TRIADS IN SKELETAL MUSCLE FIBERS OF 19-DAY FETAL RATS

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INTRODUCTION

The interfibrillar spaces of skeletal muscle fibers in adult vertebrates contain two distinct membranous systems, the T system and the sarcoplasmic reticulum. Most of the T system is oriented transversely between the fibrils. Occasionally, longitudinal connections between the transversely oriented T system are seen (14). The sarcoplasmic reticulum is, in general, oriented longitudinally between the fibrils. However, the terminal segments of the sarcoplasmic reticulum face the T system and show transverse orientation at this level. Two terminal segments of sarcoplasmic reticulum and a tubular element of the T system between these segments have been called a triad (13). A triad is found near each junction of the A and I bands in reptiles, birds, and mammals. Numerous investigators have noted that the space between the T system and the apposed sarcoplasmic reticulum is about 100 A and is remarkably constant. Revel (14) described rather regularly spaced, dense bridges traversing this space in bat muscle fibers. Similar dense bridges were reported (19) for rat muscle fibers, and evidence that these bridges are connections between the T system and sarcoplasmic reticulum has been presented (20). In a recent electron microscope study (1) on morphogenesis of rat skeletal muscle, sarcoplasmic reticulum was observed between the developing fibrils in 16.5–20-day rat fetuses; but triads were not reported in that study. In light microscope studies on skeletal muscle fibers from newborn mice and fetal guinea pigs, Veratti (17) observed an irregular reticulum in longitudinal sections. He found a regular reticulum, transversely oriented, in similar longitudinal sections of fibers from adult mice (17). The contrast in appearance of reticulum in light microscope studies when reticulum profiles in fibers from newborn and adult mice are compared led to the suggestion that development of transversely oriented triads might occur in rodents after birth. Electron microscope studies on skeletal muscle fibers from 19-day rat fetuses have shown a striking sparsity of transversely oriented triads and a more numerous distribution of longitudinally oriented triads.

METHODS

Gastrocnemius and psoas muscles were removed from 19-day rat fetuses and fixed in 3% glutaraldehyde by the method of Sabatini et al. (16). Bundles of fibers from these muscles were postfixed in OsO_4 by the Palade method as modified by Caulfield (3). The fibers were dehydrated in alcohol, embedded in Maraglas, and sectioned with an LKB microtome. In some cases, sections were triple-stained with lead, uranyl acetate, and lead; in others, fibers were first stained with uranyl acetate during dehydration, and then the sections were triple-stained. A modification of the Reynolds method (15) was used for lead staining. Sections exhibiting gray interference colors were examined with a Siemens Elmiskop IA electron microscope.

RESULTS

The observations reported here were made on eight 19-day rat fetuses. The sparsity of triads is emphasized by the fact that in about 400 electron microscope examinations of longitudinal sections and cross-sections of gastrocnemius and psoas muscle fibers, structures identified as triads were seen in only 45 sections. These triads were usually oriented longitudinally and rarely transversely. The electron micrograph illustrated in Fig. 1 shows an unusually large number of triads. In most of the 45 sections showing triads, only one triad per section was found. In Fig. 1, four of the triads (stars) are oriented along the longitudinal axis of the fiber and the fifth triad (asterisk) is oriented transversely. In all cases, the triads are located near the junctions of the A and I bands. Three dense structures (*X* in Fig. 1) are similarly located. It seems reasonable to assume that these dense structures might represent partially formed or unfavorably sectioned triads. The sarcotubular system (*ST*) shown in Fig. 1 is typical of profiles seen in longitudinal sections of fibers. A prominent feature of this system is a labyrinth of tubules in the form of a network. The network is found in the interfibrillar space at all levels of the sarcomere.

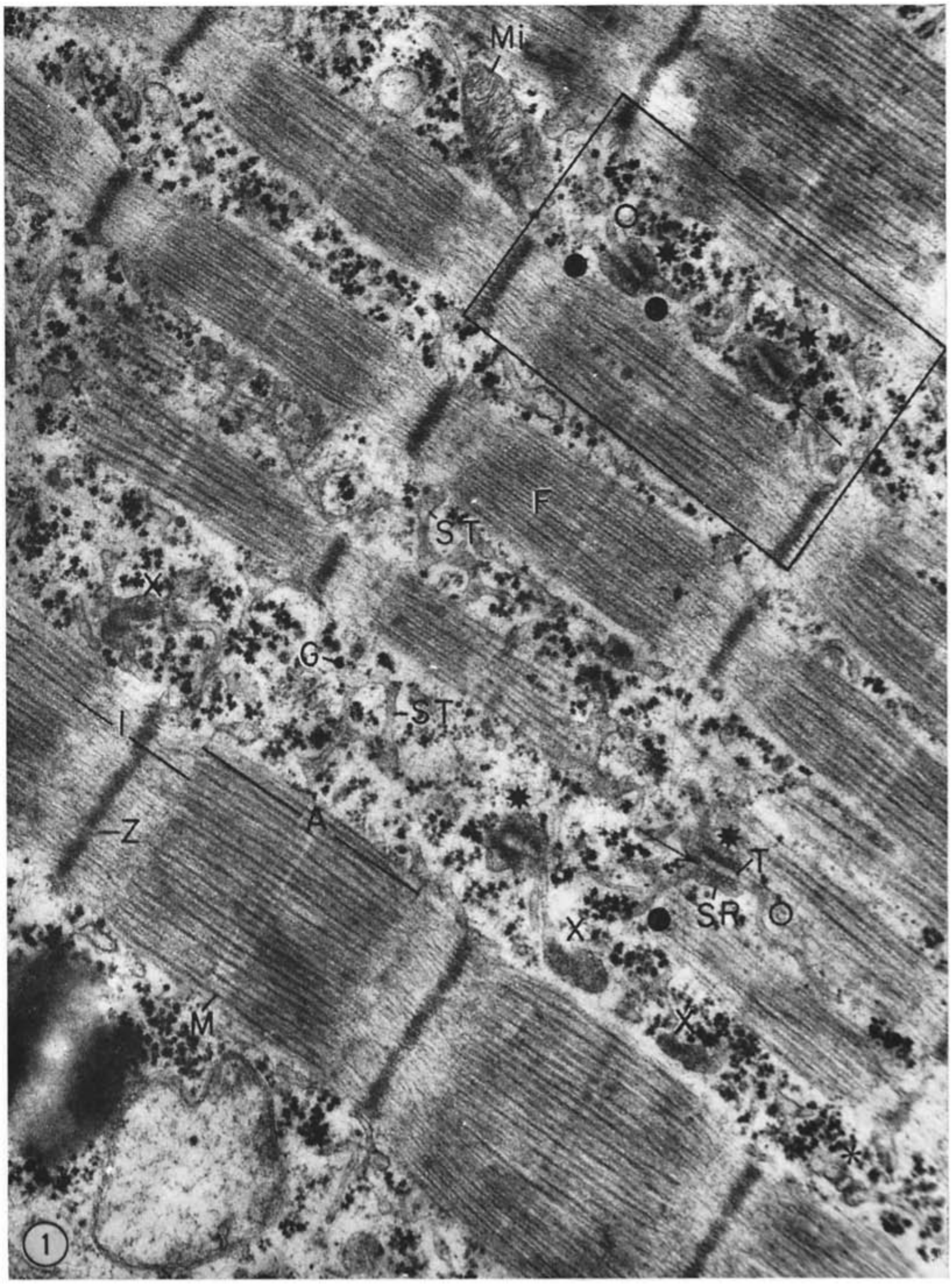

FIGURE 1 Electron micrograph of a gastrocnemius muscle fiber from a 19-day rat fetus showing a longitudinal section of several fibrils (*F*). The stars indicate longitudinally oriented triads, and the asterisk designates a cross-section of a triad that might be transversely oriented. Densely stained structures (*X*) which are apparently connected with the sarcotubular system (*ST*) are seen at some points. The open circles (○) are placed above apparent extensions of the T system (*T*) beyond the level of the triad. The closed circles (●) are placed beneath apparent extensions of the sarcoplasmic reticulum (*SR*) beyond the level of the triad. The arrows are directed toward spaces between apposed membranes of *SR* and *T*. The interfibrillar space contains a thick distribution of dense granules (*G*). *Mi*, mitochondrion; *A*, A band; *I*, I band; *M*, M line; *Z*, Z line. Sections triple-stained with lead, uranyl acetate, and lead. × 30,000.

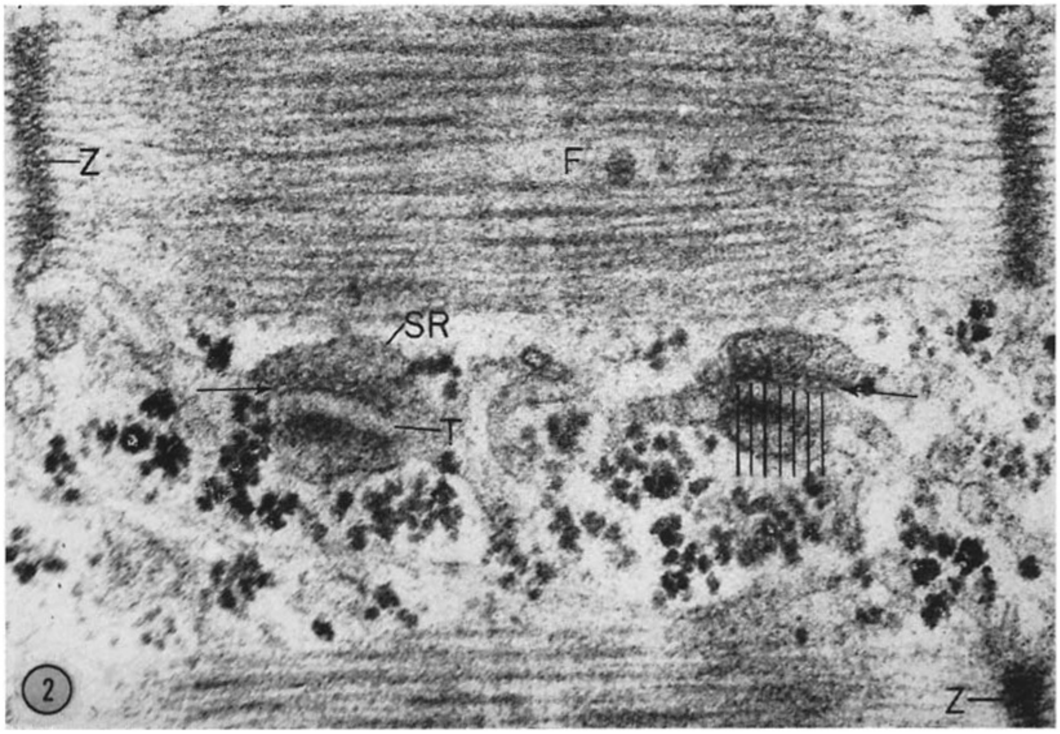

FIGURE 2 A high magnification of the area enclosed in the rectangle in Fig. 1 showing three characteristics of the triad. The horizontal arrows are directed toward spaces between *SR* and *T*. The vertical lines are directed toward densely stained bridges across the space between apposed membranes of *SR* and *T*. Electron-opaque material is seen within *SR*. Other labels as in Fig. 1. × 70,000.

The relationship of the network of tubules to the T system (central element of the triad) is uncertain. It is true that extensions of the T system (open circles) beyond the level of the triads are seen, but connections of this system with the network of tubules have not been observed. However, connections between extensions of triad lateral elements (closed circles) and the network of tubules are indicated in Fig. 1. Additional support for the view that lateral elements of triads are connected with the network of tubules is found in other micrographs.

Fig. 2 illustrates three structural characteristics that can be used for identification of a triad. First, the space (horizontal arrows) between apposed membranes of the central element and the lateral elements of the triad is about 100 A. Second, dense bridges (vertical lines) traversing this space at rather regular intervals can be seen at points at which the plane of section is favorable. Third, the lateral elements of the triad (*SR*) are more intensely stained than the central element. A more striking illustration of the three characteristics of the triad is shown in Fig. 3. In the left side of Fig. 3, extensions of the central element beyond the level of the lateral element are clearly shown (open circles). In addition, membrane-like structures can be seen by close inspection of the lateral element in the left side of Fig. 3. The structure designated by *X* in Fig. 3 might be an early stage in the development of a lateral element or an unfavorable section of a lateral element.

Observations on cross-sections reveal considerable variation in diameter of skeletal muscle fibers in the 19-day rat fetus. The average diameter is about 8 μ, with the greatest diameter being no more than 12 μ. This fiber size represents a much smaller cross-sectional area than that in the adult fiber, with an average diameter of 40 μ. However, fibril size in the fetus more closely approximates that observed in the adult rat. The diameter of fibrils may be as much as 1 μ, which is somewhat

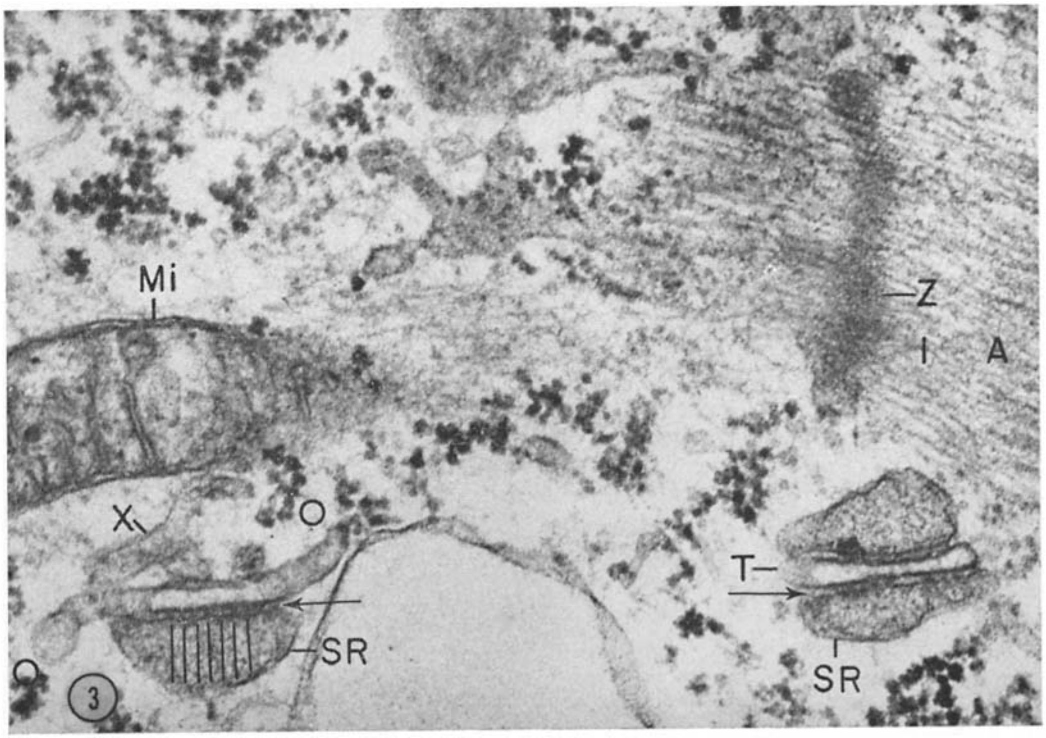

FIGURE 3 Electron micrograph of a psoas muscle fiber from a 19-day rat fetus showing a section somewhat oblique to the longitudinal axis of the fiber. Although the triad in the left side of the figure is not in the usual position relative to the A-I junction, it shows well the characteristics of a longitudinally oriented triad. Note the membrane-like structures within the lateral element in the left side of the figure. *A*, A band; *I*, I band; *X*, structure apparently attached to T system; O, extensions of T beyond apposed membrane of SR; *Mi*, mitochondrion. Other labels as in Fig. 2. Preparation as in Fig. 1, except for uranyl acetate stain during dehydration. × 70,000.

greater than the average diameter of fibrils in the adult rat. In fibrils of all sizes seen in the 19-day rat fetus, the length of the A band is about the same as that found in adult muscle fibers. The M line and Z line are well developed (Fig. 1) and resemble those seen in adult fibers.

Sarcotubules (*ST*) like those illustrated in Fig. 1 sometimes show dense projections on the external surface of their membranes. Although such projections are not infrequently seen, none of them is illustrated in Figs. 1–3. It is noteworthy that the periodicity of these projections is similar to the periodicity of the bridges between central and lateral elements of triads.

DISCUSSION

It is obvious from comparison of observations from numerous electron microscope studies on adult mammalian muscle fibers in the literature with the observations reported here that triad development is very incomplete in the 19-day rat fetus. All fibrils in adult mammalian muscle fibers are encircled by triads located near each A-I junction. The lateral elements of triads are connected by networks of tubules. One network of connecting tubules encircles the fibril at the A-band level, another at the I-band level. The tubules in the networks that connect lateral elements are usually oriented along the longitudinal axis of the fiber. However, many transverse tubules are seen in these networks (14). In adult mammalian muscle fibers, the networks of tubules are almost completely separated by the transversely oriented triads. In 19-day fetal rat muscle fibers, the network of tubules (*ST* in Fig. 1) usually extends uninterrupted along successive sarcomeres. The longitudinally oriented triads do not form an effective barrier, and transversely oriented triads are seldom present. It is noteworthy that the longitudinally oriented triads are usually found near the A-I junctions, i.e., at levels at which transverse triads are located in adult fibers. Therefore, it must follow that the longitudinal triads either undergo reorientation to the transverse direction or suffer displacement by newly formed transverse triads as fiber development progresses toward the adult structure. It will be of interest to attempt to find out the time and nature of complete triad development in rat muscles by observations on late fetal and early postnatal animals.

When the triadic junctions in fetal rat muscle (Figs. 2 and 3) are compared with those in adult rat muscle (19, 20), they are remarkably similar. Therefore, it seems likely that the mechanisms of triadic junction formation are the same in longitudinal triads of the fetus and in transverse triads of the adult. As pointed out in the study on adult rat muscle (20), the periodicity of bridges traversing the space between the apposed membranes of triadic central and lateral elements is the same in longitudinal and in transverse sections of the triad. This observation along with others led to the conclusion that the junctions of triads contain structures homologous to sealed compartments in a honeycomb. This conclusion implies that the bridges between the central element and the lateral elements are well organized and complex. Before the studies on fetal muscle were begun, no basis for speculation about the source of structural material for the bridge was available. Our tentative view, based upon observations on fetal muscle fibers, is that the processes involved in formation of projections on the external surface of sarcotubules may also be involved in formation of bridges between sarcotubules and the T system. Indirect evidence for this view is similarity of periodicity of projections and bridges. There is no direct evidence.

The striking enlargement of sarcotubules frequently seen at the level of triad formation, as illustrated in Figs. 2 and 3, suggests that morphogenic processes within the lateral elements are enhanced at the site of triadic junction development. The dense material in lateral elements of fetal muscle fibers usually appears to be amorphous. The evidence for membrane-like structures within the lateral elements of these fibers (Fig. 3) is not so good as the evidence for membrane-like structures within the sarcoplasmic reticulum of adult muscle fibers (18). In that study (18) on adult muscle fibers, it is clear that the increased density within the sarcoplasmic reticulum is due, at least in part, to membrane-like structures.

Page (11) has observed that the triads in slow muscle fibers of the frog are sparsely distributed in the interfibrillar space and are oriented either transversely or longitudinally at the level of the Z line. This sparsity of triads is the only similarity that can be seen between frog slow muscle fibers and rat fetal muscle fibers. As already noted, the longitudinal and transverse triads in fibers of the rat fetus are usually located near the A-I junctions. The M lines are well defined, and the Z lines show connections with the thin filaments that are typical of fast muscle fibers (Fig. 1). Two distinct sizes of sarcotubules, comparable to those described for frog slow fibers, are not distinguishable in ordinary electron micrographs of rat fetal muscle fibers. To distinguish the T system from the sarcoplasmic reticulum in the rat fetus, one might possibly trace this system by placing electron-opaque particles in the extracellular fluid and allowing time for them to enter the T system prior to preparation of the fibers for electron microscope examination. Furthermore, "calcium staining" like that described by Revel (14) provides another possibility for identification of the T system.

If the triads are involved in inward conduction of excitation from the surface of the muscle fiber, it is reasonable to suppose that a well developed transverse orientation of triads like that seen in the adult fiber would be the most effective structural basis for this inward conduction. Involvement of triads in inward conduction of excitation has been suggested (13) and substantially supported (8, 9). Continuity of the T system (central element of the triad) with the sarcolemma and the extracellular space implicates this system specifically with inward conduction of excitation (6, 10, 19). Other observations (5, 7, 12) suggest that the lateral elements of the triad are associated with release and accumulation of calcium involved in contraction and relaxation of muscle. Therefore, it seems reasonable to assume that the transverse triads in skeletal muscle fibers of adult animals might be closely related to speed of muscle contraction and duration of contraction time. Findings that the transverse triads, so typical of adult rat muscle, are sparse in the 19-day rat fetus pose a question about the speed and duration of contraction of fetal muscle. The light microscope studies of Veratti (17), showing the irregular orientation of reticulum profiles in muscle fibers of newborn mice and a regular transverse orientation in fibers of adult mice, suggest that changes in triad orientation might occur after birth. Close (4) has reported that the extensor digitorum longus of the newborn rat shows an increase in speed of contraction and a decrease in contraction time of the twitch response during the first month after birth. Similar results from studies on fast leg muscles of kittens were reported earlier by Buller, Eccles, and Eccles (2). It would be of interest to find out whether growing young rats and kittens show changes in triad orientation that might be correlated with the increase in speed of contraction and the decrease in contraction time of the twitch response.

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Note:

After submission of this report for publication, two papers (*J. Cell Biol.*, 1967, **35**:405; *J. Cell Biol.*, 1967, **35**:445.) showing triads in embryonic skeletal muscle fibers of chicks have appeared.

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