Effects of Short-term Denervation and Subsequent Reinnervation on Motor Endplates and the Soleus Muscle in the Rat*

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Summary. The rat sciatic nerve was locally frozen, and changes in the nerve, motor endplates, and the soleus muscle were examined for up to 6 weeks by light and electron microscopy. The wet weights of denervated soleus muscles compared with contralateral values progressively declined to a minimum at 2 weeks after injury (60.7±2.5%) and began to reverse following 3 weeks. The sciatic nerve thoroughly degenerated after freezing. However, numerous regenerated myelinated and thin nerve fibers were observed at 3 weeks. They were considerably enlarged but still smaller than normal counterparts at 6 weeks postoperatively. Nerve terminals containing synaptic vesicles of endplates disappeared at day 1 and mostly reappeared at 3 weeks (about 70% of the endplates). All endplates examined were reinnervated at 4, 5, and 6 weeks. On the other hand, postsynaptic folds of muscle fibers seemed to be only slightly influenced by denervation or reinnervation. Ultrastructural alterations of myofibrils, in particular the loss of register, immediately appeared after denervation, spread progressively, peaked at 2 weeks, ameliorated following reinnervation, and became significantly normalized at 6 weeks after freezing. The proportion of type II fibers in the soleus muscle similary showed an increase and a decrease with a short delay in response to denervation and reinnervation, respectively. This study clearly demonstrated that the nerve supply affects the ultrastructural integrity of skeletal muscles. In addition, changes in the endplates and the soleus muscle evaluated in this study after short-term denervation are largely reversible following reinnervation.

Denervation causes a great variety of muscle changes such as severe atrophy (PELLEGRINO and FRANZINI, 1963; JAWEED et al., 1975; HERBISON et al., 1979; VIGUIE et al., 1997; NNODIM, 1999), reduction in fiber cross-sectional area, ultrastructural disorganization of fibers (PELLEGRINO and FRANZINI, 1963; TOMANEK and LUND, 1973; LU et al., 1997), changes in fiber type ratio (KARPATI and ENGEL, 1968; BISHOP and MILTON, 1997) and transient satellite cell proliferation (MURRAY and ROBBINS, 1982a, b; LU et al., 1997). Most investigations have focused on the effects of denervation, while relatively few studies have dealt with regenerative processes. JAWEED et al. (1975) observed the denervated and reinnervated rat soleus muscle at the light microscopic level. BODINE-FOWLER et al. (1996) injured the rat sciatic nerve by phenol and observed the time course of muscle atrophy and recovery, but the denervation was incomplete. It is reported that long-term denervated (2–4 months) muscles recover poorly, perhaps due to the deposition of interstitial collagen (LU et al., 1997). However, not enough attention has been paid to the time course of alterations in muscles after reinnervation. Moreover, changes in the fiber type ratio after denervation remain controversial (KARPATI and ENGEL, 1968; CHIEN and CHU, 1995; BODINE-FOWLER et al., 1996; BISHOP and MILTON, 1997). To study these problems, we froze the sciatic nerve of rats and examined degenerative as well as regenerative changes in endplates and the soleus muscle from immediately after the nerve injury until recovery.

MATERIALS AND METHODS

Twenty-seven 8-week-old female Wistar rats weighing 160–182 g were used. Rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The skin covering the buttock was cut on one side. The sciatic nerve was exposed and separated from surrounding tissues. The nerve was frozen and thawed a few times by contact with a stainless rod a few mm in diameter cooled by liquid nitrogen. Care was taken not to injure other tissues. Freezing was chosen as the method of denervation since this method uniformly and definitively damages nerve fibers with reinnervation more likely than in other procedures such as nerve crushing or transection with a suture. The nerve became white when frozen, and the proximal margin of the frozen portion was loosely tied with a black thread for marking. Contralateral hindlimbs were untreated and used as one type of control. Additionally, three normal 8-week-old rats were also used as controls, in particular to survey the type II fiber ratio in the normal soleus muscle. Food and water were supplied *ad libitum*. In the treated animals the degree of paralysis of the hindlimbs was examined every day. The animals were sacrificed by an overdose of inhaled diethyl ether at 1 or 3 days, or 1, 2, 3, 4, 5 and 6 weeks (three rats each) after nerve freezing. Soleus muscles from both legs and accompanying sciatic nerves of the frozen sides were removed *en bloc*. The distance between the black thread for marking and the nerve entrance into the soleus muscle was measured. Soleus muscles were dissected free of extramuscular tissues, weighed, and cut into proximal and distal halves. Distal halves which were somewhat smaller than the others were used for light microscopy. Midbelly portions of proximal halves were used for electron microscopy since endplates were aligned transversely at the midbelly region.

Histochemical analysis (ATPase staining)

The soleus muscles were mounted vertically on a cork plate in tragacanth gum jelly of appropriate softness (e. g. 7%; Karpati and Engel, 1968) to obtain cross sections. The mounted muscle was then frozen by immersion in melting isopentane cooled in liquid nitrogen.

Transverse sections (10 μm) were cut in a cryostat cooled to $-25°C$, affixed to gelatin-coated slides, and stained by hematoxylin and eosin for general observation. Cryosections were also stained for a myosin adenosine triphosphatase (ATPase) reaction according to Guth and Samaha (1970) with some modifications, and muscle fibers were classified into type I and type II fibers. In brief, sections were preincubated with a solution either at pH 4.3 for 15 min to stain type I fibers or at pH 10.4 for 10 min to stain type II fibers, and histochemically stained in a medium containing 5 mM sodium-ATP, 0.18 M calcium chloride and 0.1 M sodium barbital, pH 9.4. In the present study, no attempt was made to distinguish between subtypes of type II fibers due to technical difficulties. After the ATPase reaction, a whole cross section of each soleus muscle was photographed at a magnification of 20 for fiber-type ratio analysis. The population of type II fibers was expressed as a percentage of the total fiber number. The central region of the soleus muscles at 2 weeks after denervation (the most atrophic condition) was photographed at a magnification of 50 and all muscle fibers delineated by entire fiber boundaries were measured for cross sectional areas using computers and NIH Image software.

Electron microscopy

Midbelly regions of the soleus muscles and sciatic nerves (both proximal and distal to the frozen site) were immediately immersed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and cut into small rectangular or cylindrical pieces to facilitate orientation during embedding and sectioning. Specimens were fixed in the same fixative for 6 to 20 h, postfixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) overnight for better postfixation of central regions of the sciatic nerve trunk at 4°C, rinsed in 10% saccharose three times (10 min each), and stained *en bloc* in 3% aqueous uranyl acetate for 1 h at room temperature. Then samples were dehydrated in an ascending series of ethanol, replaced by propylene oxide and flat embedded in epoxy resin. Three blocks were selected at random from each soleus muscle. Semithin sections were cut, stained with 0.5% toluidine blue in 0.5% borate and observed by a conventional light microscope for location of the endplates. This attempt, however, was unsuccessful. Ultrathin sections were then cut longitudinally so that each section would more likely encounter endplates since these are aligned in a narrow zone across the midbelly portion. Ultrathin sections were stained with uranyl acetate and lead citrate and observed by a JEM-1200 electron microscope. All endplates observed were photographed and analyzed.

Data were expressed as mean±standard deviation. Comparisons of means of muscle wet weight and fiber type II ratio were made among the different animal groups using Fisher's protected least significant difference method. The level of statistical signifi-

cance was set at 95% ($P < 0.05$).

RESULTS

After nerve freezing, rats remained ambulatory though they dragged the foot on the frozen side. Loss of active movement at the ankle and toe was observed. About 3 weeks after the operation paralysis was alleviated and voluntary extension of digits was noticed. At 4, 5 and 6 weeks, no paralysis of the hindlimb persisted, although flexion of the ankle joint was still weaker than on the contralateral side.

The distance between the black marker thread and entrance of the nerve into the soleus muscle was 21.7 ± 2.7 mm.

Wet weight of soleus muscles and fiber cross-sectional areas

Soleus muscle wet weights in normal 8-week-old rats were 59.6 ± 3.5 mg; there were no left-right differences. To minimize individual and age differences in treated rats, denervated/reinnervated soleus muscle wet weights were expressed as percentages of their contralateral soleus muscles. The extent of muscle atrophy at each time interval before and after freezing is shown in Table 1.

Cross-sectional areas of muscle fibers at 2 weeks of denervation measured as follows: type I fibers of the frozen side (1146.7 ± 340.0 μm^2; n=668), type I fibers of the contralateral side (2388.9 ± 641.3 μm^2; n=269), type II fibers of the frozen side (651.7 ± 214.1 μm^2; n= 320) and type II fibers of the contralateral side (1831.0 ± 424.8 μm^2; n=98). The relative cross-sectional areas compared with the contralateral counterparts were 48.0% in type I fibers and 35.6% in type II fibers. However, the extent of atrophy of type II

fibers is probably underestimated since some type I fibers—which are generally thicker than type II fibers—changed to type II and the ratio of this type increased postoperatively (Table 1).

Fine structural changes in the sciatic nerve

In normal portions proximal to the frozen site, sciatic nerve cross sections showed numerous instances of ovoid and ellipsoid myelin surrounding light areas of axons at their centers.

In portions distal to the frozen site, light microscopy revealed that the whole nerve was uniformly damaged and swollen 1 day after freezing. Intervals among nerve fibers were widened. At the electron microscopic level, myelin sheaths were deformed and axons were occasionally difficult to observe. At 3 days, deformation of myelin and loss of axons were apparent both at the light and electron microscopic levels. Myelin degenerated and Schwann cells contained variably large vacuoles and lamellar inclusions (Fig. 1). At 1 week, light microscopy revealed that most myelin sheaths had clearly degenerated and were densely stained even at their centers where axons were located. Electron microscopically, most myelin sheaths formed irregularly whorled structures. Vacuoles of varying sizes were found within Schwann cells. At 2 weeks after the treatment, most degenerated myelin sheaths had disappeared, leaving only some densely stained parts. A considerable number of macrophages, probably containing myelin debris, were observed. Light central areas indicating axons were few. In contrast, at 3 weeks numerous thin formations of myelin reappeared with small light centers inside. At 4 and 5 weeks, the number and size of these formations increased. At 6 weeks, the sciatic nerve was full of well regenerated myelin sheaths and axons. The myelin sheaths thickened and axons

Table 1. Relative wet weight, number of nerve terminals, and type II fiber ratio in soleus muscle.

	Before freezing	1 day	3 days	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Relative wet weight (%)	96.8± 7.5	97.4± 0.1	87.8± 0.1	66.7± 0.9**	60.7± 2.5**	74.5± 16.3**	76.0± 19.7*	89.4± 2.1	92.7± 4.0
No. of nerve terminals/ No. of endplates examined	20/20	0/16	0/20	0/16	1/17	19/27	19/19	22/22	19/19
Type II fiber ratio (%)	13.0± 4.6	not measured	not measured	23.0± 2.3*	34.9± 4.6**	41.3± 4.0**	29.6± 12.0**	19.5± 3.6	18.0± 9.4

*$P < 0.05$, **$P < 0.01$ compared with specimens before freezing.
The number of nerve terminals was not statistically analyzed.
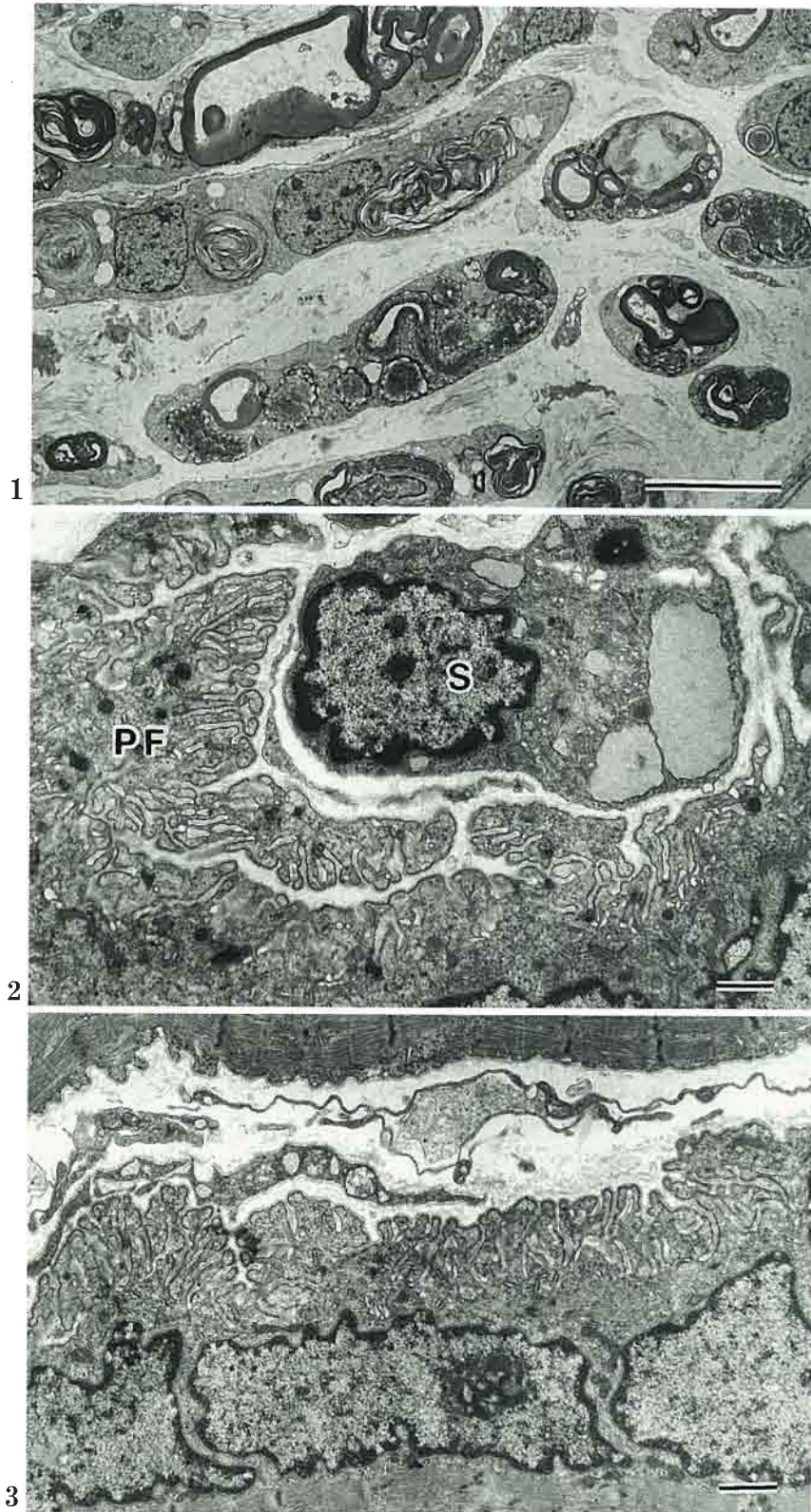
Fig. 1. Electron microscopic view of sciatic nerve at 3 days after freezing. Most myelin sleaths and axons are degenerated. Bar=5 μm.
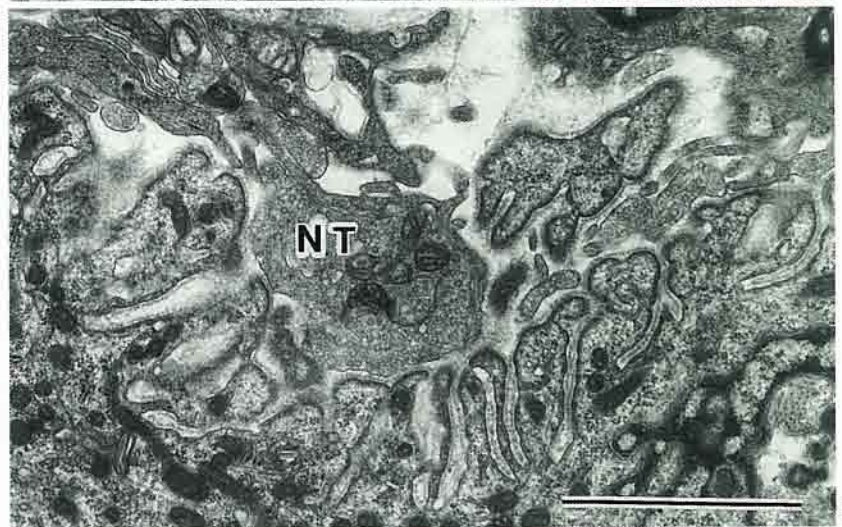
Figs. 2 and **3.** Electron micrographs of motor endplates after nerve freezing. Bars=1 μm.
Fig. 2. At 3 days. No nerve terminals are recognizable. Elements of rough endoplasmic reticulum within a Schwann cell (S) are greatly expanded. *PF* postsynaptic folds.
**Fig. 3.** At 1 week after freezing. No nerve terminals are seen.
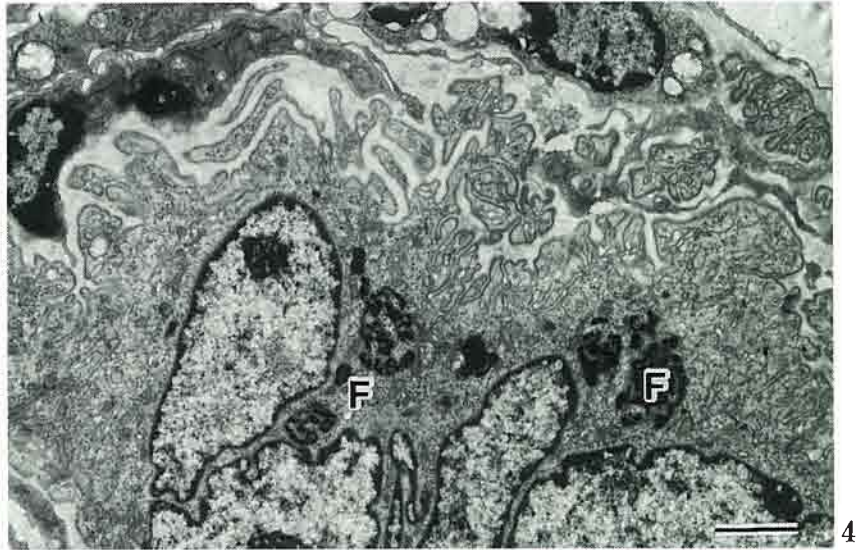
Figs. 4-6. Electron micrographs of motor endplates after nerve freezing. Bars=2 μm.
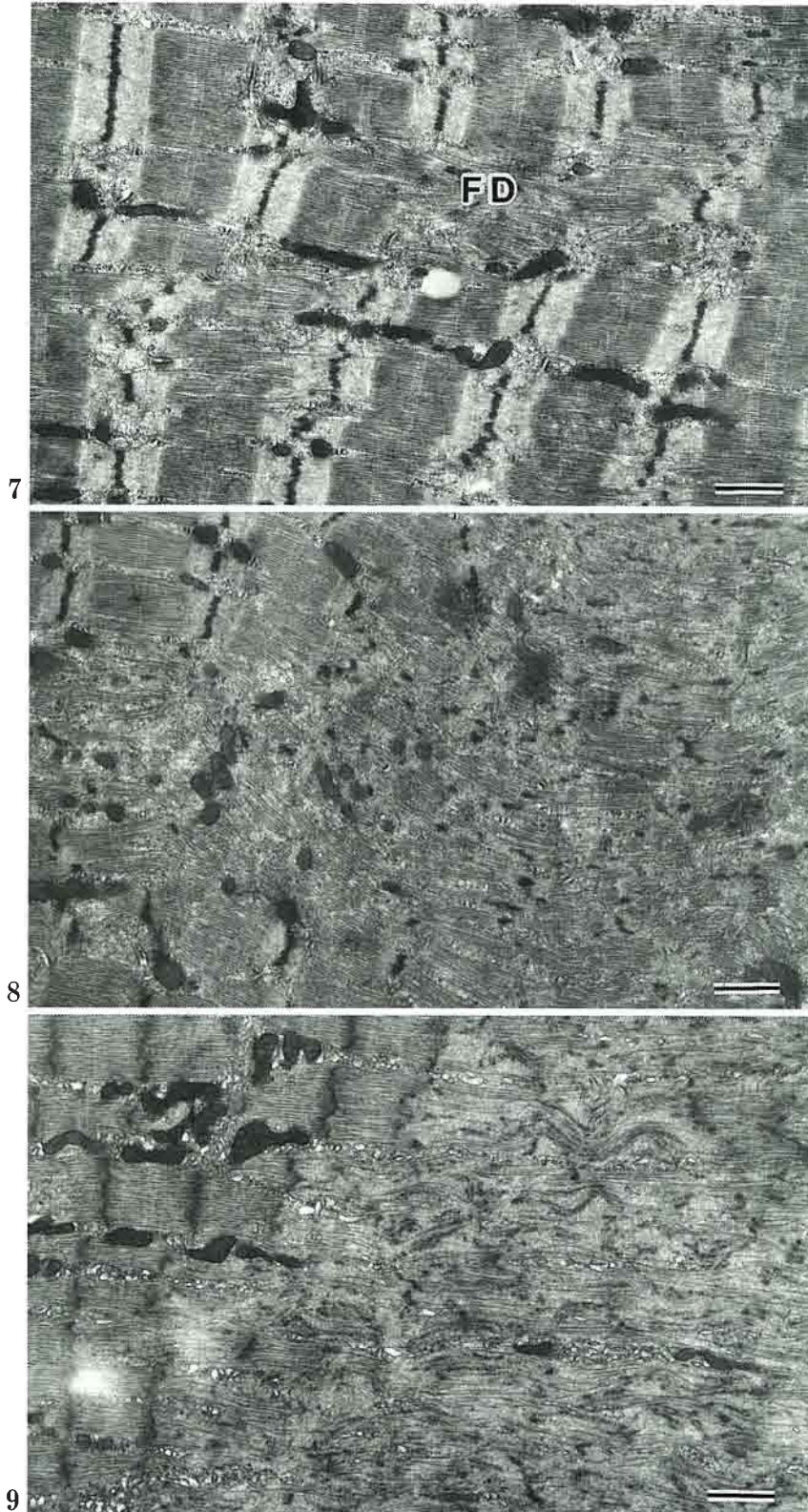
Fig. 4. At 2 weeks. No nerve terminals are observable. Postsynaptic folds seem more complicated possibly due to oblique sectioning. Fragmented and condensed nuclei (*F*) are seen.

Fig. 5. At 3 weeks. A regenerated nerve terminal (*NT*) containing synaptic vesicles is seen.

Fig. 6. At 5 weeks. Regenerated nerve terminals (*arrows*) contain numerous synaptic vesicles.
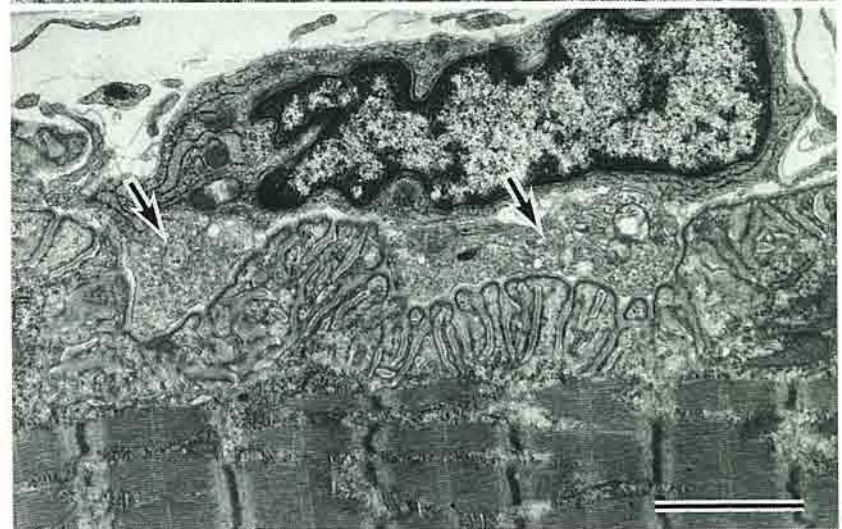
Figs. 7-9. Electron micrographs of the soleus muscle after freezing. Bars=1 μm.

Fig. 7. One day after nerve freezing. Focal disruption (FD) of Z bands and loss of myofibrillar register are seen.

Fig. 8. At 3 days. Z bands are broken into fragments and structures of sarcomere are disorganized in areas on the right.

Fig. 9. At 1 week. Sarcomeres are severely disintegrated in the middle and right areas.
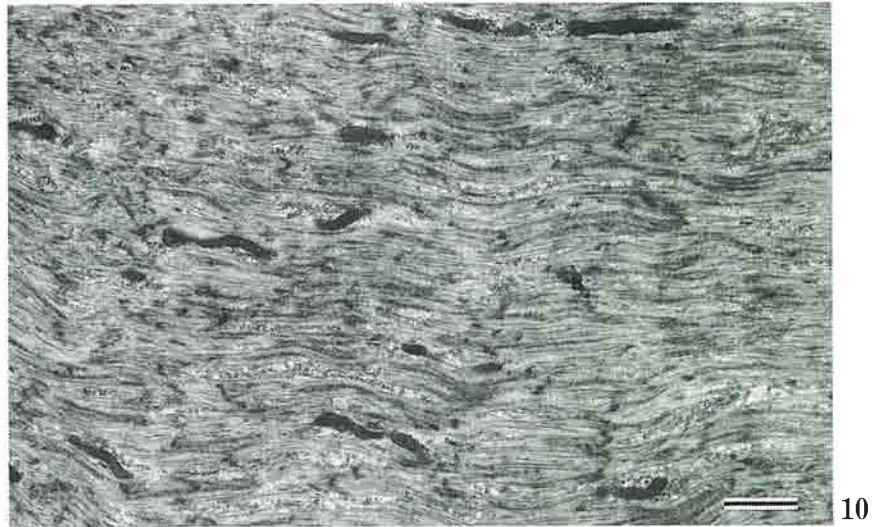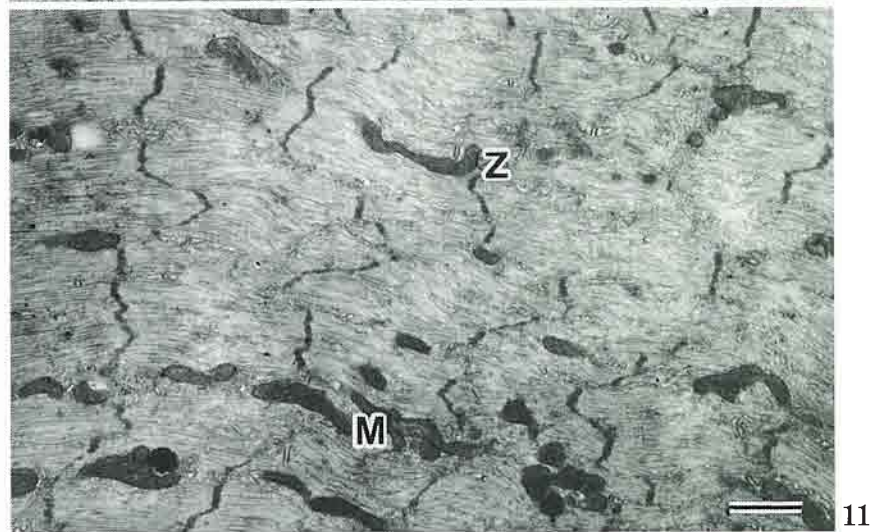
Figs. 10-12. An area of the soleus muscle. Bars=1 μm.

Fig. 10. At 2 weeks. The structure of sarcomeres is totally disorganized.

Fig. 11. At 2 weeks after nerve freezing. In less severe lesions, Z bands (Z) are not disrupted but highly wavy. A and I bands are not discernible. M mitochondria.

Fig. 12. At 3 weeks. Sarcomeres are deformed and Z bands are interrupted in many sarcomeres.

Figs. 13 and **14.** Electron micrographs of the soleus muscle after freezing. Bars=1 μm.
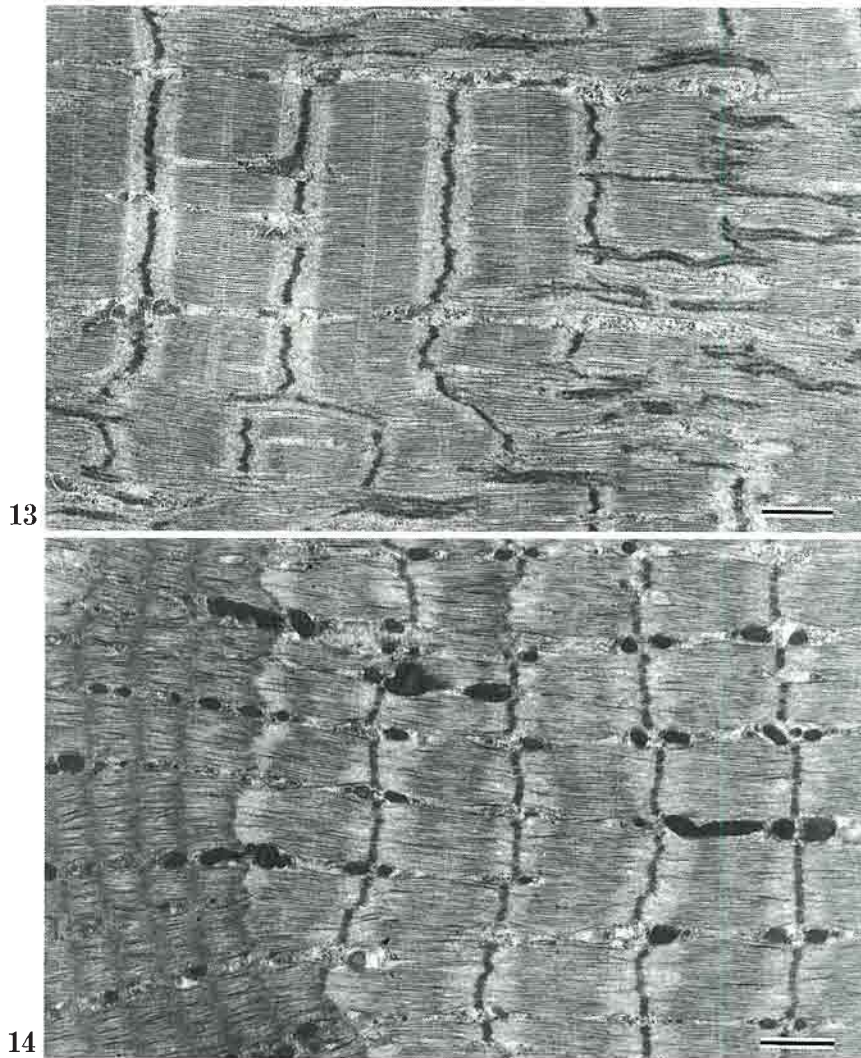**Fig. 13.** At 5 weeks. Most sarcomeres are in register. Deformation of Z bands can be seen in some areas.
Fig. 14. At 6 weeks after freezing. Sarcomeres not in register are very few, but overcontraction (left) and overextension (right) are seen.

enlarged, though still smaller than those of normal nerves.

Fine structural changes of motor endplates

The numbers of soleus muscle endplates found at various time intervals are shown in Table 1. In normal soleus muscles, nerve terminals were observed to lie within a depression of the muscle fiber surface, their upper halves being covered by a Schwann cell. The presynaptic nerve terminals contained numerous synaptic vesicles and a number of mitochondria. From the depression of the muscle fiber, the sarcolemma extended deeply inward, sometimes branching into an elaborate specialization of postsynaptic folds.

At 1 and 3 days after freezing, none of the very few nerve terminals which were found contained synaptic vesicles. Elements of rough endoplasmic reticulum were greatly expanded within Schwann cells (Fig. 2). At 1 week, no presynaptic nerve terminals were found. Postsynaptic folds were devoid of any overlying nerve terminal. Most postsynaptic folds remained essentially unchanged in structure (Fig. 3). At 2 weeks, no nerve terminals were found except for one regenerated endplate (Table 1). No changes were noticed at postsynaptic regions, whereas some folds seemed slightly more complicated in folding and branching. Condensation and fragmentation of nuclear chromatin (Lu et al., 1977) were observed in some nuclei (Fig. 4). At 3 weeks, nerve terminals were found in about 70% (19 out of 27) of the endplates examined. These terminals were smaller, and synaptic vesicles of normal size and shape were fewer than in control samples (Fig. 5). At 4, 5 and 6 weeks, all endplates had vesicle-laden nerve termi-
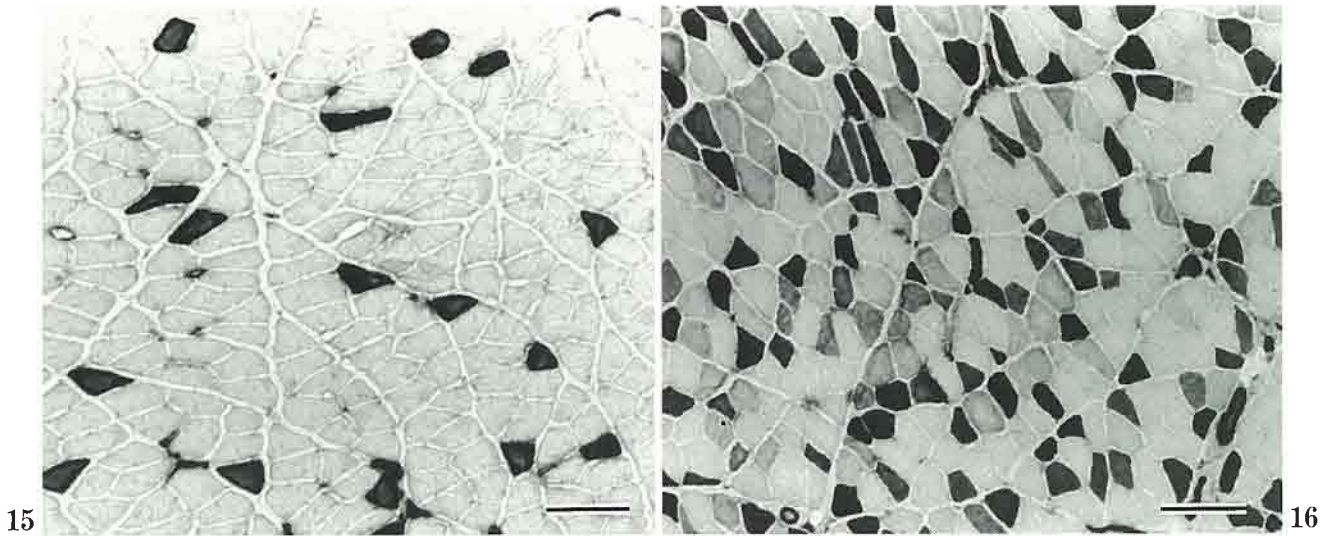
Figs. 15 and 16.   Light micrographs of the soleus muscle at 3 weeks after nerve freezing, stained by alkaline pretreated ATPase reaction. Bars=100 μm. **Fig. 15.** Contralateral soleus muscle. Darkly stained type II fibers are small in number. **Fig. 16.** Experimental side of the same animal shown in Figure 15. Densely stained type II fibers are significantly numerous. Note muscle fibers with various intermediate densities, classified as type II fibers in the present study.

nals that were still smaller than their normal counterparts (Fig. 6). No new formation of developing postsynaptic folds was observed in any specimen.

Ultrastructural changes in soleus muscle fibers

Fine structural alterations of fibers appeared after denervation but decreased after reinnervation, with some variation. Careful observations revealed that changes in muscle fibers could be divided into two types; 1) focal hypercontraction and/or overextension of myofibrils (Fig. 14) and 2) loss of myofibrillar register (Figs. 7–13).

In hypercontracted and overextended (overstretched) regions, sarcomeres were shortened or lengthened, respectively. A and I bands were often indistinct, whereas Z bands were usually continuous and traceable even if they were deformed and highly curved. Hypercontracted and overextended structures were often combined and varied in number and size from one time interval after freezing to another. In regions where myofibrillar register was lost, the normal lateral alignment of myofilaments was incompletely or completely lost. Z bands were disrupted into fragments or short segments and randomly distributed. The structure of sarcomeres was so disorganized that A and I bands were hardly discernible. When changes were less severe, Z bands were highly wavy or streaming rather than interrupted.

At 1 day, myofibrils with their Z bands not in register were only focally observed (Fig. 7). At 3 days and 1 week, disorganized myofibrils progressively increased in number and size (Figs. 8, 9). At 2 weeks, these disintegrated myofibrils were most frequently and widely encountered (Figs. 10, 11). Such lesions tended to concentrate within a relatively small number of fibers and sometimes mixed with portions of hypercontraction and overextension. Abnormal sarcomeres not in register began to decrease after 3 weeks (Fig. 12), were further reduced at 4 and 5 weeks (Fig. 13), and were very rare at 6 weeks. Hypercontracted and overextended lesions generally ran parallel with the extent of loss of register with respect to both number and size, but considerable deviations from this were occasionally observed, even at 6 weeks (Fig. 14).

Neither an increase of interfibrillar collagen nor degeneration of capillaries was evident. Condensed and fragmented nuclei and degenerated fibers were rarely found.

Changes in muscle fiber type ratio

The type II fiber ratio ranged from 10 to 17% in normal and contralateral soleus muscles. The type II fiber ratios of denervated sides were consistently higher than control levels. Type II fibers increased and reached a maximum at 3 weeks (Figs. 15, 16) after denervation and gradually decreased thereafter, but were still higher at 6 weeks (Table 1).

DISCUSSION

The present study clearly demonstrated that ultrastructural alterations, in particular loss of lateral alignment of myofibrils, and other parameters of muscles are closely correlated with the nerve supply. Frozen sciatic nerves regenerated well, and varying changes including ultrastructural changes of the soleus muscle after denervation were found to recover, either immediately or slowly, after reinnervation.

From an ultrastructural point of view, a great amount of myelin and approximately 70% of nerve terminals are considered to have regenerated between 2 and 3 weeks after freezing. It can be roughly estimated that the sciatic nerve takes about 3 weeks to grow a distance of 22 mm, which is comparable to the data by JAWEED et al. (1975), and to a reported speed of 10 mm in 7–8 days (PACHTER and EBERSTEIN, 1989).

Changes of the motor endplate

For light microscopic observation of endplates, the activity of cholinesterase, i. e., an enzyme present at the sarcolemmal surface, has frequently been employed (KANAYA, 1988; PACHTER and EBERSTEIN, 1992; WANG et al., 1997). These reports suggest that postsynaptic folds are well preserved for a considerable period and then gradually deteriorate with increasing postdenervation time. At the electron microscopic level, morphometric changes have been documented at the postsynaptic areas under various conditions, such as denervation (PACHTER and EBERSTEIN, 1989), denervation plus passive exercise (PACHTER and EBERSTEIN, 1983), limb immobilization (PACHTER and EBERSTEIN, 1986; FAHIM, 1989; WANG and LIU, 1995), and tenotomy (PACHTER and SPIELHOLZ, 1990). In the present study, postsynaptic folds of a few endplates at 1 and 2 weeks seemed slightly more complicated in folding and branching. This finding may be attributed to possible oblique or tangential sectioning of endplates since muscle fibers were cut longitudinally.

With respect to the regeneration of neuromuscular junctions, regenerated nerve terminals in the present study are speculated to have reunited with the remaining postsynaptic folds. KORNELIUSSEN and SOMMERSCHILD (1976) and FAHIM (1989) observed new nerve terminals which made contact with developing postsynaptic folds. We did not confirm such a finding, however. Nerve fibers damaged by freezing may more easily reach preexisting postsynaptic structures as both parts retain continuity after injury.

Changes of soleus muscle wet weight

Wet weights of the soleus muscle showed a progressive decrease in proportion to the time after denervation (JAWEED et al., 1975; BODINE-FOWLER et al., 1996; the present study). Denervation produces more atrophy than immobilization or tenotomy (HERBISON et al., 1979; KANAYA, 1988) due not only to muscle disuse caused by paralysis but also to the deprivation of trophic substances (DAVIS et al., 1985). Type II muscle fibers are generally reported to undergo preferential atrophy after total denervation (KARPATI and ENGEL, 1968; KANAYA, 1988; LU et al., 1997, the present study) and after selective motor nerve cutting (WANG et al., 1997). In contrast, BODINE-FOWLER et al. (1996) stated that the slow fibers (type I) of the rat soleus muscle showed more atrophy than the fast fibers (type II). TOMANEK and LUND (1973) and HERBISON et al. (1979) found that type I fiber atrophy was equal in degree to type II fiber atrophy. TOMANEK and LUND (1973) stated that the magnitude of atrophy should be interpreted with reference both to the muscle and to the time period after denervation. Furthermore, the effect of muscle fiber type change should be considered as described in Results.

Following reinnervation, wet weights steadily increased. However, it is known that the magnitude of gain in muscle weight does not directly reflect functional recovery. The ability to generate muscle tension needs a much longer time to recover (IRINTCHEV et al., 1990).

Ultrastructural changes in muscle fibers

Various changes in fibers have been reported after denervation. Similar ultrastructural alterations of fibers such as hypercontraction, overextension, and loss of myofibrillar register have been reported to follow eccentric contraction (FRIDEN and LIEBER, 1998; THOMPSON et al., 1999) as well as reloading after spaceflights or suspension unloading (RILEY et al., 1992; KRIPPENDORF and RILEY, 1994). FRIDÉN and LIEBER (1998) considered that a loss of myofibrillar register results from cytoskeletal disruptions on the basis that desmin immunoreactivity is lost in some fibers. THOMPSON et al. (1999) thought that the focal contraction was attributed to calcium homeostasis loss and that the lateral displacement of filaments was caused by a disorder of titin. In the present study, the loss of myofibrillar register was clearly related to denervation. As is well known, desmin has an important role in maintaining the lateral alignment of sarcomeres. In addition, the disorganization of myofibrils and Z bands is found in some skeletal

muscles of desmin knockout mice (MILNER et al., 1996; LI et al., 1997). Denervation may thus elicit the loss of myofibrillar register possibly via disorganization of its desmin cytoskeleton.

Changes in muscle fiber type ratio

The normal soleus muscle of the rat is more than 75% type I fibers (ARMSTRONG and PHELPS, 1984; CHIEN and CHU, 1995). This value increases with age (NARUSAWA, 1985; HORI et al., 1998) and changes by varying procedures. It is generally believed that a decrease in muscle activity facilitates a transformation of fiber type from slow to fast (HORI et al., 1998). This increase in type II fibers is observed not only after denervation but also after cast immobilization, unloading, and spinal cord injury (GROSSMAN et al., 1998; TALMADGE et al., 1999). After denervation, however, findings on the change in fiber type ratio are extremely controversial. It is reported that type II fibers of the soleus muscle after denervation decrease in mice (BISHOP and MILTON, 1997), hardly change in rats (KARPATI and ENGEL, 1968) or in rabbits (d'ALBIS et al., 1994), increase in rats (CHIEN and CHU, 1995; the present study), and increase only in the soleus muscle in guinea pigs (TOMANEK and LUND, 1973). BISHOP and MILTON (1997) observed that nerve crushing at its entrance into the soleus muscle resulted in a considerable decrease of type II fibers in mice, while soleus muscles denervated 4 mm more proximally exhibited only a small decrease in type II fibers. Alterations of fiber type ratio after denervation (with or without subsequent reinnervation) are claimed to be permanent (BISHOP and MILTON, 1997) or temporary (BODINE-FOWLER et al., 1996). In the present study, the type II fiber ratio markedly increased and began to reverse after reinnervation with some delay (1 week or more). This finding agrees with those of KARPATI and ENGEL (1968), TOMANEK and LUND (1973) and CHIEN and CHU (1995), although the extent of increase considerably differs. The discrepancy in the extent of increase is perhaps, at least in part, due to the increase in hybrid fibers containing both myosin heavy chains I and II at varying ratios within the same fiber (JAKUBIEC-PUKA et al., 1990; CHIEN and CHU, 1995). Such hybrid fibers are sometimes difficult to clearly classify by ATPase reaction alone into type I or type II fibers. The delay of fiber type changes may be attributable to the time needed to produce enough new myosin to predominate in the myosin pool within each muscle fiber. The mechanism of muscle fiber type change remains to be elucidated, and the following factors should be taken into consideration: animal species, the muscle examined,

site of nerve injury, duration after denervation, and whether or not the muscle is reinnervated.

In conclusion, the nerve supply has an essential relation with the morphological and probably functional integrity of skeletal muscle. Denervation resulted in a loss of myofibrillar register, sarcomeric hypercontraction and overextension, severe muscle atrophy and other changes. These changes evaluated in this study are to a great extent reversible after short-term denervation, although some alterations such as fiber type ratio and contractile function may need more time to approach normal values.

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