RELATIONS BETWEEN STRUCTURE AND FUNCTION IN RAT SKELETAL MUSCLE FIBERS

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ABSTRACT

The fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus muscle of the rat consist of heterogeneous fiber populations. EDL muscle fibers differ in size, mitochondrial content, myoglobin concentration, and thickness of the Z line. The sarcoplasmic reticulum, on the other hand, is richly developed in all fibers, with only small variation. Myofibrils are clearly circumscribed at both the A and I band level. The soleus muscle is composed primarily of fibers with moderate mitochondrial content and myoglobin concentration. In most fibers the sarcoplasmic reticulum is poorly developed, with the exception of the portion of reticulum in phase with the Z line. As a consequence the myofibrillar fields are amply fused together. Contacts between sarcoplasmic reticulum and T system are discontinuous and may occur in the form of "dyads" instead of the typical triad structure. In a small proportion of soleus muscle fibers the organization and development of the sarcoplasmic reticulum is similar to that of EDL muscle fibers, with prominent fenestrated collars at the H band level. In these fibers mitochondria are larger and more abundant. The results are correlated with physiological studies on motor units in the same and in similar rat muscles. It is suggested that the variable structural pattern of rat muscle fibers is related to two distinct physiological parameters, speed of contraction and resistance to fatigue.

INTRODUCTION

Structural and enzymatic differences among mammalian skeletal muscle fibers have been demonstrated by electron microscopy and with histochemical techniques (24, 8, 28, 9, 27, 26). The functional significance of these findings is, however, not yet clear; conflicting interpretations have been reported and difficulties have been experienced in attempting to correlate ultrastructural and histochemical features with functional properties (cf. 13, 8, 11, 12). The lack of a comprehensive picture which integrates the various data is also reflected in the variety of classification systems of fiber types, based on different physiological, morphological, or histochemical criteria whose mutual relations have not yet been defined (cf. 19 and 10).

These difficulties may be overcome by using more direct approaches to the study of structural and functional relations. Although physiological studies on isolated muscle fibers have not been performed in mammals, the contraction properties of the motor units comprising several mammalian muscles have been investigated in detail (e.g. 13, 18, 3). In addition, the homogeneity of motor units with regard to histochemical type of fiber has been demonstrated, and important correlations have been established between histochemical characteristics and physiological properties of the motor

units (5). In the light of these studies, we have reexamined in rat skeletal muscle the relations between histochemical pattern and ultrastructural features of the various types of fibers and functional properties of the motor units, using fast and slow muscles which have been previously well characterized physiologically with respect to motor unit composition.

MATERIALS AND METHODS

Extensor digitorum longus (EDL) and soleus muscles from 2-month old Wistar rats were used. Succinate dehydrogenase activity was demonstrated on fresh-frozen sections with nitro-blue tetrazolium (17). Myoglobin was demonstrated with benzidine in tissue fixed in glutaraldehyde (14). For electron microscopy the muscles were fixed at rest length *in situ* in 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, with 2 mM calcium chloride. Small bundles of fibers were separated during subsequent washing in buffer with 0.2 M sucrose and postfixed in 1% OsO_4 in phosphate or cacodylate buffer. Most specimens were stained en bloc with 0.5% uranyl acetate in aqueous solutions before dehydration (7) or in absolute alcohol during dehydration. Other muscles were treated with 5% sodium tungstate in 0.2 M sucrose after fixation in glutaraldehyde, without subsequent osmication and uranyl acetate staining en bloc. This was found to result in better preservation of myofibrillar components, especially of the Z line. Embedding was done in Epon. Sections were cut on an LKB microtome (LKB Instruments, Inc., Rockville, Md.) with glass knives, mounted on uncoated grids, and stained with uranyl acetate and/or lead citrate. Occasionally, longitudinal and transverse sections were cut consecutively from the same bloc in such a way that the face of the pyramid trimmed for longitudinal cutting became one side of a new pyramid oriented for cross-sectioning. Sections were then collected on Formvar-coated, single-hole grids. The fibers seen in a longitudinal section could thus be easily identified as incomplete fibers at the edge of the consecutive transverse section (see Fig. 14). Specimens were examined in a Siemens Elmiskop 1A microscope.

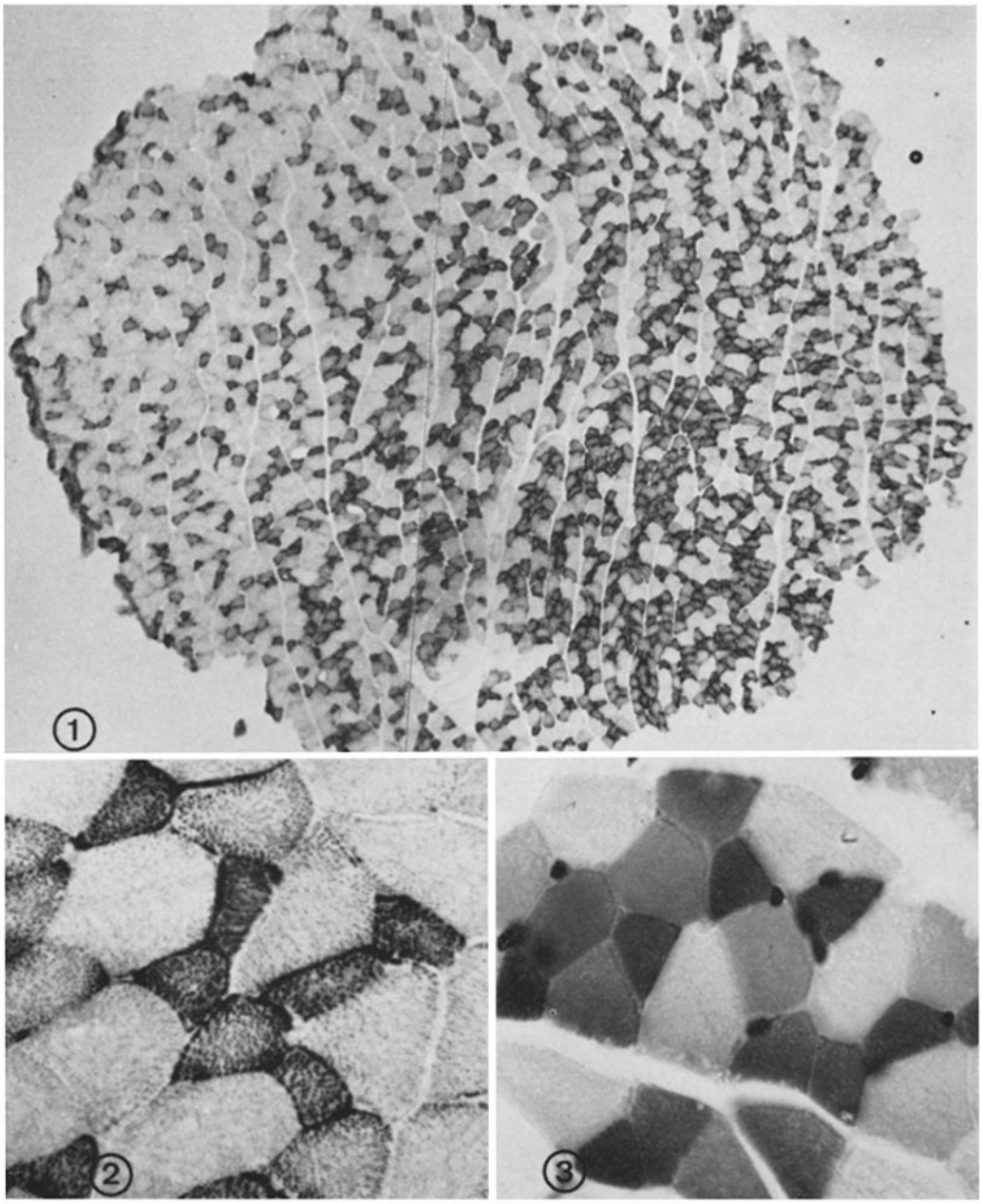
RESULTS

Extensor Digitorum Longus

The histochemical pattern after staining for succinate dehydrogenase activity in EDL is illustrated in Figs. 1 and 2. Large fibers with very low activity, small fibers with intense activity, and a spectrum of intermediate forms are variably mixed, with limited grouping. A comparable pattern of staining is seen in sections incubated for demonstration of myoglobin (Fig. 3). The distribution of the different fibers is not uniform throughout the muscle: mitochondria-rich fibers occur in greater concentration in the deep medial areas and towards the proximal head. At the mid-belly the percentage of large mitochondria-poor fibers is about 42% of the population. The various forms of mitochondria-rich fibers comprise about 58%, the smallest fibers with highest activity accounting for almost half of this value.

A corresponding pattern of fiber types is observed with the electron microscope. The variable mitochondrial content is the most prominent differential feature among the various fibers (Figs. 4, 5). The mitochondrial volume, as measured by point-counting analysis (29) on cross-sections of complete fibers, ranged from less than 5% to more than 25% of the total fiber volume. The variation in volume was continuous and does not suggest the existence of an intermediate type as a discrete class of fibers. The size, shape, and pattern of disposition of mitochondria in the different fibers are similar to those described in analogous fibers of the rat diaphragm and semitendinosus muscles (8, 9). The sarcomere organization is correspondingly variable (Fig. 6). Small mitochondria-rich fibers have a much thicker Z line than large fibers (over 1000 A vs. about 550 A, with intermediate values for intermediate fiber types). In addition, the sarcomeres are slightly longer and the M line is wider and less prominent in mitochondria-rich fibers.

In transverse sections (Fig. 5) it is apparent that in all EDL muscle fibers, independent of the variable mitochondrial content, the sarcoplasmic reticulum forms an extensive network surrounding the myofibrils at both the A and I band level. At the mid-A band the longitudinal tubules are fused into fenestrated collars (Fig. 7 *a*). In addition, perforated sacs are frequently observed at the edges of the A band (Figs. 7 *a–c*). Small variations in the form of the sarcoplasmic reticulum, similar to those described by Gauthier in the semitendinosus muscle (9), are found in the mitochondria-rich fibers at the H band level (Fig. 7 *d*). At the I band, the sarcoplasmic reticulum consists of tightly meshed networks of tubules and flattened sacs extending as single or double layers from the junctional cisternae to the Z line, in close association with glycogen masses (Figs. 6 and 7). Longitudinal prolongations of the junctional cisternae



FIGURES 1 and 2 EDL, succinate dehydrogenase. Fig. 1 is a transverse section through the mid-belly of the whole muscle and illustrates the heterogeneous fiber composition of the muscle and the uneven distribution of the different fibers. In Fig. 2 a small field is seen at higher magnification. The intensity of the reaction varies inversely as the size of the fibers. Fig. 1, × 55; Fig. 2, × 225.

FIGURE 3 EDL, myoglobin. Various grades of staining intensity are seen in the different fibers, with a pattern corresponding to that shown in Fig. 2. Red blood cells are also strongly stained. × 225.

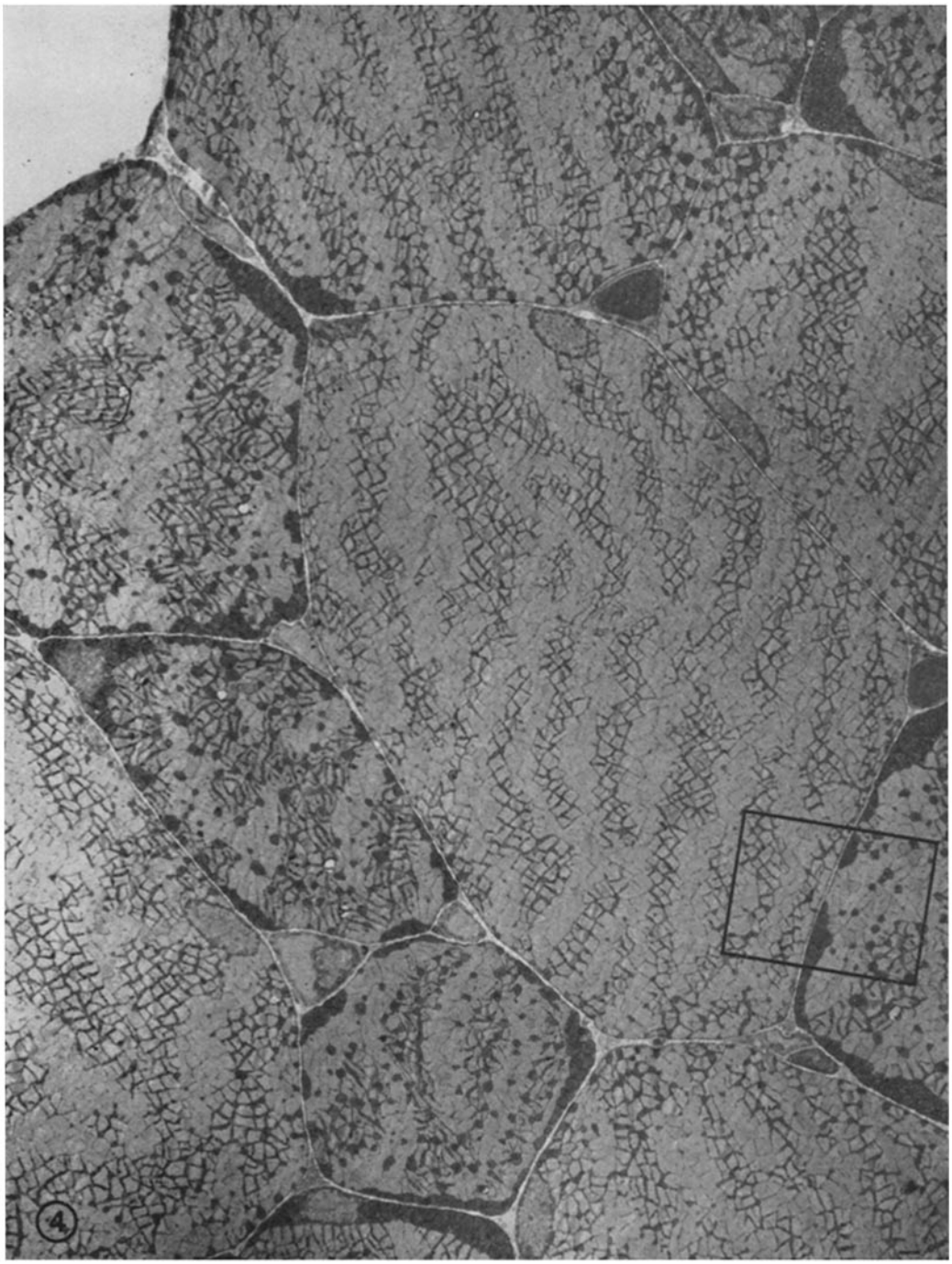


FIGURE 4 EDL. Low-power electron micrograph of a transverse section. The figure illustrates the diversity in fiber size and in the quantity and distribution of mitochondria among the different fibers. Conspicuous aggregates of large mitochondria are seen at the cell periphery in the small fibers. Round mitochondria, corresponding to cross-sectioned profiles of longitudinally oriented mitochondria, are also prominent among the fibrils in the same fibers. The area enclosed in the rectangle is shown at higher magnification in Fig. 5. Scale marker = 1 μ. $\times$ 3000.

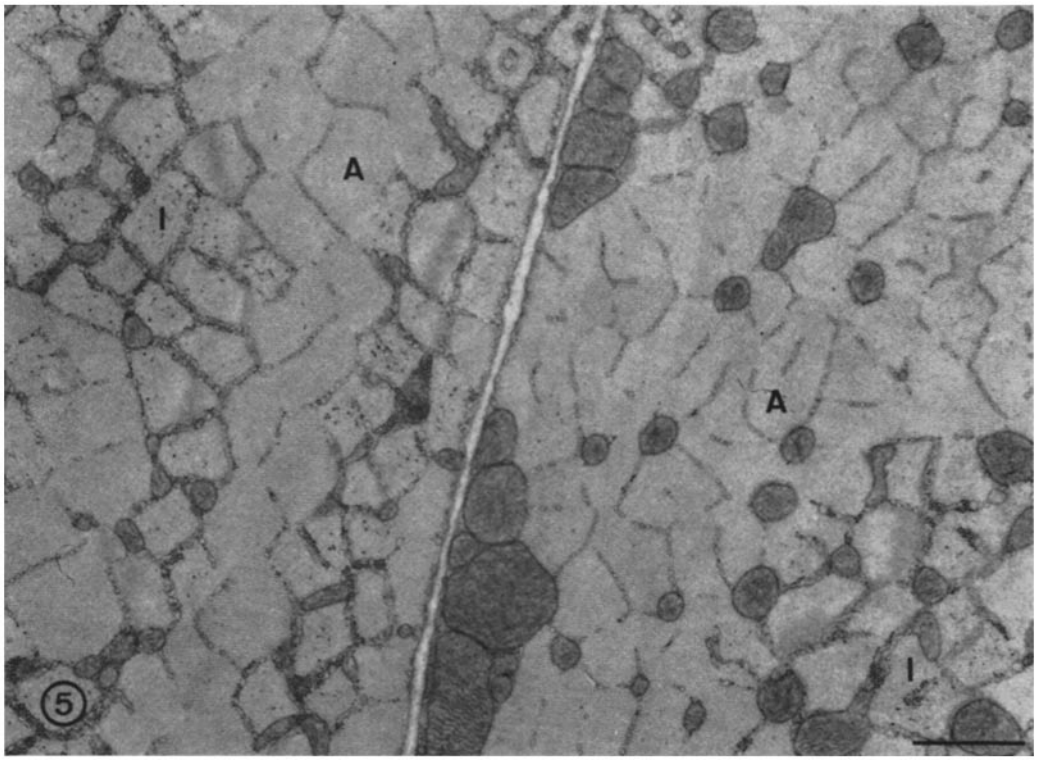


FIGURE 5 EDL. Higher magnification of the marked area of Fig. 4, showing portions of two adjacent fibers with different mitochondrial content. The sarcoplasmic reticulum appears to be richly developed in both types of fibers. At the *I* band, single or double layers of sarcoplasmic reticulum flanked by glycogen deposits encircle regularly the myofibrils. At the *A* band level, the fibrils are outlined by extensive rows of vesicular profiles, corresponding to cross-sectioned profiles of the fenestrated collars. Scale marker = 1 μ. × 15,000.

filled with characteristic granular material are also frequently seen (Figs. 7 *b* and *e*).

Soleus

The soleus muscle is composed primarily of fibers with moderate succinate dehydrogenase activity and of a small percentage of fibers (about 20–25%) with a more intense reaction (Fig. 8 *a*). These different fibers are randomly mixed in all parts of the muscle, without preferential distribution. A correspondent pattern of staining is revealed with the histochemical method for the demonstration of myoglobin (Fig. 8 *b*).

The fine structure of the fiber type most commonly encountered in soleus is illustrated in Figs. 9–12. The general organization of the sarcotubular system is similar to that of EDL, but the sarcoplasmic reticulum is much less developed. T tubules are often flanked only along one side by junctional cisternae, so that contacts occur not infrequently in the form of "dyads" instead of the familiar triad structure (Fig. 12). Sparse longitudinal tubules extend from the junctional cisternae into the A band, with limited branching at the H band level (Figs. 9, 10, 12). As a consequence, the contractile mass is not subdivided into clearly circumscribed fibrils. At the level of the Z line, by contrast, extensive fenestrated collars are disposed as continuous single or double sheets around the myofibrils (Figs. 9, 11). In most soleus muscle fibers the mitochondrial content is comparable to that found in EDL muscle fibers of intermediate size, but the distribution of mitochondria differs somewhat. The long, thin mitochondria running transversely at both sides of the Z line represent the predominant form in the soleus (Fig. 9). They are extensively branched and form complex networks surrounding the fibrils. Thin

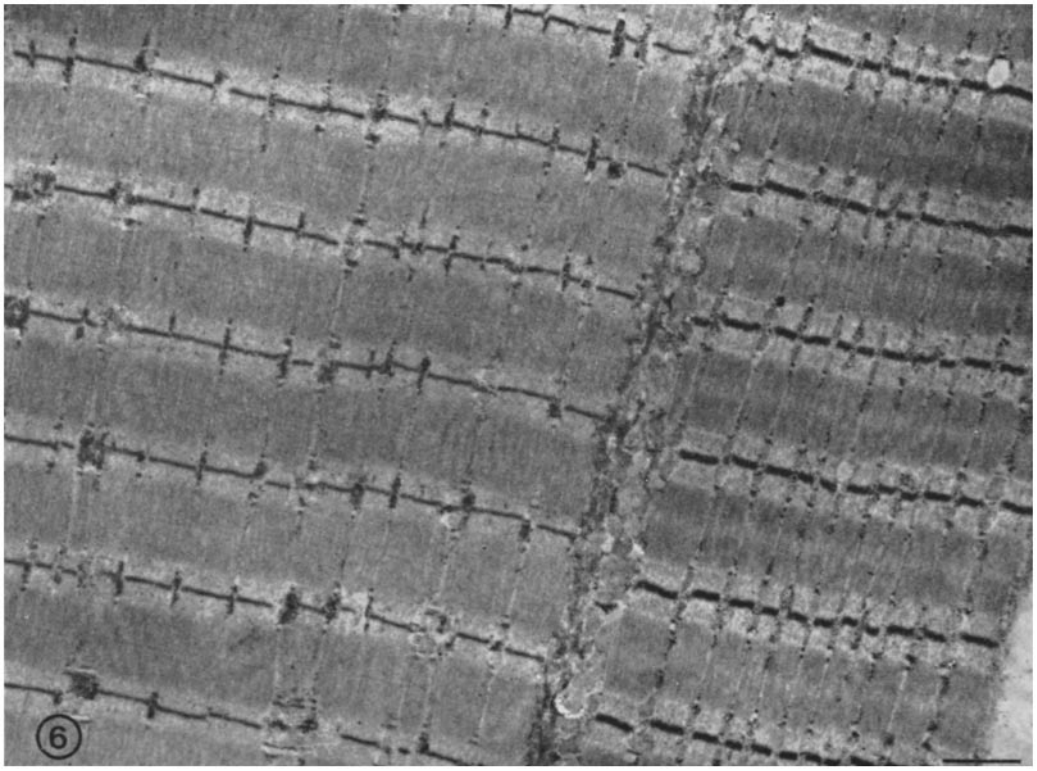
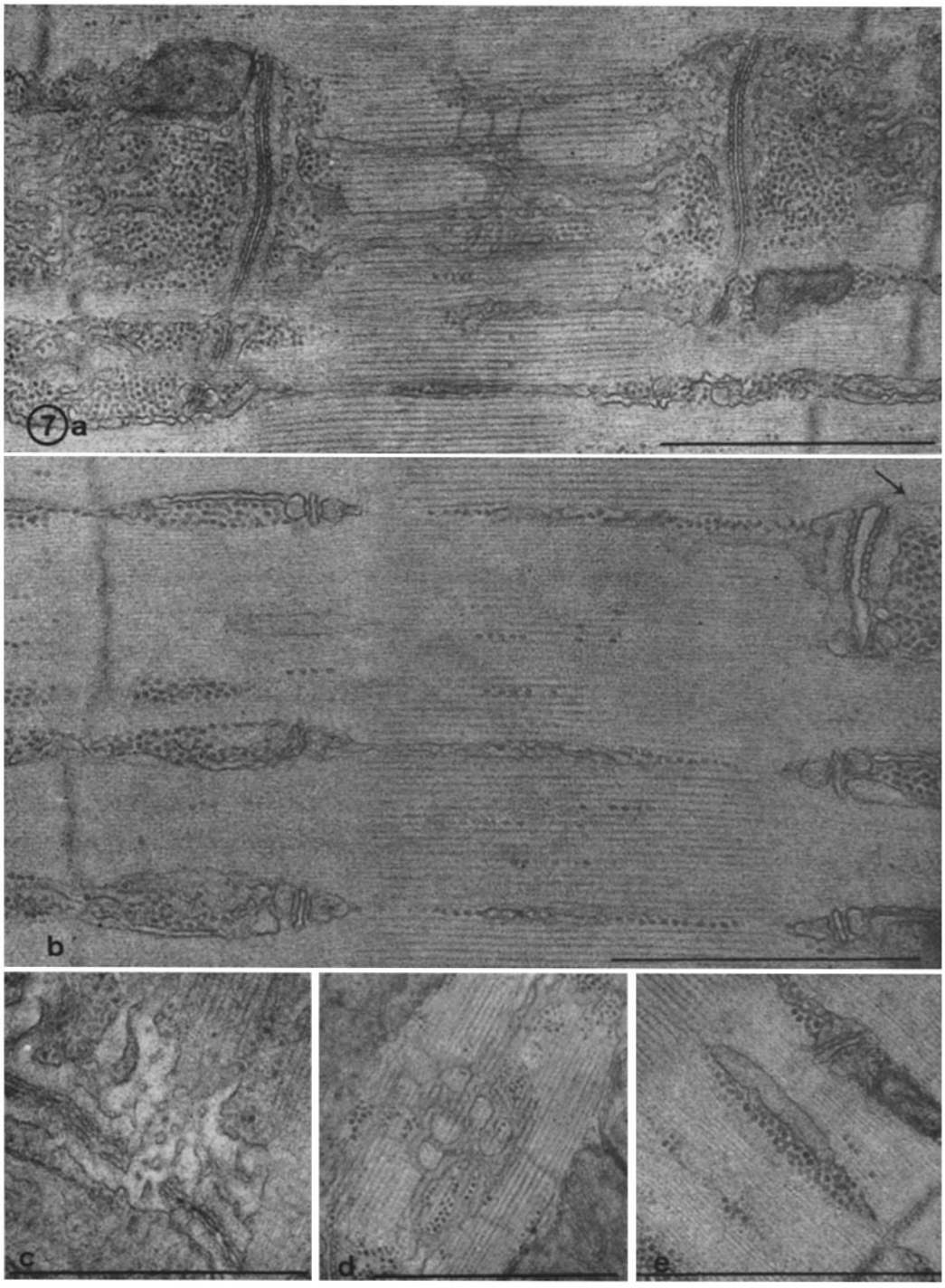


FIGURE 6 EDL. Longitudinal section. Two extreme fiber types are seen. The small mitochondria-rich fiber at right has thicker Z-lines and slightly longer sarcomeres in comparison with the large fiber at left. Tungstate treatment after glutaraldehyde, without postosmication. Scale marker = 1 μ. × 7500.

longitudinal prolongations of these mitochondria extend into the A band with variable frequency (Figs. 9, 10). Subsarcolemmal accumulations of round mitochondria are also present. The sarcomere organization is similar to that of small mitochondria-rich fibers of EDL with wide Z and M lines (Fig. 12).

A small proportion of soleus muscle fibers (about 10–15% of the total fiber population) stand out because of a significantly richer development of the sarcoplasmic reticulum (Figs. 13, 14). In transverse sections these fibers appear similar to mitochondria-rich EDL muscle fibers. The myofibrils are smaller and more regularly delineated than in other soleus fibers. Particularly at the H band level long rows of vesicular profiles of the sarco-

FIGURES 7 *a–e* EDL. Longitudinal sections illustrating organization of sarcotubular system. T tubules divide the sarcoplasmic reticulum into two networks which are seen in face view (*a*) or in profile (*b*). Fig. 7 *c* shows a connection between these two portions of the sarcoplasmic reticulum. At the A band the longitudinal tubules are confluent into fenestrated collars not only at the mid-sarcomere level, but also near the junctional cisternae (*a–c*). Extremely thin tubules appear to connect, at sites, portions of the H band fenestrated collar (*a*). A lacework of narrow tubules with circular pattern (*d*) is characteristically found at the H band in mitochondria-rich fibers. At the I band, elongated narrow sacs connect the junctional cisternae to a perforated network in register with the Z line (*a, b*). Longitudinal extensions of the junctional cisternae at the I band are shown in Figs. 7 *b* (arrow) and 7 *e*. Scale marker = 1 μ. *a*, × 36,000; *b*, × 45,000; *c*, × 45,000; *d*, × 36,000; *e*, × 45,000.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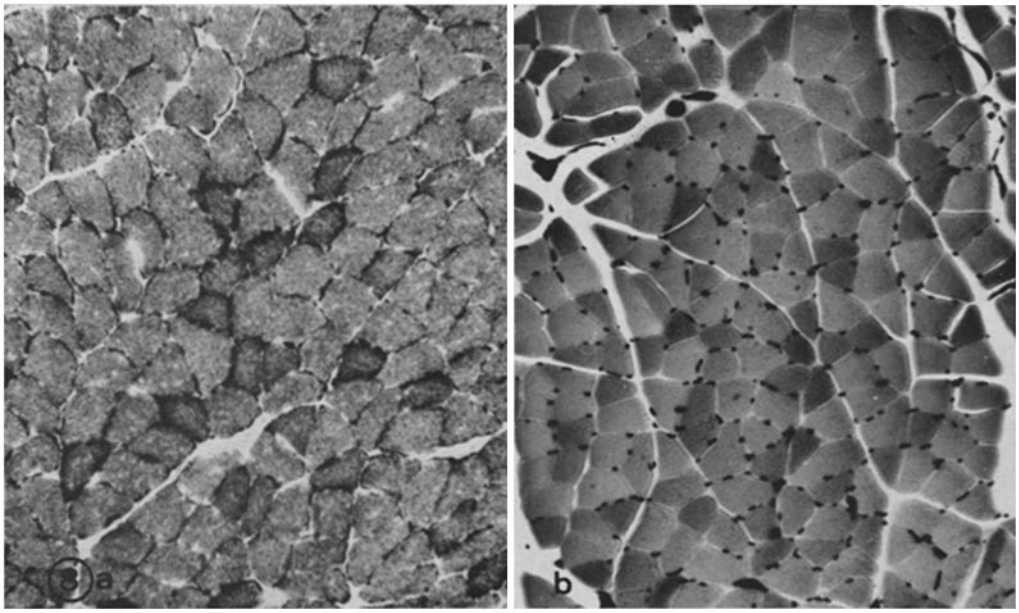


FIGURES 8 *a* and *b* Soleus. Cryostat sections incubated for histochemical demonstration of succinate dehydrogenase (*a*) and myoglobin (*b*). Most fibers display moderate succinate dehydrogenase and peroxidase activity. A small proportion of fibers show higher degrees of staining. × 158.

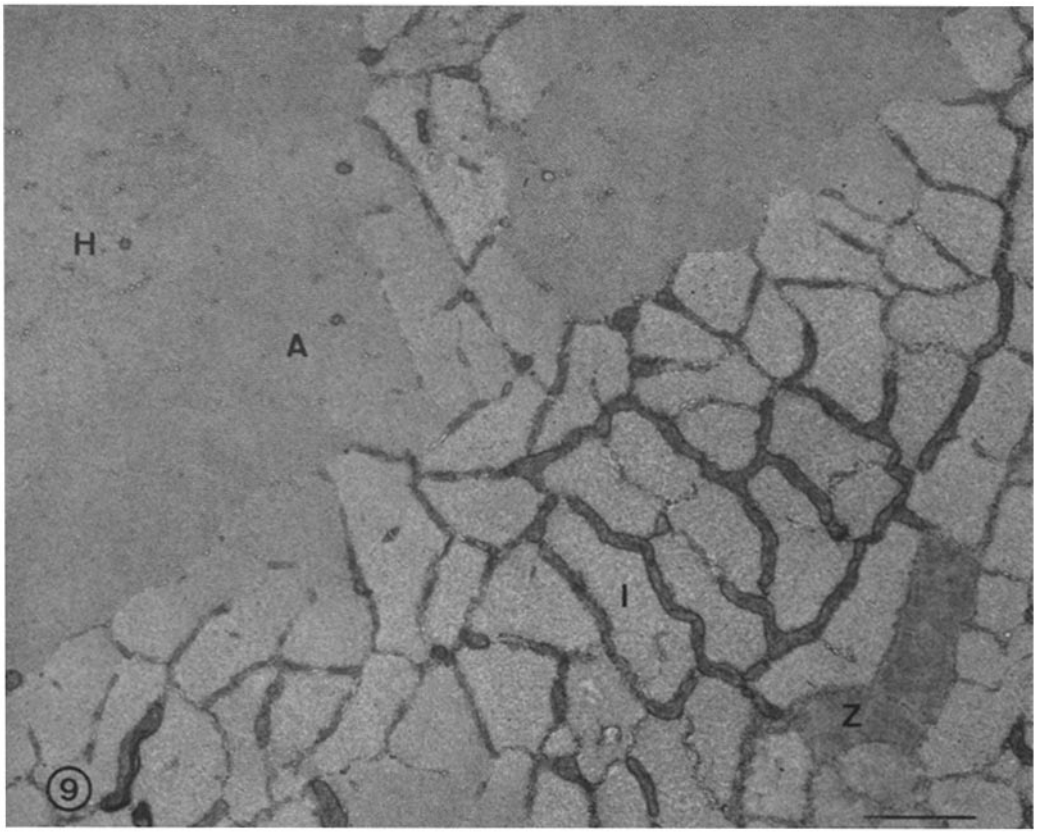


FIGURE 9 Soleus. Transverse, slightly oblique section. The myofibrils are encircled by sarcoplasmic reticulum profiles at the Z line (*Z*) level, outlined mainly by elongated mitochondria at the I band (*I*), and fused into an almost continuous mass at the A band (*A*). *H*, H band. Scale marker = 1 μ. × 15,000.

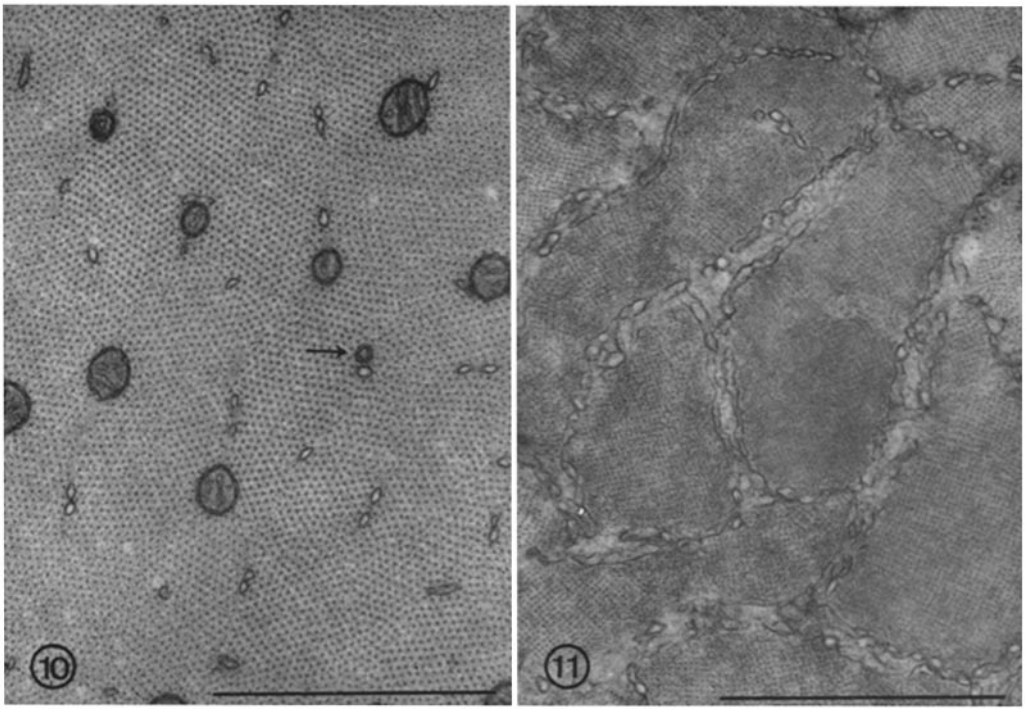


FIGURE 10 Soleus. Transverse section through the H band. The sarcoplasmic reticulum is represented only by sparse vesicular profiles interspersed in the myofibrillar fields. Round profiles of mitochondria, corresponding to longitudinal extensions of the mitochondrial network at the I band, are also seen. Some of them are extremely thin and without cristae (arrow). Scale marker = 1 μ. × 37,500.

FIGURE 11 Soleus. Transverse section through the Z line. The square pattern of the thin filaments is clearly apparent. Single or double sheets of sarcoplasmic reticulum elements surround the myofibrils. Scale marker = 1 μ. × 37,500.

plasmic reticulum outline the myofibrills. In corresponding longitudinal sections these fibers display more extensive and frequent fenestrated collars at the H band. The mitochondrial complement is also more abundant in these fibers: large mitochondria with tightly packed cristae are especially prominent in subsarcolemmal spaces and in longitudinal rows among the fibrils.

DISCUSSION

In a detailed physiological study on the contraction properties of motor units in rat EDL and soleus muscles Close (3) found that EDL is composed only of fast motor units, whereas in soleus the majority of motor units are slow with a minor proportion (about 10%) of intermediate units. We have shown that EDL contains a largely variable population of fibers, ranging from small, mitochondria-rich fibers with broad Z line to large fibers with low mitochondrial content and thin Z line through a continuous spectrum of intermediate forms. The apparent discrepancy between the physiological uniformity of EDL motor units, as regards speed of contraction, and the structural heterogeneity of its fiber population can be explained in two ways. One possibility is that each individual fast motor unit in EDL is composed by a mixture of mitochondria-rich and mitochondria-poor fibers. But this hypothesis contrasts with several lines of evidence supporting the view that motor units are homogeneous in their fiber composition (15, 5, 6). A second possibility seems therefore more likely, namely that various types of fast motor units are present in EDL, composed of sets of fibers similar in size and mitochondrial content within each unit but differing from unit to unit. This interpretation is supported by recent physiological and histochemical studies of

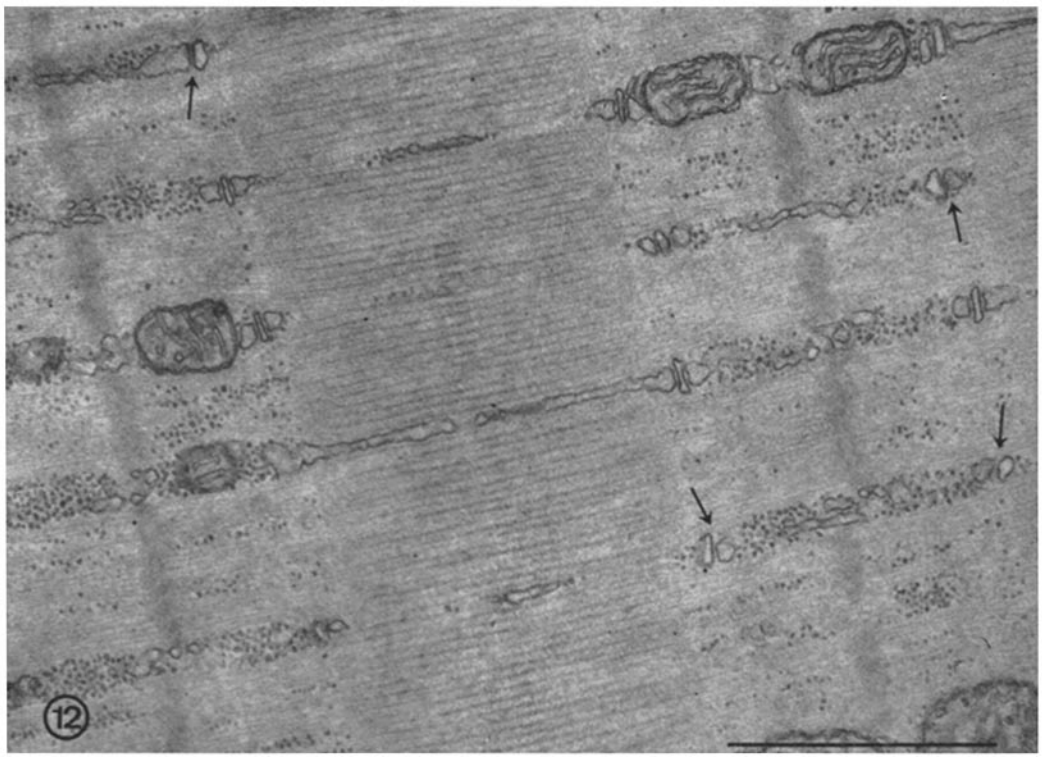


FIGURE 12 Soleus. Longitudinal section. Triads and dyads (arrows) are regularly disposed at the A-I boundary. Sparse longitudinal tubules extend into the A band. Profiles of sarcoplasmic reticulum are consistently found at the Z line level, more rarely in the other regions of the I band. Paired mitochondria can be seen at both sides of the Z line. Scale marker = 1 μ. × 36,000.

individual motor units in the rat tibialis anterior, a muscle very similar to EDL as to contraction properties of motor units and histochemical pattern of fibers (5). Motor units composed of small mitochondria-rich fibers or of intermediate fibers were found to be as fast as motor units composed of large mitochondria-poor fibers. The assumption that both mitochondria-rich and mitochondria-poor fibers are fast in EDL is also consistent with the comparably rich development of the sarcoplasmic reticulum in all fibers of this muscle, independent of variations in size, mitochondrial complement, and thickness of the Z line. By contrast, in most soleus muscle fibers the development of the sarcoplasmic reticulum is far less prominent: junctional cisternae form only discontinuous contacts with the T system, and longitudinal tubules are sparse and less extensively branched at the H band level. The small percentage of fibers with more richly developed sarcoplasmic reticulum in soleus compares fairly well with the small proportion of motor units with relatively fast speed of contraction. The variable development of the sarcoplasmic reticulum appears thus to be the only demonstrable ultrastructural feature which bears a direct relationship to the speed of contraction[1]. These findings conform with current views on the role of the sarcoplasmic reticulum in the mechanism of muscle contraction and relaxation (reviewed in reference 23) and are in line with ultrastructural observations on vertebrate muscles with differing contractile properties (25, 2, 20–22). It is noteworthy that variations in the quantity and complexity of sarcoplasmic reticulum membranes

[1] Differences in the intrinsic properties of the contractile material can contribute to the different speeds of contraction of EDL and soleus muscle fibers. Barany (1) has shown that actin-activated myosin ATPase activity of rat EDL is about two and one-half times that of soleus. However, we were unable to find any specific structural basis for these biochemical differences.

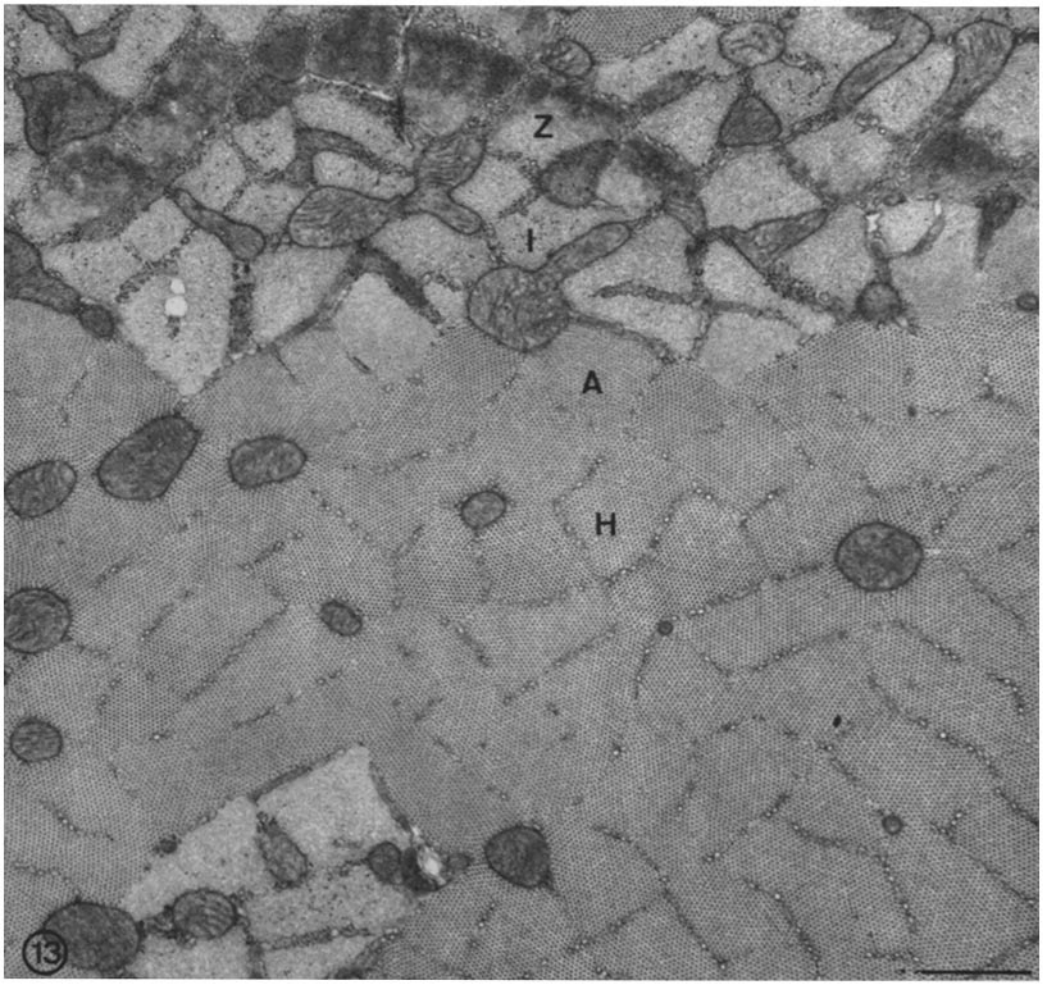
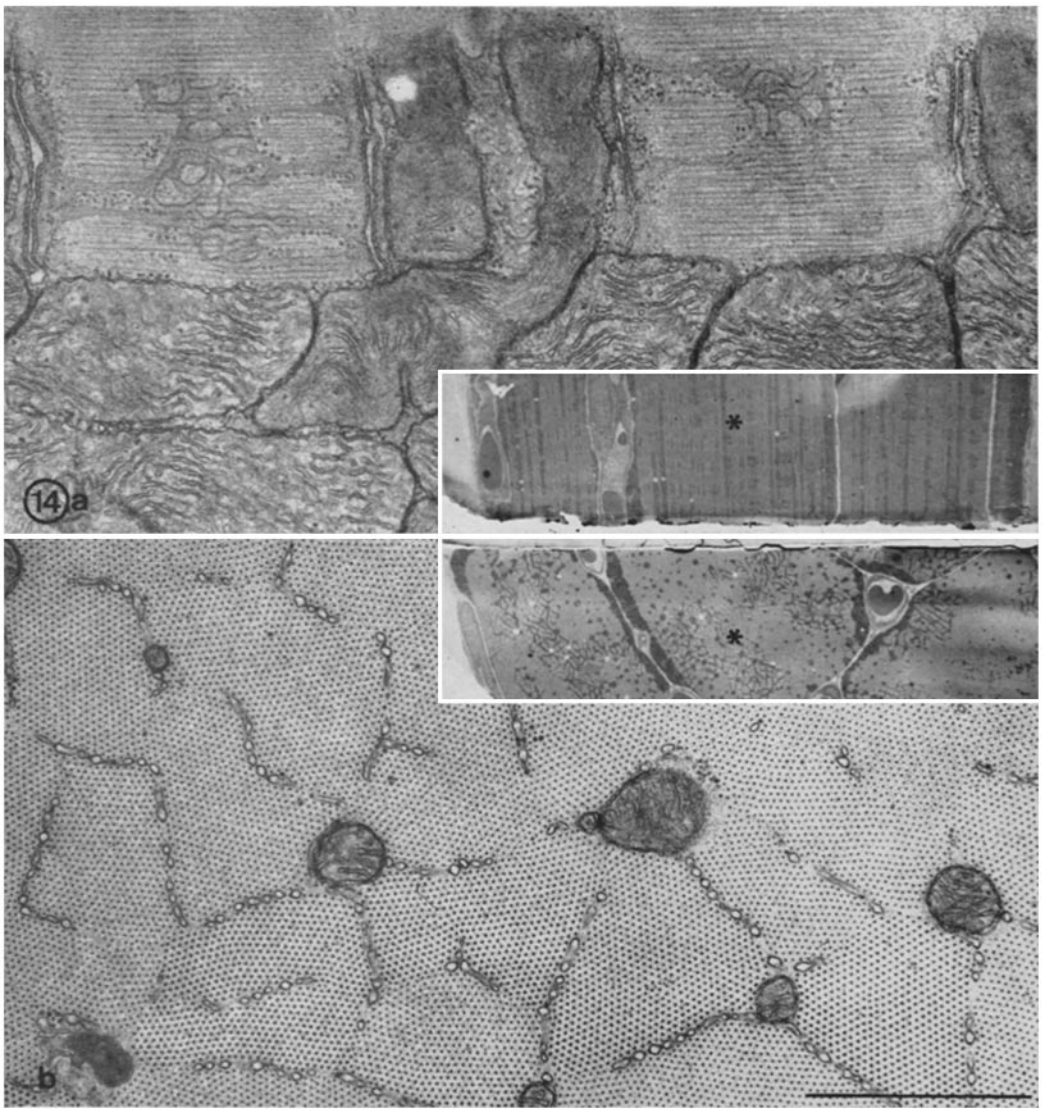


FIGURE 13 Soleus. Cross-section through a fiber with unusually rich development of the sarcoplasmic reticulum. The myofibrils are regularly outlined by sarcoplasmic reticulum profiles at both the I and A band (*I, A*) level. Mitochondrial profiles are large and numerous. *Z*, Z line; *H*, H band. Scale marker = 1 μ. × 18,000.

among rat muscle fibers do not involve the portion of reticulum in phase with the Z line, which is similarly well developed in every fiber type investigated.

As to the functional significance of the diversities in mitochondrial content and sarcomere organization, in particular the thickness of the Z line, among the various EDL muscle fibers, important indications can be inferred from the studies of Edström and Kugelberg (5). These authors found that, in the fast tibialis anterior muscle, motor units composed of large mitochondria-poor fibers undergo rapid decline of tension, dependent on intrinsic muscular fatigue, following repetitive stimulation, whereas units composed of small mitochondria-rich fibers show no fatigue. Intermediate units show a spectrum of intermediate reactions. Abundant mitochondrial complement and broad Z line thus appear to be related, in fast rat muscle fibers, to great resistance to fatigue. It is of interest that these features are also found in muscle fibers of soleus, a muscle likewise capable of maintaining tension on prolonged stimulation (16). The relationship of mitochondrial content with the resistance to fatigue suggests that exhaustion of energy supply is an important factor



FIGURES 14 *a* and *b* Soleus. Consecutive longitudinal and transverse sections through the same fiber (marked by asterisk in the *insets*) with rich development of the sarcoplasmic reticulum. Extensive fenestrated networks which overlie the sarcomeres at the H band level (*a*) are seen in cross-section as rows of vesicular profiles surrounding the fibrils (*b*). Chains of large mitochondria extend longitudinally along successive sarcomeres. Scale marker = 1 μ. *a*, *b*, × 30,000; *insets*, × 1100.

in the development of fatigue. The slight but significant discrepancy between the very thick Z line and the moderate mitochondrial content of most soleus muscle fibers, in comparison with mitochondria-rich muscle fibers of EDL, may be related to the lower energy expenditure in maintaining tension by slow muscle fibers, with low actomyosin ATPase activity (cf. 4).

In conclusion, we would suggest that the structure of rat muscle fibers is an expression of two main functional parameters, speed of contraction and resistance to fatigue, the latter being presumably a measure of adaptation to continuous or sustained activity. Each of these factors appears to affect selectively distinct morphological characteristics of the muscle cell. The assumption that

these two functional parameters are independently variable leads to the prediction that diverse combinations of structural features may be found in various kinds of skeletal muscle fibers as adaptation to differing functional demands. If this interpretation is correct, the classifications of rat muscle fibers based on single functional or structural properties (such as fast-slow, white-intermediate-red, A-B-C, type 1-type 2) should be integrated into more complex multiple systems which take into account the interaction of more than one variable.

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