COORDINATED DEVELOPMENT OF THE SARCOPLASMIC RETICULUM AND T SYSTEM DURING POSTNATAL DIFFERENTIATION OF RAT SKELETAL MUSCLE

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ABSTRACT

An electron microscope study has been carried out on rat psoas muscle, during the early postnatal stages of development. Among the several subcellular components, the sarcotubular system undergoes the most striking modifications during this period. In muscle fibers of the newborn rat, junctional contacts between the T system and the SR are sparse and are, mostly, longitudinally or obliquely oriented. The T tubules do not penetrate deeply into the muscle cell, as indicated by the predominantly peripheral location of the triads and the persistence, at these stages of development, of a highly branched subsarcolemmal system of tubules. Diadic associations of junctional SR elements with the plasma membrane are also occasionally observed. The early SR elaborations incompletely delineate the myofibrils, at both the A- and I-band level. Longitudinal sections show irregularly oriented SR tubules, running continuously over successive sarcomeres. Flattened junctional cisterns filled with granular material are sparse and laterally interconnected, at circumscribed sites, with the SR tubules. Between 1 and 2 wk postpartum, transversal triadic contacts are extensively established, at the A–I band level, and the SR network differentiates into two portions in register with the A and I band, respectively. At 10–15 days after birth, the SR provides a transversely continuous double sheet around the myofibrils at the I-band level, whereas it forms a single discontinuous layer at the A-band level. The relationship that these morphological modifications of the sarcotubular system may bear to previously described biochemical and physiological changes of rat muscle fibers after birth is discussed.

INTRODUCTION

The time-course of biochemical and functional changes in skeletal muscle during development varies considerably according to the animal species. In mammals which, like the rat and rabbit, are relatively immature at birth, marked changes in the rate of hydrolysis of ATP by myosin (47), in the protein (18, 36) and enzymatic composition (24) of the sarcoplasmic fraction, and in the isoenzyme composition of lactic dehydrogenase (see reference 8), take place in the early postnatal stages of development. Accompanying these biochemical modifications are also changes in the speed of contraction (5, 6) and in the contracture response to caffeine (14, 16), which underline muscle functional specialization into fast and slow types.

In comparison with the considerable amount of available information on the biochemistry and physiology of muscle development, knowledge of the correlated changes in muscle ultrastructure is,

in its present state, very limited. In view of the role played by the T system in conduction of the excitatory impulse (see reference 32), and by the SR in muscle relaxation (see reference 50) and carbohydrate metabolism (see reference 1), of special interest, from a correlative viewpoint, are early observations in the light microscope by Veratti (48) which indicate marked changes of the reticular apparatus, later identified with the sarcotubular system (39, 2), in muscle fibers during ontogenesis.

These morphological aspects of muscle development are now amenable to reinvestigation with the greater resolution afforded by the electron microscope. In the present study[1] the ultrastructure of muscle fibers, with regard particularly to the sarcotubular system, has been studied in psoas muscle of rats at different stages of development, from birth to 15 days postpartum, i.e. at ages when rapid increases in glycolytic enzymes (1) and changes in physiological properties (5) occur in skeletal muscle of this species. Since biochemical differentiation of skeletal muscle was correlated with the onset of motile activity (24), it was also of interest to compare the differentiation of the sarcotubular system during ontogenesis in vivo with muscle differentiation in vitro, as recently illustrated in the elegant studies of Ezerman and Ishikawa (10) and Ishikawa (22) on developing chick myotubes.

MATERIALS AND METHODS

Albino rats of a highly inbred Wistar strain were used. Psoas muscle from 1-, 3-, 10-, and 15-day-old animals was fixed at rest length *in situ* in 4% glutaraldehyde-0.1 M phosphate buffer, pH 7.2, and postfixed in 1% OsO_4 in the same buffer. Embedding was carried out in an Epon mixture consisting of Epon 812 47%, DDSA 25%, MNA 28%, and DMP-30 2%. Thin sections were cut on an LKB microtome with glass knives. The sections were mounted on uncoated grids and stained with aqueous uranyl acetate and/or lead citrate. The specimens were examined in a Siemens Elmiskop I electron microscope operated at 60 kv.

[1] Preliminary reports were presented at the International Conference convened by the Muscular Dystrophy Associations of America Inc. at Arden House, Harriman, New York, 1966 (1), and at the 4th Regional Conference of Electron Microscopy, Rome, 1968 (4).

RESULTS

Psoas Muscle of the Rat at Birth

GENERAL FEATURES: The psoas muscle of the rat at birth is composed of fibers which, despite the wide variation in size, all exhibit well differentiated myofibrils with the same banding pattern as seen in adult rat muscle (Fig. 1). These basic aspects of muscle fiber differentiation are reflected also by the peripheral location (Fig. 2) reached by most nuclei at this stage of development, from the central position which is characteristic of the earlier stages of muscle cell differentiation during embryogenesis.

Distinguishing features of muscle fibers from newborn rats in respect to fibers at more advanced stages of development are the richness of ribosomes and the greater prominence of the subsarcolemmal sarcoplasm, both absolutely and relative to the intermyofibrillar sarcoplasm (Fig. 2). Ribosomes, polyribosomes, and segments of rough-surfaced membranes are particularly abundant in the thinner, less mature fibers (Fig. 3). In the larger fibers, accumulations of ribosomes and rough-surfaced membranes are localized mainly at regions beneath the sarcolemma and in the perinuclear sarcoplasm, where they are seen in close association with clusters of oval or slightly elongated mitochondria (Figs. 1, 2, 8). There is a lesser tendency to observe, at this stage, rows of longitudinally oriented mitochondria lying in the intermyofibrillar spaces (Fig. 1). As compared with muscle fibers of the adult rat, even more marked differences concern the intermyofibrillar membrane system, collectively denoted sarcotubular system (see reference 29 for terminology). Although the sarcotubular system varies somewhat in extensiveness between muscle fibers, according to their varying size and degree of maturation at birth, its incomplete differentiation constitutes the most characteristic feature of psoas muscle of the newborn rat.

SARCOPLASMIC RETICULUM: Cross-sections of fibers from psoas muscle of newborn rats show SR elements providing only a discontinuous layer around the myofibrils, at both the A- and I-band level (Figs. 3, 4). Accordingly, myofibrils appear very irregular in shape and are amply fused together. These features are at variance with those seen at more advanced stages of development when myofibrils appear, in cross-section, more regularly round in shape and are completely

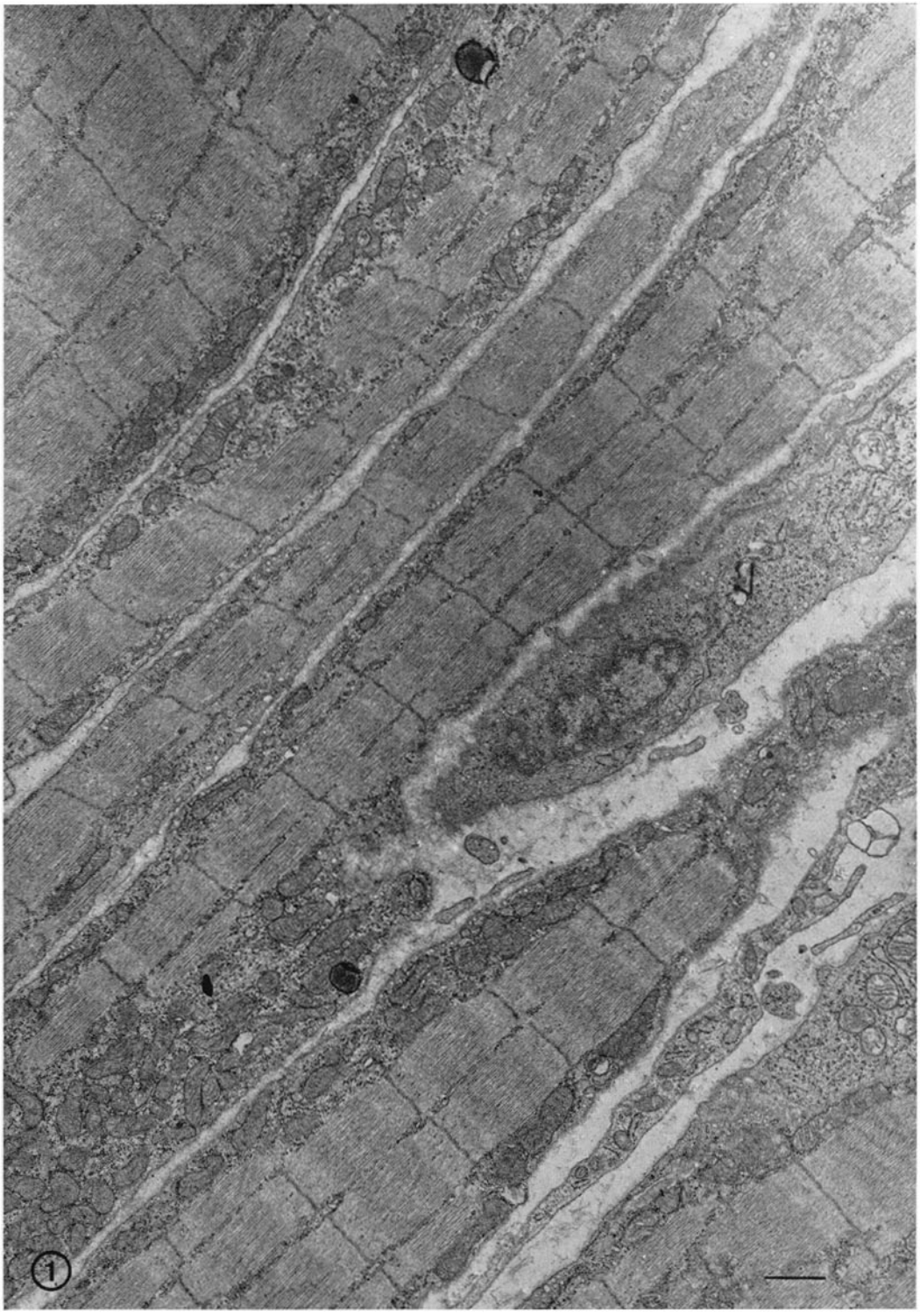

FIGURE 1 Longitudinal section of psoas muscle from newborn rat. The figure illustrates at low magnification the large variability in fiber diameter and prominence of the sarcolemmal sarcoplasm at these early stages of development. × 9,000. Scale mark on all micrographs: 1μ.

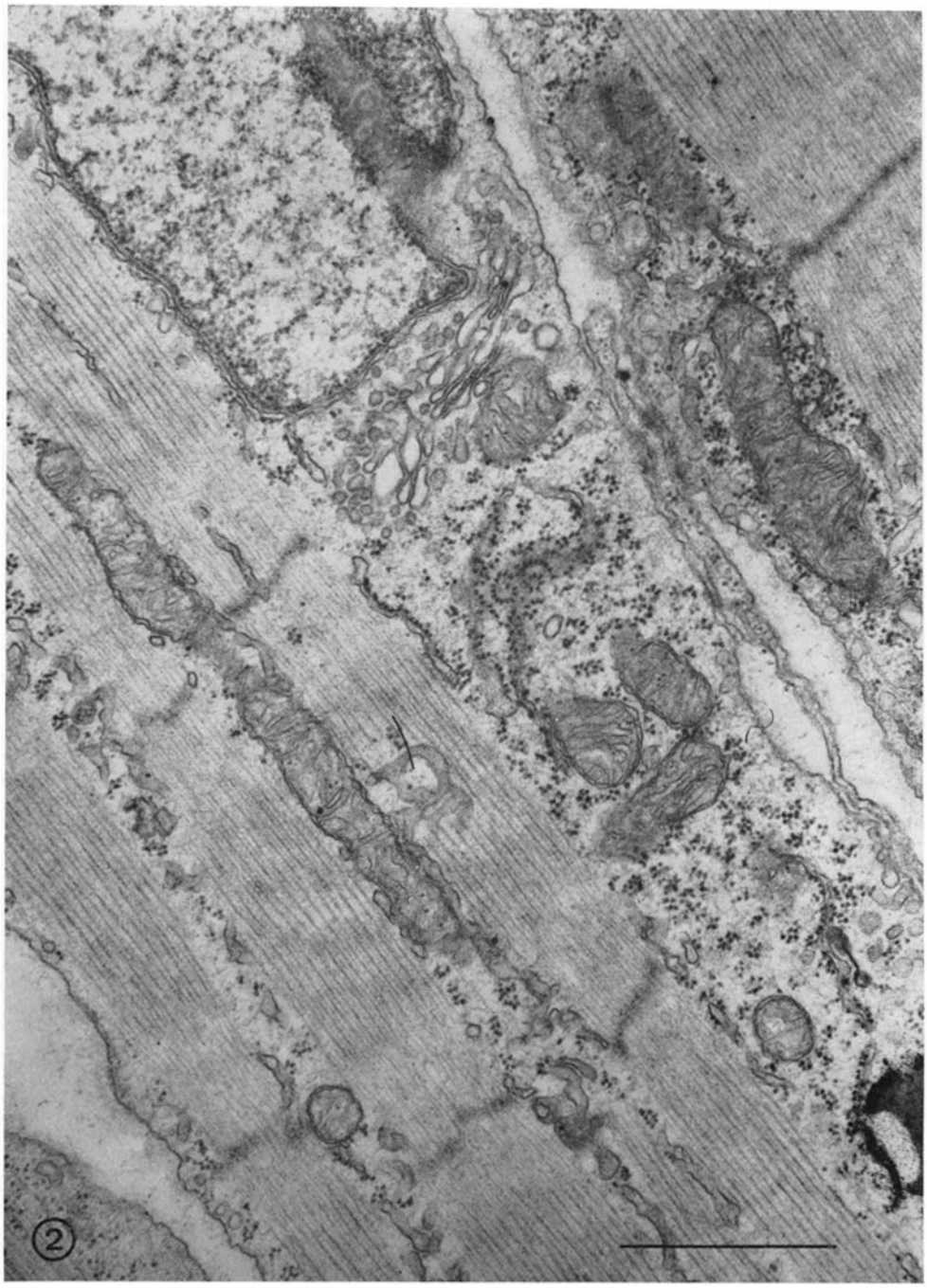

FIGURE 2 High-power view of longitudinal section of psoas muscle fiber from newborn rat showing peripheral, perinuclear area with prominent Golgi apparatus, clusters of mitochondria, elongated profiles of rough-surfaced membranes, and polyribosomes. × 30,000.

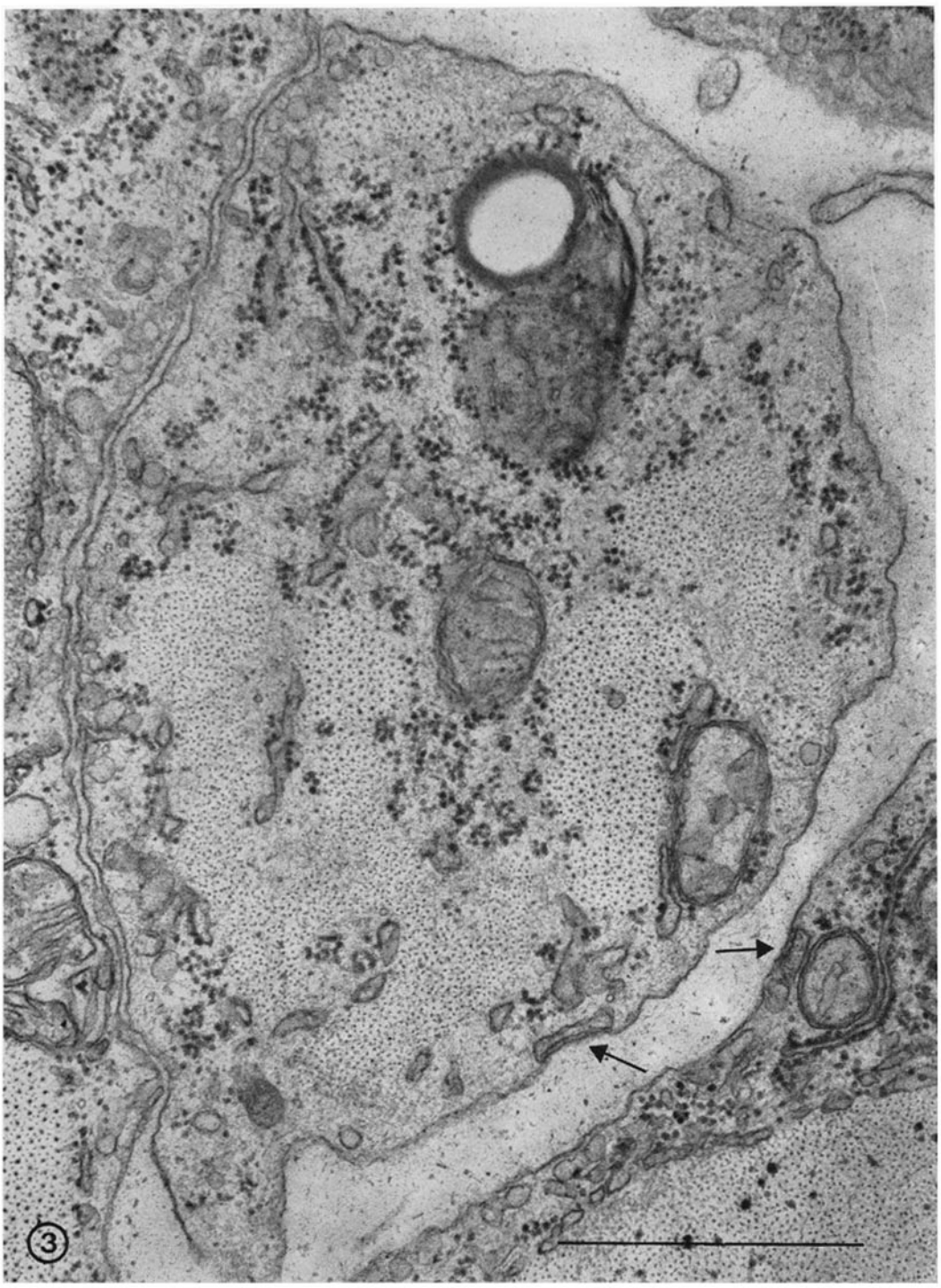

FIGURE 3 Cross-section of small, immature muscle fiber of newborn rat psoas. Early elaborations of the SR with attached ribosomes, and free ribosomes and polyribosomes are irregularly interspersed among bundles of contractile filaments. Arrows: couplings of flattened filled cisterns with the plasma membrane in the same and in an adjacent fiber. × 45,000.

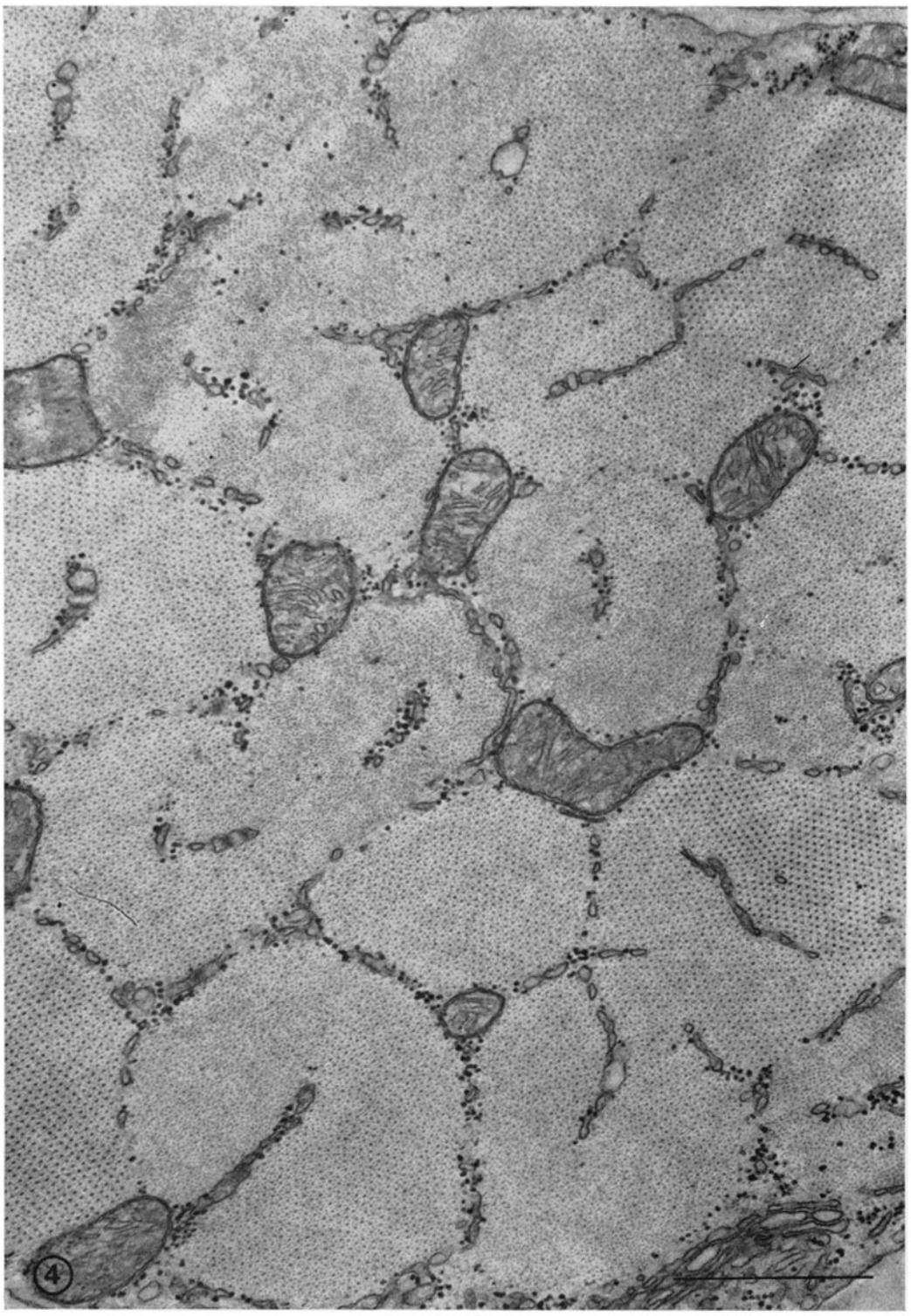

FIGURE 4 Cross-section of larger more differentiated psoas muscle fibers of newborn rat. The SR is more regularly organized, as compared with the less differentiated fiber shown in Fig. 3. The SR elements are more developed but still mark incompletely the periphery of myofibrils which appear irregular or ribbon-like, at both the A- and I-band level. × 30,000.

FIGURE 5 Grazing view of the perimyofibrillar SR network in muscle fiber from newborn rat, showing the irregular disposition of the SR elements without differentiation into distinct regions in register with the sarcomeres. × 32,000.

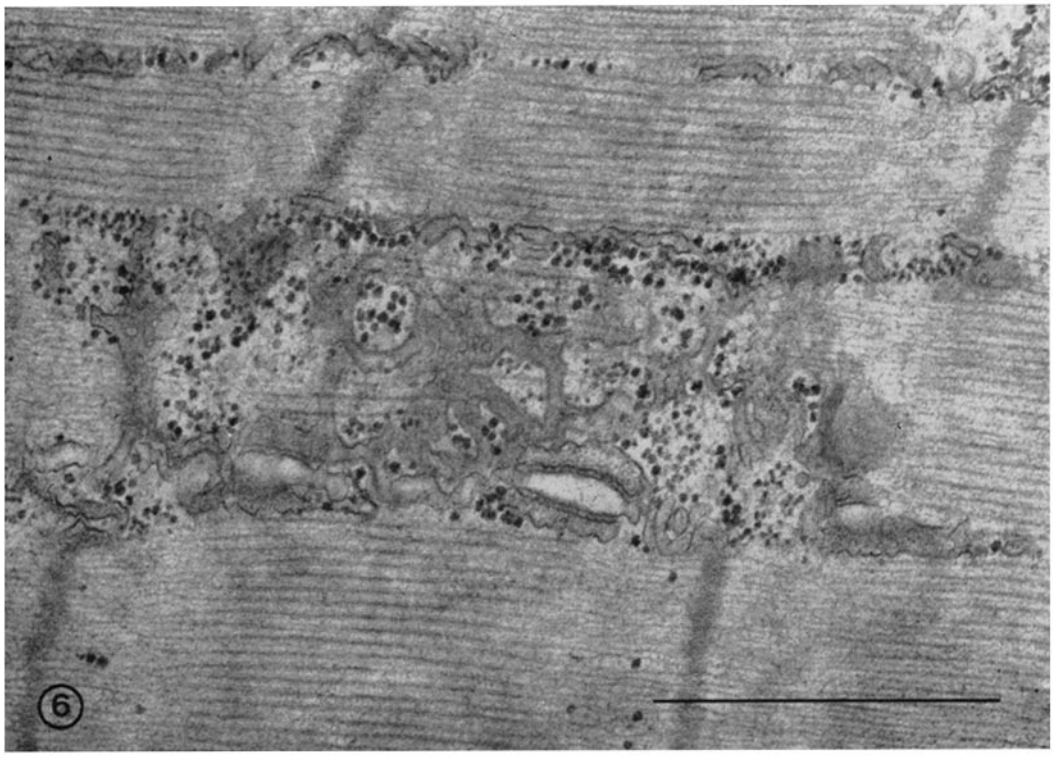

FIGURE 6 Grazing view of the SR overlying a single sarcomere of muscle fiber from newborn rat. The SR network shows a greater degree of complexity, as compared with that in the fiber illustrated in Fig. 5. The SR tubules appear to be confluent into a perforated sheet, at the mid-A-band region. A similar fenestrated structure is seen positioned at the Z-band region. A longitudinally oriented triad is also observed. × 45,000.

encircled, at the I-band level at least, by the SR network (Fig. 11). On the other hand, these morphological aspects of rat muscle fibers at birth are reminiscent of the "*Felderstruktur*" of the slow-tonic fibers of amphibia (34, 21).

Longitudinal sections show that these early elaborations of the SR have not reached the very regular repeating pattern, in register with the banding of the myofibrils, that is typical of adult mammalian muscles (39). As illustrated in Fig. 5, the sheets of SR intervening between adjacent myofibrils consist of a predominantly, irregularly oriented network of tubules running continuously over the A and I band, without being interrupted by transversely oriented terminal cisternae. At the A-I band boundary, the tubular network is, however, seen at sites to be laterally interconnected with dilated sacs filled with granular material which are lying in a plane oblique or parallel to the major axis of the fiber, and which are morphologically homologous to the terminal cisternae of the differentiated SR (see below).

Occasionally, there is also evidence of a initial differentiation of the SR into a distinct region at the mid-sarcomere and mid-I-band level (Fig. 6) where there is a tendency for the irregularly running tubules to become united into perforated sheets (see references 39 and 46).

T System and Triads

In immature psoas muscle, triads are observed to be identical with those seen in adult muscle, with respect to morphological appearance of the three-component structure and of the intervening junctional area, and likewise are positioned near the A-I band boundary.

However, important differences concern the number of triads found in sections and the orientation of the triads in respect to the major axis of the fibers. In psoas muscle of the newborn rat, and

FIGURE 7 Longitudinal section of two adjacent muscle fibers of newborn rat. The subsarcolemmal sarcoplasm containing mitochondria, glycogen granules, and ribosomes appears to be particularly prominent in the fiber shown in the upper part of the figure, where the section is partly tangential to the fiber surface. Only one peripherally located, longitudinally oriented triad is observed in the section (arrow). Subsarcolemmal vesicles and clear tubules are indicated by small arrows. × 23,000.

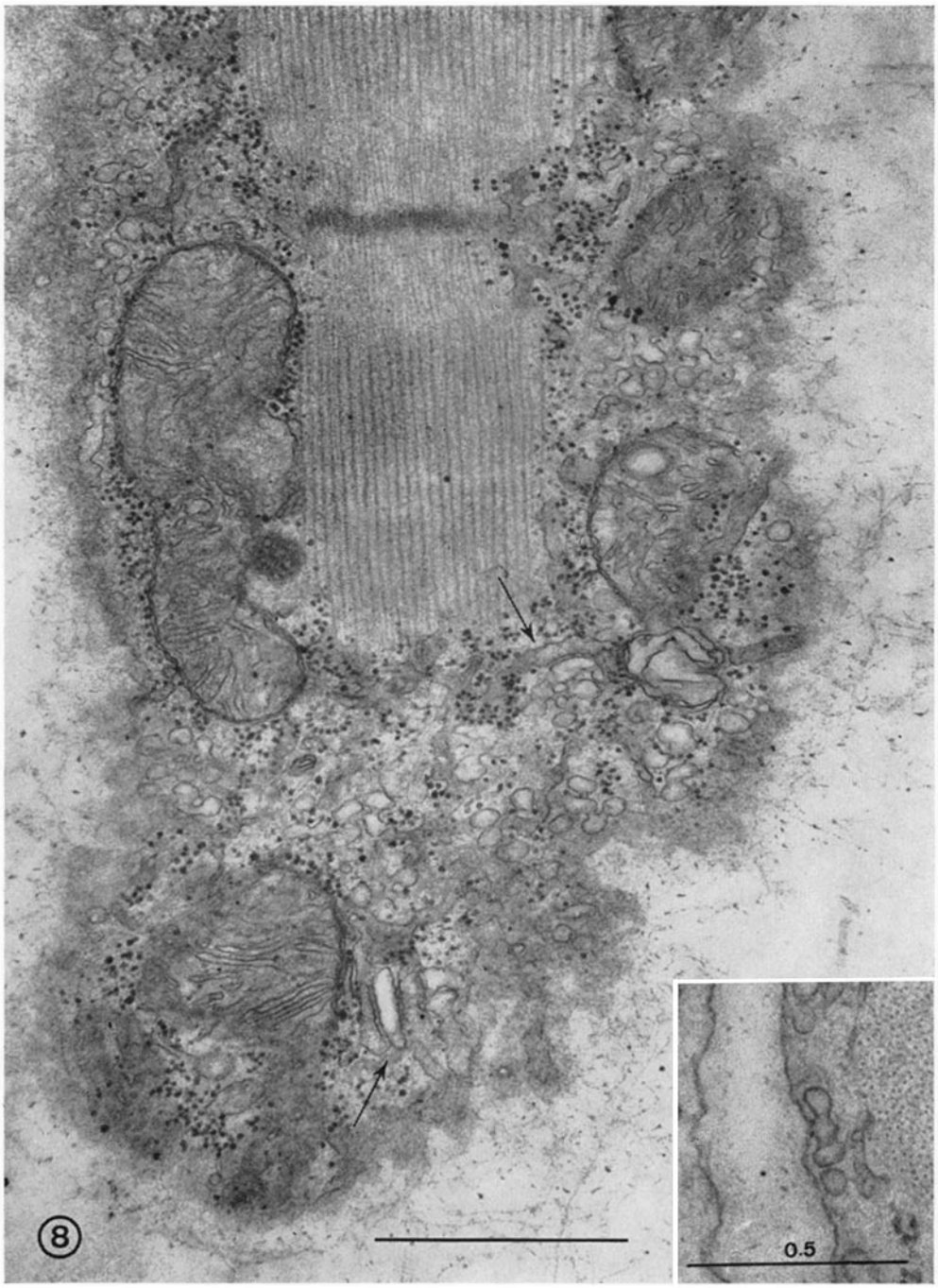

FIGURE 8 Longitudinal section of muscle fiber from newborn rat illustrating an extensive system of branching tubules in the subsarcolemmal sarcoplasm. A diadic coupling between one of these peripheral tubules and a filled SR cistern, and a typical triad are indicated by arrows. Ribosomes and polyribosomes are present between the vesicles. × 35,000. Inset: cross-section of muscle fiber of newborn rat showing a subsarcolemmal tubule opening into extracellular space. × 60,000.

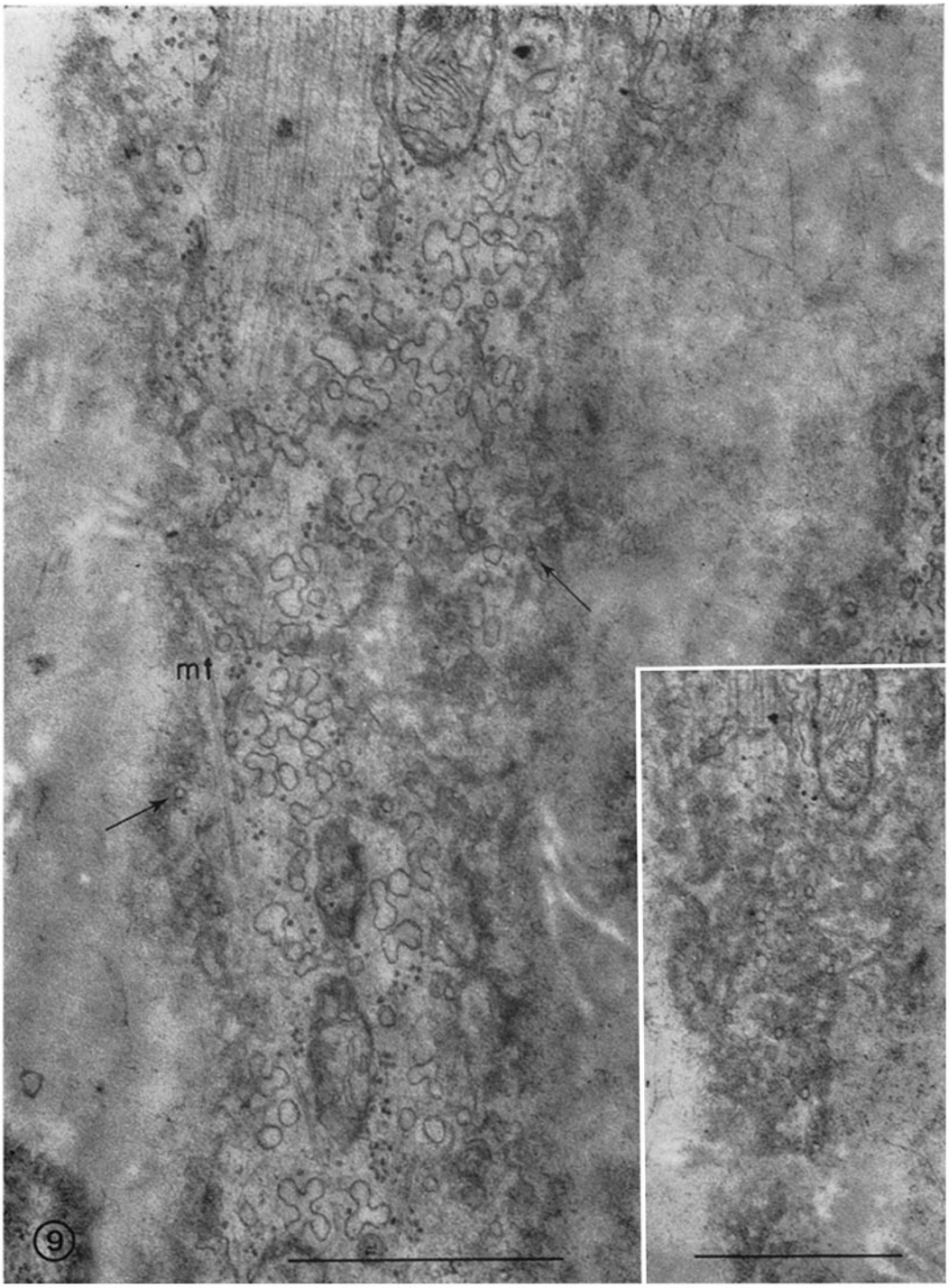

FIGURE 9 Face view of subsarcolemmal tubules with multilobed appearance. Round openings in the sarcolemma are indicated by arrows. *mt*: microtubule. Inset: serial section showing round openings in the sarcolemma surrounded by basement membrane material. × 40,000. Inset, × 30,000.

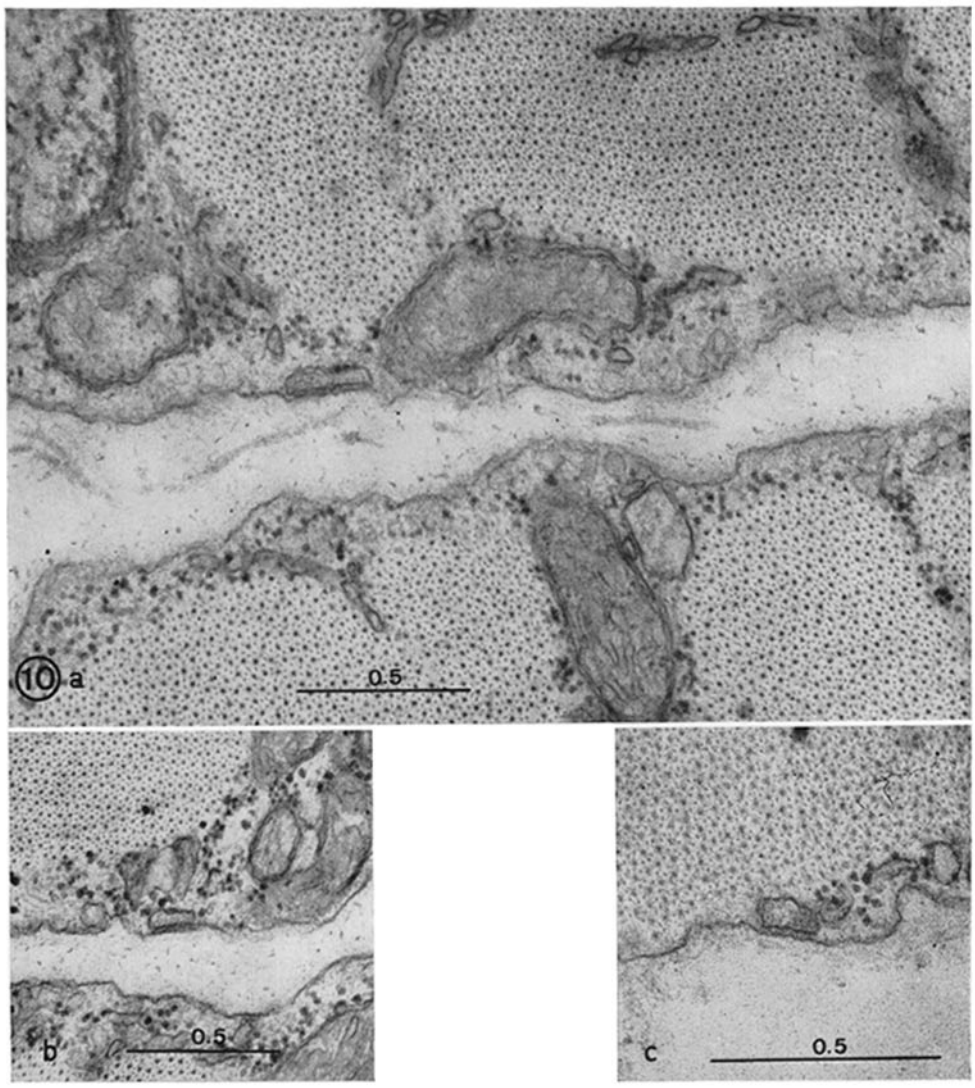

FIGURE 10 *a–c* Cross-sections of different fibers from newborn psoas showing diadic couplings of the plasma membrane with large vesicles filled with dense granular matrix. *a*, × 45,000. *b*, × 40,000. *c*, × 60,000.

as observed by Walker and Schrodt (49) in rat foetuses at term, triads are sparse and appear to be longitudinally or obliquely oriented in longitudinal sections. In our material, the few triads were also located mainly at the cell periphery (Figs. 6–8).

In addition to these differences between adult muscle and neonatal muscle relating to the varying degree of development and orientation of clearly identifiable T tubules and of "junctional" SR (see reference 44 for terminology), further differences concern the existence, at these early stages after birth, of an extensive tubular system beneath the sarcolemma (Figs. 8 and 9) which is occasionally seen to make diadic contacts with filled SR vesicles (Fig. 8). Interestingly, diadic associations of SR elements with the plasma membrane are also observed at these stages (Fig. 10 *a–c*).

The peripheral tubules follow a very tortuous

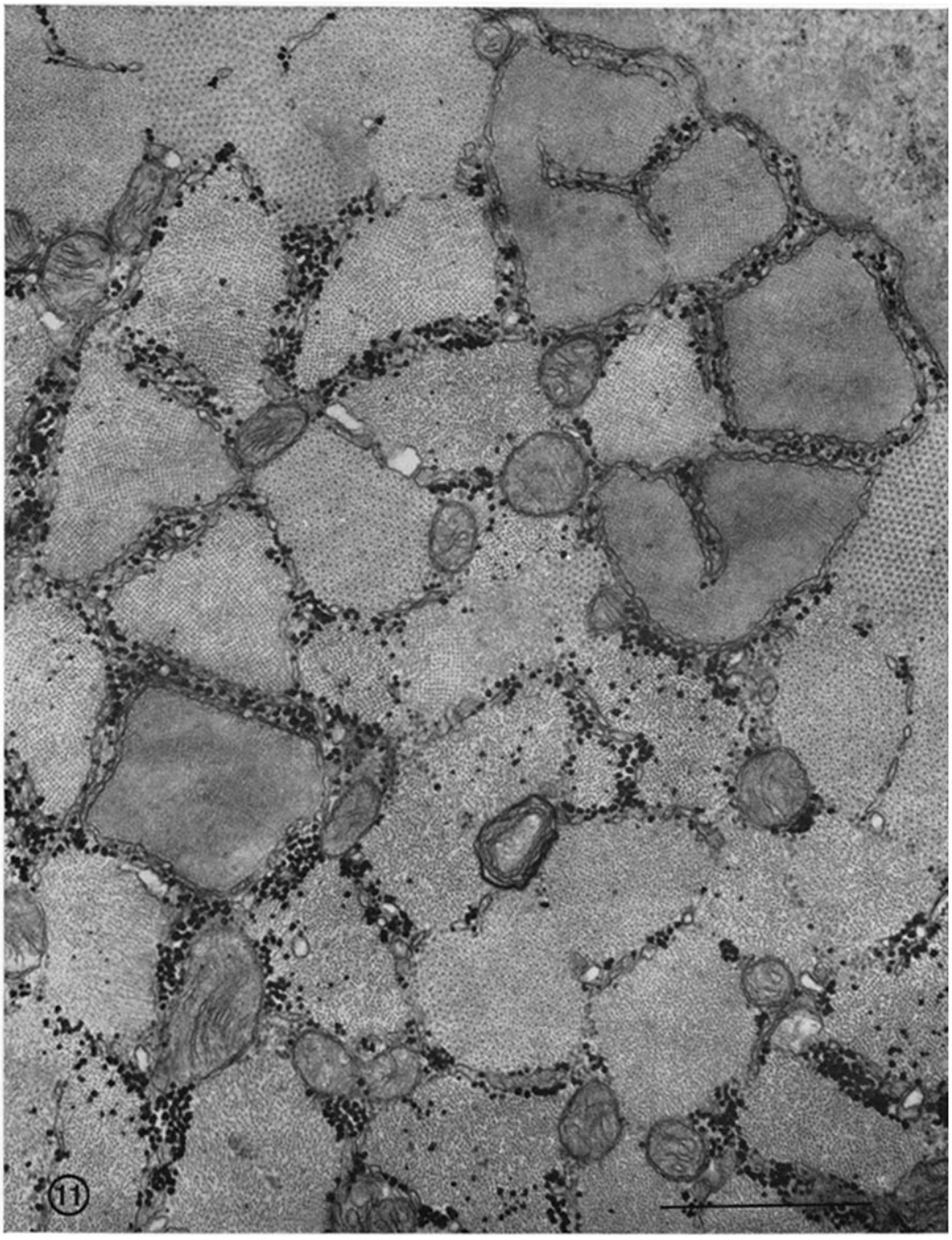

FIGURE 11 Cross-section of psoas muscle fiber of 10-day-old rat. Myofibrils are encircled by an extensive SR network. At the I-band level, especially near the Z line, the SR consists of a double sheet with an intervening space filled with glycogen particles. At the A-band level, a single SR sheet is shared by two adjacent myofibrils. Intermyofibrillar mitochondria appear as round profiles, i.e. do not exhibit at this stage transversal extensions embracing the myofibrils. × 30,000.

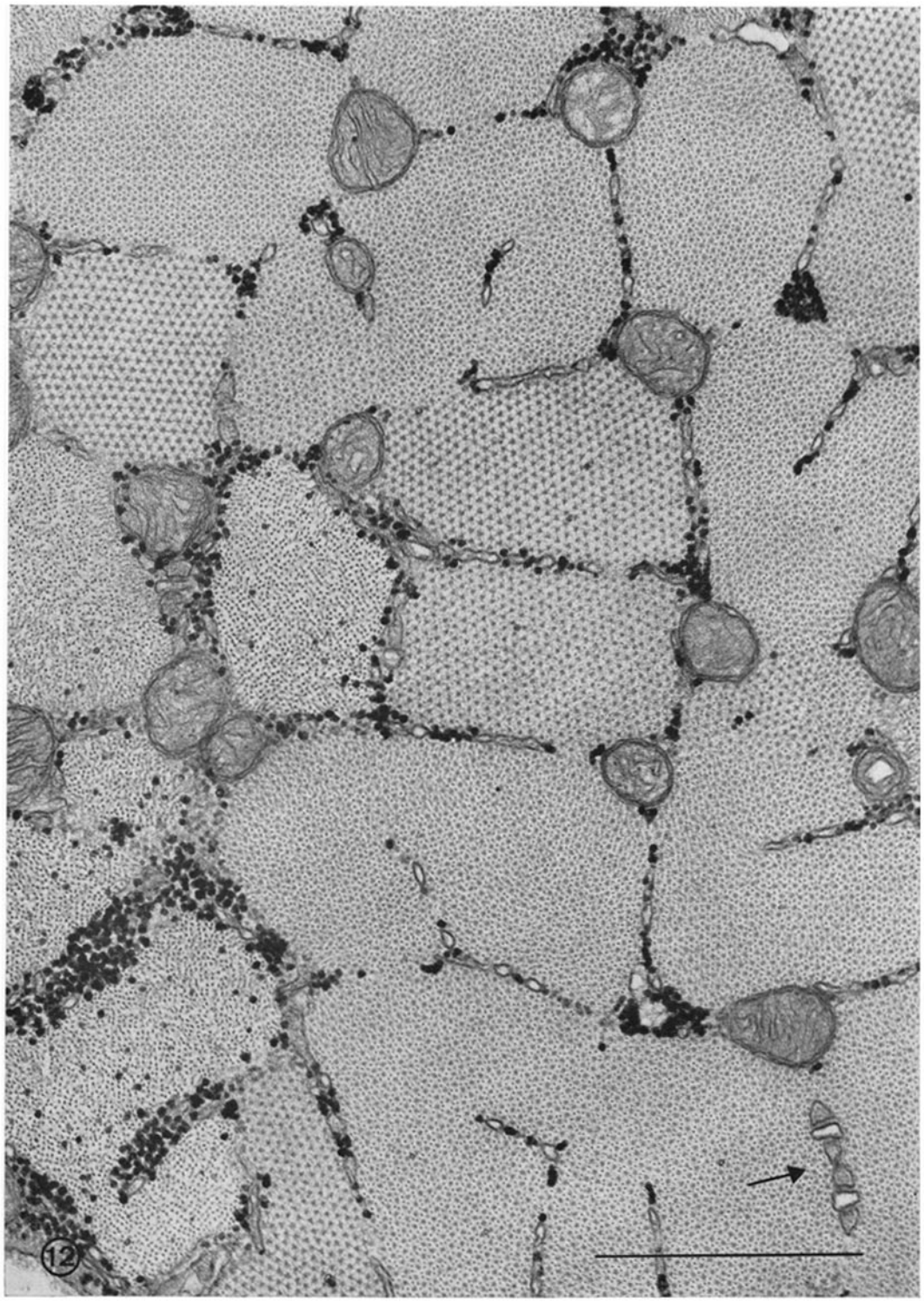

FIGURE 12 Cross-section of muscle fiber from 10-day-old rat. The SR marks completely the periphery of the myofibrils at the I-band level, whereas it provides a discontinuous sheet around the A band. Arrow: longitudinally oriented triads. × 40,000.

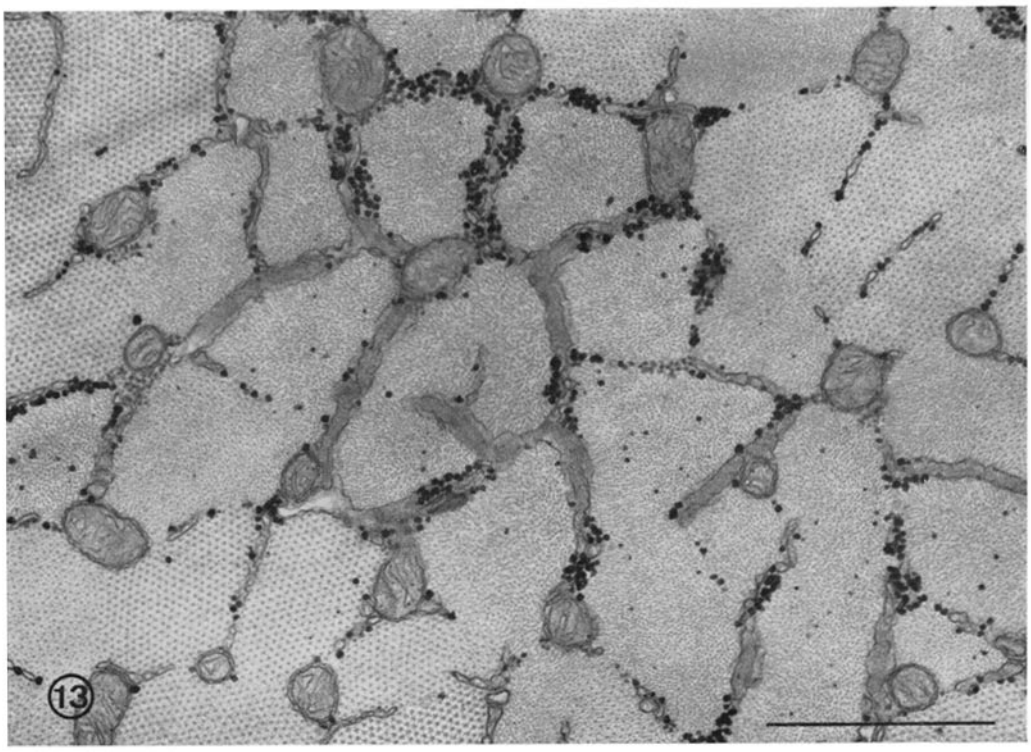

FIGURE 13 Cross-section of muscle fiber from 10-day-old rat showing several transversely oriented, filled SR cisterns which are seen at sites to overlie profiles of empty T tubules. × 30,000.

course, along which saccular dilatations or blind-ended outpocketings are characteristically seen. Serial sections tangential to the sarcolemma show round openings in the plasma membrane (Fig. 9) which probably represent communications of the tubular system with extracellular space, although continuity of the tubule membrane with the plasma membrane is only occasionally observed (inset to Fig. 8).

Morphological Changes of Psoas Muscle during the Early Postnatal Stages of Development

A sustained rate of increase in weight and an appreciable increase in fiber diameter are observed in rat muscles only 1 wk postpartum, following an initial lag-phase (see reference 25).

Between 1 and 2 wk after birth, there occurs a definite increase in diameter of psoas muscle fibers, which is associated with an increase in number of the constituent myofibrils and with marked proliferative changes of the sarcotubular system.

The greater development of the SR component in muscle fibers from 10-day-old, as compared with newborn animals, is particularly evident in cross-sections. At 10 days, as shown in Figs. 11–13, the SR elements provide an extensive network around the myofibrils, at the I-band level. At the mid-I-band, a double layer of SR with an intervening space filled with glycogen granules is observed, like that described in mature fibers of rat "white" muscles (13). Incomplete delineation, at the A-band level, of myofibrils, by interspersed profiles of longitudinally oriented, narrow tubules, still persists at these stages of development, but is not at variance with the aspects seen in adult psoas muscle (S. Schiaffino, unpublished observations).

A related striking modification that takes place in muscle at these periods and is best seen in longitudinal sections concerns the much larger number of triads per unit area, in muscle of 10-day-old animals (Fig 14). Concurrently, most triads appear

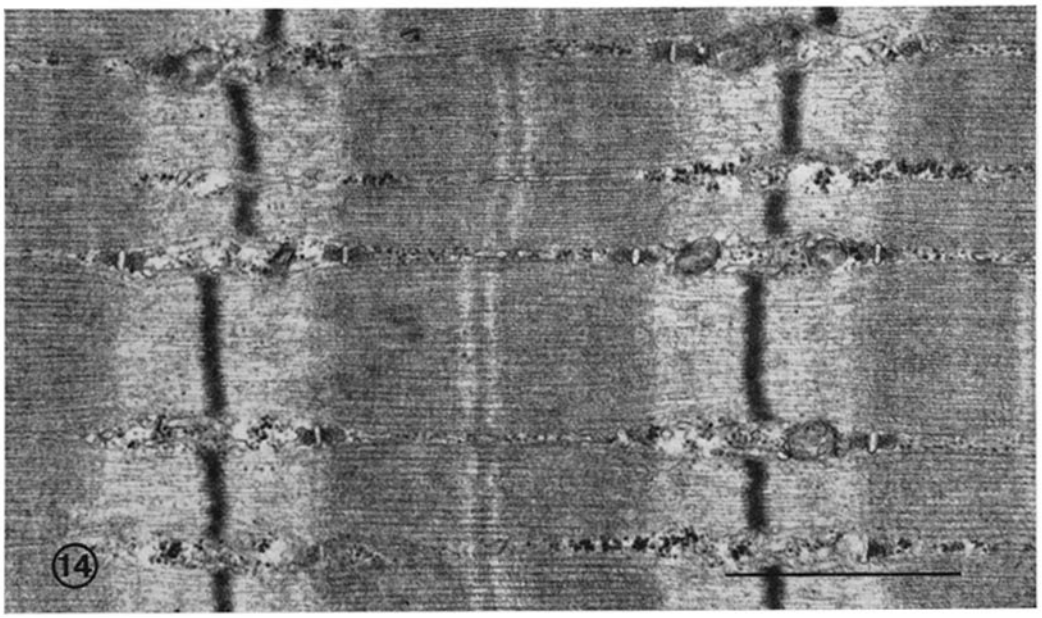

FIGURE 14 Longitudinal section of psoas muscle fiber from 10-day-old rat. Many triads with transversal orientation are seen, regularly positioned at the A-I band boundary. × 30,000.

to have attained the transversal orientation that is typical of adult rat muscle. Only few T-tubule extensions are still observed at this stage, running obliquely or longitudinally with respect to the long axis of the fibers. The persistence, at circumscribed sites, of this immature disposition accounts for the occasional finding of triads in transverse sections also (Fig. 12).

Under the conditions attending these early stages of differentiation, mitochondrial modifications were found to be comparatively much less marked, except for a lesser tendency for mitochondria to occur in large clusters in the subsarcolemmal sarcoplasm in fibers from 2-wk-old rats, as compared with newborn animals. Appreciable changes in the number of the intermyofibrillar mitochondria, or variations in the number of mitochondria among the several fibers, were not observed throughout the period investigated. On the other hand, the intermyofibrillar mitochondria in fibers of 10-day-old rats maintained, mostly, elongated shape and straight longitudinal orientation, as seen at earlier stages of development. Not until 15 days after birth were L-shaped mitochondria with lateral branches observed extending transversely at the I-band level.

Changes in Fractional Volume of Myofibrillar and Sarcoplasmic Components during Postnatal Development

The volumes occupied by the myofibrillar and sarcoplasmic components were determined on electron micrographs of randomly chosen cross-sections of muscle fibers by differential point-counting (51). The volume occupied by the ground substance (i.e. the cell matrix interspersed among myofibrils, sarcotubular elements, and mitochondria) was estimated from the difference between the fiber volume (with the exclusion of nuclei) and the summed volumes of the myofibrils, mitochondria, and sarcotubular system. Values were expressed as per cent of the fiber volume and, in the case of the sarcoplasmic components, of the volume of the extramyofibrillar sarcoplasm. As shown in Fig. 15, cytoplasmic growth of muscle fibers which occurred in the first 2 wk after birth appeared to be associated with a relatively greater increase in volume of the contractile material, as compared with the total sarcoplasm. However, the several sarcoplasmic compartments underwent considerable changes in their relative proportions, at these periods. In line with the morphological observations reported in the previous section, a

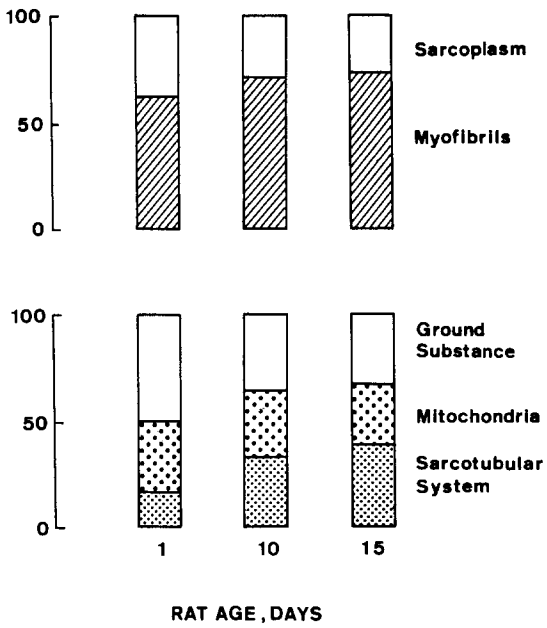

FIGURE 15 Postnatal changes in fractional volume of myofibrillar and sarcoplasmic components of psoas muscle fibers. Each value is the mean of 25 determinations on electron micrographs of randomly sampled cross-sections of psoas muscle fibers. Volumetric measurements were carried out by differential point-counting analysis (51). Upper figure: fraction of fiber volume (per cent). Bottom figure: fraction of sarcoplasm volume (per cent).

large increase in the percentage fractional volume was found only for the sarcotubular system, whereas the mitochondrial compartment showed less changes and the ground substance was considerably reduced (Fig. 15). When referred to the total cytoplasmic volume, the relative volume (mean values of 25 determinations on different sections ± SD) occupied by the sarcotubular system rose from 6.4 ± 2.3% in fibers at birth to 9.4 ± 2.1% and 10.6 ± 2.5% in fibers of 10- and 15-day-old animals, respectively.[2]

DISCUSSION

It was first reported by Veratti (48), on the basis of observations in the light microscope of skeletal muscles from several species, that the silver-impregnated reticular apparatus of the fiber, later identified with the sarcotubular system (39, 2), undergoes marked modifications during ontogenesis. Immature muscle fibers of chick and lizard embryos and of mammalian foetuses (guinea pig, rabbit, and mouse) were found to exhibit sparse reticulum, with very irregular disposition. The morphogenetic process leading to the highly organized reticulum seen in adult muscle was remarkably similar among the various species investigated. However, differences were noted, in respect to the age at which these differentiation changes were completed. In those animals, such as the guinea pig, which are relatively mature at birth, the fiber reticular apparatus had attained, at this age, morphological features similar to those seen in adult animals. On the other hand, an embryonic type of organization of the reticulum still persisted, a few days postpartum, in skeletal muscle of the mouse whose ontogenic development is less advanced at birth.

The present electron microscope observations on psoas muscle of the rat, whose degree of maturation at birth compares to that of the mouse, are in conformity with and further extend these early findings by Veratti (48). In muscle fibers of newborn rats, as in fibers of 19-day rat foetuses (49), the most prominent differential feature that we have found in respect to mature muscle fibers concerns the structural organization of the sarcotubular membranes. Two distinguishable sets of tubules with occasional triadic couplings could be recognized in these early stages, despite their irregular disposition, on the basis of differing morphological features and separate morphogenesis. With the use of these parameters as criteria for

[2] Under the conditions used in the present measurements, the relative volumes of the two components of the sarcotubular system, viz. the SR and the T system, could not be separately determined, owing to the frequent overlapping of profiles of T tubules and SR terminal cisternae in cross-sections. However, it is reasonable to assume, on general grounds, and this assumption is, in part, supported by published data on frog muscle (31), that even in rat muscle, in which the T system is relatively more extensive than in the frog muscle, the volume of this system may be small in respect to that of the SR. Thus, changes in volume of the SR would be considered to make a major contribution to the over-all increase in volume of the sarcotubular system during postnatal development of rat muscles. Because of the further difficulties arising from the irregular organization of the sarcotubular system at the earliest stages of differentiation and from its modifications in pattern after birth, a suitable model could not be developed to determine the accompanying changes in surface area of the SR and T tubules.

classification, the two distinguishable tubular components of these early elaborations of the sarcotubular system were identified as precursor forms of the SR and T system of mature muscle fibers, respectively.

As concerns the process of formation of T tubules, our present observations with newborn rats are in agreement with recent findings obtained from study of chicken muscle cells in tissue culture (10) and, in general, they conform to the early proposal made by Porter (38), that the T system has a separate origin in respect to the SR.

Several lines of evidence support the view that the T system is a sarcolemmal derivative (12, 20). In muscle fibers of newborn rats, at a stage when T tubules are rarely found deep within the cell, we observed a peripheral extensive system of short, branching tubules whose limiting membrane appeared, at circumscribed sites, to be continuous with the sarcolemma. An analogous tubular system has previously been illustrated in chick myotubes by Ezerman and Ishikawa (10) who unequivocally proved its origin from the plasma membrane by an invagination process, on the basis of experiments with ferritin.

The interpretation offered by Ezerman and Ishikawa (10), that the sarcolemmal tubular system they described represents an early form of T system, is further substantiated by our present observations. As reported in the Results section, we were able to find typical junctional areas between the closely apposed membranes of some of these tubules and of cistern-like SR elements which exhibited a granular content and should, therefore, be regarded as morphologically homologous to the lateral elements of the triads. The functional homogeneity of this tubular membranous system with respect to the plasma membrane is emphasized by the existence, at the same stages of development, of diadic associations of SR elements with the plasma membrane directly.

Diadic couplings of SR elements with the sarcolemma appear to be peculiar to the very early stages of development in rat skeletal muscle. They have never been observed in muscle fibers of adult vertebrates, although, interestingly, they have been reported in skeletal muscle of invertebrates (4) and are a normal feature of mammalian heart muscle where they were referred to as "subsarcolemmal cisterns" (40) or "peripheral couplings" (44).

With regard to the origin of the SR, which is characteristically smooth in mature muscle fibers, the derivation of SR membranes from rough-surfaced ER is supported by the present findings of smooth SR elaborations in continuity with ribosome-studded membranes in fibers from newborn rats, and is also in agreement with our earlier observations in denervated frog muscle (29). An analogous continuity of smooth and rough intracellular membranes was recently reported by Ezerman and Ishikawa (10) in chick myotubes in tissue culture.

Altogether, the results obtained in the present investigation seem to indicate that, in respect to morphogenesis of the sarcotubular system components, there exists a considerable degree of structural similarity between the early stages of muscle ontogenesis in vivo and the differentiation of muscle cells in vitro (10, 22). It should be inferred from this, therefore, that in rat muscle fibers, until birth at least, these aspects of differentiation are relatively independent of a regulatory influence of the nerve. A possible difficulty for this interpretation arises in view of the complete absence from our material of three-dimensional arrays of T-tubule networks, as observed by Ishikawa (22) in myotubes differentiating in vitro. Ishikawa has suggested that these structures originated as the consequence of uncoordinated development of the T system, with the lack of the morphogenetic influence of the nerve, under the prevailing conditions in tissue culture. However, overdevelopment of T tubules in myotubes in vitro may, alternatively, be explained as a nonspecific structural reaction, since analogous T-system formations were reported to occur in muscle fibers in response to a variety of pathological stimuli (7, 42, 9), in addition to denervation (35).

Differentiation of rat muscle fibers from birth to 2 wk postpartum appears to involve a coordinated development of the T system and the SR. The basic features of this process concern the T system's progressing more deeply into the cell and becoming transversely oriented, and the SR's being separated into morphologically distinct regions in register with the sarcomeres. In immature muscle fibers, junctional cisternae are sparse and are laterally interconnected with an irregular tubular network having longitudinal continuity over several sarcomeres. With further development, the junctional SR cisternae increase in number and attain, with respect both to the SR tubular network and to the T system, transversal positioning on each side of the A-I band boundary. As a consequence, the SR becomes divided into

two distinct, regularly repeating portions, in register with the A band and the I band, respectively. Parallel to the differentiation of the terminal cisternae, the longitudinal SR gains in extensiveness and complexity (see Results).

It would appear reasonable to deduce, from the structural changes of the sarcotubular system observed during the early postnatal stages after birth, that maturation of rat muscle fibers is attended by a progressive increase in the surface area of the T-system in respect to the outer fiber surface, and by an increase of the junctional surface area between the T-system tubules and the terminal cisterns of the SR. In addition, measurements of the fractional volume occupied by the SR indicate that this volume is augmented considerably during development. Parallel to the growth of the reticulum membranes, matrix material appears to accumulate in the membrane-limited spaces of the SR, in a coordinate fashion.

The nature of the dense material which characteristically is seen inside the SR of mature muscle fibers has been investigated only poorly, and we also do not know whether this material may vary in composition between the terminal cisternae of the SR, where it has granular appearance, and the longitudinal tubules, where it is more homogeneous. It has recently been suggested that part of this material may be mucopolysaccharides or glycoproteins (37), which would provide calcium-binding sites for calcium uptake and storage activities of the SR in connection with muscle relaxation. On the other hand, it has been demonstrated by electron-microscopic histochemical methods that two glycolytic dehydrogenases are localized in the lumen of the intact SR (11), and phosphofructokinase activity has been found to be associated with isolated SR fragments from frog muscle (26–28). These findings and additional morphological considerations have suggested to us that enzyme proteins concerned with the Embden-Meyerhof glycolytic pathway may be compartmented within the SR (1).

It is, therefore, of interest that phosphofructokinase, aldolase, and lactate-dehydrogenase activities of rat leg muscles are low at birth and increase rapidly during early postnatal stages of development, these changes paralleling, in time-course, the increase in volume of the SR (1).

Physiological data are not presently available to allow classifying rat psoas muscle as a fast muscle. However, several lines of evidence indicate that psoas muscle of the adult rat is fairly homogeneous and composed mainly of "white" fibers.

Firstly, we have found (S. Schiaffino, unpublished results) that in fibers of adult psoas muscle the SR is markedly well developed as in fast extensor digitarum longus (EDL) muscle, and that the pattern of organization of the SR in these two muscles is also similar to that of the "white" medial head of the gastrocnemius muscle and of the white, fast (17) bulbocavernosus muscle of the rat, described previously by Tice and Engel (46) and by Gori et al. (13), respectively. Common features, as far as the SR disposition in "white" muscle fibers is concerned, are the existence of a double sheet of SR overlying the I band, and of a fenestrated collar at the mid-sarcomere and at the mid-I-band region. As reported in the Results section, these particular arrangements become almost completely expressed in psoas muscle fibers at about 10–15 days after birth. Secondly, the histochemical pattern after staining for myofibrillar ATPase (30) appears to be characterized, in both the psoas muscle and EDL of the rat, by a greater prevalence of type II fibers with high ATPase (S. Schiaffino and P. Settembrini, unpublished results), a pattern which is at variance with that of the slow soleus muscle which is composed almost completely of type I fibers with low ATPase (23). Thirdly, electrophoretic separation of lactate-dehydrogenase isoenzymes from psoas muscle extracts has shown (C. Valfrè, C. Angelini, and A. Margreth, unpublished results) a similarly greater prevalence of M forms in the psoas muscle and EDL, in contrast with predominance of the H type of enzyme in the soleus. Since there is a fairly general consensus that a certain degree of correlation exists (15) between development of the SR (21, 35), myosin ATPase activity (43, 3), pattern of lactate-dehydrogenase isoenzymes (8), and speed of contraction of mammalian muscle fibers (5), it would seem to be suggested, therefore, that rat psoas muscle may be composed, mainly, of fast fibers.

Pending the obtaining of more direct data on the physiological properties of rat psoas muscle, knowledge of the role played by the T system in the inward spreading of excitation into the muscle cell and by the SR in regulation of the contraction-relaxation cycle (see reference 33) raises the question of whether structural changes of the sarcotubular system, during the early stages of postnatal development, may have a bearing on the physiological differentiation of muscle fibers.

Indeed, the present morphological observations encourage the interpretation that changes in the intrinsic speed of shortening of rat muscles during postnatal development may be related not only to changes in the contractile system (see reference 6), but also to changes in the excitatory system and the excitation-contraction coupling as well. Pertinent to this suggestion may be the findings that the resting membrane potential increases in rat muscles during the first month of life (19), and that calcium uptake activity in isolated SR fragments, from rabbit muscles at least, shows a similar time-course (45).

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