CHANGES IN THE SARCOPLASMIC RETICULUM AND TRANSVERSE TUBULAR SYSTEM OF FAST AND SLOW SKELETAL MUSCLES OF THE MOUSE DURING POSTNATAL DEVELOPMENT

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ABSTRACT

The sarcoplasmic reticulum (SR) and transverse tubular system (TTS) of a fast-twitch muscle (extensor digitorum longus-EDL) and a slow-twitch muscle (soleus-SOL) of the mouse were examined during postnatal development. Muscles of animals newborn to 60 days old were fixed in glutaraldehyde and osmium tetroxide and examined with an electron microscope. At birth the few T tubules were often oriented longitudinally, but at the age of 10 days most of them had a transverse orientation. In the EDL, the estimated volume of the TTS increased from 0.08% at birth to 0.4% in the adult; corresponding values for the SOL were 0.04% at birth and 0.22% in the adult. A similar relative change was observed in surface area of the TTS during development. Calculated on the basis of a 30 μm diameter fiber, the surface area of the TTS in the EDL increased from 0.60 cm^2 TTS/cm^2 fiber surface in the newborn to 3.1 cm^2/cm^2 in the adult, compared with 0.15 cm^2/cm^2 at birth to 1.80 cm^2/cm^2 in the adult for the SOL. The SR in the newborn muscles occurred as a loose network of tubules that developed rapidly within the subsequent 20 days, especially at the I band level. The volume of the SR increased in the EDL from 1.1% of fiber volume at birth to 5.5% in the adult. In the SOL the change was from 1.7% to 2.9%. The SOL approached the adult values more rapidly than the EDL, although the EDL had more SR and T tubules. Fibers of both EDL and SOL muscles showed variation in Z line thickness, mitochondrial content, and diameter, but over-all differences between the two muscles in amount of SR and TTS were significant. It is considered that the differing amounts of SR and TTS are closely related to the differing speeds of contraction that have been demonstrated for these two muscles.

INTRODUCTION

Previous work on postnatal development of mammalian fast and slow muscles has been concerned mainly with biochemical and physiological features. There has been little work on the comparative ultrastructure of fast and slow muscles during development, although ultrastructural changes have been described in a few muscles (e.g. 33, 39, 40), and several studies have been done on the fine structure of various fibers in adult muscles (16, 23, 27, 30, 32, 34). No quantitative comparison has been made of the rate of development of physiologically important fiber components in mammalian fast and slow muscles.

The present study was therefore undertaken to

provide a quantitative description and comparison of the developmental changes in the sarcoplasmic reticulum (SR)[1] and transverse tubular system (TTS) of fibers in a fast-twitch muscle (extensor digitorum longus-EDL) and in a slow-twitch muscle (soleus-SOL). Estimates were made of the relative volumes occupied by the SR and TTS, and of the surface area of the TTS, at various stages in development. Such an analysis is required to provide information on the differentiation of these muscles into fast and slow types, and on the changes that occur in physiological performance and in electrical membrane properties during development.

One problem confronting such a study is the heterogeneity of muscle fiber types known to exist in many adult mammalian muscles (9, 16, 17, 31, 36). Various schemes of classification, based mainly on histochemical results, have been put forward. For example: fibers have been classified as "red," "white," and "intermediate," using a test for mitochondrial enzyme (succinic dehydrogenase) activity (23, 36). Edgerton and Simpson (11) have provided a more extensive classification based on tests for both myosin ATPase activity and mitochondrial enzyme activity. Their primary categories are "red," "white," and "intermediate," with a secondary grouping into "slow" and "fast." Ultrastructural differences are also known to exist in the different fiber types (16, 23, 32).

We could not find a previous classification of fibers in mouse SOL and EDL muscles. However, we have studied the contraction speeds of single, directly stimulated surface fibers in the adult muscles (Luff and Atwood, in preparation). The mean twitch-contraction times for EDL and SOL fibers are 6.55 ± 2.2 (SD) msec and 17.05 ± 4.7 (SD) msec, ($P > 0.001$), respectively; there is little overlap in values. Clearly the individual fibers, as well as the whole muscles, differ greatly in contraction speed. We did encounter some intramuscular variation in ultrastructure of fibers in muscles at the older stages of development, especially in Z line thickness and mitochondrial content. However, there was a clear-cut difference between most SOL and EDL fibers in the extent of the SR and TTS. Such differences have been shown in rat SOL and EDL fibers (32). In addition, Z lines in fibers of the EDL were usually about 400 A in thickness (ranging up to 750 A), whereas in fibers of the SOL the Z line thickness was always about 750 A. Since the physiological and ultrastructural observations showed clear differences between the fiber populations of the two muscles, we felt justified in using the fast and slow terminology in comparing the development of the TTS and SR in "typical" fibers of the two muscles.

[1] *Abbreviations used in this article:* EDL, extensor digitorum longus; SOL, soleus; SR, sarcoplasmic reticulum; TTS, transverse tubular system.

MATERIALS AND METHODS

Male albino mice obtained from a colony maintained in the Department of Zoology at the University of Toronto were used throughout the investigation. EDL and SOL muscles were examined at 11 age points: newborn, 2, 3, 5, 7, 10, 15, 20, 30, 40, and 60 days old.

The muscles were dissected and fixed slightly stretched on cork blocks in 6.2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 2 mM $CaCl_2$ (26), washed in buffer with 10% added sucrose, and postfixed in 1% osmium tetroxide with 2 mM $CaCl_2$. In some cases the tissue was stained *en bloc* with uranyl acetate (18). Some muscles were also fixed in the presence of ruthenium red; 0.5 mg/ml of the stain was added to both the glutaraldehyde and osmium fixatives (19, 22).

Longitudinal and transverse sections were cut, mounted, and stained with uranyl acetate and lead citrate (38). The grids were examined in a Philips EM 200 microscope. Measurements were made from micrographs taken at calibrated magnifications.

The volume and surface area of the TTS were calculated by first finding the mean myofibrillar cross-sectional area and circumference at each age for each muscle. Next, the total length of TTS en-

TABLE I

Percentage Incidence of Triads

Age	EDL	SOL
days		
Newborn	16	6
2	12	16
3	31	19
5	38	21
7	44	29
10	50	24
15	68	58
20	71	63
30	90	56
40	93	64
60	91	68

circling all the myofibrils was calculated for unit area of cross-section of the fiber. Account was taken of the failure of the T tubules to fully encircle all the myofibrils, particularly in the younger stages (see Table I). The incidence of triads was determined as described by Eisenberg and Eisenberg (13); in mature SOL fibers the incidence was lower than the 84% reported for frog fibers, while in mature EDL muscles the incidence was higher. From the cross-sectional dimensions of the T tubule, and from the total length of TTS per unit area of fiber cross-section, it was possible to calculate both volume and surface area of the TTS. Peripheral nuclei and mitochondria were excluded from the calculations.

In calculations of SR volume, the mean cross-sectional dimensions of the SR tubules were determined at the A and I band levels for the fibers selected for measurement. Next, the cross-sectional area of the SR at the A and I band levels was found by multiplying the number of SR tubules per unit area of fiber cross-section by the mean cross-sectional area of the SR tubules. The SR volume was found by multiplying the SR area per unit fiber cross-sectional area at the levels of the A band, I band, and terminal cisternae by the fractional sarcomere length for each level (25). Added together, these figures give the total SR volume per sarcomere for unit area of fiber cross-section.

RESULTS

Development of TTS

The typical association between the SR and the T system as found in the adult, and referred to as a triad (28), was observed throughout postnatal development in both SOL and EDL fibers. However, as noted previously in rat muscles (10, 33, 39) the orientation and frequency of occurrence of the T tubules were very different in the newborn animal and the adult (Fig. 1). In the fibers of very young animals both the T tubules and the associated "terminal cisternae" of the SR were oriented longitudinally as well as transversely, whereas in the adult both structures were always oriented transversely, or nearly so (see Fig. 8).

In newborn and 2-day old animals the fibers were small in diameter (5–10 μm) but the contractile apparatus appeared well developed. The sarcoplasm was packed with myofibrils and the nuclei were generally in a peripheral position; numerous mitochondria occurred, both peripherally and between the myofibrils. The T tubules occurred infrequently and appeared to be distributed randomly within the fiber. They were observed to run longitudinally for at least half a sarcomere. They could easily be identified by their appearance and by their relationship with ele-

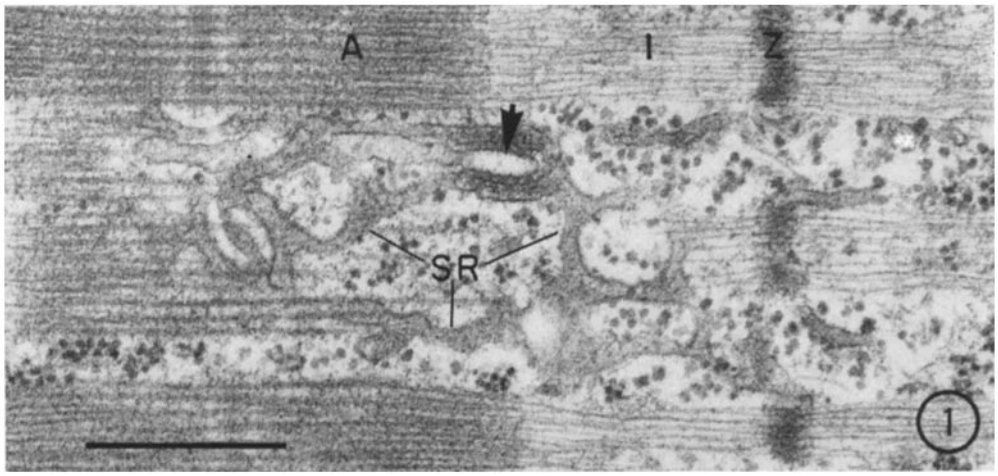


FIGURE 1 Longitudinal section from an EDL muscle of a 2 day old mouse. The arrow indicates a longitudinally oriented T tubule making typical contact with the surrounding sarcoplasmic reticulum (*SR*). The T tubule is well defined with a clear lumen and at this age may be associated with one or more tubules of the SR. The latter have granular contents and are always a fixed distance (115 A) from the T tubule. The "beaded" appearance between the two tubules is quite characteristic. Note also the diffuse nature of the SR. The dark granules are mainly glycogen. *A*, A band; *I*, I band; *Z*, Z line. Bar = 0.5 μm. × 52,500.

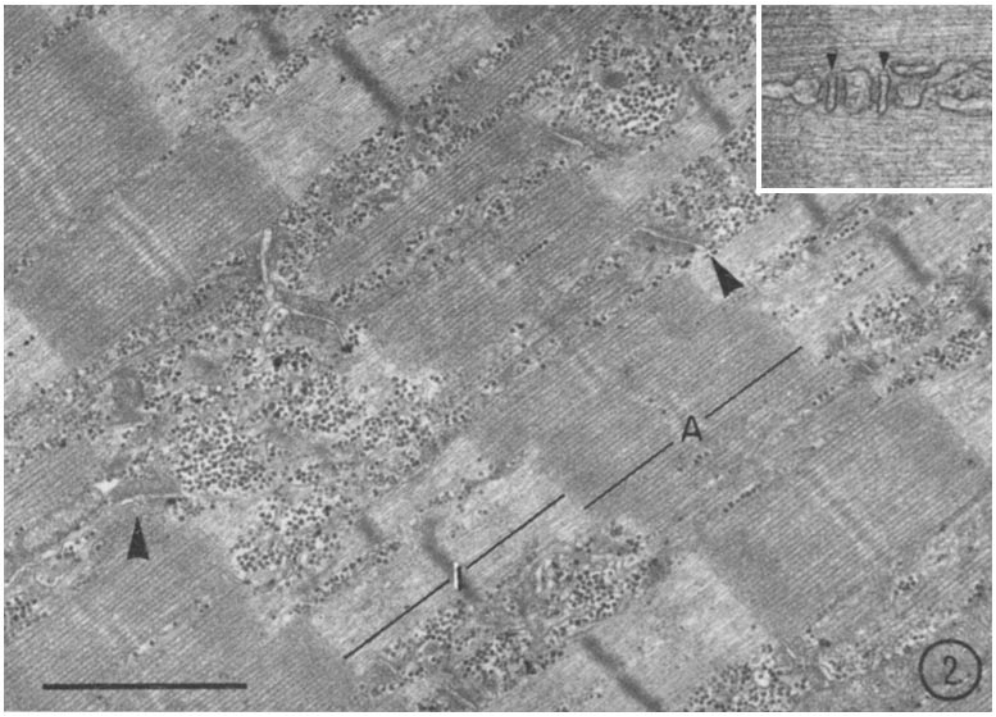


FIGURE 2 Longitudinal section from an EDL of a 7 day old mouse. T tubules occur in various orientations. The arrows indicate one in longitudinal and another in oblique orientation. *A*, A band; *I*, I band. Bar = 1 μm. × 27,000. The *inset* is of a longitudinal section from an EDL at 30 days and shows a pentad, i.e., two T tubules (small arrows) with an element of the SR between them. This arrangement was encountered occasionally. × 52,000.

ments of the SR (Fig. 1). T tubules formed diadic, as well as triadic, contacts with the SR in these early stages (cf. reference 33).

By the age of 7 days (Fig. 2) the T tubules were more plentiful and were oriented in all directions: longitudinally, obliquely, and transversely. During this period the fiber diameter increased, from 6 μm at birth to a mean of 8 μm in the EDL, and from 8 μm at birth to a mean of 13 μm in the SOL.

At 20 days the TTS in both the EDL and SOL fibers had approached the adult condition. In the EDL typical triads occurred, two per sarcomere in nearly every intermyofibrillar space at the level of the A-I boundary (see, for example, Fig. 16). In the SOL fibers, the incidence of triads was lower (see Table I). The cross-sectional size of the T tubule was the same in both muscles, i.e. about 250 A by 1000 A.

Fig. 2 (inset) shows a "pentad" (cf. references 25, 30), comprising two T tubules in association with three SR elements. This doubling up of the T tubules was not uncommon, though it was only apparent in the older stages where the T system was more highly developed.

Fig. 3 shows the calculated volume of the TTS for fibers of the EDL and SOL. At birth, TTS volume was 0.04% of fiber volume in the SOL and 0.08% in the EDL. These values increased considerably during the 15 days after birth. The adult value (0.22% of fiber volume) was attained between 15 and 20 days of age in the SOL. In the EDL, the TTS volume increased to an adult value of 0.40% at 30 days of age.

The surface area of the TTS was calculated as a ratio of fiber surface area for a 30 μm diameter fiber (Fig. 4). The 30 μm value is close to the mean fiber diameter for both the EDL and SOL in the adult animal, although it is important to note that considerable variation in fiber diameter occurs in both muscles (21, 32).

In SOL fibers, the ratio of TTS surface area to unit fiber surface area rose from 0.15 cm^2/cm^2 in the newborn mouse to 1.80 cm^2/cm^2 in the adult. In the EDL fibers this ratio increased from

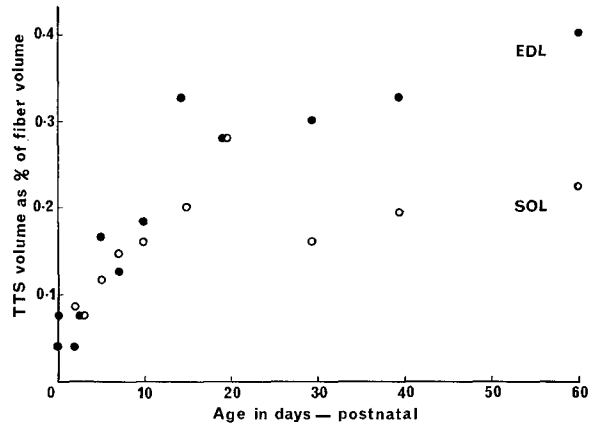


FIGURE 3 The TTS volume expressed as a percentage of fiber volume is plotted against the age of the animal in days. Measurements for the EDL are shown by the filled circles, and measurements for the SOL by the open circles. Note that the data points for the SOL attain a "plateau" more rapidly than those for the EDL.

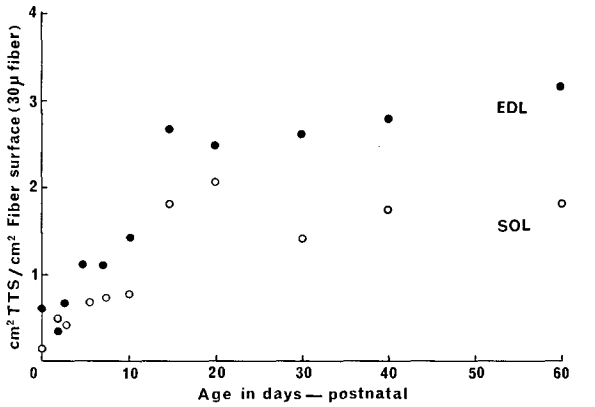


FIGURE 4 The ratio of TTS surface area to fiber surface area for a 30 μm fiber is plotted against age of the animal in days. As in Fig. 3, the filled circles are for the EDL and the open circles are for the SOL.

0.60 cm^2/cm^2 at birth to 3.10 cm^2/cm^2 in the adult. Both EDL and SOL fibers appeared to attain the adult values by 20 days of age, or slightly later. The increase in the ratio resulted mainly from the development of the T system around the myofibrils at the A-I boundary. The difference in TTS surface area between fibers of the two muscles was due to the less complete occurrence of the TTS in the SOL fibers. There was approximately 90% triad occurrence in the EDL of the adult but only about 60% triad occurrence in the SOL (Table I).

Further statistical analysis of the differences in TTS volume and surface area was carried out using *t* tests, since both muscles were somewhat heterogeneous in fiber type. There is no significance difference in values for SOL and EDL muscles of the mice in the 0–10 day old group. For mice in the 10–60 day old group, a paired *t* test showed a significant difference (0.1% level) between the SOL and EDL sets of data. Thus, in spite of fiber heterogeneity, there is a clear difference in the TTS of sampled fibers in the two muscles.

Development of the SR

The development of the SR in mouse EDL and SOL muscles was qualitatively similar to that described in rat psoas muscle (33). However, there were important quantitative differences between mouse EDL and SOL muscle.

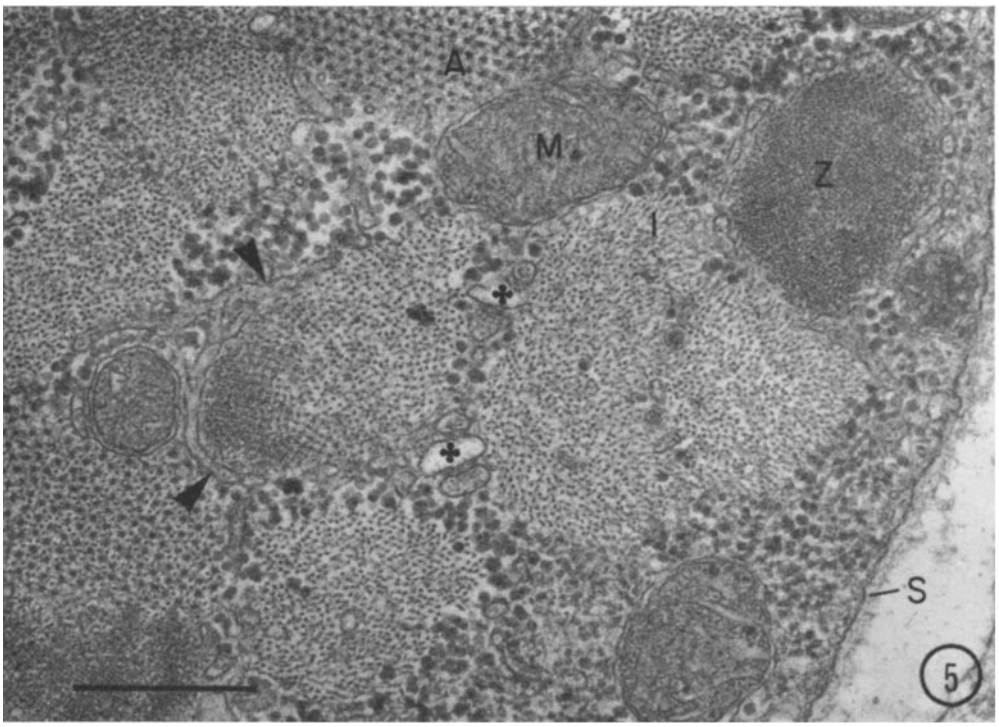


FIGURE 5 Transverse section of SOL fiber from a 2 day old mouse. Note the encircling band of SR (arrows) at the level of the Z line (*Z*). *A*, A band; *I*, I band; *S*, sarcolemma; *M*, mitochondrion. Two longitudinally oriented T tubules (stars) in typical triad formation arrear in the center. Bar = 0.5 μm. × 59,400.

In the EDL and SOL fibers of newborn and very young mice the SR forms a random interconnecting network of tubules around and along the myofibrils, common to the A and I band alike. Fig. 1 shows this in face view. The SR tubules form a single layer between the myofibrils (Fig. 5). A similar formation of the SR was found by Shimada, Fischman, and Moscona (35) and by Ezerman and Ishikawa (14) in cultured chick skeletal muscles. The only specialization of the SR noted at this stage was the occurrence of tubular "collars" around the myofibrils at the level of the Z line (Fig. 5), similar to the association between the SR and Z line described by Allen and Pepe (1) and by Walker, Schrodt, and Bingham (40) in neonatal rat muscle. The latter authors also claimed that there were actual connections between the SR and the Z line.

From birth up to the age of 20 days there was a considerable increase in the amount of sarcoplasmic reticulum, particularly at the level of the I band. Fig. 6 shows the SR in a fiber of the EDL at the age of 7 days. An increase in the amount o SR over that at 2 days is evident. The beginnings of a fenestrated collar of SR can be seen at the level of the H band.

Fig. 7 shows the SR volume calculated as a percentage of total fiber volume at different ages. The total change was less in SOL fibers than in EDL fibers. The volume of SR increased from 1.7% at birth to 2.9% in the adult SOL fibers and from 1.1% at birth to an adult value of 5.5% in EDL fibers.

In the SOL fibers the adult value of the SR volume was reached by the age of 30 days, while in the EDL fibers the SR volume appeared to increase throughout the period of investigation. However, it is considered that the value at 60 days represents the adult value, as examination of fibers from an 80 day old animal yielded values similar to those at 60 days.

Statistical comparison (by *t* test) of SOL and EDL values for SR volume in adult (60 day old) and neonatal (0–2 day old) mice showed a highly

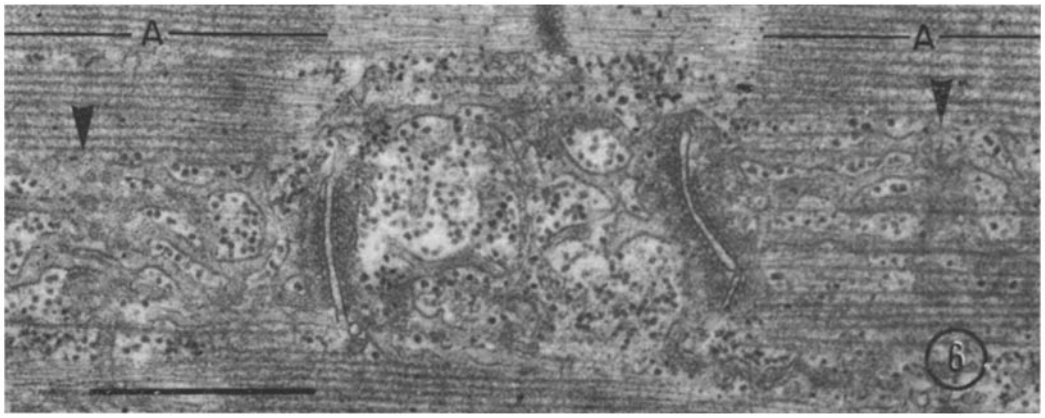


FIGURE 6 Longitudinal section of an EDL fiber from an 8 day old mouse. Arrows indicate an early stage in the development of the fenestrated collar in the middle of the A band (*A*). Note also the general increase in amount of SR and also the higher incidence of T tubules in comparison with muscles of younger animals (Fig. 1). Bar = 1 μm. × 29,100.

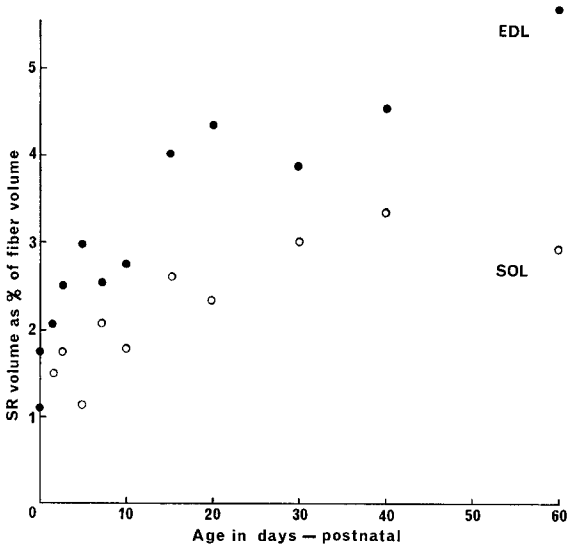


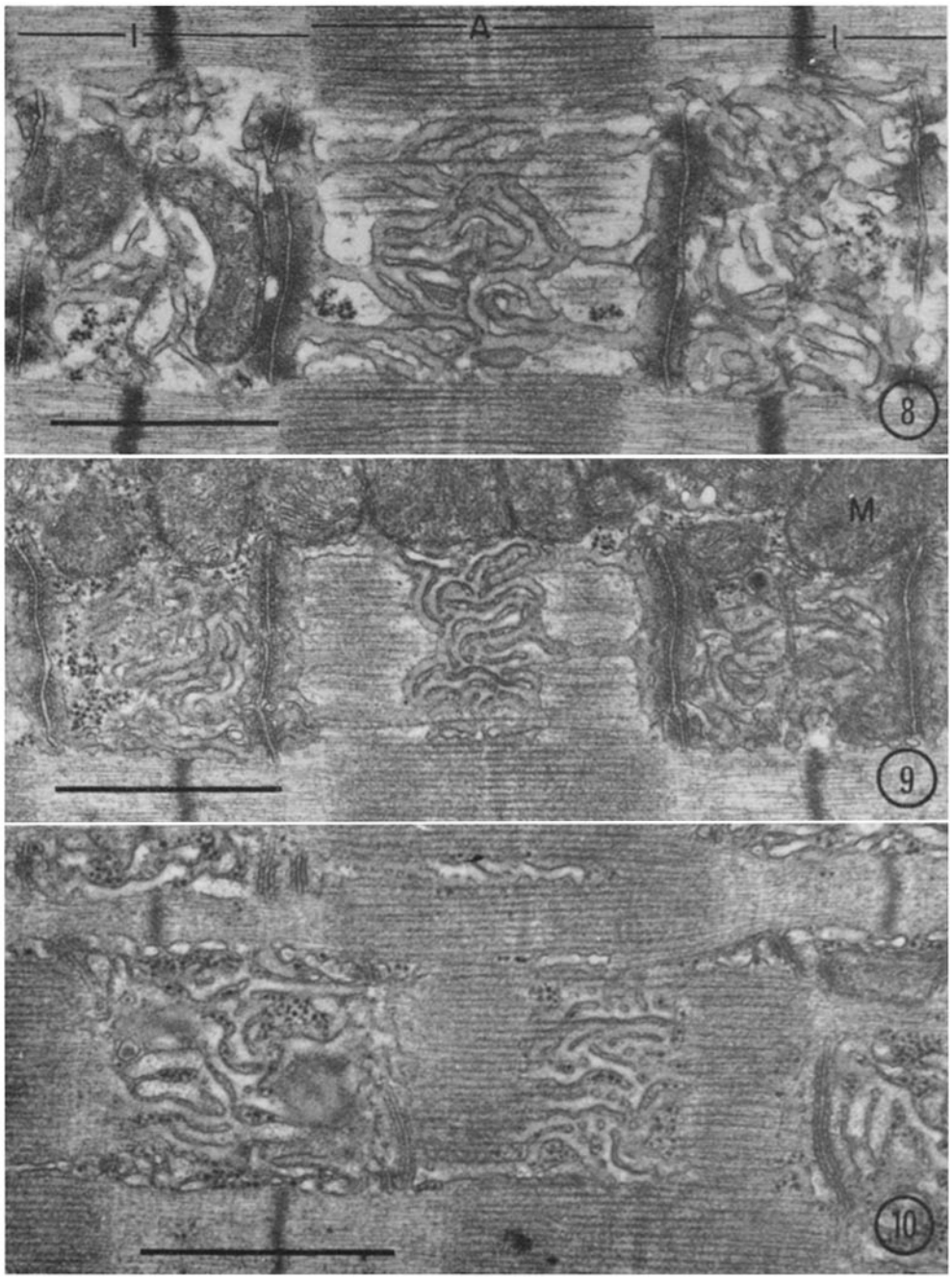
FIGURE 7 SR volume expressed as a percentage of fiber volume and plotted against age of the animal in days. Filled circles are for the EDL; open circles are for the SOL. Note the earlier plateau in the SOL curve.

significant difference (0.1% level) between adult values, and no significant difference between neonatal values. Also, adult values differed significantly from neonatal values (1% level) in both SOL and EDL muscles. Thus, sampled SOL and EDL fibers differ in SR volume in adult muscles, even though intramuscular heterogeneity of fiber type was observed (see Figs. 8, 9, and 10).

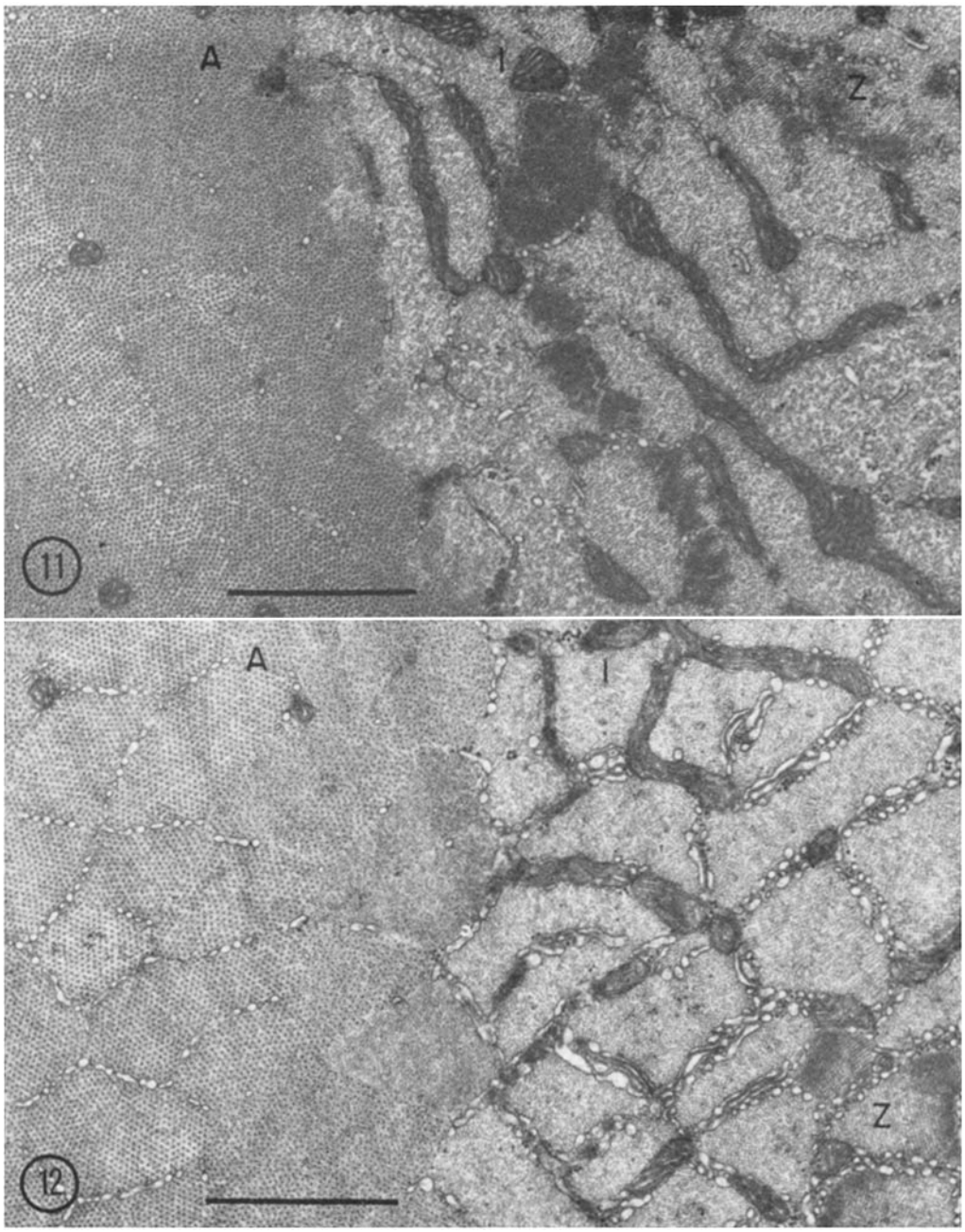
SR in Adult Muscles

The extensive proliferation and penetration of the T system during the first 15 days has the effect of dividing the SR at the level of the A-I boundary into an I band portion and an A band portion.

In the adult the organization of the SR in the I band portion is similar to that observed in the early stages of development (Figs. 1 and 6). The



FIGURES 8, 9, and 10 Longitudinal sections of EDL fibers from a 30 day old mouse. These all show the SR in face view. Note the convoluted formation of the SR tubules at the I band level (*I*) and the "collar" of SR in the center of the A band (*A*), with a few longitudinal connections to the terminal cisternae at the ends of the A bands. The SR in Fig. 8 has suffered some swelling during fixation. Many more circular fenestrations occur in the A band collar in Fig. 10 than in Fig. 8. Also, it is interesting to note that the Z line is thickest in Fig. 8 and thinnest in Fig. 10. Fig. 9 is intermediate in both features. *M*, mitochondrion. Bar = 1 μm. × 31,200 for Figs. 8 and 9, × 34,900 for Fig. 10.



FIGURES 11 and 12 Slightly oblique transverse sections from fibers of SOL and EDL, respectively (30 day old mouse). In both figures the section passes from the middle of the A band (*A*) through the I band (*I*) and into the Z line (*Z*). This clearly demonstrates the difference in amount of SR between the two muscles. There is very little SR in the region of overlap of the thick and thin filaments. Bar = 1 μm. × 26,500.

SR forms a sleeve of loosely interconnecting tubules, some of which are oriented longitudinally, around the myofibril.

In the A band portion of the adult fibers the form of the SR differs from that in the immature stages (Figs. 8, 9, and 10). In the H zone the SR forms a single-layered fenestrated sheet. Towards the ends of the A band, the SR is reduced to a few longitudinally oriented tubules connecting the central region to the terminal cisternae. The fenestrated sheet or collar at the H zone was first observed in the sartorius muscle of the rat (28) and later in the mouse (2) and the gastrocnemius of the rat (37).

The regional distribution of the SR can be seen best in Figs. 11 and 12. These are slightly oblique transverse sections of fibers from the SOL (Fig. 11) and EDL (Fig. 12) of a 40 day old mouse. It is possible to observe the profile of the fiber from the Z line to the center of the A band. Within the I bands of both fibers the SR is multilayered, and more abundant than in the early stages. At the I band and Z line levels there are only two layers of SR in the SOL fiber (Fig. 11), compared with two to four layers in the EDL fiber (Fig. 12). Also, relatively less SR appears in the A band region of the SOL fiber.

Differences in amount of SR in other fast and slow mammalian muscles have been noted by previous authors (27, 34).

Peachey (25), in a detailed examination of the organization of the SR and T system in fibers of the frog's sartorius muscle, showed occasional instances of longitudinal connections of the SR around the T tubule. A similar feature was observed in mouse muscle fibers (Fig. 13). In the young stages where the triads were often longitudinally oriented, the SR was continuous across the A-I boundary. However, in the adult where the triads were transversely oriented, longitudinal connections between the A and I systems of the SR were still observed on many occasions. As Peachey (25) pointed out, this would make the SR continuous from one sarcomere to the next

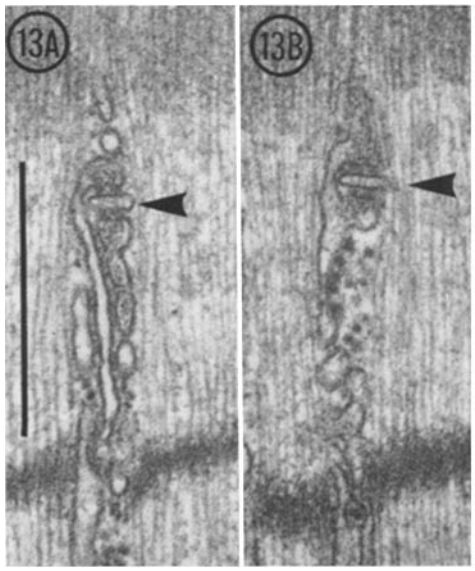


FIGURE 13 Longitudinal sections from an EDL of a 30 day old mouse. This shows two examples of SR continuity around the T tubule (arrows). In A, a separate SR tubule runs from the I region around the T tubule to join the terminal cisternae of the next sarcomere. In B, the SR is continuous between the terminal cisternae. Bar = 0.5 μm. × 72,600.

Sarcolemmal Invaginations

In longitudinal sections of ruthenium red–treated material which cut the sarcolemma tangentially, lobed invaginations of the sarcolemma were noticed (Fig. 14). While these invaginations appeared to be distributed randomly on the surface of the fiber, it was considered that they might be connected in some way with the T system. Fig. 15 shows a T tubule connecting directly with one of the sarcolemmal invaginations. This example is from a ruthenium red–treated muscle, in which the stain penetrated only a short distance from the surface of the fiber. Several other T tubules were also observed to connect with sarcolemmal invaginations. In some cases the T tubule was observed to run obliquely or longitudinally just before it reached the A-I region of a surface myofibril, at which point it became transversely oriented (Fig. 16).

These observations are similar to those of Rayns et al. (29) who studied surface features of guinea pig skeletal muscle by freeze-etching techniques and by use of lanthanum as an extracellular marker. They reported that T tubules approaching the surface of the muscle often change orientation, and terminate in subsarcolemmal "caveolae," which are apparently the same as the invaginations seen in mouse muscles in the present study.

Z Line

At the age of 7 days the mean thickness of the Z line in fibers of the EDL and SOL was similar:

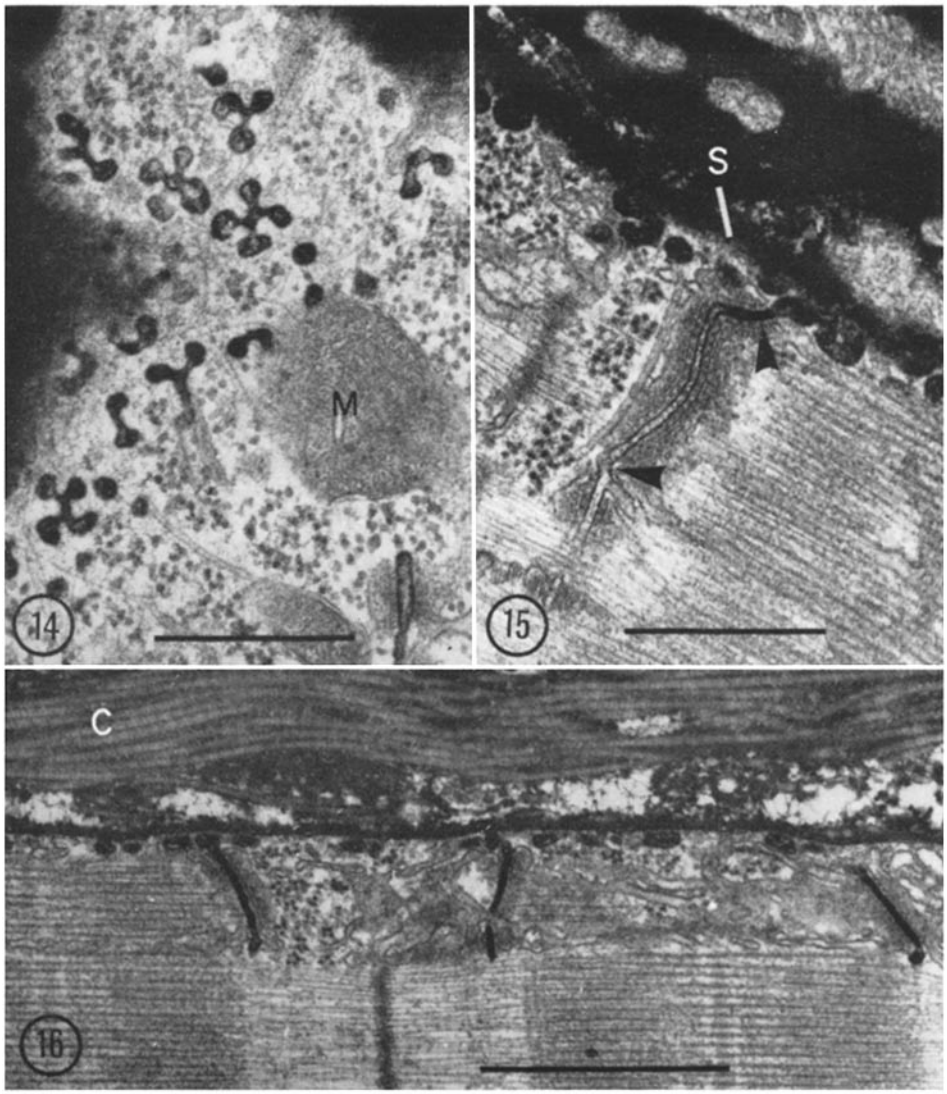


FIGURE 14 Longitudinal section cutting the surface of a muscle fiber tangentially, and showing lobed sarcolemmal invaginations, which appear dark due to ruthenium red treatment. EDL of 30 day old mouse. *M*, mitochondrion. Bar = 0.5 μm. × 53,200.

FIGURE 15 Longitudinal section of ruthenium red–treated EDL of a 30 day old mouse. Although only partially stained, a T tubule (arrows) is shown connecting directly with a sarcolemmal invagination. *S*, sarcolemma. Bar = 0.5 μm. × 53,200.

FIGURE 16 Longitudinal section of a ruthenium red–treated EDL from a 30 day old mouse. The sarcolemma and three T tubules are stained, and two of the T tubules are connected with sarcolemmal invaginations. Note how the T tubules do not run through to the surface at right angles but go in various directions near the surface. *C*, collagen fibers. Bar = 1 μm. × 33,400.

740 A and 790 A, respectively. By the age of 30 days the mean value for the EDL fibers had fallen to 370 A (range, 295–750 A), compared to 740 A (range, 650–820 A) in the SOL fibers.

It was found that the Z line thickness in the SOL fibers remained reasonably constant during postnatal development, whereas in the majority of EDL fibers it was reduced considerably between the ages of 10 and 15 days. However, variation in Z line thickness appeared in fibers of adult muscles, as shown in Figs. 8, 9, and 10. This was particularly true of the EDL muscle. Variation in Z line thickness was one of the more important sources of fiber heterogeneity. This feature has been discussed by other authors (16, 32).

Mitochondria

The mitochondria occurring between the myofibrils in the fibers of both the EDL and SOL muscles in the adult were often found to have a strap-like appearance. They were usually restricted to a position either side of the Z line within the I band, and were rarely observed to run continuously across the Z line. Mitochondria 4 μm and 5 μm long were observed twisting between the myofibrils at right angles to the longitudinal axis of the fiber (Fig. 11); they were seen occasionally to branch. Mitochondria of this form occurred in significant numbers relatively late in development. Some elongation of mitochondria in a transverse direction was first noted in the SOL at 10 days of age and in the EDL at 15 days. Up to this point they always appeared circular or oval when the fiber was cut in transverse section. In the SOL fibers, significant extension of the mitochondria in the transverse direction was noted by the age of 20 days, but in the EDL fibers this was not obvious until 40 days of age. Such strap-like mitochondria have been reported by several authors (2, 5, 28, 34).

Variation in mitochondrial content occurred in fibers of mouse EDL muscles, as in rat EDL (32). There was less variation among SOL fibers.

Fractional Volume Changes

Fig. 17 shows the various fiber components expressed as a proportion of total fiber volume of the EDL and SOL fibers of newborn and 60-day old mice. Development proceeds by a relative displacement of the sarcoplasmic volume and the mitochondria by the myofibrils and, to a limited extent, by the SR (see also reference 33). At birth, the myofibrillar volume was greater and the sarcoplasmic volume less in the EDL than in the SOL, whereas at 60 days of age the situation was reversed.

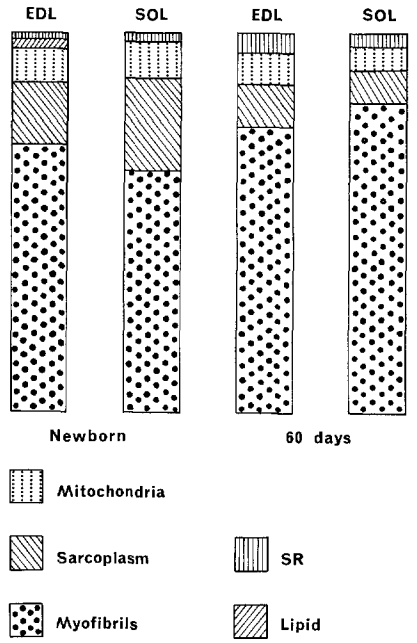


FIGURE 17 Summary of the relative volumes of the various components of EDL and SOL fibers from newborn and 60-day old animals.

DISCUSSION

In the SOL and EDL muscles of the mouse at birth the fibers, although small in diameter, were packed with well-differentiated myofibrils. However, SR was poorly developed and the TTS was sparse and rudimentary. There was little difference between SOL and EDL fibers at this stage. Within the succeeding 15 days both membranous components developed almost to the adult level. In adult muscles the SOL fibers contained significantly less SR and TTS than the EDL, although the development of these components to the adult condition was faster in the SOL. These observations support the idea that the fast EDL fibers are more highly differentiated than slow SOL fibers.

The SR has been shown to arise in the first instance from rough endoplasmic reticulum which is plentiful during the very early development of the fiber (8, 14). After birth, when a rapid increase in the amount of SR occurred, there was very little if any rough endoplasmic reticulum within

the fibers. Presumably at this stage the SR arises by direct synthesis.

The underdeveloped nature of the TTS in muscles of young animals may adversely affect their physiological performance. Possibly the spread of excitation throughout the fiber would be less efficient than in the adult muscle, with contractile activity restricted to superficial myofibrils and those close to a triad. However, since these fibers are small it is also conceivable that substantial activation of the contractile material could occur without a well-developed TTS, as argued by Peachey (24) for small muscle fibers of *Amphioxus*.

The less-developed TTS of SOL fibers might contribute to the slower speed of contraction of this muscle in comparison with the EDL.

It was pointed out by A. F. Huxley (20) that there is a possible relationship between the density of SR and a muscle's speed of shortening. This relationship might explain in part the different contraction speeds of EDL and SOL fibers. Comparative evidence in support of this argument can be derived from the cricothyroid muscle of the bat (30) which is an extremely fast-acting muscle and which contains considerably more SR than any other mammalian muscle examined so far. The same argument could be applied to changes in contraction speed of the muscles during early development (6, 7).

Recent evidence by Barany (3) tends to contradict this argument. He has shown that the myosin-ATPase activity of the EDL of the mouse is approximately twice that of the SOL. He considers that the different speeds of contraction are a characteristic of the ATPase activity of the myosin in any given muscle. However, Edgerton and Simpson (11) argue that myosin ATPase can only be a gross indicator of the contraction time of a fiber. It is likely that both the mysoin-ATPase activity and the amount of SR affect the speed of contraction of a muscle. In many mammalian fibers the two features are probably correlated.

It is interesting to note in this context that if the increase in the amount of SR as shown in Fig. 7 is compared to the changes in the EDL and SOL of the rat given by Close (7), the time courses of the changes are roughly parallel. In both cases the changes are virtually complete by the age of 20 days. The morphological and physiological changes are very likely related. Unfortunately, to our knowledge, changes in myosin-ATPase activity in the fibers of developing EDL and SOL muscles of the mouse have not been described.

Falk and Fatt (15) attributed the relatively high values for membrane capacitance of skeletal muscle fibers to the TTS, which does not occur in nerve fibers. On this basis it would be expected that the capacitance resulting from the TTS would be greater in the EDL fibers than in the SOL fibers. Similarly, the contribution of the TTS to the membrane capacitance would be expected to increase during development, assuming that the nature of the T tuble membrane remains the same. Recently, we have measured total membrane capacitance in mouse EDL and SOL muscle fibers, and have found a mean value of 5.3 $\mu F/cm^2$ for EDL fibers, compared with 3.1 $\mu F/cm^2$ for SOL fibers (Luff and Atwood, unpublished).

The difference between these values correlates with the difference in surface area of the TTS measured in the present study. Thus, it is likely that the EDL and SOL fibers have different membrane electrical properties by virtue of the quantitative differences in TTS and SR.

One point which was not explored in the present study concerns the relationship between fiber type (as determined by myosin-ATPase activity and mitochondrial enzyme activity) and the TTS and SR volumes. Barnard et al. (4) and Edgerton and Simpson (11, 12) have shown that red and white fibers of many muscles (including rat EDL) have relatively high myosin-ATPase activity and are probably fast twitch, whereas fibers classified as intermediate (such as those of the guinea pig soleus muscle, and about 4% of those in the rat EDL) have relatively low myosin-ATPase activity and are probably slow twitch. At present, we do not know the fiber type composition of the mouse EDL and SOL muscles. However, if these muscles are similar to those of the rat, we might expect a preponderance of red and white fast muscle fibers in the EDL, and of intermediate slow fibers in the SOL. Some ultrastructural features, such as Z line thickness and mitochondrial content are correlated with the red, white, and intermediate appearance of the muscle fibers in some cases (16, 23). Fibers of the mouse EDL showed variations in Z line thickness known to be characteristic of red and white fast fibers. However, both mitochondria-rich fibers with thick Z lines and mitochondria-poor fibers with thin Z lines had relatively well-developed SR and T system, as in the rat EDL (32). This provides some justification for

lumping the results obtained from randomly sampled fibers, but it would still be of interest to follow the differentiation of red, white, and intermediate fibers of the same animal.

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