FINE STRUCTURE OF EXTRAOCULAR MUSCLE IN RABBIT

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It has been established that the extraocular muscles of several mammalian species possess two different types of muscle fibers, twitch and slow fibers, with distinct differences in their respective fine structures (1–8). In this investigation we have examined the fine structure of isolated and identified extraocular muscle fibers in rabbit.

Though there has been general agreement on the differences between the fine structures of the two fiber types, it remains unclear whether the slow fibers are characterized by an M line. Extraocular muscle fibers in rhesus monkey which have fine structural characteristics similar to those of slow fibers in frog skeletal muscle have been reported by Cheng and Breinin (5) and Miller (6) to lack an M line, whereas in cat such fibers have been described by Hess (7) as clearly possessing an M line.

Thus the morphological definition of slow fibers in terms of their fine structural characteristics has varied in previous investigations. According to Hess (1, 7), reliable criteria for the identification of slow fibers are irregular and ill-defined myofibrils, a virtually absent T system, zigzag Z lines, and rudimentary postjunctional folds. Page (9) and Miller (6) rely, rather, on the absence of an M line for identification of slow fibers.

There is complete agreement, however, that slow fibers have multiple motor nerve endings (1, 2, 6, 7, 9). In the present study, therefore, we first classified extraocular muscle fibers, on the basis of their motor nerve endings, into either singly innervated or multiply innervated fibers. The respective fibers were then examined under the electron microscope.

Our findings indicate that the fine structural characteristics of the singly and multiply innervated fibers in the extraocular muscle of rabbit are essentially similar to those previously described for the twitch and slow fibers, respectively, in other species. No M line was observed, however, in the multiply innervated fibers.

MATERIALS AND METHODS

Rabbit superior rectus muscle was exposed under general anesthesia and fixed *in situ* by dripping 4% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) for 15 min. Specimens approximately 10–15 mm in length and 1–2 mm in thickness were then teased from the surface as well as the interior of the muscle. The teased specimens were incubated in Karnovsky's (10) modified acetylthiocholine solution (pH 6.0) for 10 min at 4°C, washed with 0.2 M sucrose in the phosphate buffer for 15 min, and again fixed with the glutaraldehyde solution for 1 hr. The stained specimens were examined under a binocular light microscope and again teased by fine glass rods; thereby individual fibers were isolated. The cholinesterase sites at the motor nerve endings were visualized by brown reaction products of copper ferrocyanide; this permitted classification of the muscle fibers as either singly or multiply innervated.

Two types of clearly identifiable fibers were isolated, by the fine teasing, for subsequent examination in the electron microscope. The first type had single, large, and well-lobulated endings (Fig. 1) similar in appearance to "en plaque" endings of the twitch fiber (1, 2). The second type had multiple, small endings distributed along the length of the fiber (Fig. 2), corresponding in appearance to "en grappe" endings associated with slow fibers (1, 2, 11).

Six such singly innervated and eight such multiply innervated fibers were isolated and post-fixed with 1% osmium tetroxide, dehydrated with a series of graded alcohols, and embedded in Epon. These individual specimens were cut with a diamond

knife on a Reichert's ultramicrotome, and thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I.

RESULTS

The observations of muscle fibers with single (Fig. 1) and multiple (Fig. 2) motor nerve endings in rabbit superior rectus were found to be in agreement with studies in other species (1, 2, 7). As regards the distribution of these two types of fibers within the muscle, the multiply innervated fibers were observed more frequently in specimens taken from the surface of the muscle, whereas the singly innervated fibers were more numerous in specimens from the muscle interior. However, there was no distinct separation of the two types of fibers, and they were observed side by side frequently in both the interior and surface portions of the muscle.

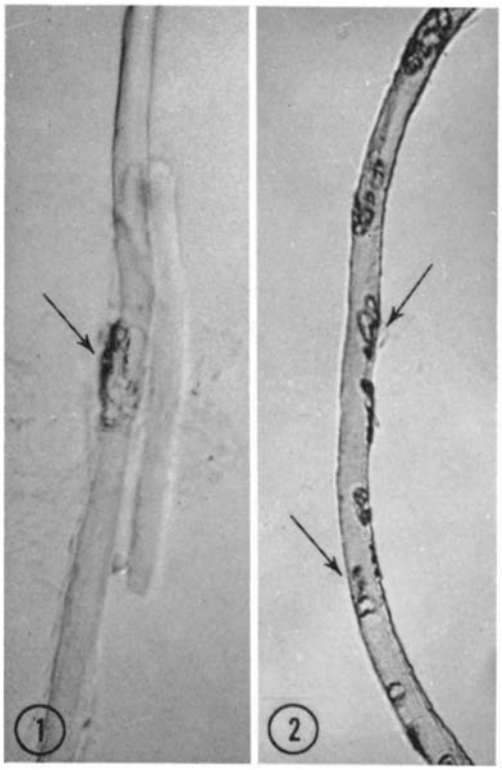

FIGURE 1 Light micrograph of an isolated muscle fiber of rabbit superior rectus, showing a single motor end plate (arrow) visualized by cholinesterase stain. Adjacent to this fiber is seen a piece of another muscle fiber. × 400.

FIGURE 2 An isolated extraocular muscle fiber showing multiple endings (arrows) distributed along the length of the fiber. × 400.

Under the electron microscope, the singly innervated fibers possessed a straight Z line, clearly delineated myofibrils, a well-developed T system, and a clearly defined H zone with a distinct M line (Fig. 3). The multiply innervated fibers showed a zigzag Z line, ill-defined myofibrils, and a poorly developed T system (Figs. 4, 5). Although a faint H-zone was recognizable occasionally in this type of fiber, no clear indication of an M line was observed (Figs. 4, 5).

The neuromuscular junctions of these two types of fibers in the rabbit superior rectus were also found to be essentially the same as those observed in other species (3, 4, 7). In the singly innervated fibers the terminal axon branchlets were located in a synaptic groove where the muscle plasma membrane showed numerous, distinct, junctional folds. In the multiply innervated fibers the terminal axon was located on a very shallow groove or on the flat surface of the muscle fiber where the muscle plasma membrane showed a few rudimentary invaginations (Fig. 5).

DISCUSSION

The M line has been reported to be present in extraocular muscle slow fibers of cat and absent in those of monkey and rabbit. The discrepancy might be a species difference. Other species differences have been reported between the extraocular muscles of various mammals, such as the presence of muscle spindles in man, higher apes, cattle, sheep, and goat and their apparent absence in rabbit and cat. Indeed, the M line is not always to be found in the slow fibers of skeletal muscle; it is absent from frog (7, 9, 12) but present in chicken (7).

Another possible explanation of the M line discrepancy would be that multiple endings are present on more than one kind of fiber (12, 13), of which one kind may have the M line and one kind may not. In the present study, only those multiply innervated fibers with typical en grappe endings were selected for fine structure examination. In addition to such fibers with numerous and closely spaced endings, other fibers with but a few and widely spaced endings were also observed. The latter fibers were not included in this study, however, and conceivably may have

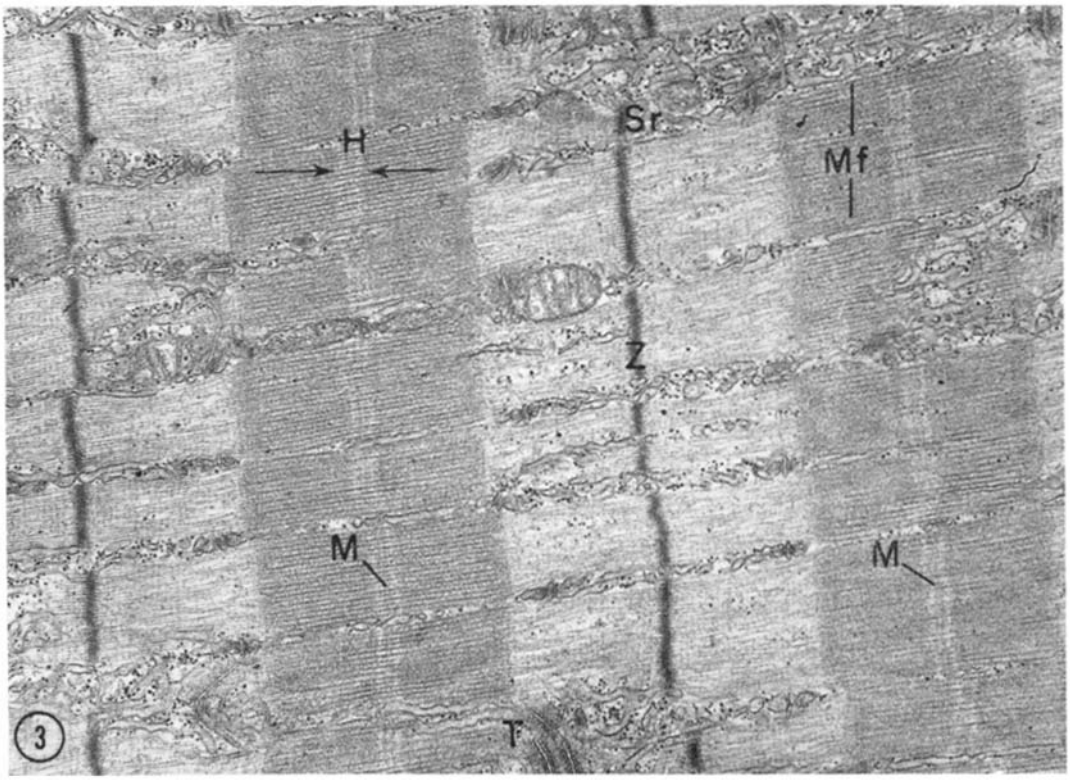

FIGURE 3 Electron micrograph of a longitudinal section of a singly innervated muscle fiber (identified by cholinesterase stain) showing straight Z line (*Z*), well-developed T system (*T*), myofibrils (*Mf*) well delineated by sarcoplasmic reticulum (*Sr*), and distinct H zone (*H*, arrows) with M line (*M*). × 22,000.

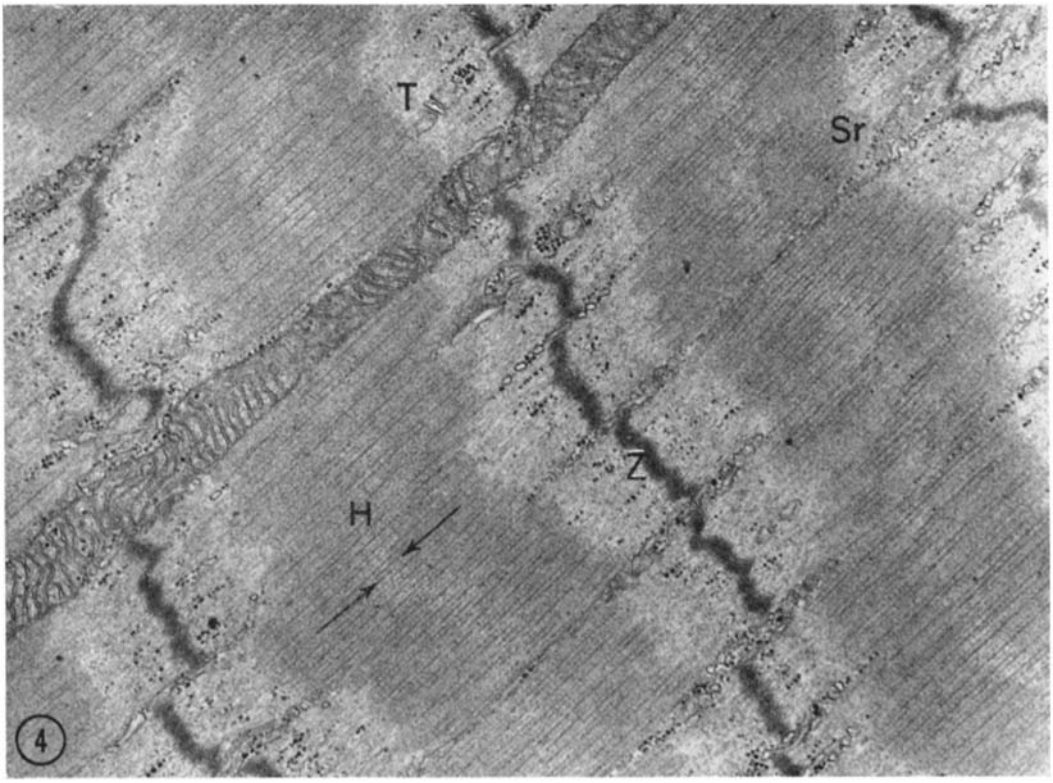

FIGURE 4 Longitudinal section of a multiply innervated muscle fiber (identified by cholinesterase stain) showing zigzag Z line (*Z*), poorly developed T system (*T*), ill-defined myofibrils with sparse sarcoplasmic reticulum (*Sr*), and faint H zone (*H*, arrows). × 26,000.

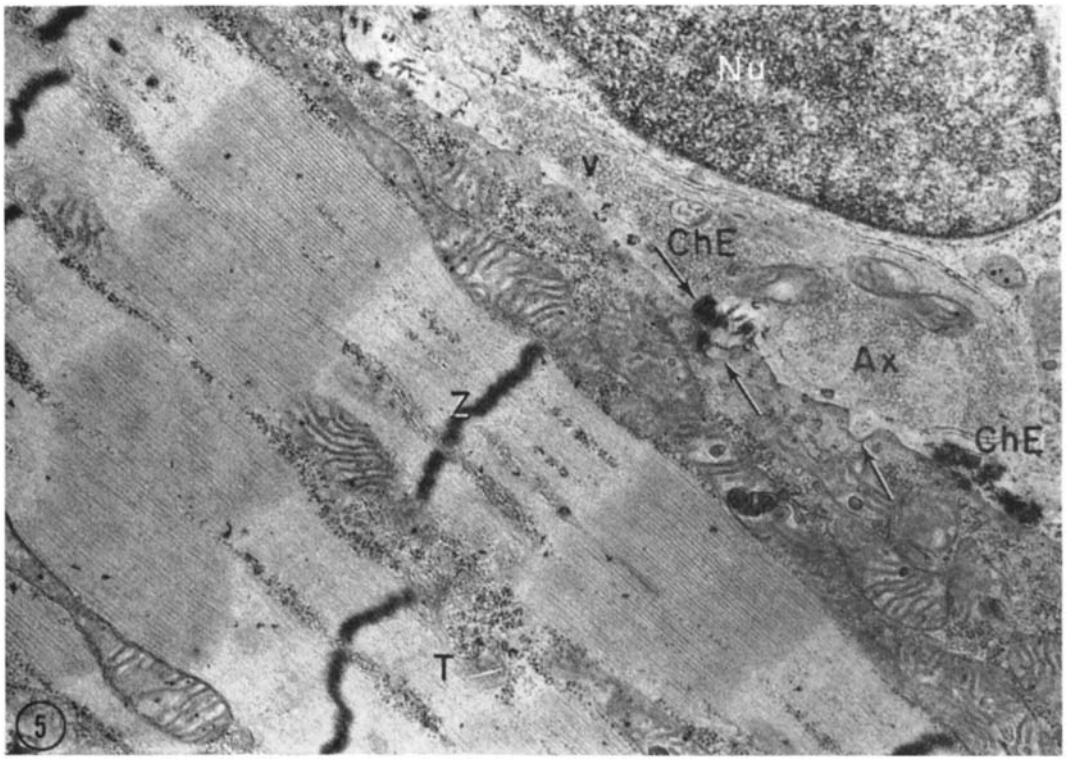

FIGURE 5 Longitudinal section of a multiply innervated fiber (identified by cholinesterase stain) showing a neuromuscular junction. A terminal axon (*Ax*) containing vesicles (*v*) is associated with a nucleated Schwann cell (*Nu*). This axon contacts on the surface of the muscle fiber whose plasma membrane forms only a few slight invaginations (arrows). In the synaptic gap are precipitated dense particles, presumably deposits of copper ferrocyanide, i.e., the reaction product of cholinesterase (*ChE*). The muscle fiber shows a zigzag Z line and a transverse tubule (*T*). × 25,000.

fine structural characteristics which differ from those of the fibers which have the typical en grappe type of ending.

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