque granules within the lumina of these terminal dilatations is of interest since such deposits may represent calcium or a calcium-binding material (53). Although the precise nature of this granular material is still unknown, Philpott and Goldstein (48) suggested that similar material found in terminal cisternae of extrafusal fibers may be a mucopolysaccharide or a glycoprotein which would provide calcium-binding sites for uptake and storage activities.

Other functional activities of the SR, aside from contraction-relaxation, may also be greatly enhanced by the presence of such terminal dilatations. ATPase activity, for example, has been localized within the SR lumen as well as on the apposed SR cisternal membranes at triadic junctions (14). In addition, glycogen synthetase activity, necessary for the formation of glycogen particles outside of the SR, has been localized within the SR lumen (2). It is noteworthy that the abundance of glycogen in chain fibers and its frequent accumulation in the vicinity of the SR dilatations reflect this possibility.

The abundance of large mitochondria in chain fibers and their relative paucity and small size in bag fibers has been reported by other workers (8, 9, 33). A similar difference in mitochondrial content was first reported in frog extrafusal muscle

where mitochondria are more abundant in "twitch" fibers than in "slow" fibers (41). Furthermore, several unusually fast-contracting muscles, such as the bat cricothyroid (50), the fish swimbladder muscle (18), the rabbit thyroarytenoid (24), and certain fast muscles of the mammalian diaphragm (22), also contain an unusual abundance of mitochondria. From the foregoing, Gauthier and Padykula (22) have suggested that mitochondrial abundance may be directly related to the frequency of contraction rather than to the duration of the contractile response. Since mitochondria are known to provide a readily available supply of energy to the cell, the possibility, therefore, exists that energy requirements may be greater in chain fibers in which the abundance and large size of mitochondria may be directly related to a high frequency of contractile activity.

The intimate relationship of mitochondria to terminal SR cisternae in chain fibers may have functional significance since it was emphasized that similar mitochondrial-SR relationships were not encountered in bag fibers. Workers in the past have noted the close proximity of mitochondria and SR in a variety of extrafusal muscle fibers (1, 50, 68). Intimate contacts between mitochondria and SR cisternae, for example, were reported in mouse skeletal muscle (1) and in the fast-contracting bat cricothyroid muscle (50). Direct continuity between mitochondria and SR membranes in rat cricothyroid muscle has also been reported (68). Furthermore, it is known that mitochondria in cardiac muscle cells are able to accumulate calcium against a concentration gradient (6), and it has been shown that both mitochondria and the SR in cardiac muscle actively withdraw calcium from the sarcoplasm during relaxation (16). Several workers, in addition, have implicated mitochondria in movements of calcium during the contraction-relaxation cycle in the heart (26, 42). In view of this, it is tempting to speculate that the intimate mitochondrial-SR associations observed in chain fibers may perform a similar function in the regulation of calcium movements during contraction and relaxation. The frequent presence of conspicuous dense granules in mitochondria of chain fibers may reflect this possibility since such granules are known to be sites of concentrated divalent cations such as calcium (43, 67). Hence, the possibility exists that calcium metabolism (i.e., storage, release, and uptake) may involve both mitochondria and the SR during the contraction-relaxation cycle in chain fibers, and may be considerably greater and more efficient in these fibers than it is in bag fibers.

In comparing bag and chain fibers in the past, many workers have used the M band as a distinguishing criterion, noting its absence in bag fibers and its presence in chain fibers (8, 9, 12, 33, 55). In the present study, it was emphasized that both fiber types contained M bands, although they were rather inconspicuous in longitudinal sections of bag fibers. Since, at low magnifications, M bands of bag fibers frequently appeared ill defined or absent (see Fig. 4), it is conceivable that past workers may have simply missed these structures upon cursory inspection or with the use of other fixation and staining techniques. It is the author's contention that M bands of the bag fiber, although poorly defined and appearing as a double density in longitudinal section, are, indeed, normal features since their presence was consistent in every bag fiber examined. They are not merely species-specific since similar double densities identified as M bands have also been seen in bag fibers of the cat (28). Moreover, in transverse section, M bands of both fiber types exhibited typical triangular profiles (see Figs. 1 and 2), indicating that the M bridges interconnecting the A filaments in these regions are present in the bag as well as in the chain fiber. It is, therefore, possible that the M band is less pronounced in longitudinal sections of the bag fiber simply because the thickening of the A filaments in this region is less than that of a corresponding region of the chain fiber.

Although the precise function of M bands in muscle is still rather obscure, it has been suggested that they may play a significant role in providing transverse anchorages for the thick filaments, keeping them in proper alignment during forceful contractions (32). If this is so, well developed M bands may be a more necessary requirement for muscle fibers which exhibit rapid or vigorous twitch contractions, while slower contracting muscle fibers may not need them. This may explain the presence (in longitudinal section) of typical well-developed M bands in chain fibers, and poorly developed "atypical" M bands in bag fibers.

Such a difference in M-band structure may also have some bearing on the ability of intrafusal muscle fibers to undergo contracture. Fehr (19) examined the effects of succinylcholine on intact spindles of the cat. He suggested that intrafusal

fibers underwent contracture after intravenous injections of succinylcholine. In addition, he maintained that contracture and release from contracture occurred more rapidly in bag fibers than in chain fibers. Smith (63) noted that addition of succinylcholine to the bathing medium in which spindles of the rat were immersed resulted in strong, prolonged contractures of bag fibers. He noted that, after onset of contracture, direct electrical stimulation failed to cause further movement in these fibers. From the foregoing, it is possible that in a fiber with less rigid and poorly defined M bands (as in the bag fiber), the I-band myofilaments at the two ends of a sarcomere may be more inclined to completely pass by each other in the mid–H zone region during contracture. On the other hand, in fibers with rigid and well-defined M bands (as in the chain fiber), such an overlap of I-band myofilaments at the mid–H zone region may be less apt to occur. Future ultrastructural studies on drug-induced contractures of intrafusal muscle fibers may offer the opportunity to answer certain questions involving roles of the M band in maintaining the structural integrity of the sarcomere.

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