A comparison of the fine structure of extraocular and interosseus muscles in the monkey

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The fine structure of the lateral rectus muscle in the rhesus monkey was compared with that of the palmar interosseus muscle. Since the interosseus possessed muscle spindles, it was classified into extra- and intrafusal muscle fibers. Although both of these fibers corresponded to the twitch fiber, the intrafusal fibers were smaller in diameter (15 to 30 μm) than the extrafusal (50 to 60 μm), and both the transverse tubular system (T system) and the sarcoplasmic reticulum were less developed in the intrafusal than in the extrafusal fibers. The lateral rectus, however, lacked muscle spindles but possessed the slow fiber as well as the twitch. The slow fibers, smaller in diameter (9 to 15 μm), showed irregular, ill-defined myofibrils and poorly developed sarcoplasmic reticulum, and no T system. The twitch fibers, larger in diameter (25 to 30 μm), which showed regular arrangement of myofibrils and well developed T system, were further classified into two subtypes: the fiber with numerous mitochondria (red) and the fiber with few mitochondria (white). The physiological significance of the ultrastructural characteristics in each type of muscle fiber was discussed.

Recently, it has been shown that there is a close relationship between function and structure of skeletal muscle fiber. The twitch fiber which responds to a single motor impulse to give a propagated action potential has regular and distinct myofibrils. The transverse tubular system (T system)1 which extends from the sarcolemma toward the interior of the muscle fiber2, 3 is well developed in the twitch fiber; however, the slow fiber appears unable to propagate action potentials but undergoes a graded contraction or contraction by repetitive impulses. This slow fiber has irregular, ill defined fibrils and no T system.4-6 In addition, the neuromuscular junction of the slow fiber shows a fine structure different from that of the twitch fiber.6-8

Both slow and twitch fibers have been identified in the skeletal muscles of lower vertebrates6, 9, 10 and the extraocular muscles of mammals,9, 5-10 but the slow fiber has not thus far been observed in the skeletal muscles of mammals. The question arises as to whether the skeletal muscles of mammals consist of more than just the twitch fiber. In addition, the physiological
The significance of the slow-fiber system in the extraocular muscles remains unexplained.

A morphological comparison of extraocular muscle with skeletal muscle in mammals is one approach to these problems. The muscles of the hand seem to be suitable for comparison with extraocular muscle, since both types of muscle are rather small and act in a very delicate and complex manner.

In the present study, the fine structure of the lateral rectus muscle in the rhesus monkey is compared with that of the palmar interosseus muscle, in an attempt to develop information correlating function and structure of the slow and twitch muscle fibers.

Materials and methods

The lateral rectus and the palmar interosseus muscles were obtained during anesthesia from four rhesus monkeys, presumably young. The muscles were pinned onto a dental-wax sheet to prevent them from contracting during fixation. They were fixed by cold 4 per cent glutaraldehyde buffered with 0.1M phosphate (pH 7.3) for 15 minutes, then cut into small pieces and fixed again with the glutaraldehyde for about one hour. The specimens were washed briefly with the phosphate buffer, post-fixed with 1 per cent osmium tetroxide (pH 7.3) for one hour, dehydrated by a series of graded alcohols and embedded in Epon 812. The thin sections were cut with a glass or diamond knife (LKB-Ulrotome), stained with uranyl acetate and lead citrate, and observed with a Siemens Elmiskop 1. Adjacent thick sections were stained with conventional methylene blue solutions (pH 9.2) for light microscopy.

Results

Interosseus muscle. The interosseus muscle fibers were separated into two groups, the extrafusal and the intrafusal fibers, since this muscle contained several muscle spindles.

Extrafusal fiber. In transverse section, the diameters of the fibers ranged from 50 to 60 μm, and all of them showed regular arrangement of the myofibrils which were well delineated with sarcoplasmic reticulum (fibrillar structure) as shown in Figs. 1 to 3. Each myofibril, round or ovoid, showed similar dimension, 0.4 to 0.9 μm, in diameter (Fig. 3). Myofibrils which were cut through the A-band showed regular arrangement of two arrays of myofilaments, thick filaments and thin filaments; in the fibrils cut through the I-band, the thin filaments were packed irregularly and, as they approached the Z-line, they became thicker and were packed in a square array (Fig. 4).

In longitudinal section, the fibers again showed distinct myofibrils, straight Z-line, distinct M-line, and numerous triads in the fibrils cut through the I-band, the thin filaments were packed irregularly and, as they approached the Z-line, they became thicker and were packed in a square array (Fig. 6). It was obvious, therefore, that these muscle fibers correspond to the twitch fiber. The triads were observed at both edges of the A-band and they were shown in various orientations, depending on the plane of section. When the section passed through the median part of the myofibrils, the triads were cut across their narrow dimensions and appeared a shown in Fig. 6. The central element of each triad usually

Plate I shows photomicrographs (Figs. 1 and 2) and electron micrographs (Figs. 3 to 5) of transverse sections from the extrafusal fibers in the interosseus muscle.

Figs. 1 and 2. Transverse sections stained with methylene blue showing “fibrillar structure” in each muscle fiber. (Fig. 1, ×400; Fig. 2, ×1,000.)

Fig. 3. Electron micrograph of the transverse section, showing regular arrangement of myofibrils (mf), each of which is distinctly surrounded by sarcoplasmic reticulum (sr), mitochondria (mi), and glycogen particles (g). (×16,500.)

Fig. 4. High magnification of the myofibrils, showing double arrays of thick and thin myofilaments in the fibril cut through the A-band (A) and irregular arrangement of thin filaments in the fibrils cut through the I-band (I). Note that thin filaments, as they approach the Z-line (Z), become thicker and packed in a square array. tc, terminal cisterna. (×36,000.)

Fig. 5. High magnification of oblique transverse section, showing a transversely oriented tubule (t) running close to the sarcolemma (S). pv, pinocytotic vesicles. (×42,000.)
Figs. 1-5. For legend see opposite page.
Figs. 6 and 7. For legend see opposite page.
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appeared as a single tubular element, having the same dimension, about 250 Å in diameter (transverse tubule or T system). The outer elements of the triads, the terminal cisternae, were larger dilated sacs whose surfaces were flattened where they faced the transverse tubules. They were filled with a diffuse granular material. The membranes of the two terminal cisternae facing the T system were scalloped or indented regularly (Fig. 6, inset). In addition, this junctional region, about 200 Å in thickness, between the two adjacent membranes of transverse tubule and terminal cisterna, was bisected by an intermediate line, averaging 20 to 30 Å in thickness (Fig. 6, inset). This structure of junctional complex seemed to be the same as "tight junction" (zonula occludens), and thus it confirmed the result in human rectus abdominis muscle.

When the plane of section coincided with the surface of myofibrils, the profile of the network of sarcoplasmic reticulum was well shown, as seen in Fig. 7. The T system ran across myofibrils and was continuous as far as the triad remained in the plane of the section. Although direct connection between the T system and the sarcolemma has not been identified, the transverse tubules were often observed very close to the sarcolemma (Figs. 5 and 7). It seemed, therefore, likely that the T system is continuous to the sarcolemma, as described in other skeletal muscles.

Mitochondria between myofibrils were usually observed alone or with a pair which faced adjacent to the Z-line (Figs. 6 and 7). Glycogen particles, 200 to 300 Å in diameter, were scattered in the sarcoplasm; they were between the fibrils, or sometimes inside the fibrils, but were never observed inside the T system or sarcoplasmic tubules (Figs. 3 to 7).

Intrafusal fiber. The muscle spindles of the interosseous were surrounded with the distinct capsule (Figs. 8 and 9) which consisted of 6 to 8 thin elongated concentric tubular sheets of the specialized fibroblasts, "capsular-sheet cells" (Fig. 10). Inside the capsule, there were from 6 to 8 intrafusal muscle fibers (Figs. 8 and 9). The diameters of the intrafusal fibers at the polar region of the spindle ranged from 15 to 30 μ, being approximately half the size of the extrafusal fibers, but all of them showed almost the same fine structure (Fig. 8). In transverse section, they showed irregular arrangement of myofibrils; the sarcoplasmic reticulum was less developed as compared with that of the extrafusal fibers, so that each myofibril was not distinct (Fig. 11). The arrangement of thick and thin myofilaments in the fibrils was almost the same as in the extrafusal fibers (Figs. 11 to 13).

In longitudinal section, these fibers showed distinct M-line and straight Z-line. The triads were also observed, and their structure was similar to that of the extrafusal (Figs. 13 to 15), thus confirming the results in other muscle spindles. The number of triads in the intrafusal fibers was, however, markedly less than in the

Plate II shows electron micrographs of longitudinal sections from the extrafusal fibers in the interosseous.

Fig. 6. Longitudinal section passed through the median part of the myofibrils (mf) showing straight Z-line (Z), distinct M-line (M), and numerous triads (one of which is indicated by square line). Each myofibril is well delineated by longitudinal tubules (lt) of the sarcoplasmic reticulum, glycogen particles (g), and mitochondria (mi). Inset shows higher magnification of the triad indicated by the square line. Note the intermediate line (i) between the two adjacent membranes of the transverse tubule (T) and the terminal cisterna (tc). (×40,500; inset, ×82,500.)

Fig. 7. Longitudinal section passed through the surface of myofibrils (mf) near the sarcolemma (S), showing the profile of the network of the sarcoplasmic reticulum (sr) and well-developed T system (T). Large mitochondrion of U-shape is observed beneath the sarcolemma. cf, collagen fibrils. (×30,000.)
Figs. 8-10. For legend see page 542.
extrafusal, so that the T system appeared poorly developed in the intrafusal fibers. The network of the sarcoplasmic reticulum was also poorly developed in the intrafusal fibers (Fig. 16).

Mitochondria in the intrafusal fibers were more frequently observed: They were often found in chains or in groups, between myofibrils as well as beneath the sarcolemma (Figs. 8 and 14). In summary, the intrafusal fibers corresponded to the twitch fiber because of the presence of the T system, but differed from the extrafusal fibers in the poor development of the T system and sarcoplasmic reticulum, and in the abundance of mitochondria.

**Lateral rectus muscle.** Although numerous thick sections were examined by light microscope, no muscle spindles were found in this muscle, which confirmed the results of other authors.10

In transverse section, two kinds of muscle fibers with different diameters were observed: the small fibers, 8 to 15 μ in diameter, and the large fibers, 25 to 50 μ in diameter (Fig. 17). The small fibers, in transverse section, showed an irregular arrangement of myofibrils. The sarcoplasmic reticulum was so poorly developed that each myofibril was irregular, larger, and indistinct (afibrillar structure), as shown in Fig. 18. The arrangement of myofilaments at the A-band was the same as in the interosseous muscle, while the thin filaments at the Z-line were not so regularly arranged as they are in the interosseus (Fig. 18). In longitudinal section, they showed ill defined myofibrils, zigzag Z-line, but no M-line (Figs 19 and 20).

The network of sarcoplasmic reticulum was poorly developed, surrounded the fibrils incompletely, and lay roughly parallel to the fiber axis. Sometimes small tubules were observed, about 250 to 300 A in diameter; they appeared to run transversely or obliquely across the fibril (Figs. 19 and

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Plates III and IV show photomicrographs (Figs. 8 and 9) and electron micrographs (Figs. 10 to 16) from the polar regions of muscle spindles in the interosseous.

**Fig. 8.** Transverse section stained with methylene blue, showing a distinct capsule of muscle spindle (C), intrafusal muscle fibers (if), extra- and intrafusal myelinated nerve fibers (en, in). Note the distinct difference of diameters between the intrafusal and extrafusal (ef) muscle fibers. (x450.)

**Fig. 9.** Longitudinal section showing the profile of the muscle spindle. if, intrafusal fiber; ef, extrafusal fiber; C, capsule; in, intrafusal myelinated nerve. (x450.)

**Fig. 10.** High magnification of the spindle capsule at the area shown by the arrow in Fig. 8, showing thin, elongated, concentric, capsular-sheets cells. Note numerous pinocytotic vesicles (pv) and caveolae (cv) in these cells. The cell indicated by the arrow faces inside the spindle. N, nucleus; mi, mitochondria; gr, granules. (x28,000.)

**Fig. 11.** Transverse section of the intrafusal fibers showing ill-defined myofibrils (mf) and poorly developed sarcoplasmic reticulum (sr). Note the myofibrils which were cut through the A-band, showing regular arrangement of thick and thin filaments. (x37,000.)

**Fig. 12.** The thin filaments are packed irregularly in the myofibrils cut through the I-band (I); as they approach the Z-line (Z), they become thicker and arranged in square array. (x53,000.)

**Fig. 13.** Transverse section of the myofibril (mf) cut through the edge of the A-band (A), showing a triad which consists of central transverse tubule (T) and two adjacent terminal cisternae (tc). (x61,000.)

**Fig. 14.** Longitudinal section of the intrafusal fiber beneath the spindle capsule, showing straight Z-line, distinct M-line, and a cluster of mitochondria (mi) and triads (T). Numerous collagen fibrils (cf) are packed between the capsular sheets (C), and the endomysial cell (ec) covers the surface of the intrafusal fiber. (x13,000.)

**Fig. 15.** Higher magnifications of the two triads, one of which is shown in Fig. 14, showing the transverse tubules (T) and the adjacent terminal cisternae (tc). (x29,000.)

**Fig. 16.** Poorly developed sarcoplasmic reticulum (sr) between ill-defined myofibrils (mf). Z, Z-line; M, M-line. (x22,000.)
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However, the triads, which are characteristic of the twitch fiber, were not observed in this type of fiber (Figs. 19 and 20). The T system, therefore, appeared to be absent. All these findings showed that the small fibers correspond to the slow fiber, as described by many authors. Mitochondria in these slow fibers were distributed almost evenly between myofibrils, usually with a pair facing the Z-line (Figs. 18 and 19).

On the other hand, the large fibers in transverse section showed a regular arrangement of myofibrils which were well delineated with sarcoplasmic reticulum (fibrillar structure), as shown in Figs. 21 to 23. In longitudinal section, they showed straight Z-line and numerous triads (Figs. 24 to 26). These large fibers, therefore, appeared to correspond to the twitch fibers. It seemed, however, possible to divide the large fibers into two subtypes: those containing a large number of mitochondria and those with few (Fig. 21). In transverse section of the former, the peripheral aggregation of the mitochondria imparted a rimmed appearance under the light microscope, and their aggregation between myofibrils appeared as coarse granules distributed in the interior of the muscle (Fig. 21). Under the electron microscope, the well-developed sarcoplasmic reticulum surrounded each myofibril distinctly, and the average diameter of the myofibrils was 0.6 to 0.8 μ. In longitudinal section, these fibers showed straight Z-line and numerous triads. The M-line, however, was not observed in this type of fiber (Figs. 24 and 25). The transverse tubules, approximately 250 to 300 Å in diameter, were located near the edge of the A-band. The intermediate line between the two adjacent membranes of the transverse tubule and the terminal cisterna was confirmed also in this fiber (Fig. 25, inset). Numerous large mitochondria with abundant thick cristae, 350 to 400 Å in thickness, formed chains or columns running longitudinally between myofibrils. Those chains of mitochondria sometimes extended over more than 10 sarcomeres, about 18 μ in length (Fig. 24). Lipid droplets were often closely associated with the aggregation of mitochondria (Figs. 22 and 25). The ultrastructural characteristics of these fibers corresponded to those of the red fiber described in the soleus muscle and diaphragm.

The second subtype of the large fibers differed remarkably from the red fibers with respect to the amount of mitochondria. They contained far fewer mitochondria, and did not show such an aggregation of mitochondria between fibrils or beneath sarcolemma as were seen in the red fiber. Mitochondria in these fibers were usually located adjacent to the Z-line, were smaller and thinner, and contained fewer cristae. Lipid droplets were rare. In transverse section these fibers showed regular arrangement of the myofibrils, which were well delineated with the network of sarcoplasmic reticulum (Fig. 23). In longitudinal section, they showed straight Z-line, distinct M-line, and many triads (Fig. 26). The triads were also located at the edge of the A-band and showed the same structure as in the red fibers.

The fine structure of these fibers was almost the same as that of the extrafusal fibers of the interosseus muscle; therefore, it corresponds to the white fiber described in the gastrocnemius, diaphragm, and adductor magnus muscles.

Discussion

In this comparison of the fine structure of extraocular and interosseus muscles, attention is drawn to the two intracellular components: the T system and mitochondria. The former, which consists of the central components of the triads, extends from the sarcolemma across the myofibrils and forms a transversely oriented network between myofibrils. This T system is thought to be the pathway for the inward conduction of the effect of depolarization of the surface membrane of muscle fiber. Moreover, it is assumed that the membrane depolarization could progress through the tight junction, as described in this study,
Figs. 17-20. For legend see page 547.
Figs. 21-24. For legend see page 547.
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From the T system into the terminal cisternae. The slow fiber, which appears unable to propagate action potentials, has been found to lack the T system—although one investigator suggested that a tubular network similar to the T system is present in the slow fiber. The present study revealed absence of the T system in the slow fiber of the lateral rectus muscle in the rhesus monkey.

On the other hand, it is of interest that the T system and the sarcoplasmic reticulum are poorly developed in the intrafusal fibers of the intersens, and it has been shown that intrafusal fibers, though capable of propagating action potentials, contract more slowly than do extrafusal fibers. Consequently, the lack of the T system, or poor development of the T system and sarcoplasmic reticulum, seems to be one

Plates V, VI, and VII show photomicrographs (Figs. 17, 21) and electron micrographs (Figs. 18 to 20 and 22 to 26) of the lateral rectus muscle in rhesus monkey.

Fig. 17. Transverse section stained with methylene blue, showing two types of fibers with different diameters: the small fibers (arrows) and the large fibers. Note rimmed appearance of the peripheral margin and the coarse granules disseminated in the sarcoplasm of the large fibers. (×450.)

Fig. 18. Higher magnification of transverse section from the small fiber (slow fiber) showing ill-defined myofilaments (mf) and poorly developed sarcoplasmic reticulum (sr). Two types of myofilaments are regularly arranged in the fibrils cut through the A-band, while thin filaments (arrow) are irregularly packed in the fibrils cut through the Z-line (Z). Mitochondria (mi) are almost evenly distributed among the myofilaments, and glycogen particles (g) are scattered in sarcoplasm. (×37,000.)

Fig. 19. Longitudinal section of the slow fiber, showing zigzag Z-line (Z), no M-line, and ill-defined myofilaments (mf). Sarcoplasmic reticulum (sr) is poorly developed and no triads are observed. Mitochondria (mi) are distributed, generally facing the Z-line with a pair. (×15,500.)

Fig. 20. Higher magnification of longitudinal section of the slow fiber, showing no M-line and poorly developed sarcoplasmic reticulum (sr) between myofilaments (mf) or beneath sarcolemma (S). Note a small tubule (t) running transversely across the fibril. A, A-band; I, I-band; Z, Z-line. (×35,000.)

Fig. 21. Transverse section stained with methylene blue, showing two subtypes of the large fibers: the fiber containing numerous mitochondria (red fiber, R) and the fiber with few mitochondria (white fiber, W). (×1,000.)

Fig. 22. Higher magnification of the transverse section from the red fiber, cut through the A-band, showing aggregation of numerous mitochondria (mi) beneath sarcolemma (S) and regular arrangement of myofilaments (mf) well delineated with network of sarcoplasmic reticulum (sr). Lipid droplet (l) is often observed among mitochondria. (×17,000.)

Fig. 23. Transverse section of the white fiber cut through the A-band, showing no aggregation of mitochondria beneath sarcolemma (S) and regular arrangement of myofilaments (mf), each of which is well delineated with longitudinal tubules (l) of sarcoplasmic reticulum. (×38,000.)

Fig. 24. Longitudinal section of the red fiber, showing long chains of mitochondria (mi), one of which extends over more than 6 sarcomeres, between myofilaments (mf). Each myofilament is distinct, the Z-line is straight, and numerous triads (arrows) are observed at the edge of the A-band. (×12,000.)

Fig. 25. Longitudinal section of the red fiber, showing well-developed T system (T), and numerous mitochondria (mi) with closely packed thick cristae (er) beneath sarcolemma (S) as well as between myofilaments (mf). L, lipid droplet; tc, terminal cisternae; g, glycogen particles. (×19,000.) Inset shows higher magnification of the triad which is indicated by square line. Note two intermediate lines (i) between the two adjacent membranes of the terminal cisternae and the transverse tubule. (×54,000.)

Fig. 26. Longitudinal section of the white fiber, showing straight Z-line (Z), M-line (M), distinct myofilaments (mf), well-developed sarcoplasmic reticulum (sr), and numerous triads (arrows). Mitochondria (mi) are thinner and fewer in the white fiber than in the red. (×15,500.)
of the factors which cause slow contraction of muscle fiber.

The amount and distribution of mitochondria differ among various muscles. So-called red muscles, such as the soleus muscle, contain a high amount of mitochondria; white muscles, such as gastrocnemius, have fewer mitochondria. It is generally believed that red muscle, though capable of propagating action potentials, contracts more slowly than white muscle. The exact relationship between the amount of mitochondria and the speed of contraction in each type of muscle fiber, however, is still controversial. Therefore, the functional differences between the red and the white fibers in the extraocular muscle require further study.

In spite of its delicate function, the interosseus muscle possessed only the twitch fibers. The plentiful nerve supply and the highly developed propulsive function with numerous muscle spindles may be responsible for the delicate function of this muscle. In contrast, the extraocular muscles of certain animals (e.g., rabbit, cat, dog, or rhesus monkey) lack the muscle spindle. Nevertheless, these muscles, especially the rhesus monkey, are required to act in a very delicate and complex manner. This may be subserved not only by the plentiful nerve supply, but also by the presence of a variety of muscle fibers, twitch (red and white) and slow, in the extraocular muscle. The suggestion that the twitch fibers of the extraocular muscle may be associated with rapid scanning or shift movements of the eye, while the slow fiber may be involved in the slow sustained contraction required for maintaining the eye position, has yet to be confirmed.

However, it is evident from the present study that the development of the T system, sarcoplasmic reticulum, or mitochondria varies considerably in each type of the extraocular muscle fiber and that these intracellular components must be noted among the factors correlating function and structure of extraocular muscle fiber.

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