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Reinnervation of Motor Endplate-Containing and Motor Endplate-Less Muscle Grafts[1]

DAVID BADER
Department of Anatomy, University of Michigan, Ann Arbor, Michigan 48109

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Damaged muscle fibers degenerate and are phagocytosed, but their connective tissue sheaths and myogenic cells survive. New myofibers regenerate within the connective tissue sheath and functional reinnervation is restored. The present study compared regenerating myofibers and their reinnervation in muscle grafts where the original motor endplates were removed and when they were present. In regenerating muscles, where the endplates were present, axons made neuromuscular connections with the myofibers. High levels of CAT activity, characteristic of cholinergic innervation, were reached and maintained in these muscles. Also, motor endplate-containing grafts developed characteristic muscle fiber types and nerve-evoked muscle contraction was observed. In preparations lacking the original endplate region, CAT activity initially rose to high levels, but with time diminished to 17% of control, and muscle fiber types did not develop. Muscle contraction after nerve stimulation was not observed in these grafts. It was concluded that, although a small number of motor endplates are formed in grafts devoid of original motor endplates, the presence of the motor endplate region in regenerating muscle is critical for development and maintenance of myofibers and the axons that contact them.

INTRODUCTION

When the continuity between motor nerves and muscle fibers is disrupted, the various neural and muscular components undergo changes to reestablish connection. If motor nerves are damaged by crushing or cutting, sprouts grow out from the damaged nerve and make contact with muscle fibers (Gutmann and Young, 1944; Zacks, 1973). Conversely, if the muscle fibers are damaged and the nerves are left uninjured, as is the case after injection of methylbupivacaine (Marcaine) into a muscle, the muscular components will regenerate and reestablish neuromuscular contact (Jirmanova and Thesleff, 1972). Finally, when both the nerve and muscle are damaged motor endplates (MEP) are reconstructed by the regenerated nerve and muscle (Bennett *et al.*, 1974; Marshall *et al.*, 1977).

Sanes *et al.* (1978) have demonstrated that the original MEPs of skeletal muscle play an integral role in the reinnervation of injured skeletal muscle. They have shown that nerves make precise contact with the basal lamina of original MEPs of injured and X-irradiated muscle even in the absence of muscle fibers and that nerves undergo synaptic differentiation at the original MEP. Very little, if any, synaptic differentiation of nerves occurred at places other than the original MEP.

Regenerating muscle has been shown to be dependent to reinnervation for growth and differentiation. Carlson and Gutmann (1974 and 1975a) have demonstrated that regenerated myofibers are dependent on reinnervation for muscle fiber-type differentiation. If muscle grafts are kept denervated, muscle fibers atrophy and differentiation of fiber types does not take place.

The aim of this study was to determine whether or not the original motor endplate region of a muscle is involved in the reestablishment of neuromuscular connections

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in regenerating skeletal muscle. The MEP zone of the soleus (SOL) muscle, which exists as a single band, was cut from the muscle and the resulting MEP-less muscle was grafted and studied for the presence or absence of MEPs and nerves at various times after surgery. Morphological, histochemical, physiological, and biochemical methods were used to compare myoneural interactions in MEP-less grafts and SOL grafts which contained original MEPs. In this way it could be determined to what degree the original zone of MEPs was involved in the reinnervation of skeletal muscle grafts.

MATERIALS AND METHODS

Experimental Design

All experiments were conducted on 150- to 200-g male Sprague-Dawley rats (Charles River). The rats were anesthetized with ether. The soleus (SOL) muscle was chosen because the motor endplate (MEP) zone can be removed by cutting a small, midportion from the muscle, thus making a graft devoid of original MEPs.

In one leg, the SOL was removed and the MEP zone was cut out. The excised MEP zone was tested histochemically for acetylcholinesterase (AChE) activity to determine the completeness of MEP zone removal (described below). The MEP-less graft was then transferred to the bed of the ablated extensor digitorum longus (EDL) muscle and sutured to the proximal and distal stumps of the EDL tendon (Fig. 1). The EDL bed was chosen for grafting because it is shorter than the soleus bed and can better accommodate the shorter MEP-less grafts. In the contralateral leg, the so-

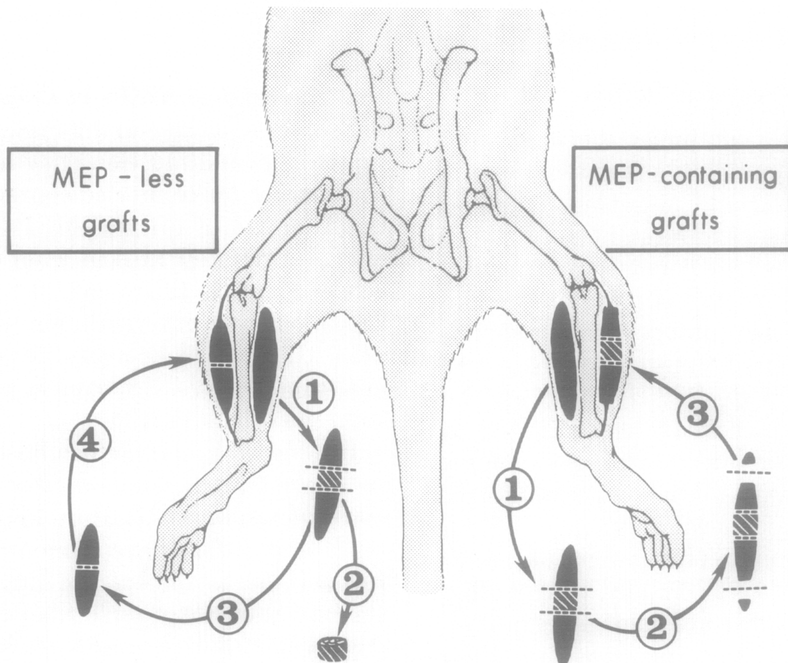

FIG. 1. Experimental design for preparation of MEP-less and MEP-containing grafts. *Left, MEP-less grafts.* (1) The SOL muscle is taken from its bed and the MEP zone is cut from the muscle. (2) The excised MEP zone is tested histochemically for AChE activity to check the completeness of MEP removal. (3) The two remaining pieces are placed together and (4) grafted into the bed of the ablated EDL. *Right, MEP-containing grafts.* (1) The SOL muscle is taken from its bed and the MEP zone is cut from the muscle. (2) The three pieces are placed together. (3) The ends of the graft are cut so that the graft will fit the EDL bed and the MEP-containing graft is placed in the bed of the ablated EDL.

leus MEP zone was cut out as previously described but was returned to the muscle to be grafted (Fig. 1). This was done to test whether cutting the muscle grafts would inhibit the reinnervation of muscle fibers. This MEP-containing graft was then placed in the ablated EDL bed and sutured to the EDL tendons. The ends of the MEP-containing graft were cut down so that the muscle would fit into the EDL bed. The initial weights of the MEP-less and MEP-containing grafts were approximately 100 mg. Initially, the pieces of the two types of grafts were sutured together, but it was discovered that nonsutured pieces would adhere satisfactorily to each other and, therefore, this procedure was omitted. The fascia and skin were sutured with surgical thread and the animals were maintained under standard laboratory conditions.

At various times after surgery, ranging from 0 to 60 days, MEP-less and MEP-containing grafts were taken from the rats, weighed, and processed for morphological, histochemical, biochemical, or physiological tests.

Cholinesterase/Nerve Stain

A stain for acetylcholinesterase (AChE) activity and silver impregnation for nerves (Goshgarian, 1977) was used in this study. With this technique, AChE activity can be assayed singly or combined with the stain for nerves. The AChE stain alone was used on the excised MEP zone from MEP-less grafts to test whether all MEPs were removed at grafting. This was done either by freezing the excised piece in cooled isopentane (−140°C), cutting serial sections on a cryostat, and staining, or by staining the piece as a whole mount. If unstained muscle appeared on both sides of the area of AChE activity, the entire MEP zone was considered to be removed (Fig. 3). The total number of MEPs in three excised pieces of MEP zones was counted in serial longitudinal sections which were cut at 32 μm and stained for AChE activity. At least three MEP-less and MEP-containing grafts were frozen in cooled isopentane, serially sectioned at 32 μm, and stained with the AChE/nerve stain at 4, 10, 19, 23, 25, 30, 35, 40, and 60 days after surgery. Tetraisopropylpyrophosphoramide (concn 2×10^{-4} *M*, Iso-OMPA [Sigma Co.]), which is an inhibitor of nonspecific cholinesterases, was used on some sections from these grafts. Little difference between Iso-OMPA and non-Iso-OMPA-treated sections was observed.

Choline Acetyltransferase (CAT) Activity

CAT is an enzyme characteristic of cholinergic innervation and has been used as a marker for the degree of reinnervation in skeletal muscle regeneration (Max and Rifenberick, 1975; Carlson *et al.*, 1979). At 4, 16, 20, 30, 34, and 50 days after surgery, at least five MEP-less and MEP-containing grafts were taken and a 10% homogenate of each graft was made in 10 m*M* EDTA/1% Triton X-100, pH 7.6. Five EDL muscles were homogenized and assayed for CAT activity as a control because the cut motor nerve of the EDL would presumably reinnervate the muscle grafts into the EDL bed. Ten microliters of muscle homogenates were assayed in 10 μl of Na_2HPO_4, 100 m*M*; NaCl, 200 m*M*; EDTA, 40 m*M*; choline Cl, 8 m*M*; acetyl-CoA, 0.2 m*M*; [^{3}H]acetyl-COA, 0.1 m*M*; eserine SO_4, 100 m*M* for 30 min at 35°C. ([^{3}H]Acetyl-CoA, specific activity of 1.3 Ci/m*M*, was purchased from New England Nuclear.) The CAT assay described above is not totally specific for choline acetyltransferase because of the contamination of enzymes such as carnitine acetyltransferase. Therefore, in all cases, parallel incubations were run with choline deleted from the reaction mixture. Values obtained from these incubations were assumed to be attributable to muscle enzymes other than CAT and were subtracted from the valves obtained from assays containing choline substrate. All data presented here represent values obtained from the buffer/

substrate containing choline minus values obtained from the control with choline deleted. Scintillation mixtures were prepared according to Fonnum (1975) and counted in a Beckman scintillation counter at 32% efficiency. All muscles and grafts were assayed in triplicate. In the five control EDL muscles tested, a value of 54.3 ± 8 pmole/mg wet wt/hr was obtained.

Histochemistry

At 60 days after surgery, five MEP-less and five MEP-containing grafts were frozen in cooled isopentane. The muscles were cut transversely at 10 μm in a cryostat. The serial sections were stained for myofibrillar adenosine triphosphatase (ATPase) at a base preincubation of pH 10.1 (Padykula and Herman, 1955) and for succinic dehydrogenase (SDH) activity (Nachlas *et al.*, 1957). Uninjured SOL and EDL muscles, stained for ATPase and SDH activity as described above, served as controls.

Physiological Tests

Four MEP-containing and MEP-less grafts were tested at 34 and 60 days after surgery for neuromuscular transmission and isometric contractile properties. Due to the degree of adhesion of free grafts to surrounding tissues and the variable level of entry of nerves into the grafts, intact nerve/muscle preparations could not be consistently removed from the leg without damage to the nerve. Therefore, neuromuscular transmission in MEP-containing and MEP-less grafts was tested *in situ* by electrical stimulation of the nerve to the graft after denervation of all other leg muscles. After isolation of the muscle graft, the nerve was repeatedly stimulated up to 80 V with hook electrodes while muscle contractions of grafts were monitored with a dissecting microscope. Upon completion of these tests, grafts were removed and isometric contractile properties were measured. The grafts were attached to an isometric force transducer and suspended between platinum electrodes in a muscle bath containing Krebs bicarbonate saline. The muscle bath was aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. Preparations were stimulated transversely with unidirectional square wave pulses of 200 μsec duration. The current flow and the muscle length were adjusted to give a maximal twitch tension. Time to peak twitch tension (TPT), and time for twitch tension to decline to half the maximal valve (½ RT), were measured. The stimulation frequency was then increased in 50-Hz intervals from 100 Hz until a maximum isometric tetanus tension (Po) was obtained.

Electron Microscopy

During the course of this study, it was found that a small number of AChE/nerve complexes were present in later stage MEP-less grafts. Therefore a preliminary electron microscopic study was conducted on 60-day MEP-less grafts to determine if, ultrastructurally, these complexes resembled MEPs from previously published studies of mammalian skeletal muscle. Three 60-day MEP-less grafts were fixed in phosphate-buffered glutaraldehyde (2%). Fixed tissues were treated for AChE activity with the method of Karnovsky (1964), and areas of AChE staining were postfixed in 2% osmium tetroxide, dehydrated, and embedded in Epon/Araldite resin. Thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Graft Weights

Previous studies of freely grafted muscle have shown an initial loss in graft weight followed by a gain in muscle mass in the third and fourth weeks after surgery (Carlson and Gutmann, 1975a). Grafts which are kept denervated throughout regeneration do not regain weight after the initial loss of muscle mass. As depicted in Fig. 2, the initial weights of the two types of grafts were approximately 100 mg. Both MEP-

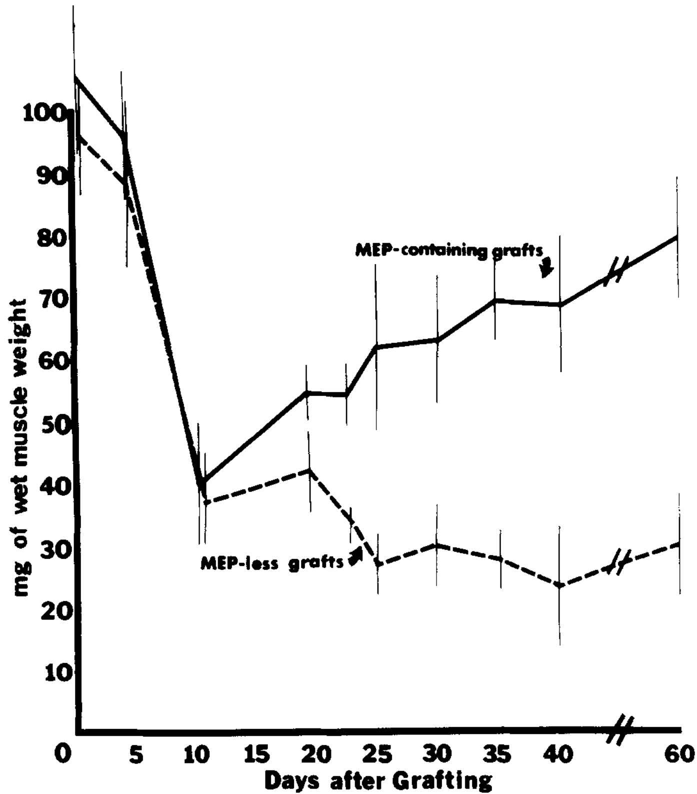

FIG. 2. Weights of MEP-containing and MEP-less grafts. Both types of grafts initially lose weight. MEP-containing grafts regain muscle mass after the third week but the weight of MEP-less grafts remains low.

containing and MEP-less grafts drop in weight until 19 days, at which time the MEP-containing grafts increase in weight through 60 days, while MEP-less grafts do not gain weight after 23 days.

AChE/Nerve Stain

Controls. When the excised piece of muscle from MEP-less grafts was stained for AChE alone, the MEPs of the muscle fibers could be seen as a row of claw-like structures (Fig. 3). The number of MEPs counted in the midportion removed at surgery was 3412 ± 387 (SD) in three cases when counted in serial sections. With or without Iso-OMPA treatment, little AChE activity was seen in the portions of freshly grafted MEP-less SOL muscles. MEPs were present in the midsection of MEP-containing grafts.

MEP-containing grafts. Four days after surgery in MEP-containing grafts the standard pattern of degeneration and regeneration seen in mammalian skeletal muscle grafts was observed (Carlson, 1973). Areas of AChE staining which correspond to orig-

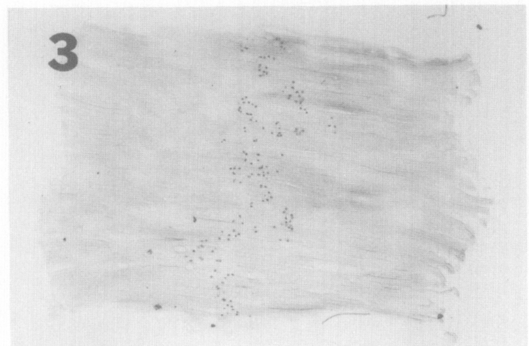

FIG. 3. Typical section through the excised MEP zone. Numerous MEPs are seen when stained for AChE (pretreated with Iso-OMPA). The resulting MEP-less graft, 30 days after surgery, is seen in Fig. 6. × 9.

inal MEPs were present in degenerated portions of the grafts (Fig. 4). By 10 days, the grafts consisted primarily of cross-striated regenerating muscle fibers and nerves were first observed at this time. The number of nerves in MEP-containing grafts generally increased throughout the course of regeneration. Although the precise time of contact between the MEP and the nerve cannot be determined with the light microscope, numerous MEP–nerve complexes were observed at 23 days after surgery (Fig. 5). In the five 40-day MEP-containing grafts the number of MEPs was 2138 ± 469 (SD). Sixty-day MEP-containing grafts generally had numerous nerves and small areas of connective tissue interspersed in the grafts.

MEP-less grafts. MEP-less grafts do not vary in appearance from MEP-containing grafts during the first 30-days after grafting except for their lack of staining for AChE (Fig. 6). As with the MEP-containing grafts, nerves were first seen in MEP-less grafts at 10 days when the muscle fibers already had cross striations. Increasing numbers of nerves were present in 25- and 30-day MEP-less grafts. In 40-day grafts, muscle fibers were generally thinner than those seen in 40-day MEP-containing grafts, but isolated thick muscle fibers were observed. The average number of MEPs in five 40-day MEP-less grafts was 46 MEPs/muscle ± 22 (SD).

Sixty-day MEP-less grafts had predominantly small atrophic muscle fibers and an increased amount of connective tissue and fat. When compared to 60-day MEP-containing grafts of MEP-less grafts at 34 days after surgery, few nerves were present in

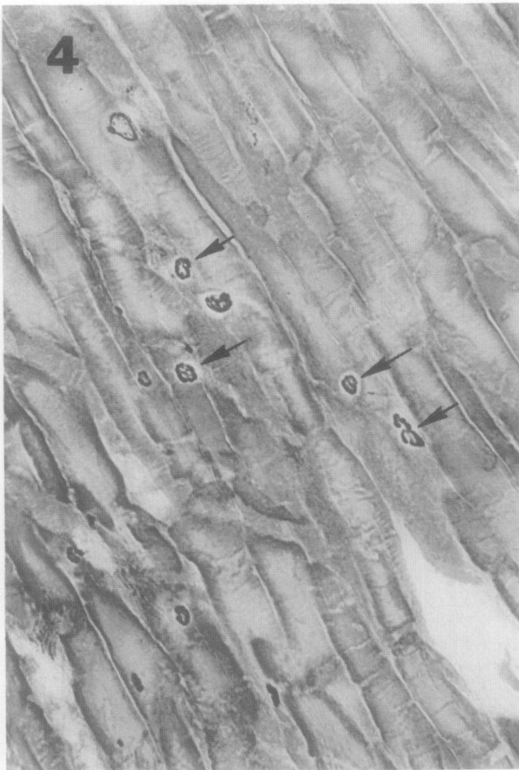

FIG. 4. Four-day MEP-containing graft stained for AChE. AChE reaction product (arrows) corresponding to MEPs observed in the degenerated muscle of MEP-containing grafts. AChE/nerve stain. × 175.

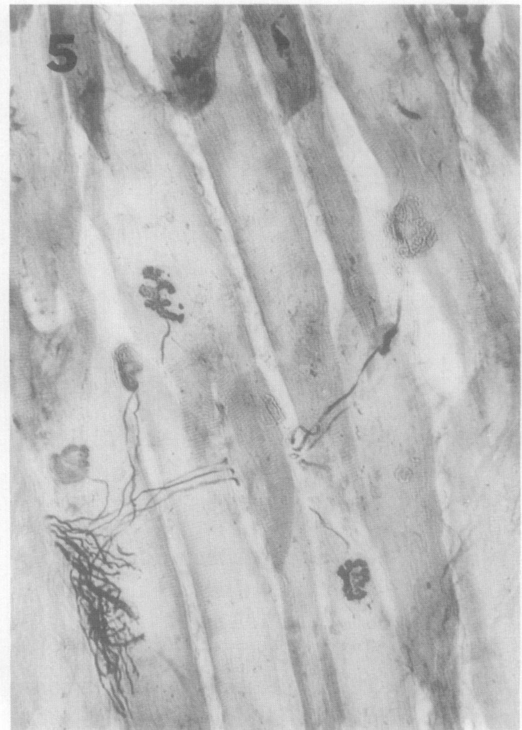

FIG. 5. In this 23-day MEP-containing graft numerous nerve/MEP structures are seen in the regenerated muscle. AChE/nerve stain. × 450.

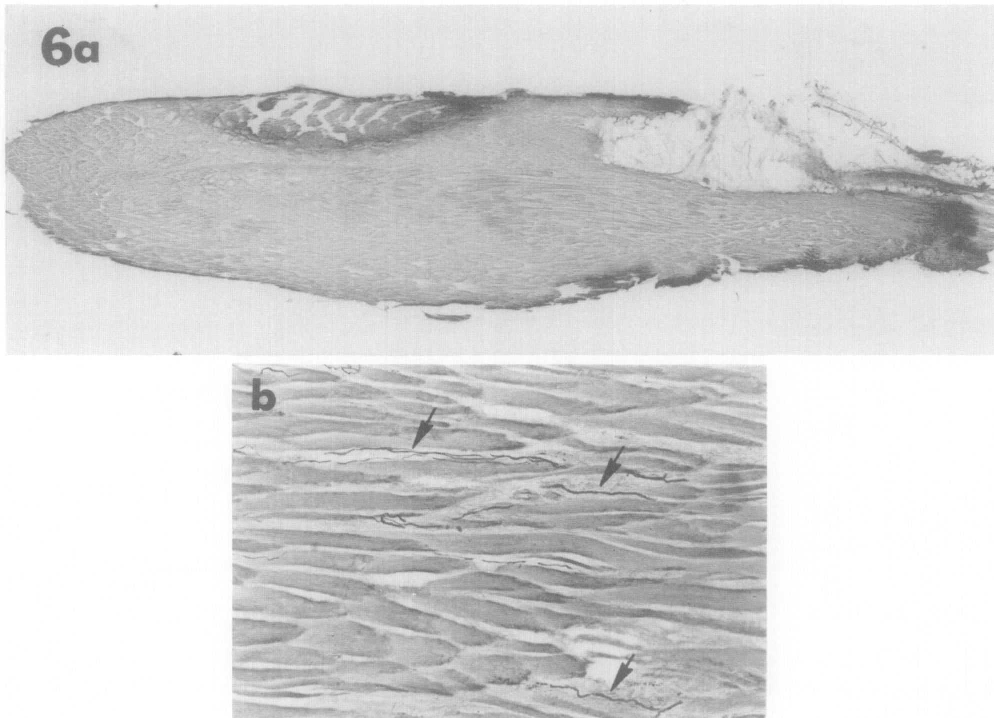

FIG. 6. Typical section of a MEP-less graft at 30 days after surgery. (a) Muscle fibers of the graft are seen longitudinally but fibers are devoid of AChE activity. × 12. (b) A higher power view of a portion of the graft seen in (a) shows nerves (arrows) interspersed in muscle fibers. × 125. AChE/nerve stain, Iso-OMPA pretreatment.

these grafts. MEPs were observed in these grafts but their numbers were comparable to the number of MEPs seen at 40 days after surgery in MEP-less grafts.

Choline Acetyltransferase Activity

Initially both MEP-containing and MEP-less grafts lose CAT activity due to the denervation caused by surgery, but by 16 days after surgery, approximately 20% of normal CAT activity of the EDL muscle was regained in both types of grafts (Fig. 7). CAT activity in both MEP-containing and MEP-less grafts increased until 34 days. This correlated with morphological evidence that nerves increase in number in grafts up to this time. At 50 days, MEP-containing grafts had increased to 51% of normal EDL CAT activity, but MEP-less grafts decreased to 17% of control CAT activity (Fig. 7).

Fiber Types

MEP-containing grafts. Examination of cross sections of MEP-containing grafts showed that muscle fibers were large in diameter (Figs. 8a and b). Myosin ATPase staining indicated the presence of different fiber types. The pattern of ATPase staining of grafts resembled that seen in EDL muscles. (The SOL muscle has a checkerboard appearance of fast and slow muscle fiber types, whereas the EDL has an almost homogeneous pattern of fast fibers (Carlson and Gutmann, 1975a). This is indicative of the fiber-type conversion that was seen in SOL muscles grafted into the EDL bed (Carlson and Gutmann, 1974). MEP-containing grafts had predominantly homogeneous ATPase staining. Different fiber types could also be observed in sections stained for SDH activity, with some fibers stained more heavily than others.

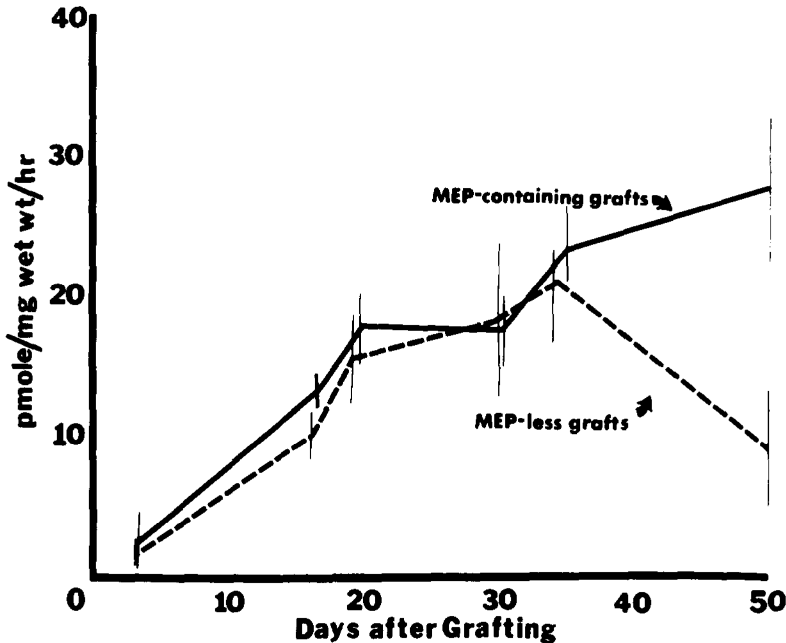

FIG. 7. Choline acetyltransferase (CAT) activity in MEP-containing and MEP-less grafts expressed as pmole/mg wet wt/hr. (Control EDL activity was 54.3 ± 8 pmole/mg wet wt/hr.) CAT activity in both MEP-containing and MEP-less grafts is 4% of control at 4 days after surgery. Both types of grafts gain in CAT activity until 34 days after surgery. At 50 days, MEP-containing grafts increase in CAT activity, but MEP-less grafts fall to approximately 17% of normal.

MEP-less grafts. In contrast to MEP-containing grafts, muscle fibers of MEP-less grafts were generally small and atrophic (Figs. 8c and d). Fibers stained intensely for ATPase activity but SDH staining was very weak or nonexistent. Occasionally, a large fiber lightly stained for ATPase was present among the numerous small fibers. The pattern of ATPase/SDH staining was indicative of denervated muscle (Carlson and Gutmann, 1975a).

Physiological Studies[2]

In three of the four MEP-containing grafts, at 34 days after surgery, distinct muscle contractions of the graft were observed upon nerve stimulation. In the 60-day MEP-containing group, three of the four grafts had distinct contractions upon stimulation and the remaining graft had slight contractions. None of the 34-day MEP-less grafts had any sign of muscular contraction when the nerve was stimulated and only one 60-day MEP-less graft showed slight contraction upon nerve stimulation.

No significant differences ($P < 0.05$) were observed in the isometric contractile properties of MEP-less or of MEP-containing grafts between 34 and 60 days after transplantation. Therefore, data collected at the two different time periods were pooled and compared to each other (Table 1). Compared to the MEP-containing grafts, the MEP-less grafts had a significantly ($P < 0.05$) longer TPT and ½ RT and a greater P_0. The larger P_0 of MEP-containing grafts is indicative of greater isometric tension. The number of myofibers in three 60-day MEP-containing and MEP-less grafts used in physiological studies were counted in histological sections of these grafts. No significant difference was found in the number of fibers present in the two types of grafts

[2] These data were collected in collaboration with John A. Faulkner.

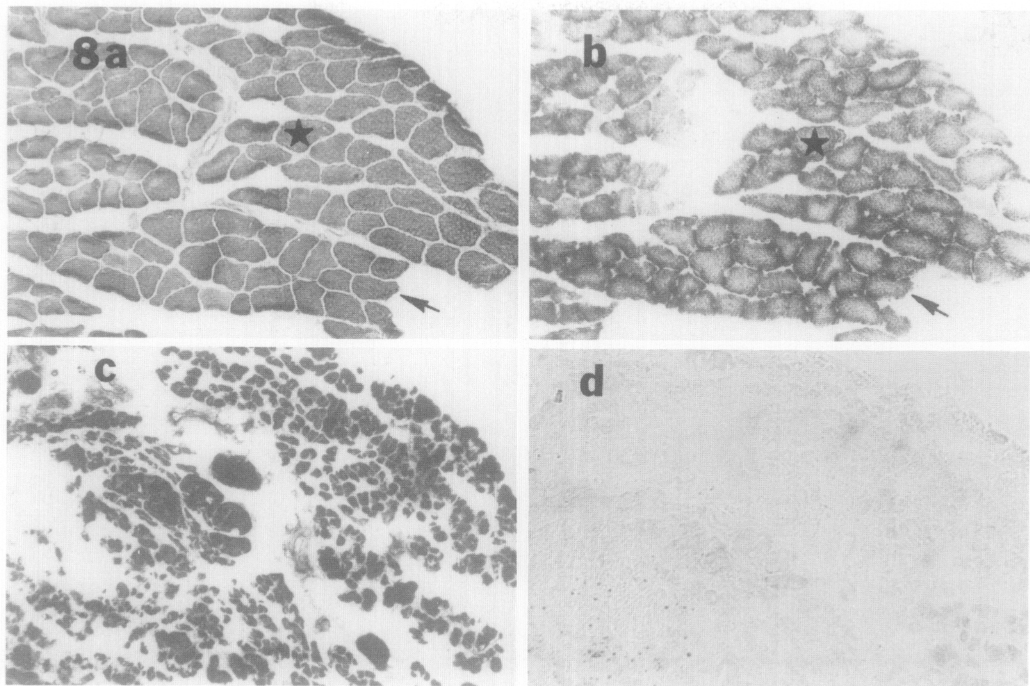

FIG. 8. Histochemistry of 60-day MEP-containing and MEP-less grafts. (a) MEP-containing graft stained for ATPase activity shows that the dark fiber type is predominant. The asterisk and arrow mark the same muscle fibers in (a) and (b). (b) Section of MEP-containing graft adjacent to (a) stained for SDH activity shows that muscle fibers have different levels of SDH activity. (c) MEP-less graft stained for ATPase activity. Muscle fibers stain intensely for ATPase which is similar to the staining pattern of denervated muscle. Note the difference in fiber size between (a) and (c). (d) Section of MEP-less graft adjacent to (c) stained for SDH activity. Muscle fibers stain weakly, if at all, for SDH. All × 95.

TABLE 1

THE ISOMETRIC CONTRACTILE PROPERTIES OF MEP-LESS AND MEP-CONTAINING GRAFTS 35 TO 60 DAYS AFTER TRANSPLANTATION

	MEP-less grafts	MEP-containing grafts
TPT (msec)	15.2 ± 0.6*	13.0 ± 0.4
½ RT (msec)	15.2 ± 1.2*	11.2 ± 1.2
Po (newtons)	0.18 ± 0.09*	3.50 ± 1.18

* Significant difference $P < 0.05$.

(MEP-containing grafts = 3007 ± 361 (SD) fibers/muscle; MEP-less grafts = 2877 ± 317. (SD) fibers/muscle), but myofibers of MEP-containing grafts were larger than those of MEP-less grafts.

Electron Microscopy

This preliminary study was undertaken to determine whether AChE/nerve complexes in MEP-less grafts resembled MEPs from mammalian skeletal muscle from previously published studies (Jirmanova, 1975). Six AChE-positive areas in all three MEP-less grafts were examined with the electron microscope. In all cases, nerves with synaptic vesicles were present in some sections through the AChE-positive area (Fig. 9). Muscle fibers had junctional folds and postsynaptic densities associated with the nerve.

DISCUSSION

Previous studies have shown that original MEPs provide information for the growth and differentiation of axons in regenerating muscle. This study demonstrates that, although nerves initially sprout into muscle grafts devoid of original MEPs to the same degree as grafts with original MEPs, few lasting neuromuscular connections are

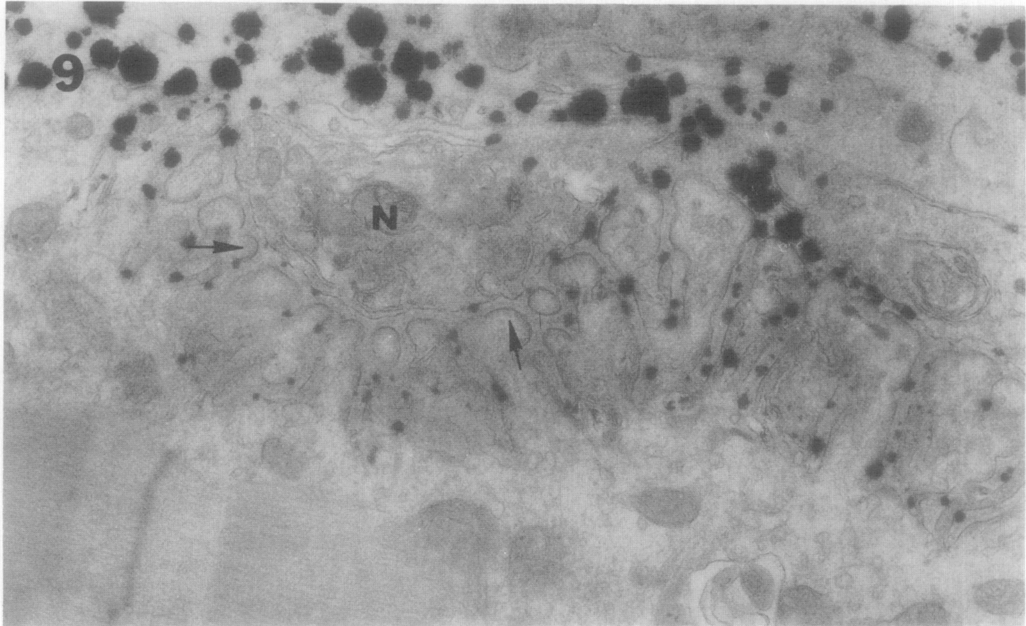

FIG. 9. Electron micrograph of a 60-day MEP-less graft. The black precipitate is reaction product from the AChE stain. The nerve (N) with synaptic vesicles is adjacent to a myofiber with postsynaptic clefts and postsynaptic densities (arrows). × 26,000. Uranyl acetate and lead citrate stain.

made in MEP-less grafts. In addition, in the absence of the original MEP zone, the vast majority of muscle fibers become atrophic and do not differentiate fiber types. Muscle contractions could be elicited in MEP-containing grafts upon electrical stimulation of the nerve and contractile speeds reached speeds of normally innervated muscle. In contrast, nerve-evoked muscle contractions were not observed in MEP-less grafts and contractile speeds resembled those of denervated muscle. Thus while a small number of new MEPs are formed in muscle grafts devoid of original MEPs, the original MEP zone is necessary for successful reinnervation of skeletal muscle grafts.

Nerves grow into both MEP-containing and MEP-less grafts. CAT biochemical analysis indicates that the degree of reinnervation is approximately the same in the two types of grafts at 34 days after surgery (approximately 40% of control CAT activity). This corresponds to previous studies of orthotopically placed EDL grafts in the rat (Carlson *et al.*, 1979). At 50 days after grafting, MEP-containing grafts have 50% of control CAT activity, whereas in MEP-less grafts CAT activity drops to 17% of control. This correlates with morphological evidence from the AChE/nerve stain that nerves are fewer in number in later stage MEP-less grafts. Marshall *et al.* (1977) have found that nerves of damaged frog skeletal muscle make precise contact with the original MEPs of the muscle and that differentiation of synaptic structure occurs in the nerve. It is possible that nerves grow into MEP-less grafts and cannot find the proper place (presumably the original MEP) to make a sustained synapse with the regenerated muscle. After this period of ingrowth, the nerves may recede from the graft. However, it cannot be ruled out that axons make transient contact with non-MEP regions of muscle fibers in muscle grafts.

Nerve-evoked muscle contraction in MEP-containing grafts also indicates the presence of neuromuscular connections in these grafts. Isometric tension (P_0) of MEP-

containing grafts is much greater than observed in MEP-less grafts and is similar to previous studies of reinnervated, sliced SOL grafts (Carlson and Gutmann, 1975b). The TPT and ½ RT of the MEP-containing grafts were not significantly different from normal rat EDL muscles (Carlson and Gutmann, 1975b). This suggests that successful reinnervation by the predominantly Type II motoneurons of the EDL nerve has occurred and that the majority of muscle fibers have differentiated into Type II fibers. This interpretation is supported by the histochemical data. Nerve-evoked muscle contraction in MEP-less grafts was not observed. Also, the slower TPT and ½ RT of the MEP-less grafts is indicative of poorly innervated muscle. Slowing of isometric contractile properties has been observed with denervation of mammalian muscle (Lewis, 1972). Thus, although approximately the same number of myofibers are present in MEP-less grafts as in MEP-containing grafts, it does not appear that MEP-less grafts are well innervated.

The eventual atrophy of muscle fibers in MEP-less grafts and failure of MEP-less grafts to regain muscle mass during regeneration were relatively consistent results in this study. MEP-less grafts do not vary in appearance from MEP-containing grafts, except for the lack of AChE staining, until 30 days after grafting. It is possible that the regenerating axon cannot make intimate and sustained contact with the muscle fibers of MEP-less grafts. An alternate explanation for the failure of MEP-less grafts is that satellite cells of the MEP zone are important in the successful regeneration of skeletal muscle grafts. Also, the presence or absence of original intramuscular nerve trunks as a nerve sprouting factor or as a pathway which axons prefer to facilitate their growth (Gutmann and Young, 1944; Carlson *et al.*, in preparation) cannot be excluded as a possible determinant on the success of reinnervation.

Several studies have dealt with the histochemical differentiation of mammalian skeletal muscle after injury (Carlson and Gutmann, 1974, 1975a). When the SOL muscle is cross-transplanted into the bed of the EDL, there is conversion of the regenerated SOL muscle to the histochemical staining pattern of the EDL (Carlson and Gutmann, 1974). In the present study, MEP-containing grafts of the SOL muscle also resemble the fiber type pattern of the EDL. This is a good indication of reinnervation of the regenerated muscle. Although MEP-less grafts have regenerated muscle fibers and nerves which grow into the graft, eventual differentiation of histochemical muscle fiber types does not generally take place. When compared to muscle fibers of MEP-containing grafts, muscle fibers of MEP-less grafts are small and stain intensely for ATPase but stain very weakly, if at all, for SDH. This lack of eventual histochemical differentiation, the general atrophy of muscle fibers, and the lack of weight gain in MEP-less grafts after initial degeneration (Fig. 2) mirrors the reactions of grafts which are continuously denervated (Carlson and Gutmann, 1975a).

Very few MEPs are seen in MEP-less grafts when compared to grafts in which original MEPs were present (46 ± 22 MEPs in MEP-less grafts versus 2138 ± 469 MEPs in MEP-containing grafts in the 40-day experimental groups). Frank *et al.* (1975) and Weinberg and Hall (1979) have demonstrated that in denervated but otherwise uninjured muscle, neuromuscular junctions will form in regions other than the original MEP region after implantation of a foreign nerve. In these studies, the original motor nerve to the muscle was disrupted after foreign nerve implantation and subsequently allowed to grow back into the muscle. They found that new neuromuscular junctions formed in regions other than the original MEP zone. In the present study, new MEPs, albeit in small numbers, are formed in grafts with no original MEPs. The structure of these MEPs appears to be

similar to MEPs of mammalian muscle in previously published studies (Jirmanova, 1975). The small number of MEPs formed in MEP-less grafts when compared to hyperinnervated muscle may be due to the delay in reinnervation or to the pattern of reinnervation in free muscle grafts. In free muscle grafts, substantial numbers of nerves are present in the graft only after two weeks (20% of normal CAT, Fig. 7). The onset of reinnervation is much faster in hyperinnervated muscle occurring in the first week. Also, the pattern of reinnervation in freely grafted muscle is highly variable (Carlson *et al.*, in preparation). Further electron microscopic investigation is needed to study the interactions of nerves and muscle in muscle grafts with and without original MEPs.

Previous studies have shown that axons reinnervate the basal lamina of original MEPs even in the absence of myofibers (Sanes *et al.*, 1978). The present study has demonstrated that the region of the original MEP is necessary for the reinnervation of skeletal muscle grafts and the differentiation of muscle fiber types in regenerating skeletal muscle. Future study of the interactions between axons, myofibers, and connective tissue elements may elucidate the regulating factors involved in the reinnervation of damaged skeletal muscle.

I would like to thank Drs. Bruce Carlson and U. J. McMahan for their helpful criticism in the revision of this paper and Drs. John Faulkner and Peter Rogers for their assistance in the physiological and biochemical studies. I would also like to thank Mr. William Brudon and Mr. John Beckerman for their fine work in the preparation of the figures and Ms. Joan Simpson for the preparation of the manuscript.

Note added in proof. During the revision of this paper, it was discovered that a previous study had also found that the original zone of motor-endplates played a vital role in the success of free muscle grafts (Thompson *et al.*, 1978). In this study of rabbit skeletal muscle grafts, successful survival of the graft occurred only when the original motor endplate zone was present.

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