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Abstract

The changes of rat muscle fiber structure and fiber types after the reunification of the nerve and cross-innervation between the nerve to M. soleus (SOL) and M. extensor digitorum longus (EDL) were cytologically studied and the following results were obtained: 1. After the reunification of the nerve, the tendency toward grouping to a single fiber type was observed, although in normal muscle, the red, white and intermediate fibers were distributed in mosaic pattern. 2. After the cross innervation, the changes of fiber types occurred; namely, in SOL, normally composed of red and intermediate fibers, the three types of fibers appeared after the cross- innervation with the nerve to EDL, which originally was composed of the red, white and intermediate muscle fibers, and vice versa. These changes were observed not only in histochemical sections, but also in the ultrastructural level by electron microscope.

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A CYTOLOGICAL STUDY OF THE EFFECT OF REINNERVATION AND CROSS-INNERVATION ON RAT STRIATED MUSCLE

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Recent histochemical and electron microscope studies (1, 2) reveal that the mammalian striated muscle is composed of the mixture of the different types of fibers. The so-called "white muscle" M. extensor digitorum longus (EDL), is composed of three types of fibers, i. e. the white, the red and the intermediate fibers, which have different contents of mitochondria, while the so-called "red muscle" M. soleus (SOL) is composed of two, the red and the intermediate ones.

In this preliminary study, the changes of muscle fiber structure and fiber types after the reunification of the nerve and cross-innervation between the nerve to SOL and to EDL were cytologically clarified.

MATERIALS AND METHODS

Adults male Wistar rats weighing 150—200 g were used for reinnervation experiments. The animals were anaesthetized with intraperitoneal injection of sodium pentobarbital, the sciatic nerve was exposed and cut at the middle of the thigh and immediately reunified with 8—0 silk or alon-alpha. The animals were killed two weeks, 2, 3, 5, 9 and 15 months after the operation.

For cross-union experiments, three week male Wister rats were used. The nerve to SOL was cross-innervated to the nerve to EDL and *vice versa*. As the control, the same nerve was transected and self-united. The animals were killed three and five months after the operation.

Muscles fixed in 10% neutral buffered formalin were washed, embedded in gelatin, and sectioned on a freezing microtome. Sections (5μ) were stained with Sudan black B and mounted in glycerogel. Control sections were extracted with acetone before staining.

For electron microscope study, thin strips of muscle were fixed in 1% osmium tetroxide buffered to pH 7.4 with veronal acetate, and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead hydroxide, and then examined with a Hitachi model HU-llA. electronmicroscope Thicker sections were stained with toluidine blue and examined with the light microscope.

RESULTS

In Sudan sections or electron microscope pictures, three types of fibers were distinguished in EDL (Fig. 1). In all three types of fibers, on both sides of Z-line, bracelet-like mitochondria encircled the myofibril at the I-band level. In the white fiber, practically all the mitochondria appeared at the I-band level. And they were smaller than those in the red and intermediate ones. The distinct feature of white fiber was that subsarcolemmal aggregation and interfibrillar chains of mitochondria were inconspicuous or absent (Figs. 9 and 11). The red fiber had a greater number of mitochondria in the subsarcolemmal space and large mitochondria which forming chains that ran longitudinally among myofibrils. The intermediate fiber resembled the red fiber, but its subsarcolemmal aggregation and interfibrillar chains were less conspicuous than those of the red fiber. These findings are similar to those in rat diaphragm (2) and intercostal muscle (3).

SOL was composed of only the red and intermediate fibers, which were larger in diameter than those in EDL (Fig. 2). No white fibers were observed in SOL.

The changes of muscle fibers after reunification of the sciatic nerve: Two weeks after the operation, the leg was found to be paralyzed and the Sudan section revealed denervation atrophy were noticed in all muscle fibers. Two months later, the function of the leg recovered relatively well, and regeneration of most fibers was observed. In these fibers, the content of mitochondria increased and the large fiber, which seemed to be transformed to typical white fiber, had a moderate amount of mitochondria on its rim.

Five months later the motion of the leg recovered fairly well, but even one year later, some disturbance of the leg persisted and no complete recovery was attained in all the animals operated. Five months after the operation, most fibers showed the structure of almost normal appearance, although some fibers were still in atrophy stage and central nuclei were occasionally observed. A distinct feature of the regenerated muscle after the nerve reunification was a tendency towards grouping of a single fiber type (Fig. 3), although in some part three types of fibers were distributed in mosaic pattern as noraml muscle.

The changes of muscle fibers after the cross-innervation: In Sudan sections of SOL, cross-innervated with the nerve to EDL five months previously, three types of fibers just as those in normal EDL were distinguishable

(Fig. 3), but there was a tendency to group into a single fiber type in some area. There appeared the fiber, lacking both rim and interfibrillar chain of mitochondria, although in normal SOL this type of fiber was not observed. In electron microscope pictures, this type of fibers mostly lacked both subsarcolemmal aggregation of mitochondria and interfibrillar chain of mitochondria. Practically all mitochondria were located at the I-band level (Figs. 10 and 12). The fine structures of this fiber were quite similar to those of the white fiber in normal EDL. The fibers which were quite similar in structure to the red and intermediate fiber were also observed in cross-innervated SOL. Their diameter was smaller than those in normal SOL and similar to those in normal EDL. In the control sections, self-united the nerve to SOL, all fibers were classified into the red and intermediated fibers and no white fibers (Fig. 5).

In Sudan sections of EDL, cross-innervated with the nerve to SOL 5 months previously, most fibers had a similar appearance as the red, and intermediate fibers in SOL; namely, they were larger in diameter than those of both types of fibers in EDL, and the content and distribution of mitochondria were just with those in SOL (Fig. 8). In the control sections, three types of fibers appeared. A tendency of a single fiber type grouping occurred in both cross-innervated and control sections.

DISCUSSION

Even one year after the reunification of the sciatic nerve, some degree of motor disturbances, for example, difficulty of complete extension of toes, remained. The difficulty of complete recovery of motor function after the nerve suture is also observed clinically. Probably one reason for such a difficulty is due to at random reconnection between motor neuron and muscle fibers, and others are the grouping of one muscle fiber type and consequently loss of mosaic pattern distribution in normal muscle. This grouping of one fiber type may be attributed to the reinnervation by the branches of a single nerve fiber to group of atrophied muscle cells, then the differentiation to the same type of fiber ensues.

The changes of the speed of contraction by cross-innervation was reported by Buller *et al.* (4) and of enzyme pattern (5, 6) or contents of mitochondria (7) were already reported. In this preliminary study, the fibers, which have the features of fine structure of the white fiber, have been demonstrated in SOL after the cross-innervation. This means that by cross-innervation the changes occur not only in the speed of contraction or enzyme pattern of muscle fibers but also in fine structure. The more precise study by electron microscopy on the changes by reunification and cross-innervation is now in progress.

SUMMARY

The changes of rat muscle fiber structure and fiber types after the reunification of the nerve and cross-innervation between the nerve to M. soleus (SOL) and M. extensor digitorum longus (EDL) were cytologically studied and the following results were obtained:

1. After the reunification of the nerve, the tendency toward grouping to a single fiber type was observed, although in normal muscle, the red, white and intermediate fibers were distributed in mosaic pattern.

2. After the cross-innervation, the changes of fiber types occurred; namely, in SOL, normally composed of red and intermediate fibers, the three types of fibers appeared after the cross-innervation with the nerve to EDL, which originally was composed of the red, white and intermediate muscle fibers, and *vice versa*. These changes were observed not only in histochemical sections, but also in the ultrastructural level by electron microscope.

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EXPLANATIONS

Figs. 1—8 Transverse sections of muscle stained with Sudan black B.

Fig. 1 EDL of normal rat. Note three types of fibers, red, white and intermediate distributed in mosaic pattern. ×100

Fig. 2 SOL of normal rat. Note this muscle is composed of red and intermediate fibers, and lacks white fiber. ×100

Fig. 3 EDL 9 months after the reunification of the sciatic nerve. Note a tendency of single fiber type grouping. ×100

Fig. 4 SOL 9 months after the reunification of sciatic nerve. Note two intermediate fibers in the center having central nuclei. ×400

Fig. 5 SOL 5 months after self-unification of the nerve to SOL. Note a similar appearance of the red and intermediate fibers as normal SOL. ×400

Fig. 6 SOL five months after cross-innervation with the nerve to EDL. Here, we can see the appearance of white fibers. ×100

Fig. 7 Higher magnification of white fiber of the same specimen as in Fig. 6. ×400

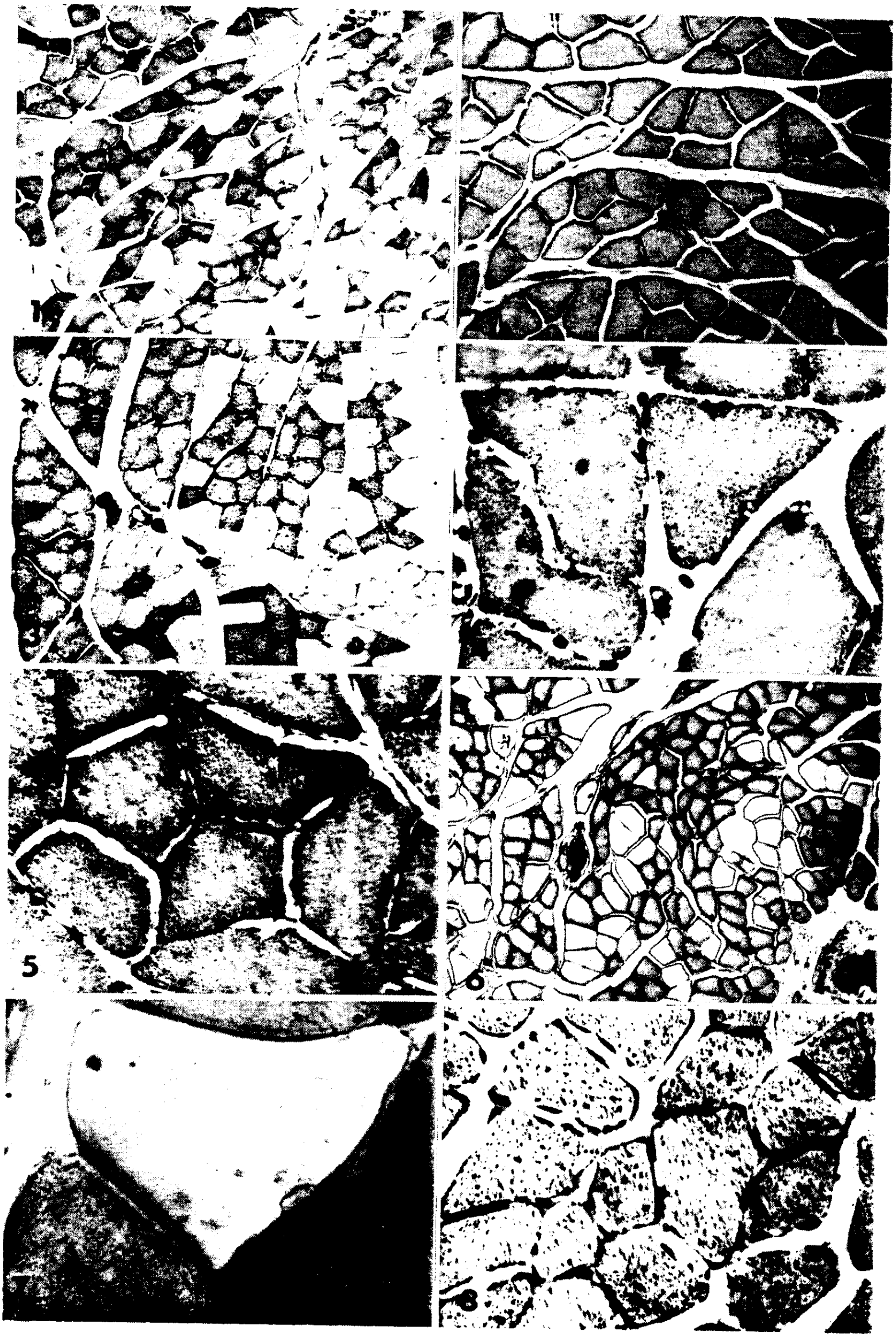
Fig. 8 EDL 3 months after cross-innervation with the nerve to SOL. This gives a similar appearance as normal SOL. ×400

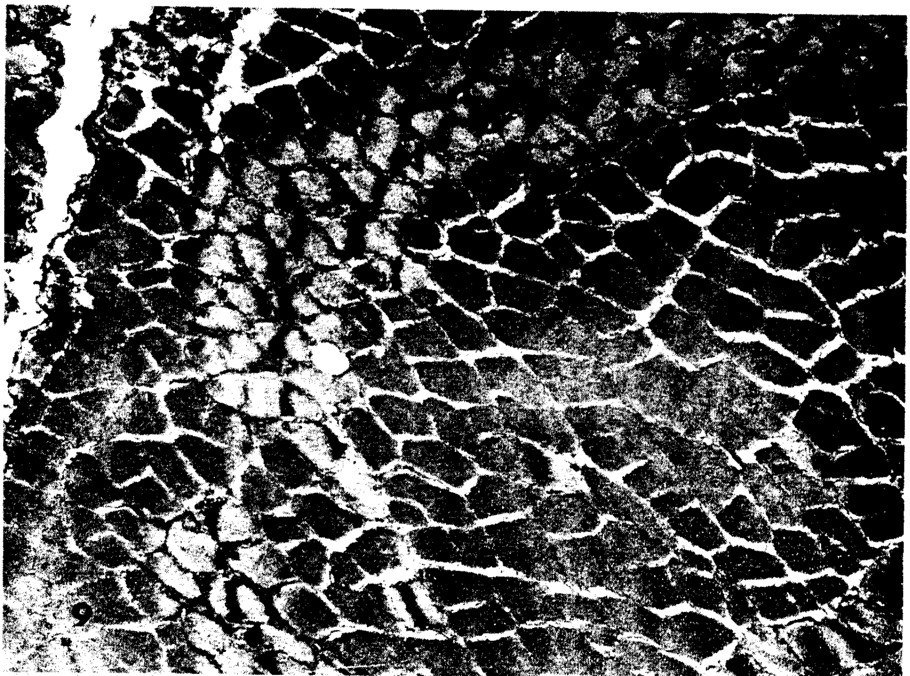
Fig. 9 An electron microscopic picture of transverse section of white fiber in normal EDL. Note the lack of both the aggregation of mitochondria in subsarcolemmal space and the chains of mitochondria in interfibrillar space. Practically all mitochondria appear in I-band level. ×5, 100

Fig. 10 A transverse section of white fiber appears in SOL cross-innervated with the nerve to EDL 5 months previously. Note the similar distribution of mitochondria as normal white fiber in Fig. 9. ×8, 600

Fig. 11 Lower half is a longitudinal section of white fiber in normal EDL. Note practically all mitochondria appear in I-band level and mitochondria are absent both in subsarcolemmal space and in A-band level. Upper half is an intermediate fiber. ×3, 000

Fig. 12 Longitudinal section of white fiber in SOL cross-innervated with the nerve to EDL. Note the structure is similar to that of the normal white fiber in Fig. 11. ×2, 000

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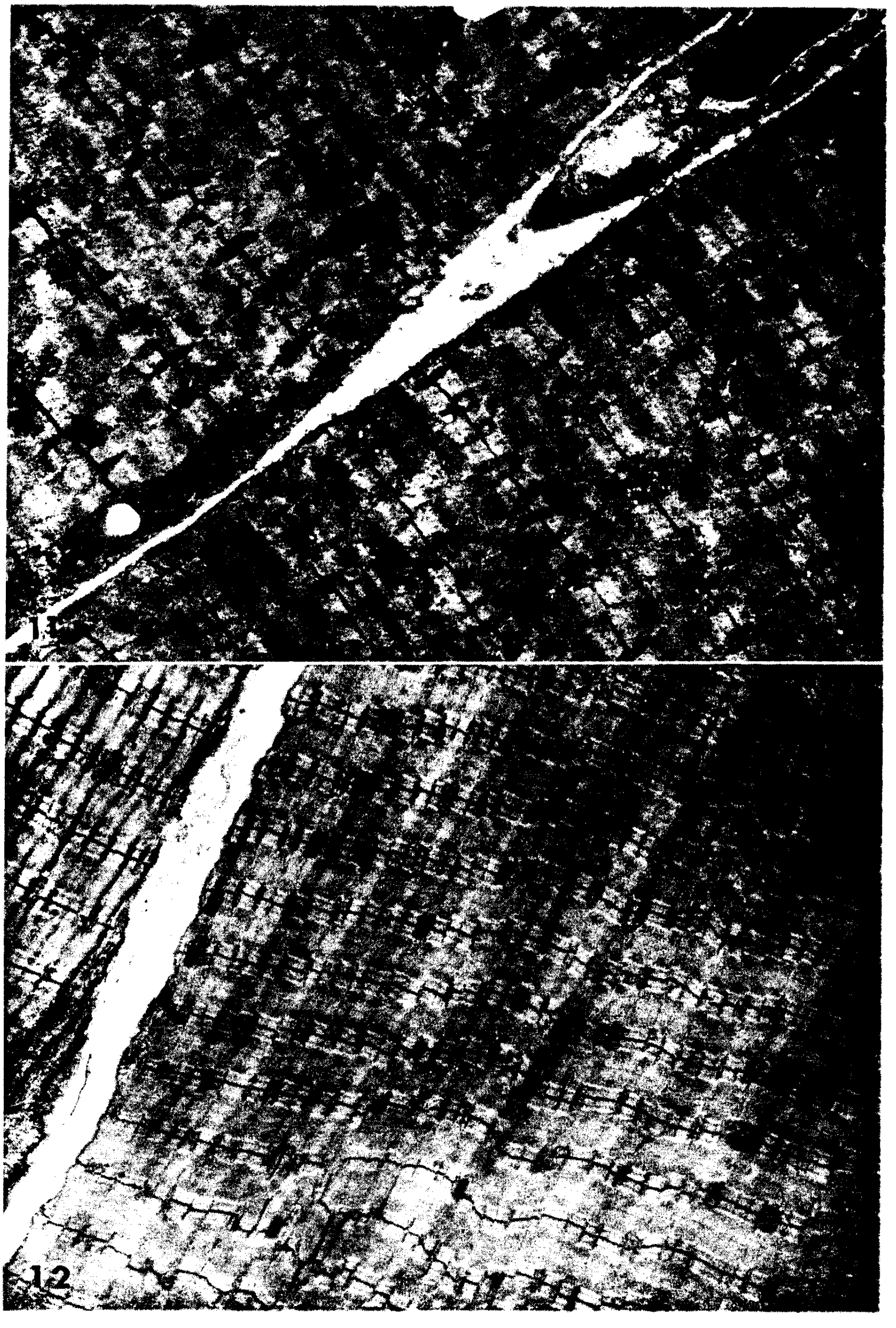
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