REGENERATIVE RESPONSE OF FAST AND SLOW TWITCH
SKELETAL MUSCLE TO DENERVATION AND DEVASCULARIZATION

By

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ABSTRACT

The contractile properties of denervated/devascularized mouse fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (SOL) were studied at 3, 6, 9, and 12 weeks post-surgery. A comprehensive examination of these physiological parameters is desired in order to establish a mouse model of regeneration. The surgical technique involved shimmying a piece of silk thread along the belly of the muscle thus severing the nervous and the vascular supply to the individual EDL or SOL muscles. The denervated/devascularized muscles were divided into two groups, reinnervated and non-reinnervated based on their twitch and tetanic tensions. During the 12 week period post-denervation/devascularization, reinnervated EDL and SOL muscles showed a gradual increase toward control values in twitch and tetanic tensions. By 12 weeks the SOL reached 107% and 98% of the control twitch and tetanic tensions. In contrast, the reinnervated EDL only recovered 52% of the twitch tension and 64% of the tetanic tension by 12 weeks post-denervation/devascularization. The non-reinnervated SOL twitch and tetanic tensions were significantly less than control ($p < 0.05$) and reinnervated values at 6 and 9 weeks but by 12 weeks they were not different from controls ($p < 0.05$). The non-reinnervated EDL produced significantly less twitch and tetanic tension at all time periods studied. At 3 and 6 weeks post-surgery the reinnervated EDL contracted very slowly, but the speed of contraction gradually increased to control values by 12 weeks. The non-reinnervated EDL was significantly slower than the control and reinnervated muscles. The reinnervated and non-reinnervated SOL were slower than control muscles at 9 weeks but they recovered to control values by 12 weeks. The

post-tetanic potentiation (PTP) of reinnervated and non-reinnervated EDL was 20% by 12 weeks and there was no PTP for reinnervated SOL. The maximum velocity of shortening (Vo) for EDL and SOL remained unchanged at all ages. The non-reinnervated and reinnervated EDL muscles were more fatigue resistant than the controls at 3 weeks post-surgery but the reinnervated EDL eventually returned to control values (at 6,9, and 12 weeks post-surgery). The reinnervated and non-reinnervated SOL were significantly less fatigable than controls at 3, 6, and 9 weeks, after which there was no difference in fatigability between the three groups. Both the reinnervated EDL and SOL muscles successfully regenerated as measured by their contractile properties. The non-reinnervated EDL resembled classically denervated muscle. The non-reinnervated SOL reveals a PTP and a fatigue pattern suggestive of reinnervation by a branch of a fast nerve.

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KEY

ACh= Acetylcholine

AChE= Acetylcholinesterase

EDL= extensor digitorum longus (fast twitch muscle)

SOL= soleus (slow twitch muscle)

NCAM's= neural cell adhesion molecules

LC1f, LC2f, LC3f= light chain fast muscle

MHC= myosin heavy chain

MHCemb= myosin heavy chain, embryonic

f_1-f_4, FM1-FM3= fast myosin isozymes.

SM1, SM2= slow myosin isozymes.

FDL= flexor digitorum longus

SR= sarcoplasmic reticulum

SER= smooth endoplasmic reticulum

rER= rough endoplasmic reticulum

LG= lateral gastrocnemius

SDH= succinic dehydrogenase

C= control

R= reinnervated

NR= non-reinnervated

D= sciatic neurectomy

CT= contraction time

Pt= Twitch tension

Po= Tetanic tension

Pt or Po /MW= Twitch or tetanic tensions normalized to muscle weight.

1/2RT= Half-relaxation time

TTP= Time-to-peak twitch tension.

Vo= Maximum velocity of unloaded shortening.

PTP= Post-tetanic potentiation.

INTRODUCTION

The physiological properties of denervated skeletal muscle have been widely analyzed using a number of techniques (nerve crush, cordotomy). The early phases of denervation, characterized by a centralization of the nuclei and a progressive increase in the disorganization of the components of a muscle cell, are basically consistent between fast and slow-twitch muscle regardless of the technique used. The corresponding physiological changes include a decreased tetanic tension (Po), a slowing of time-to-peak (TTP) and half-relaxation time (1/2RT), and an increased fatigability of the muscle fibres. The regeneration of skeletal muscle has also been examined although a complete analysis of many aspects of regeneration has yet to be done. Thus far, most of the techniques for denervation have involved whole limb immobilization in young animals. Whole limb immobilization causes atrophy of all of the muscles of the limb thus creating a very unstable environment for the regrowth of the muscles. In particular, the importance of the surrounding environment to the normal functioning of an individual muscle is well documented (Allbrook, 1962). For example, neighbouring muscle fibres provide structural support. Regeneration studies have yet to look at the regrowth of individually denervated muscles, i.e., ones whose external environment remains normal.

Vrbova and co-workers have studied the regeneration of skeletal muscle in young and adult rats using sciatic nerve section (Lowrie and Vrbova, 1984; Navarrete and Vrbova, 1984). They observed a different regenerative response of a muscle denervated at an early age as opposed to denervations conducted on adult animals. The fast-twitch muscles of young rats never completely recovered their twitch or tetanic tensions; however, these parameters fully recovered in adult denervated muscle.

A thorough examination of other contractile properties of regenerating muscle after denervating young animals has also been undertaken (Lowrie and Vrbova, 1984). However, a distinct lack of information is available on regenerating fast and slow-twitch skeletal muscle after denervating adult muscles. Additionally, to our knowledge the combined technique of denervating and devascularizing an individual muscle has never been done before. This project is an attempt to provide a more complete analysis of the physiological properties of regenerating muscle after denervating and devascularizing adult fast and slow-twitch muscle. In addition, the technique used immobilized only the individual muscles desired for study namely the fast-twitch EDL and the slow-twitch SOL muscles of the mouse. A unique feature of this technique is that it severs the nervous and the blood supplies immediately as they enter the muscle. This procedure could maximize the chances of reinnervation by leaving a very short nerve and vascular stump attached to the muscle. Burnstock et al (1983) reported a correlation between the length of time for reinnervation with the length of the nerve stump left attached to the muscle. They discovered that the shorter the stump left behind, the faster the reinnervation process. The technique used had one other potential advantage. This procedure causes a complete degeneration of the whole muscle thus taking it back to a very immature state from which the new muscle can develop. This is important because any surviving muscle fibres may alter the measured contractile parameters; therefore, masking the true recovery of the denervated/devascularized muscles.

LITERATURE REVIEW

DEVELOPMENT OF MOTOR NERVES

Nerve and muscle development initially are independent processes but eventually they become highly dependent upon each other once they have acquired the properties that allow them to interact with each other - ie: release of Acetylcholine (ACh)(nerve) and the response to ACh (muscle).

The neuroepithelium lining the embryonic neural tube contains germinal cells undergoing rapid proliferation to produce neurons (Burnstock, O'Brien, and Vrbova 1983). All of the cells within the neuroepithelium undergo mitosis with one of the resulting daughter cells eventually developing into a neuroblast. This neuroblast, now incapable of further division, migrates away from the germinal layer to form a layer just underneath the mantle. It will eventually become the white matter of the spinal cord. From their position around the central canal, the neuroblasts send out processes which will be the first nerve fibres. The formation of these nerve fibres begins with the differentiation of the neuroblasts to form a column of motor cells running the length of the spinal cord. Outgrowths from these neuroblasts form the axons of motor neurons. As the animal grows during early embryonic life, the developing muscle mass extends further from the cord and the nerves elongate. Later, other axons grow along the paths traced by these first nerves. During normal development the nerves reach the muscle at the myotube stage. The contact of the nerve with the muscle causes a rapid development of the subneural apparatus (postsynaptic membrane and associated AChE) (Burnstock et al, 1983). As the motility of the animal increases most of the localization of chemosensitivity, elaboration of the subneural apparatus and the desensitization of the extrajunctional membrane are related to the

activity of the neuromuscular junction. As soon as the end plates are visible on the embryonic muscle, the characteristic pattern of innervation has been completed. Vrbova, Gordon, and Jones (1978) suggest that the activity of the muscle desensitizes the extrajunctional muscle membrane to ACh. Within the end plate region, ACh released from the nerve stimulates the release of proteolytic enzymes into the synaptic cleft. These enzymes then digest the superfluous nerve terminals causing them to retreat from the end plate region.

Wallerian Degeneration

The cell body of a neuron is the major site of macromolecular synthesis and it is the transportation of material produced in this region and sent to the nerve terminal that is critical for the synthesis of various substances, like neurotransmitters, and finally neural transmission. The separation of the axon from the cell body results in Wallerian Degeneration. This obviously removes the nutritional supply to the nerve terminal, a job normally accomplished by a slow transport system (1-5 mm/day), which also transports actin, tubulin and the subunits of neurofilaments, and a fast transport system (400 m/day), shipping membranous organelles for normal membrane turnover, for transmitter synthesis and secretion, and for axonal metabolism. Consequently, without further transmitter synthesis, neuromuscular transmission continues only as long as the remaining transmitter lasts (Burnstock et al, 1983).

Microscopically, changes can be seen within the cell body as a result of axotomy. There is a swelling and a migration of the nucleus to an eccentric position and an increased nuclear and nucleolar size. Also apparent is the disappearance of the Nissl substance which normally constitutes clusters of ribosomes and ordered arrays of rough

endoplasmic reticulum (rER). The altered appearance of the ribosomes indicates that a change in protein synthesis may be occurring, thus chromatolysis may be a response to help regenerate the lost axon. The final stages of Wallerian Degeneration involves the dissolution and disorganization of the neurotubules and the neurofilaments with the final disintegration of the axons and their phagocytosis. An increased proliferation of Schwann cells is also apparent. With a decreased nutritional supply and the loss of mitochondria the axon can no longer maintain its necessary structural characteristics in order to carry out its function (Burnstock et al, 1983).

Reinnervation and regeneration are feasible because the cell soma can initiate structural, metabolic and functional changes necessary for the repair of cellular damage and the regeneration of the lost axon (Vrbova et al, 1978).

In addition to the changes with the ribosomes, there also appears to be an altered content of neurofilaments. The normal axon contains neurofilaments, neurotubules, actin-containing filaments (neurofilament triplet protein) and microtubules with the neurofilaments in greater proportions than neurotubules. When an axon is cut the amount of neurofilament triplet protein decreases so that the number of microtubules and microfilaments increases relative to the number of neurofilaments. This is a sign of dedifferentiation to an immature state similar to the embryonic neuron where the cytoskeleton of the growth cone and the newly formed axon is composed almost entirely of microtubules and microfilaments. In a sense, the proximal end of the cut axon becomes a growth cone. Burnstock et al (1983) suggest that the growth cone in regenerating axons arises specifically as a result of the reorganization of fibrillar proteins which accumulate in the proximal ends of cut axons during the latent period before regenerative axonal

elongation (See section on regeneration). Therefore, it seems reasonable to suggest that the rate of regeneration in mammals would be equal to the rate of transportation of the proteins necessary to make actin and tubulin, or 3-4 mm/day. The increase in tubulin and actin is appropriate for growth since actin is required for the motility of the growth cone and for axonal elongation.

Regeneration of the Nerve

The turning point in the regenerative process begins when the nerve contacts the muscle. As mentioned, axotomy promotes the dedifferentiation of the neurons resulting in the formation of a growth cone. The contact of nerve with muscle halts that growth and promotes the maturation of the nerve which leads to synaptic transmission.

In embryogenesis, the growth potential initially is an inherent property of the neurons and elongation proceeds as a consequence of the supply of material from the cell body. The branching nerve fibres search for a guiding structure to enable them to find the peripheral stump. As part of this the Schwann cells divide and fibroblasts begin to accumulate. It is these structures that help bridge the gap between the central and peripheral stumps. Their mechanical guidance causes the nerve to grow in the proper direction. The motor axons then reach the original motor end plate and synaptic transmission begins. The nerve to muscle contact is critical to regeneration, without it the diameter of the axon remains small and therefore conduction velocity is reduced (Burnstock et al, 1983).

Covault, Cunningham, and Sanes (1987) have suggested the presence of special matrix-bound factors which aid in stimulating axonal growth near the synapse. Their results were derived from the study of innervated and denervated muscles with the latter releasing large quantities of these

factors that stimulate neurite outgrowth from cultured neurones. It is unlikely that pools of these factors can be maintained; therefore, it most likely is the changes in the surface of the denervated muscle fibres that is recognized by the growing axons. Increases in neural cell adhesion molecules (NCAM) and laminin-heparin sulfate proteoglycan complex have been noted in denervated muscles (which also have an increased outgrowth of neurites). The result is that 80% of the neurites contact the original synaptic site. Buller, Eccles, and Eccles (1960b) have conducted extensive studies involving cross-reinnervation leading to the discovery that contractile properties of the muscle are altered according to the nerve that supplies them. In addition, they found that after axotomy the motoneuron to fast and slow-twitch muscles returned to normal after reinnervation; consequently, as the axon size changed so did the contractile speeds of the muscles. Further detail on these denervation/regeneration studies is provided in a subsequent section.

MUSCLE DEVELOPMENT AND GROWTH

Cells within the embryonic mesoderm represent the pool of cells which proliferate to form the most primitive of muscle cells, the myoblasts. The distinguishing features of a myoblast cell are: they are cells with the ability to synthesize and assemble myosin, actin and tropomyosin into myofibrils, they eventually withdraw from the cell cycle and never enter another cell division, and they fuse with each other (Vrbova et al, 1978). Upon completion of division, the myoblasts begin to synthesize many proteins specific for striated muscle. The first such enzyme synthesized is creatine phosphokinase, followed by the synthesis and assembly into myofibrils of molecules of actin, myosin and tropomyosin. At this same point in development, ACh receptors are synthesized and

incorporated into the membrane. The myoblasts then elongate and fuse to form myotubes (Vrbova et al, 1978).

Unique to myoblasts and myotubes are special cell adhesion molecules (NCAM's), the modulation of which contributes in a significant way to muscle tissue modelling (Kelly and Rubinstein, 1986). As the animal matures, the ability of the fibres to adhere to each other, via these NCAM's, declines until they are no longer able to guide the differentiation of secondary cells. This results in the cessation of the formation of additional fibres. The remaining myoblasts then are left only with the option of fusing with other primary or secondary cells.

Just before fusion, a number of metabolic changes occurs including an increase in the number of mitochondria, the appearance of enzymes for utilizing glucose and glycogen and the development of rER. All these changes are made in preparation for contractile activity, however, their synthesis further increases when the myotubes begin contracting. The myotube at this phase has a low resting membrane potential, which increases with time as the ability of the cell membrane to transport Na^+ out of the cell improves, and the myotube can produce and conduct electrical activity along its entire length.

The muscle is able to develop only to a certain point without being innervated. It is at the myotube phase in normal embryonic development and in regeneration that the nerve seeks and attempts to connect with the myotube. Motor axons are first seen in rat trunk muscle by the 12th day of gestation. The first physiological signs of innervation are seen between days 14 and 15 (Vrbova et al, 1978), with morphologically recognizable synapses seen by the fifteenth or sixteenth day of gestation. By the eighteenth day, polyneuronal innervation reaches a maximum after which the number of nerve terminals decreases until 2 or 3 weeks after birth only 1 nerve terminal is left at each end plate.

Muscle Fibre Type Development

At birth very simple complexes are formed between the sarcoplasmic reticulum (SR) and the t-tubules. At this stage in normal development, the resting membrane potential, the speeds of shortening and the contraction times are all quite slow. In addition, there is a uniform moderate oxidative enzyme activity and a low myosin ATPase activity. These neonatal muscles also show a very high resistance to fatigue (Burnstock et al, 1983). With further maturation the "basic" muscle begins to develop specific qualities enabling one to differentiate between fast and slow muscle responses. The fully mature fast-twitch white fibres have a well developed SR network which in turn forms many triads with the t-tubules at the A-I junctions of sarcomeres. The abundance of these triads permits the rapid sequestering of calcium for short contraction times. Other characteristics of a fast-twitch muscle are: a narrow Z-line and a high myosin ATPase activity (which is stable in alkaline but labile in acid conditions, corresponding with its rapid rate of shortening). It also contains low oxidative and high glycolytic enzyme activity, and is fatigable. Fast twitch fibres also exhibit post-tetanic potentiation, a shorter after-hyperpolarization and are innervated by a large-diameter axon which enables them to maintain a greater conduction velocity. Conversely slow-twitch red fibres are innervated by small-diameter axons which are readily excited and which discharge for long periods of time but with low firing rates. These qualities make the muscle very fatigue resistant. A slow contracting muscle, like soleus, produces a smaller maximal tension as predicted from the relatively fewer number of triads, the high oxidative capacity and the lower myosin ATPase activity as compared with fast-twitch muscle (Vrbova et al, 1978).

As mentioned, complete maturation into fast-twitch or slow-twitch muscle fibres is critically dependent on the success of innervation at the

myotube stage. At birth, EDL and SOL are composed of Type II_2 oxidative glycolytic fibres; however, by two weeks of age in the rat there is a clear difference between the two fibre types (slow and fast) with a variety of enzymatic levels also evident. They have been labelled as fast-oxidative-glycolytic (FOG), fast-glycolytic (FG) and slow-oxidative (SO), depending on their metabolic properties. By adulthood, Type I_1 fibres differentiate from the Type II_2 oxidative-glycolytic fibre pool and are predominate in the SOL. In EDL, the Type II_2 glycolytic fibres differentiate from this same pool (Kelly and Rubenstein, 1986).

The myosin component of muscle is composed of fast and slow isoforms. The myosin molecule consists of six subunits. Fast muscle contains two myosin heavy chains plus 3 pairs of light chains (LC1f, LC2f, LC3f). While slow muscle contains 2 heavy chains plus 2 pairs of LC1s and LC2s. During the development of fast-twitch muscle, the type of myosin isoform changes. Fetal muscle contains MHC-emb, LC1emb-LC1f, and LC2f, but by 7-11 days of age the muscle contains MHC-neo, LC1f, LC2f and LC3f. Finally by 21 days of age, or adulthood, the fast-twitch muscle contains MHCF, LC1f, LC2f, and LC3f (Redenbach, 1985). Studies by Dhoot and Perry (1982) have revealed slow and fast forms of tropomyosin and troponin I, T and C. At birth, the fast form prevails but during differentiation some of the fibres change, switching to the synthesis of the slow forms.

It is apparent that in the early stages of embryological development muscles develop as slow-twitch muscles but that with time and differentiation certain muscles acquire qualities characteristic of what we call "fast-twitch" muscle. On the other hand, the slow muscles like SOL of the rabbit begin as slow contracting muscles and then with time they become even slower (Gutmann, Melichna, and Sycovy, 1974).

Denervation and Muscle Development

Buller et al (1960a) conducted studies on the differentiation of fast and slow muscle of the cat hind-limb. Their results indicated that all muscles in the newborn kitten were slow (contraction time (CT): 80 msec, 1/2RT=80 msec) but that within a few days some of the muscles differentiated into the fast form. In addition, they found that the slow muscle of 4 week old kittens was faster than the slow muscle of 28 week old cats (CT=70 msec, 1/2RT=75 msec). The fast muscle contraction times at birth were 80 msec, at 8-16 weeks 25 msec with 1/2RT=18 msec, and by adulthood CT=27 msec and 1/2RT=18 msec. Buller et al then transected 1-4 day old kittens, whose muscles should have been well differentiated at that age, at the first or second lumbar segment. Study of the muscles at 13 weeks post-surgery revealed that the contraction time for crureus (fast) and SOL (slow) were 33 and 32 msec respectively. The half-relaxation time for the two were 24 and 25 msec respectively (Buller et al, 1960a). These results as compared to values from normal developing fast and slow muscle suggest that there is a failure with the differentiation of slow muscle that normally occurs between 5 and 16 weeks. In fact the slow muscles resembled normal fast muscles much closer in terms of their physiology. On the other hand, the differentiation of fast muscles seemed to be unaffected by transection. This provides evidence for the suggestion that the differentiation of slow muscle is largely affected by neural influences emanating from the spinal cord. A follow-up study conducted by Buller et al (1960b) looked at the effect of neural influence on the speeds of contraction of slow and fast muscle. They conducted cross-reinnervation studies with SOL and flexor digitorum longus (FDL) as well as doing self-reinnervation experiments. Their results indicated that the speed of contraction of the SOL is accelerated when reinnervated by FDL nerve fibres, whereas there was a slowing of the

contraction speed in FDL muscle reinnervated by SOL nerve fibres. These transformations in twitch contraction characteristics also held for tetanic contraction.

Cord isolation studies (L2-S2) provided additional information (Buller et al, 1960b). Here it was found that the late phases of differentiation of slow muscle were altered (in a young kitten) yet fast muscles did not differ from normal. The cord isolation studies and transection studies together provide evidence that some type of neural differentiating influence is required for the proper development of slow muscle. This substance (nerve trophic factor) travels down the axon of the motoneuron, traverses the neuromuscular junction and then enters the muscle fibres to change their contractile characteristics (Davis and Kiernan, 1980).

Hatano, Suge, Ikuta, Miyamoto, Yoshioka, and Hiramatsu (1981) have observed a change in the shape of the individual muscle fibres after denervation. Instead of being a regular spindle-shape, the fibres assumed more of a rod shape after denervation. Type II fibres were also seen to decrease in size more than Type I fibres, weighing 50% of the control weight by the second week, 30% by the fourth week and 15-20% 3 months after denervation. The red muscle fibres also lost their succinic dehydrogenase (SDH) activity which can be visualized as a loss of the checkerboard appearance of the different types of muscle fibres (by approximately the third week). The above changes became even more extensive by one month post-neurectomy but with a peak at approximately five months where the muscle fibres appeared merely as debris and tiny fragments (Hatano et al, 1981).

DENERVATION

Denervation of skeletal muscle by cordotomy, nerve section, and nerve crush results in structural, metabolic and functional changes.

a) Morphological Changes:

One of the earliest changes to be seen is the altered position and appearance of the nuclei. Two weeks after denervation, the nuclei become rounded and swollen and their chromatin dispersed. They also take up a new position in the centre of the muscle fibre (Burnstock et al, 1983). The number of satellite cells increase (Schultz, 1984), as does the DNA content. As denervation time is lengthened, the muscle fibres begin to atrophy, first beginning at the periphery and then finally reaching the interior of the muscle fibre. Alterations in the myofilaments, mitochondria and SR can also be seen. The SR and the mitochondria follow the same pattern of change, first they increase in size or volume and then they both shrink.

b) Metabolic Changes

Generally speaking, substances used for energy production decrease and the enzymes to breakdown these substances also change thus resulting in a greatly altered metabolic state of the muscle (Leung, Jeffrey, and Rostas, 1984). Specifically, the glycogen content within rat muscle decreases after denervation whereas within the rabbit it first increases and then decreases. Several substances are known to increase, including the content of hexosamine, hexose, membrane-bound sialic acid, sialyl-galactosyl and N-acetylglucosaminyl transferase and the activities of Na^+/K^+-ATPase and guanylate cyclase. The turnover of phosphatidyl inositol is also increased but only small changes are seen in phospholipid

composition; however, the membrane content of fucose and fucosylglycoprotein transferase are known to decrease. Leung et al (1984) suggest that the changes in the glycoprotein component of membranes after denervation are to enable the muscle to respond to its new physiological environment. The major changes in these glycoproteins is that after denervation they contain more carbohydrates or they have more sugars added to the same carbohydrate chain.

c) Changes in Electrical Properties

There are several changes in the electrical properties of the nerve and muscle after denervation. There appears to be an increase in the permeability of the membrane to Na^+, manifested as a decreased resting membrane potential and therefore, an altered membrane excitability. The result of these membrane changes is a slower rate of rise and smaller overshoot of the action potential as well as the appearance of spontaneous asynchronous contractions (Hatano et al, 1981). The peak height of the action potential is lower as well (Kirsch and Anderson, 1986). Burnstock et al (1983) have also found that the resistance and the time and space constants of the membrane just outside the nerve/muscle junction of EDL and SOL of the cat were increased in the first few hours after sectioning but later this spread to the whole muscle fibre.

d) Changes in Acetylcholine Sensitivity

Denervation produces an increased sensitivity to ACh via an increase in the number of cholinergic receptors. This sensitivity also tends to spread over the entire length of the muscle as opposed to being localized to a small junctional region.

e) Changes in the Motor End Plate

Hatano et al (1981) conducted histological studies to look at the effects of degeneration on the end plates when regeneration was inhibited. After 1 week, the end plates were clearly outlined but with a slightly decreased intensity as compared to the controls. At the third week post-denervation, the intensity was moderate and there was a distinct reduction in size. By the fourth week, the end plate had changed from its characteristic round shape to a rather longer configuration. Thereafter, the outline of the end plate became increasingly difficult to see until 3 months post-denervation, at the peak of the changes, when the outline was not visible at all. The internal structure of the end plate was totally disorganized and there was a poor return of enzyme activity. Further studies at 5 months revealed an increase in the number of end plates as well as a population of end plates which reacted with normal intensity; however, the internal structure of these "new" end plates lacked the normal internal synaptic folds and they still maintained their lengthened appearance.

f) Changes in Contractile Properties

Briefly, there appears to be an increased twitch tension, a decreased tetanus tension, a prolongation of TTP and 1/2RT and a loss of the post-tetanic potentiation (PTP)(Davis, Bressler, and Jasch, 1988). Lieber, Friden, Hargens, and Feringa (1986) looked at rat SOL and EDL 1 year after thoracic spinal transection (T9). They found that EDL's force-generating capacity and contraction speed remained unchanged after this length of time. Soleus, on the other hand, showed an increased rate of contraction and relaxation times, as reflected in the decreased TTP and the increased fusion frequency. Interestingly, the cross-sectional area of SOL decreased by 50% but it was able to generate the same absolute

tension; therefore, the SOL was able to produce a greater force per unit area. It is also apparent that after cordotomy (and a year latent period) the contractile properties of slow muscle fibres become more similar to fast muscle fibres. Morphometric analysis supports this finding (Kelly and Rubenstein, 1986).

Morphometric analysis reveals that one year after cordotomy there is almost a complete conversion from Type I to Type II muscle fibres. It is important to note muscle fibre atrophy occurred in both EDL and SOL; however, Type II atrophy reached the same extent in both muscles but Type I atrophy in SOL was much more severe. One must remember that these results were gathered 1 year after cordotomy whereas most of the previous information has focussed on the first month or two after denervation.

REGENERATION

Experimental procedures such as cordotomy, nerve section and tenotomy cause degeneration changes in fast and slow muscle. With time however, a process takes over which serves to regenerate the damaged muscle and bring it back to normal functioning. Carlson (1973) has described 2 modes of muscle regeneration. They are the epimorphic mode of regeneration and the tissue mode of regeneration.

The epimorphic mode of regeneration involves a series of differentiations leading to blastemal formation before the regeneration of muscle fibres occurs. In this case, development closely resembles the phases of embryonic development, with the blastema being the key factor in the morphogenetic control.

The tissue mode of regeneration which occurs in damaged mammalian muscle and follows the degeneration of the sarcoplasm in the area of

injury. Subsequent to this, myoblasts appear associated with the inner surface of the basement membrane of the original muscle fibre. These myoblasts then differentiate into myotubes. Most of the regenerated muscle fibres eventually become arranged in a fashion similar to that of the original muscle by following the long axis of the original fibre. Allbrook (1962) conducted an interesting series of experiments where he rotated a minced muscle so it was oriented at right angles to its original axis. When the myotube lengthened past its basement membrane it turned and continued to grow parallel with the long axis of the muscle. This provided evidence that tension along the length of the muscle is a major factor in reorganizing the minced muscle for regeneration. The surrounding environment also poses limitations because dense connective tissue present in the regenerating area proves to be a difficult boundary for the new fibres to cross. As mentioned, the blastema is the key morphogenetic control in epimorphic regeneration; however, in the tissue mode of regeneration the physiological relationship or constraints that the muscle has with its surrounding tissue is presumed to be the major morphogenetic factor.

Ali (1979) examined the way myotubes are formed and his work reveals evidence for 2 modes of myotube formation. The first, the continuous theory, is based on the finding of sarcoplasmic buds growing from the damaged muscle fibres. The nuclei of these "buds" possibly originate from the surviving and presumably healthy portion of the muscle fibre. This form of regeneration predominates when the sarcolemmal sheaths are disrupted. The second form of regeneration, the "discontinuous form" stems from the discovery of mononucleated cells inside the intact sarcolemmal tube. These mononucleated or satellite cells differentiate into myoblasts which then fuse to form myotubes.

The Regenerating Muscle Fibre

The process of regeneration begins with the sprouting of the new fibres into an injured area. In order for this to occur, the damaged area must be cleared of necrotic sarcoplasm, myofibrils and other debris by phagocytic cells (Hudson and Field, 1973).

The success of the regeneration is dependent on many factors. For one, the degree of injury poses a serious threat to complete regeneration. If the muscle fibres are in an advanced stage of atrophy, the chances of complete recovery are not as good. Second, the presence of satellite cells or myoblasts from which the muscle fibres originate is critical in regeneration. Also of importance is the integrity of the sarcolemmal sheath and the endomysial framework of the necrotic segment so that the growth of the new fibres can be directed along the anatomical pathways. However, the most critical factor in muscle regeneration is the capacity of each individual fibre to regenerate. During the regenerative response, both the satellite cells and the muscle fibre nuceli become activated, the former to give rise to myoblasts and the latter to start development of the sarcoplasmic buds. Muir (1970) has divided the initial ninety-six hours of the degenerative/regenerative process into three phases:

Stage 1:   24-72 hours after injury:

At this point, one can see a pool of mononucleated myoblasts forming between the undamaged myofibres and the necrotic tissue. This process occurs first with the formation of a new generation of mononucleated myoblasts and subsequently these new myoblasts become more abundant by mitosis.

Stage 2:   72-96 hours after injury:

The new myoblasts then fuse with each other to form multinucleated cells (myotubes). It is only in the myotube phase that myofibrillogenesis becomes detectable. Carlson (1973), using electron and light microscopy, looked at the myotubes and discovered a very large nucleus with loosely scattered chromatin and a very prominent nucleolus. The cytoplasm contains numerous free ribosomes and consequently stains basophilic. These ribosomes are typical of active secretory cells and in regenerating muscle they can be seen during the most active phases of myofilament production. They therefore seem to be involved in the formation of the individual myofilament proteins. The rER and Golgi may be involved in protein synthesis and transport of materials used in building up the new basement membrane which surrounds the myotube. In normal mature striated muscle, the Golgi apparatus is quite small. Mitochondria with well defined cristae are also evident in the cytoplasm.

Stage 3.:   96 hours +

This stage involves the gradual maturation of myotubes into myofibres. Here myofibrillogenesis is more prominent and the typical cross-striations of skeletal muscle become visible. As mentioned previously, the complete development of the muscle depends upon proper motor innervation. Axonal regeneration itself proceeds at a rate of 3.2 mm/day (Barker, Scott and Stacey, 1986) with the actual contact of nerve to muscle occurring in the myotube stage. Lowrie and Vrbova (1984) suggest that the functional recovery of the muscle occurs by approximately 11 days after injury (with sciatic crush). The myofilaments first appear in the subsarcolemmal cytoplasm. Even at this early stage, Z-, A-, I-, H- and M-bands are clearly visible and the sarcomeres have already attained the lengths seen in adult muscle. In order for the mass of muscle to increase, new filaments are added to the periphery of the young

myofibrils; consequently, there is a gradient of myofibrillar size, with the thickest and oldest fibres located centrally and the thinnest and most newly formed ones located at the periphery (Allbrook, 1962). As is seen in embryogenesis, vesicular elements and the SR begin to take shape and with maturation one sees a migration of the centrally placed nuclei to the periphery of the muscle fibre. Once the nucleus has decreased in size, the nucleolus has faded and the nuclear chromatin condenses, the myotube has completed maturation into a young muscle fibre. Most important though is the fact that this new fibre closely resembles the original fibre which was damaged.

According to Allbrook (1962), 3 weeks after injury muscle regeneration is complete as suggested by the normal appearance of the muscle excluding a slightly increased content of interstitial collagen. It may also be possible to still see macrophages and fibroblasts.

The actual return of function to the muscle after nerve crush appears to occur 10-12 days after the operation (on 5 to 6 day old rats) (Lowrie, Krishnan, and Vrbova, 1982). This same study also revealed that by 2 months post-operation the SOL recovered almost completely in terms of tension production yet the fast muscles, tibialis anterior (TA) and EDL, developed only about 50% of the tension of the controls. Upon close examination, the fast muscles contained fewer muscle fibres and the ones there were, were ones with high levels of oxidative enzymes; however, the quantity of motoneurons remained similar to pre-operative counts. Therefore, nerve crush in very young rats causes changes in the fast muscles which are not caused by motor neuron death. When nerve crush is conducted on adult rats, there is a complete recovery of muscle function.

Several theories have been postulated to explain muscle fibre loss after regeneration has begun with many people supporting the concept that the new fibres are smaller in stature because of the inability of the

motoneuron to support all of its branches. This would result in incomplete reinnervation and a decreased muscle fibre weight or on actual loss of some fibres. Lowrie et al (1982) have presented another hypothesis to explain the results of degeneration and regeneration of muscle. Initial neural development produces motor units that fire at low rates, but with time some of these motor units increase their rates of firing. The muscle then develops special characteristics to suit the mode of stimulation ie: SOL-slow contracting and fatigue-resistant.

Upon reinnervation, the new immature fibres of EDL (fast) would be exposed to the high firing rates of the old fast motoneuron. This could be the cause of their degeneration. Obviously this would be not be a problem for SOL which is always innervated by a slow motor unit. Contrary to this is the finding by Davis, Bressler, and Jasch (1988) where the addition of a nerve extract to a denervated muscle aids in the development of the particular muscle fiber type.

Lowrie and Vrbova (1984) have looked at the regenerative response after sciatic nerve crush in 5-6 day old rats. They found functional recovery in SOL and EDL by the 18th day after surgery; however, at day 18 SOL and EDL only reached 55% and 70% of normal tetanic tension values respectively. By 21 days, EDL produced only 40% of its normal tetanic tension which remained at this level for the rest of the experiment. On the other hand, the tetanic tension produced by SOL reached 75% of its normal values two months after surgery. Other parameters of EDL's contractile properties changed as well. During the early stages of reinnervation the contraction and relaxation times were slower and remained so for several months. Prolonged relaxation time depends upon the speed with which Ca^{++} is sequestered by the SR. It may be that denervation affected this system so as to slow the uptake of Ca^{++}. Lowrie and Vrbova (1984) also reported changes in the fatigue

characteristics of EDL. At day 18, the muscle was as fatigable as normal muscle, but with age normal muscle becomes more fatigable. The opposite happens with reinnervated EDL, it becomes more fatigue-resistant. All of these physiological properties possibly change because with denervation and reinnervation in young animals EDL loses a large number of muscle fibres. In fact, they have lost two-thirds of their fibres by 1 month after nerve crush. This huge loss is not seen in SOL which accounts for its fairly consistent characteristics. Muscle fibre type distribution helped to account for the altered fatigue characteristics. With a NADH tetrazolium reductase stain both EDL and SOL appeared more uniformly stained with only patches of denervation at day 18. Not present in the reinnervated EDL but which normally makes up 35% of the fibre content of normal fast muscle were the pale-staining glycolytic fibres.

These same nerve crush experiments conducted in adult rats produced different results (Navarrete and Vrbova, 1984). Nerve crush in an adult induces only temporary changes in motoneuron activity. Gillespie, Gordon, and Murphy (1986) using rats, sewed the nerve to the lateral gastrocnemius (LG) muscle and then looked at the physiology of the muscle 4 to 14 months later. The contraction speed of reinnervated LG was similar to normal but the relaxation rate declined toward rates of relaxation in the control SOL. This suggests an increased slow muscle fibre content in the fast LG. In contrast, the reinnervated SOL had an increased relaxation speed, suggesting a larger proportion of fast muscle fibres than normal. Also, both muscles exerted less tetanic force than the controls (LG 45% and SOL 61% of normal values). Thus, the reinnervated muscles revealed characteristics intermediate between the normal fast LG and the slow SOL.

Albani and Vrbova (1985) conducted a series of experiments on 4 week old rats. They studied the physiology and the development of end plates in minced EDL and SOL which had been re-introduced into its own bed or

placed into the other muscles' bed ie: EDL into EDL bed or SOL bed, SOL into SOL bed or EDL bed. Their results were similar to others' in that twitch and tetanic tension values decreased and the regenerated muscles became more fatigue-resistant. The timing of muscle function recovery also correlated with that of Lowrie and Vrbova (1984)(i.e. 10-12 days after injury). The unique part of this experiment were the results on end plate development. The regenerated fibres had more than one end plate and some end plates had more than one axon terminal. On EDL, the end plates are normally found as a continuous band which stretches diagonally across the muscle when the new muscle develops. From EDL fragments, 2 groups of end plates are found. These are a proximal group, part of the remaining original end plate zone, and a distal group, long thin nerve fibres running longitudinally along the regenerated muscle. If no muscle fragments are left behind then the regenerated muscle fibres develop their own end plate zone located in the most proximal and distal ends of the muscle.

SATELLITE CELLS

Satellite cells are mononucleated cells which lie between the basement membrane and the sarcolemma of the muscle fibre. They normally are spindle-shaped structures with their long axes oriented parallel to the muscle fibre. Their numbers have been found to increase during the first 3 postnatal weeks after which they decrease in number to reach a basal level. Evidence has been collected showing the incorporation of labelled satellite cell nuclei into the growing (regenerating) muscle fibre (Carlson, 1973), suggesting the differentiation of satellite cells into myoblasts. Schultz (1984) conducted cross-reinnervation studies with SOL and EDL and looked at the effects of cross-reinnervation on the satellite cell population. His results indicate that a minimum number of satellite

cells are required for a successful reinnervation but that increasing the quantity of these stem cells does not necessarily mean that regeneration will be improved. In fact, it was apparent that extrinsic influences such as nerve supply and stretch are more influential in determining muscle mass in regenerating muscle than is the size of the satellite cell population. Schultz (1984) further suggested that the satellite cells could be the controllers of the actual rate of muscle regeneration.

MUSCLE BLOOD FLOW

In general, blood vessels run parallel to the muscle fibres; however, there is a difference in their organization between red and white fibres. The capillaries form elongated loops parallel to white muscle fibres with the transverse branches actually encircling the individual fibres. In red muscle, the loops are of equal length but the longitudinal branches of the network form sinusoids and the transverse branches have varicose dilations. Red muscle has a greater capillary network (Hudlicka, 1973).

Long-term ischaemia produces drastic effects on skeletal muscle. Within two hours after the onset of ischaemia, neuromuscular transmission is impeded and one can see a disruption of the cross-striations within the muscle fibre. After four hours of ischaemia, 10% of the fibres are damaged and by 8 hours 60% of the fibres are damaged (Hudlicka, 1973). In rat muscle grafts, the capillaries breakdown within the first 24 hours. In large vessels the endothelium disappears and the smooth muscle degenerates. By two days new blood vessels are seen at the periphery of the "muscle". They may use the persisting basal lamina of the original vessel to find their way to the muscle. Very little is known about the details of revascularization; however, suggestions have focussed on an interaction between the ends of the vascular network and the macrophages

that exist in this area. This hypothesis stems from evidence of macrophage-induced angiogenesis (Carlson, 1986).

Hudlicka and Tyler (1986) have summarized blood vessel growth during denervation and regeneration. They described how the lack of vascularization may actually help in the regeneration of skeletal muscle. Poorly vascularized grafts grow faster than well vascularized ones. Before vascularization, the Krebs cycle operates through the pentose shunt and therefore, hypoxia may affect vascular growth. Carlson (1973) using regenerated minced muscle transplants found that blood vessels started to grow 2-3 days after implantation and by 9 days vascularization was complete. He found myoblasts to be present in all of the sections previous to the capillaries. This suggests that vascularization, as long as it occurs, is not the limiting factor in muscle regeneration. Further support for this stems from the work of Carlson et al (1981). Here they compared standard grafts and nerve-intact grafts of EDL in the rat. A standard graft involves severing all connections of the muscle and then placing this muscle back into the same muscle bed or into a different one. A nerve-intact graft involves the same procedure but in this case the nerve supply to the muscle is not severed. Both groups underwent the same sequence of muscle fibre degeneration and regeneration but the return of function to the muscle was faster and greater in the nerve-intact graft as compared to the standard graft. Apparently it is the presence of an intact nerve supply that can bring a muscle graft in a rat almost back to control condition.

It is apparent that age plays a large role in the regenerative response. Both the morphological parameters of regenerating EDL and SOL in young animals have been closely examined with fairly consistent results produced. However, detailed physiological characteristics over the course of the regeneration of EDL and SOL in the adult mouse remain to be fully

described. Also, most of the denervation experiments involving whole muscle consist of immobilizing the whole hind limb. The following project will make use of a unique technique that denervates and devascularizes only EDL or SOL. The muscles, removed at various times after surgery, will then undergo an extensive physiological analysis in order to establish the physiological basis for a mouse model of muscle regeneration.

METHODS

All experiments were carried out on male mice of the C57BL/6J +/+ strain. Denervation and devascularization of the fast-twitch extensor digitorum longus (EDL) muscle and the slow-twitch soleus (SOL) muscle were carried out in mice at 4 weeks of age and contractile properties measured at 3,6,9, and 12 weeks following surgery. In addition, a sham-operated group was studied at 6 weeks post denervation/devascularization.

DENERVATION/DEVASCULARIZATION

Mice were anesthetized with sodium pentobarbitol (50mg./kg.) and anesthesia was maintained with an ether cuff. Each animal was then placed on a dissecting board and the right hindlimb supported on a perspex block and secured with surgical tape. A small incision was made in the skin overlying the approximate position of the respective muscle (either EDL or SOL). With the aid of a dissecting microscope, the connective tissue planes between tibialis anterior and EDL, or gastrocnemius and SOL were cut, thus exposing the desired muscle. A 5 cm. piece of unbraided 5-0 surgical silk was then slid under the distal tendon of EDL or the proximal tendon of SOL and was gently shimmied along the belly of the muscle to its opposite tendon. This procedure removed both the nerve and the blood supply to the individual muscle.

The animals regained consciousness within 30 minutes following surgery and were observed to run about the cage quite easily immediately after awakening.

MUSCLE DISSECTION

At 3,6,9, and 12 weeks post-surgery, animals were killed by cervical dislocation and their body weights were recorded. The right leg of each

animal was removed and pinned to a cork board and bathed in buffered Krebs solution to maintain viability of the tissue preparation and to prevent dehydration. For EDL, the fascia was cut and the overlying tibialis anterior muscle was removed. Similarly, the overlying gastrocnemius was excised to expose the SOL. Subsequently, the tendons of EDL or SOL were freed and tied with short segments of surgical silk (5-0). The ties were made as close as possible to the myotendinous junction in order to avoid stray series compliance in the preparation. The muscle was then removed by gentle dissection of the adhering fascia with fine scissors. Either the SOL or EDL was used from a single animal for measuring the contractile properties.

EXPERIMENTAL APPARATUS

The muscle was transferred to an experimental chamber (Fig 1.). The chamber consisted of a 1.43 cm. wide perspex bath embedded in a plate of hardened aluminum. With the aid of a dissecting microscope, the muscle was tied at one end to a stainless steel wire secured to a perspex block (dummy force-transducer) and the other end to a galvanometer torque motor (Cambridge, model 300H). The galvanometer was used to measure force and as a length servo-system which was extremely stiff to allow for the examination of isometric contractile properties. Both the motor and dummy force-transducers were secured to 3-way micropositioners which allowed for critical alignment of the muscle and adjustment of length. Throughout the experiment, the muscle was immersed in Krebs solution which contained NaCl 115mM; KCl 5.0 mM; $CaCl_2$ 3.1mM; $NaHCO_3$ 25mM; $NaH_2PO_4H_2O$ 1.2mM; $MgSO_47H_2O$ 1.2mM and 2gm. glucose/litre, gassed with 95% O_2 and 5% CO_2 and maintained at pH 7.4 and a temperature of $21°C \pm 1°C$.

Fig 1. Experimental apparatus: The muscle is immersed in Krebs solution in bath chamber, tied at one end to the lever arm of the motor and at the other end to a stainless steel wire extension of the dummy force transducer. It is continuously bubbled with 95% O_2 and 5% CO_2. Length adjustment is made by means of a 3-way micro-positioner.

Micrometer

Motor

Muscle

95 % O_2
5 % CO_2

3-way positioner

Brass plate

Force transducer

-30-
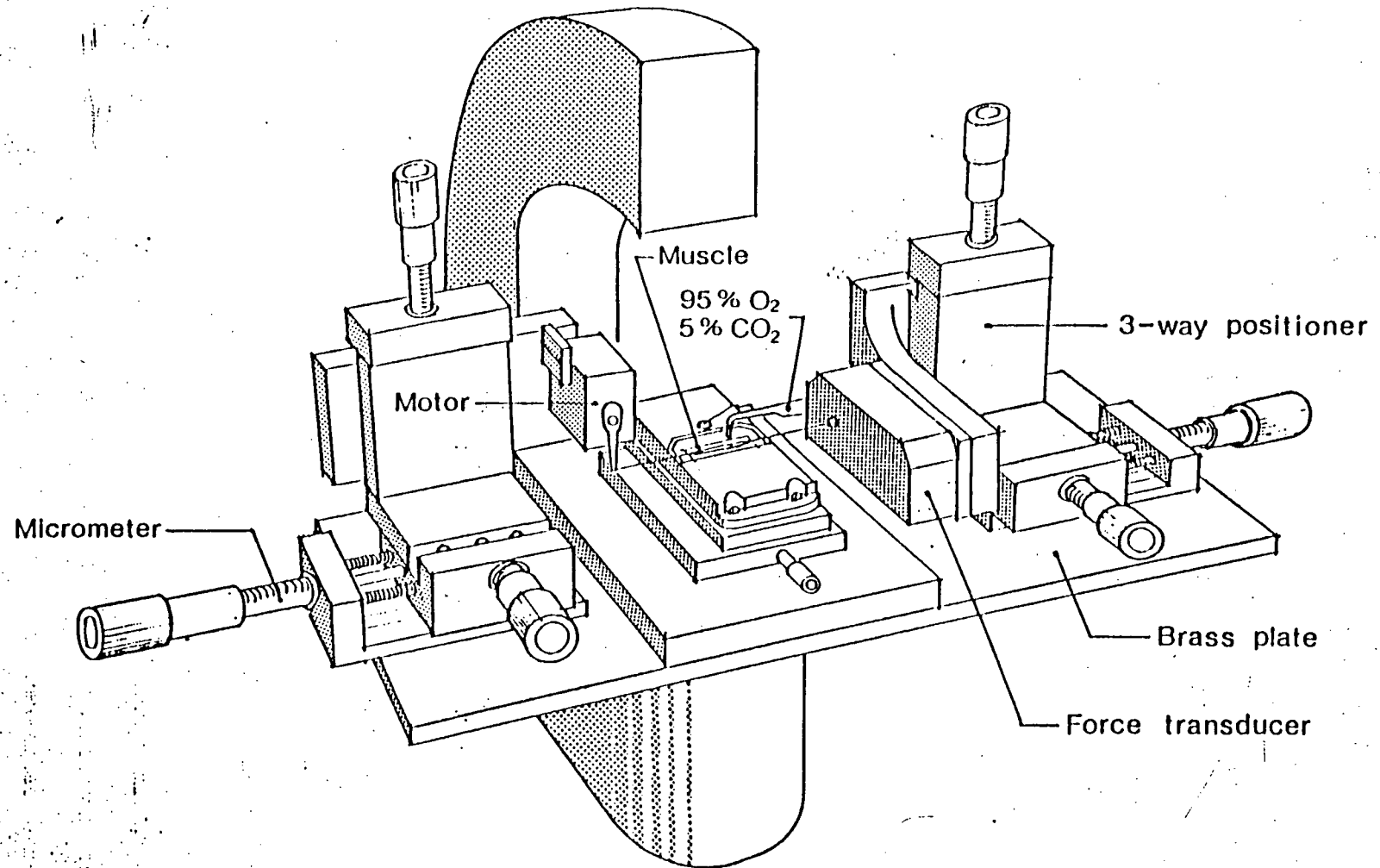
EXPERIMENTAL PROCEDURES

For purposes of comparison, muscles were mounted at the length at which the maximum isometric twitch was recorded. Length could be adjusted in 10um. increments by means of a micrometer assembly which held the motor. Stimulation consisted of supramaximal square wave pulses of 1 ms. duration. For tetanic contraction, the stimulation frequency and duration were adjusted to produce a fused tetanus. A regime of 3 twitches followed by a tetanus, with a contraction once every 90 s was used to prevent fatigue of the muscles due to repeated tetani. For each muscle, a minimum of 12 twitches and 4 tetani were recorded.

The maximum velocity of unloaded shortening (Vo) was then measured using the slack test method (Edman, 1979)(Fig 2.). This consisted of giving the muscle ramp length changes of 100 Hz. during the plateau of an isometric tetanus, which were sufficient to reduce the tension to zero and remain there until the muscle contracted to take up the slack. Releases of 4 or 5 different amplitudes were used. The time required to take up the slack is proportional to the amplitude of the length change. The slope of this relationship, determined by linear regression analysis, was used as a measure of the maximum velocity of unloaded shortening (Vo). Following Vo determination, the stimulator was turned off and the muscle was allowed to rest for 20 min. Post-tetanic potentiation (PTP) was then measured by the following procedure. The muscle was given a single stimulus (pre-twitch) and was followed 90 s later by a 1 s tetanus. After 20 seconds, the muscle was given a second stimulus (post-twitch). Both twitches were stored on a Nicolet Digital Oscilloscope, and a second oscilloscope was used to record the tetanus.

Fig 2. Determination of the maximum velocity of unloaded shortening.

A. Four different length changes and the resultant drop and then recovery of the tension. Change in length is the amplitude of the release and change in time is the time required to take up the slack.

B. Shows the line representing the least squares regression of change in length upon change in time. The slope is then divided by the muscle length to express Vo in Lo/sec.

A.

$\Delta \ell_1$

Δt_1

B.

VMAX PLOT

$V_0 =$

Δ Length (mmx10^2)

Δ Time (msec)
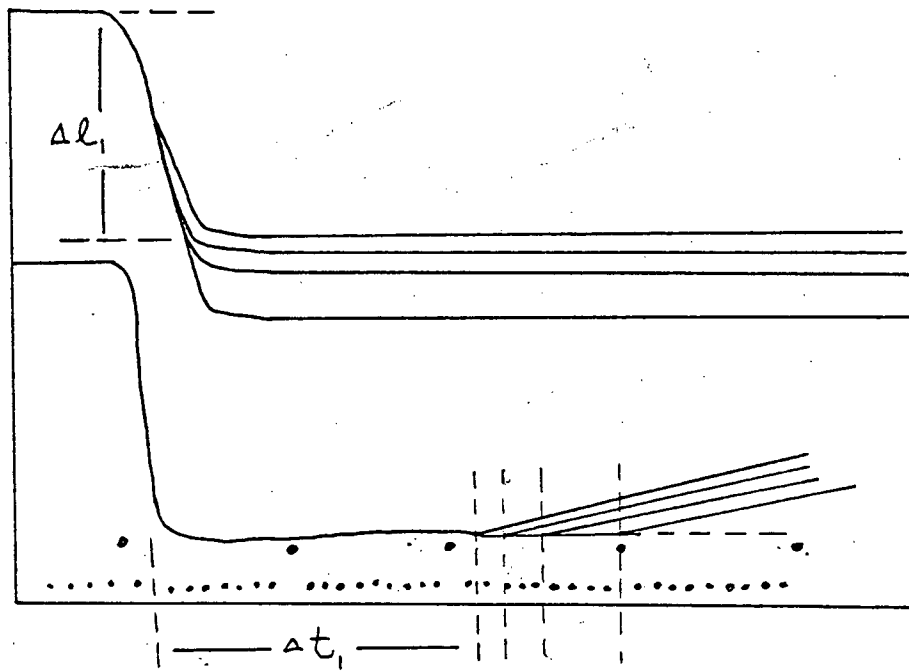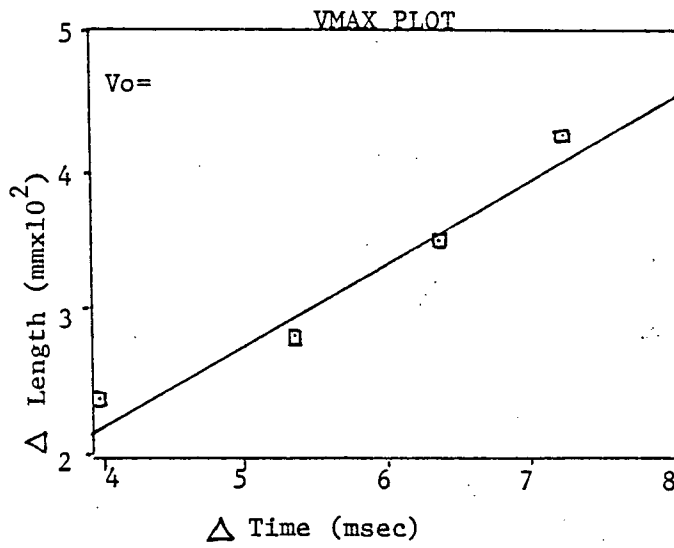
$$\frac{\text{slope} = V_0 \ (L_0/\text{sec})}{L_0}$$

Following this, the fatigue profile of the muscle was measured. This consisted of giving the muscle a 1 s tetanus at a rate of 12/min. for 5 min.

At the conclusion of each experiment, the length of the muscle was measured with fine calipers, and the muscle removed, blotted dry and weighed.

Histological Preparation

The animal was killed by cervical dislocation and the right hind leg was removed and pinned to a cork board. The connective tissue overlying the muscle was cut along the length of the muscle. During dissection of the EDL, the overlying tibialis anterior was removed to expose EDL. The SOL was exposed by removing gastrocnemius. The proximal and distal tendons were cut and the muscle was removed from the leg and placed between two layers of gauze moistened with 0.9 M saline. The muscle was then carefully embedded in mouse liver which was then mounted in gum tragacanth on a cork chuck. The sample was frozen for 30 sec. in isopentane which had been cooled to $-160°C$ in liquid nitrogen and then it was placed in a cryostat cabinet at $-20°C$ for 30 min. Serial sections of 10 um thickness were taken from the midbelly of the muscle and collected on glass cover slips. The sections were dried for 1 hour and stained with Haematoxylin and Eosin as previously described by Redenbach, Ovalle, and Bressler (1988).

ANALYSIS OF DATA

All the contractile responses were recorded on 35 mm. film with an Asahi Pentax camera which was mounted on the oscilloscope frame. The

analog signal from the tension transducer was recorded directly on the Apple IIE by the use of Scopedriver software (RC Electronics, California) and the data stored on disc. A 12 ms. delay between triggering of the analog-digital converter and the stimulator provided a baseline on the records. Custom software written for the analysis of tension data was used to calculate the twitch parameters of Pt, Pt/MW, TTP, 1/2RT, and the tetanic parameters Po and Po/MW. Maximum velocity of unloaded shortening was determined from measurements of the length and tension signals which were collected in the Nicolet Digital Oscilloscope and plotted by an x-y plotter. Least squares linear regression analysis was used to calculate the slope of the relationship of the amplitude of the length change to the duration of time taken by the muscle to take up the slack. All Vo values were expressed in muscle lengths per second (Lo/sec.). PTP and fatigue were measured directly from the film. The negatives were placed in a standard photographic enlarger and the records were analyzed from the projected images. PTP was expressed as a ratio of the pre-twitch to post-twitch tension. For the fatigue profile, each tetanic contraction is expressed as a fraction of the initial tetanic tension of the fatigue regime and plotted over time.

Data for normal and denervated/devascularized groups at 3,6,9,and 12 weeks post surgery were analysed using a two-way analysis of variance in order to assess changes with time and changes due to regeneration. Pairwise comparison at each time was done using Tukey's test. For all comparisons, a probability of $p < 0.05$ was used. The twitch and tetanic tension values for the denervated/devascularized muscles at 6, 9, and 12 weeks post-surgery fell into 2 statistically distinct groups; therefore, we divided the denervated/devascularized data into 2 groups, reinnervated and non-reinnervated. The division of the data was not done for the 3 week post-surgery animals since the differences in twitch and tetanic

tensions were not as apparent. After division of the denervated/devascularized data into two groups the statistics were conducted again in order to compare control, reinnervated and non-reinnervated data. An ANOVA was used with two factors, condition and time. The condition factor was divided into 3 levels, control, reinnervated and non-reinnervated as was the time factor with levels 6, 9, and 12, representing the groups studied. A probability of $p < 0.05$ was again used to detect statistical significance. The analysis of the 3 week post-surgery data involved the initial two-way ANOVA since this group was not divided into reinnervated and non-reinnervated. The set-up for the ANOVA of the fatigue data contained one additional factor, fatigue interval. This factor constitutes the mean$\pm$SD which is determined at 30 s intervals (for up to 320 s) for each group studied. Only the mean for each 5 s interval was plotted.

CONTROLS

Control experiments consisted of unoperated age-matched animals. In addition a series of sham experiments, 6 EDL and 6 SOL, were conducted in 4-week old male animals. The surgical procedure followed that just described except that only the connective tissue planes were cut to expose either the EDL or SOL muscle. The incision was then sutured closed. These animals were only studied 6 weeks post-surgery and physiological characteristics were compared via t-tests, to the age-matched unoperated control animals.

RESULTS

PILOT STUDY

A pilot study was conducted to determine the time course of the first appearance of regenerating muscle fibres which would indicate that reinnervation of the muscle had occurred. For this study, the surgery was performed at 4 weeks of age and then the EDL was removed for histological examination at 4, 7, and 21 days post-denervation/devascularization. An example of a normal mouse EDL skeletal muscle is shown in Fig 3. The multiple peripheral nuclei within each distinct muscle fibre is obvious as is the close association of neighboring muscle fibres. In Fig 4A. it may be seen that 4 days after surgery the site of the EDL was invaded by connective tissue and white blood cells, including neutrophils, which could be seen with Hemotoxylin and Eosin staining. Remnants of muscle fibres were also evident. In the 7 day post-denervation/devascularization EDL, connective tissue predominated but round eosin stained structures with central nuclei were also seen (Fig 4B.). These presumably were myoblasts. Finally, rows of muscle fibres with peripheral nuclei were seen 21-days post-surgery (Fig 4C.) in addition to a preponderance of fused myoblasts containing centrally-located nuclei. Connective tissue was also evident in greater quantities as compared to controls. A similar appearance of the muscle was seen in the 21-day post-denervation/devascularization SOL. The presence of mature muscle fibres at 21 days post-surgery in both the EDL and SOL, suggested that this would be an appropriate time period to begin assessing the contractile properties of these reinnervating muscles.

Fig 3. A longitudinal section of EDL muscle from a normal 21 day
old mouse. H&E stain. 650X.
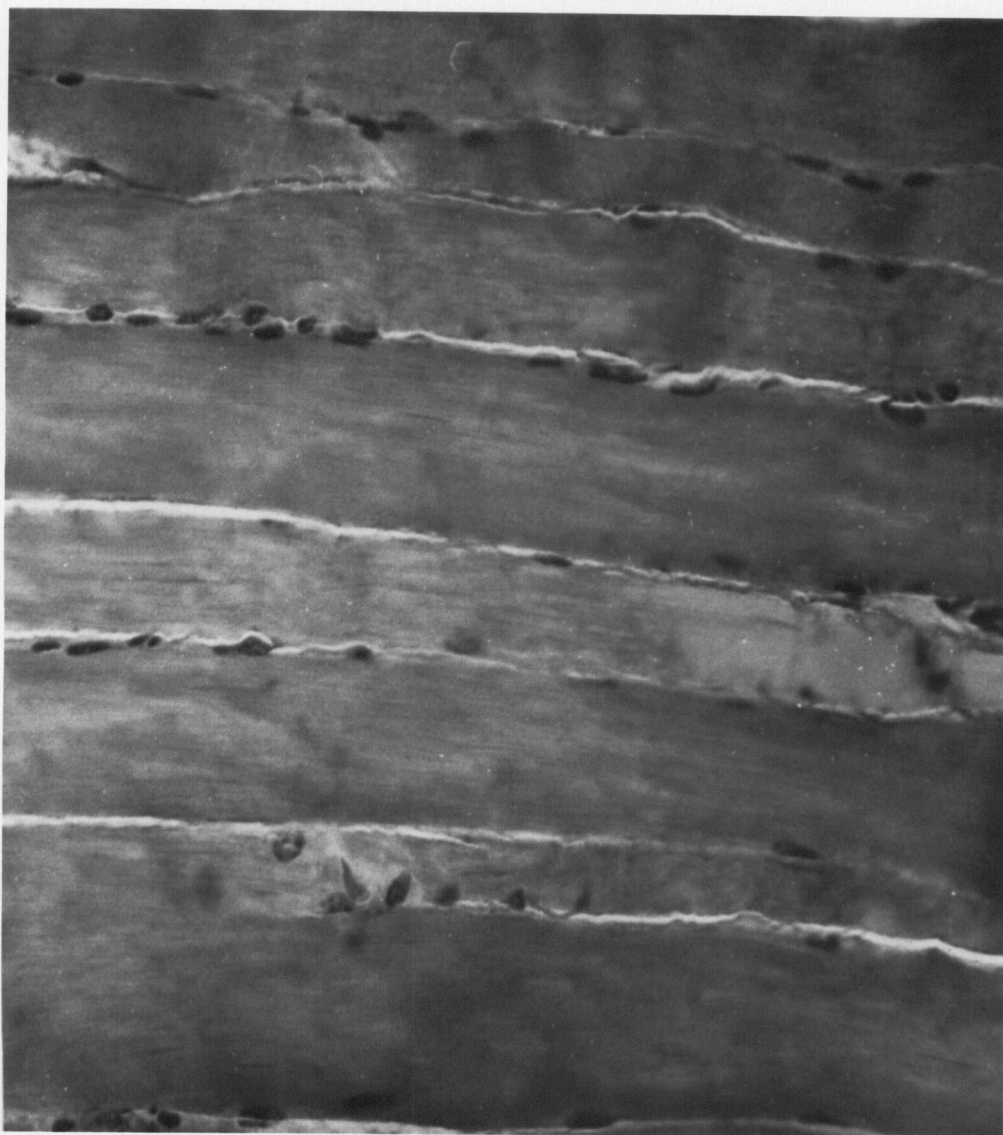
Fig 4. A section of a denervated/devascularized mouse EDL

muscle. H&E stain. 700X.

A. 4 days post-denervation/devascularization.

B. 7 days post-denervation/devascularization.

C. 21 days post-denervation/devascularization.

Following denervation/devascularization connective
tissue cells invade the muscle. Muscle fibers (large
arrow) undergo degeneration. Seven days post-surgery
myoblasts (my) appear and by 21 days post-surgery dis-
tinct mature muscle fibres can be seen (small arrow).
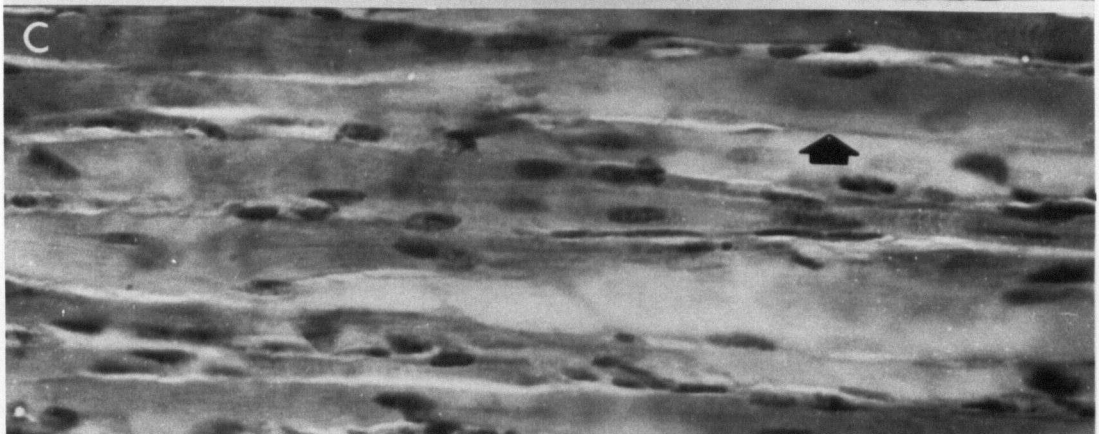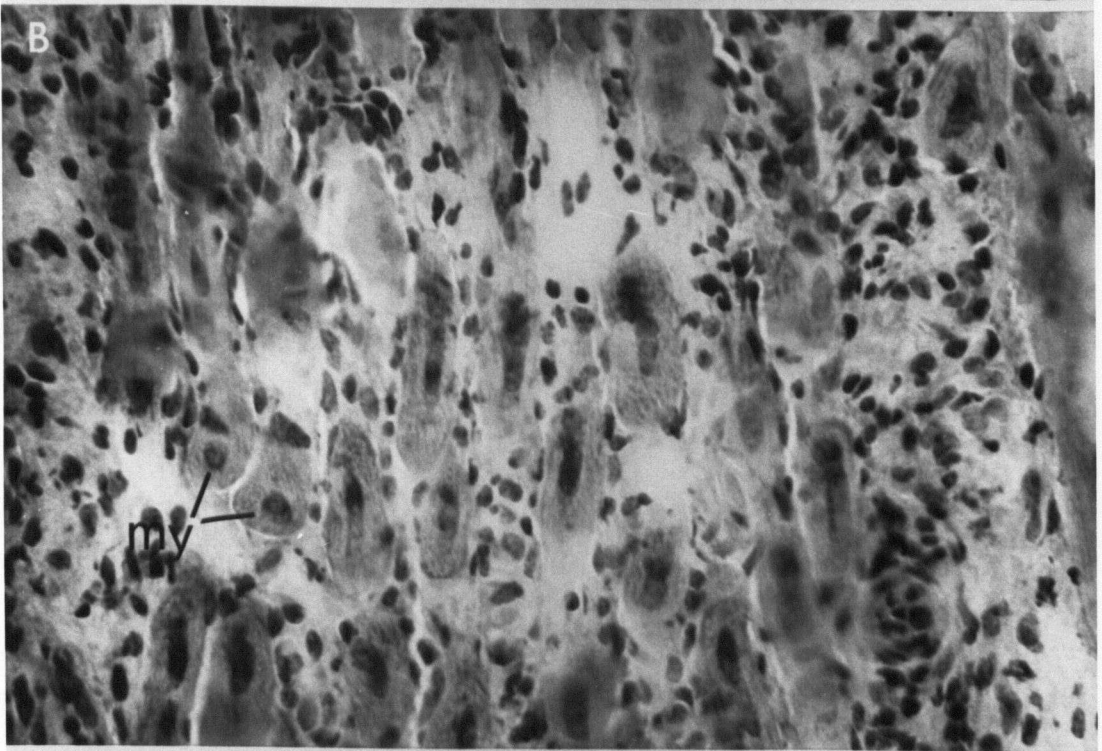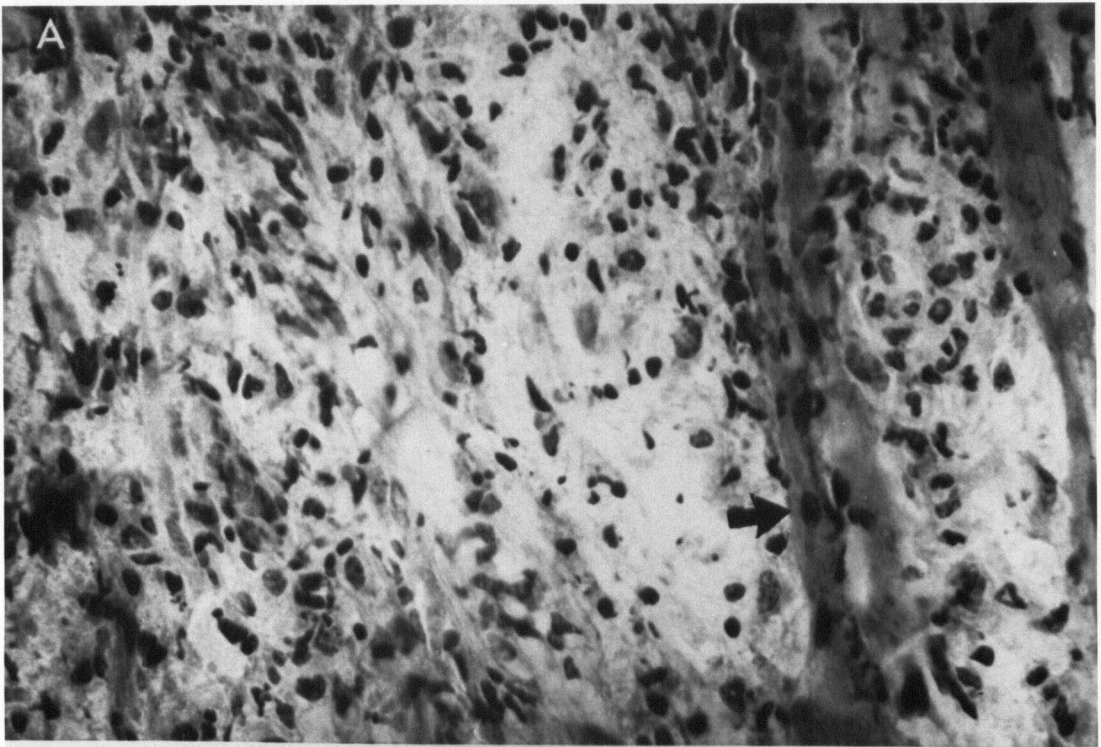
The Sham Experiments

The data from the sham experiments were compared to the data from the control experiments revealing no significant difference in absolute and normalized twitch and tetanic tensions, TTP, 1/2RT, Vo, PTP, or in their fatigability (Appendix A). As the sham experiments were only carried out for one post-operative period the results were not included with the results of the unoperated animals.

Clinical Observations

Upon initial removal of the particular muscle from the leg several features were observed. Some of the EDL or SOL muscles were very thin compared to their respective control muscles. In other cases, the respective muscle appeared to be as large as the control muscles but with a lot of connective tissue surrounding and clinging to the muscle. Subsequently the weights of the muscles were found to fall into 2 groups (see Fig 5. and Fig 6.). On several occasions, muscles that had not reinnervated, and therefore had not regenerated, were prepared for physiology yet were found to produce no tension. The total number of muscles in this group that were observed during the study was 5: 1 in each of the 6, 9, and 12 week post-surgery SOL muscles and 2, 12 week post-surgery EDL muscles.

It became apparent in assessing the results that the twitch and tetanus tensionsell into two groups for the denervated/devascularized muscles at 6,9, and 12 weeks post-surgery. The greater tension-producing muscles were designated as reinnervated and the lower tension-producing muscles were designated as non-reinnervated. The remaining contractile properties were placed into the two groups with the tension being the determining factor.

The muscle weights from the control and the denervated/devascularized

Fig 5. Weights of control and surgical EDL muscles.

(All values are means±SD).

Weights of control and surgical EDL muscles

Legend: control, den/devasc., reinnerv., non-reinerv.

Muscle weight (g) vs. Weeks post-surgery (3, 6, 9, 12)
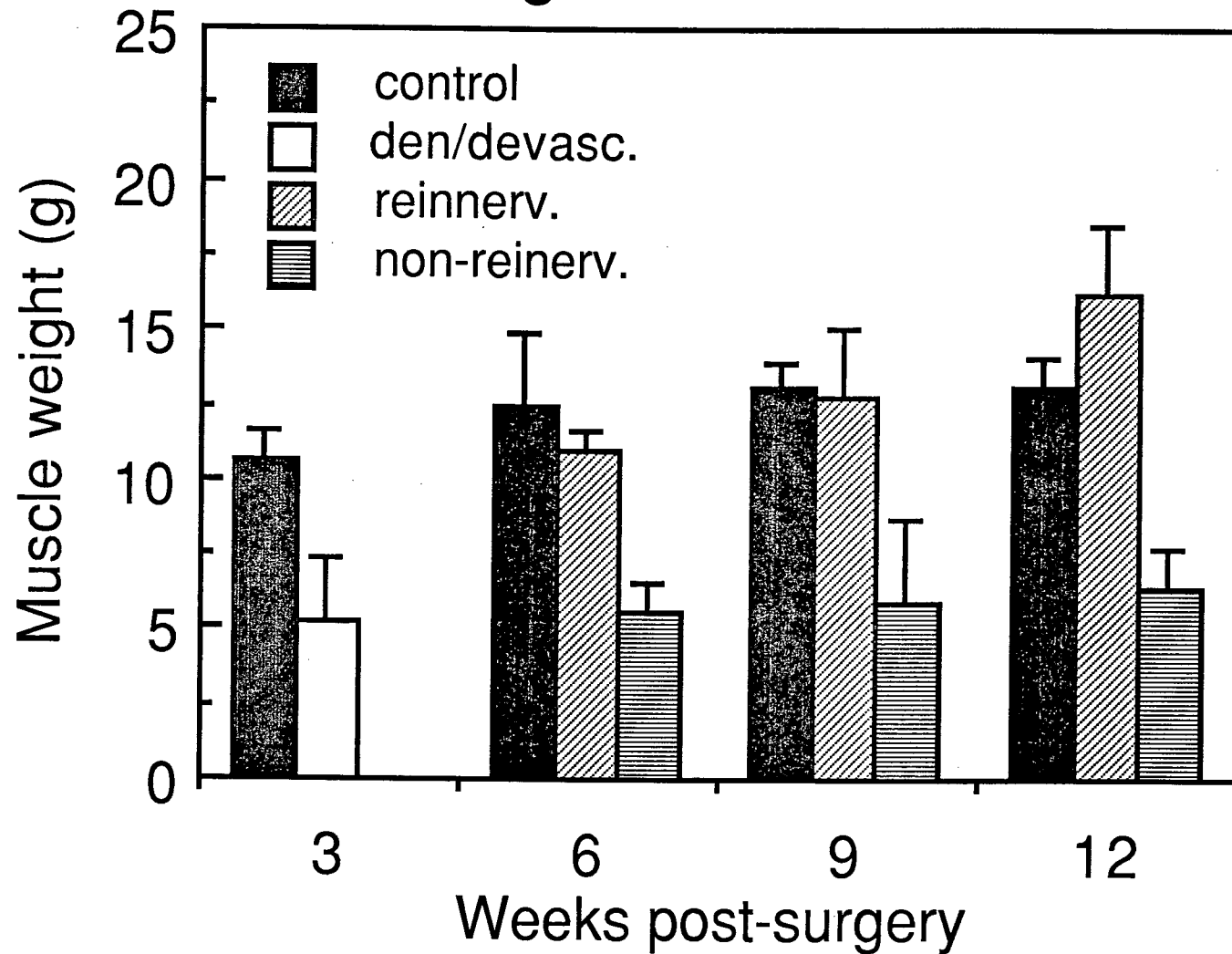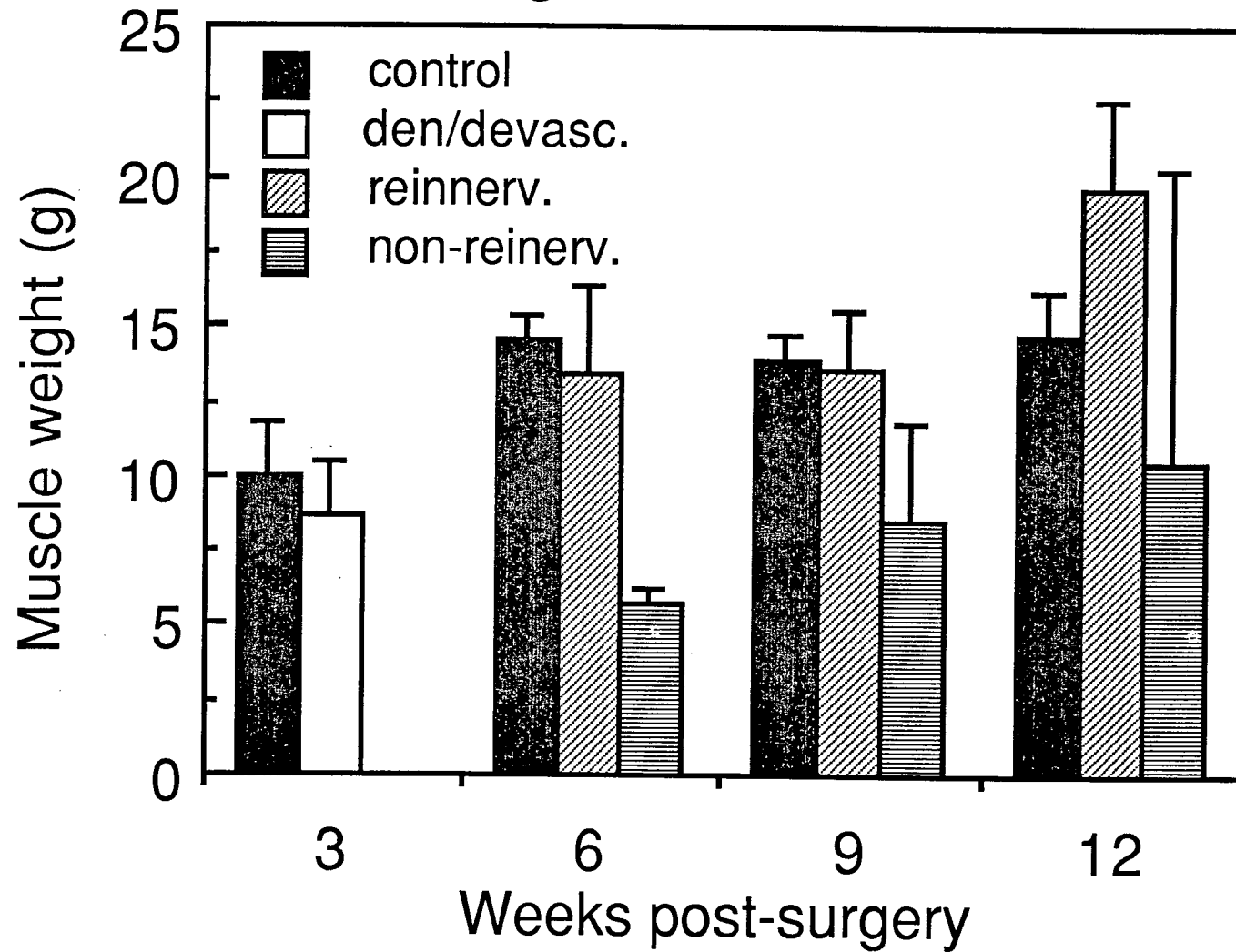
Fig 6. Weights of control and surgical SOL muscles.
(All values are means±SD).

Weights of control and surgical SOL muscles

muscles for the 4 post-operative periods are presented in Fig 5. (EDL) and in Fig 6. (SOL). There was no significant difference in muscle weights between the control and denervated/devascularized SOL at 3 weeks post-surgery (p<0.05). In contrast, at this same time the denervated/devascularized EDL muscles weighed significantly less than the controls. At 6,9, and 12 weeks after surgery, the EDL muscle weights could be divided into 2 distinct groups on the basis of isometric tension. At these time periods post-surgery the muscle weights of the reinnervated muscles were not significantly different from the unoperated EDL. The non-reinnervated muscles were significantly lighter than both the controls and the reinnervated muscles (p<0.05). The muscle weights of SOL could also be divided into 2 groups based on the isometric tension values. Similar to the EDL, at 6 weeks post-surgery, there was no significant difference between the control and the reinnervated muscle weights and the reinnervated muscle weights were significantly greater than the non-reinnnervated muscle weights. However, in the oldest age groups studied (9 and 12 weeks post-denervation/ devascularization), there was no difference in muscle weights between reinnervated and non-reinnervated SOL muscles.

Table 1 is a summary of the absolute and normalized twitch tensions of the fast-twitch EDL muscle. Both reinnervated and non-reinnervated muscles produced significantly less twitch tension than the control muscles at all time periods (weeks post-surgery); however, at 6,9, and 12 weeks post-surgery the reinnervated muscles produced significantly greater tension than the non-reinnervated muscles. In addition, by 12 weeks post-surgery, the reinnervated muscles had recovered to greater than 50% of control muscle tension. When twitch tension was normalized to muscle wet weight, the normalized twitch tension at 3 weeks following denervation/devascularization did not differ significantly from the

Table 1: Twitch tension and twitch tension normalized to muscle weight from Control (C), Reinnervated (R), and Non-reinnervated (NR) mouse EDL.

	Twitch Tension (g)			Twitch Tension/ Muscle Weight (g/mg)		
Time[a]	C (n)	R (n)	NR (n)	C (n)	R (n)	NR (n)
3[b]	8.80 ± 1.3 (6)	3.39 ± .42[c]* (6)		0.83 ± .08 (6)	0.69 ± .19 (6)	
6	8.56 ± 1.2 (6)	3.09 ± .12* (2)	1.85 ± .46+Δ (4)	0.70 ± .13 (6)	0.28 ± .01* (2)	0.30 ± .06Δ (4)
9	9.38 ± .80 (6)	4.43 ± .81* (3)	1.29 ± .71+Δ (6)	0.72 ± .09 (6)	0.39 ± .04* (3)	0.31 ± .17Δ (6)
12	10.12 ± 1.0 (6)	5.28 ± 1.2* (3)	1.55 ± 1.1+Δ (4)	0.78 ± .11 (6)	0.33 ± .09* (3)	0.70 ± .17+ (4)

a: Weeks post-surgery
b: This data was not separable into reinnervated and non-reinnervated groups, based on twitch and tetanus tensions.
c: All values are means±SD and the level of significant difference is $p < 0.05$
*: A significant difference exists between the reinnervated and the control muscles
Δ: A significant difference exists between the non-reinnervated and the control muscles
+: A significant difference exists between the reinnervated and the non-reinnervated muscles

Table 2: The twitch tension and the twitch tension normalized to muscle weight from Control (C), Reinnervated (R), and Non-reinnervated (NR) mouse SOL

	Twitch Tension (g)			Twitch Tension/Muscle Weight (g/mg)		
Time[a]	C (n)	R (n)	NR (n)	C (n)	R (n)	NR (n)
3[b]	3.38±.85[c] (6)	3.13 ± .92 (8)		0.34 ± .09 (6)	0.24 ± .09 (8)	
6	3.29 ± .53 (6)	2.51 ± .35* (5)	1.50 ± .31*Δ (2)	0.23 ± .04 (6)	0.19 ± .03 (5)	0.27 ± .08 (2)
9	3.83 ± .40 (6)	2.70 ± .20* (3)	1.32 ± .32+Δ (4)	0.28 ± .06 (6)	0.20 ± .05* (3)	0.16 ± .05+ (4)
12	3.44 ± .80 (6)	3.68 ± 1.1 (5)	1.93 ± .85 (2)	0.24 ± .07 (6)	0.19 ± .06 (5)	0.26 ± .17 (2)

a: weeks post-surgery
b: data for reinnervated and non-reinnervated muscles could not be
 divided based on twitch or tetanic tension.
c: All values are means±SD and the level of significant difference is $p<0.05$
*: A significant difference exists between the reinnervated and the control muscles
+: A significant difference exists between the non-reinnervated and the control muscles
Δ: A significant difference exists between the reinnervated and the non-reinnervated
 muscles

respective age-matched controls; subsequently, at 6,9, and 12 weeks post-surgery a significant decrease compared to controls was seen in the reinnervated muscles. Moreover, no difference was observed between reinnervated and non-reinnervated muscles at these time periods. At 3 weeks following denervation and devascularization of the slow-twitch SOL, there was no difference in absolute twitch tension between the controls and the operated muscles (Table 2). However the denervated/devascularized SOL, both reinnervated and non-reinnervated, produced significantly less twitch tension than controls at 6 and 9 weeks of age.

The non-reinnervated SOL produced significantly less twitch tension at 6 and 9 weeks than both the reinnervated and the control muscles. By 12 weeks there was no significant difference in twitch tension between the control, reinnervated and non-reinnervated muscles. The normalized twitch tensions for SOL follow a different pattern than the EDL. The only significant differences were seen at 9 weeks where the control normalized twitch tension exceeded both reinnervated and non-reinnervated values although no difference existed between the latter two.

Table 3 is a summary of the isometric tetanic tension values of the fast-twitch EDL. The tetanic tension of the denervated/devascularized muscles was significantly less than the controls at 3,6, and 9 weeks post-surgery. By 12 weeks the tetanic tension produced by reinnervated muscles was not significantly different from the tetanic tension produced by the control muscles. Moreover, the non-reinnervated muscles produced significantly less tetanic tension than the control and the reinnervated muscles at 6,9, and 12 weeks post-surgery. When the EDL tetanic tension was normalized to muscle wet weight, a significant decrease was seen between controls and the denervated/devascularized muscles at 3,6, and 12 weeks. However, at these age groups the reinnervated muscles produced more tension per muscle weight than did the non-reinnervated muscles.

Table 3: The tetanic tension and the tetanic tension normalized to muscle
weight from Control (C), Reinnerrvated (R), and Non-reinnervated (NR)
mouse EDL.

	Tetanic Tension (g)			Tetanic Tension/Muscle Weight (g/mg)		
Time[a]	C (n)	R (n)	NR (n)	C (n)	R (n)	NR (n)
3[b]	31.42±3.1[c] (6)	8.72±4.5* (6)		2.95±.39 (6)	1.62±.49* (6)	
6	36.56±3.1 (6)	15.39±.06* (2)	3.30±.53$^{+\Delta}$ (4)	3.00±.63 (6)	1.41±.10* (2)	0.54±.11$^{+\Delta}$ (4)
9	34.48±5.7 (6)	22.64±5.8* (3)	7.64±10$^{+\Delta}$ (6)	2.64±.52 (6)	1.98±.35 (3)	1.60±1.9 (6)
12	38.80±1.5 (6)	25.02±5.0 (3)	3.87±3.73$^{+\Delta}$ (4)	2.95±.26 (6)	1.54±.25* (3)	0.759±.93$^{+\Delta}$ (4)

a: weeks post-surgery
b: This data was not separable into reinnervated and non-reinnervated based
   on twitch and tetanic tensions.
c: All values are means±SD and the level of significant difference is p<0.05
*: A significant difference exists between the reinnervated and the control muscles
Δ: A significant difference exists between the non-reinnervated and the control muscles
+: A significant difference exists between the reinnervated and the non-reinnervated
   muscles

Table 4: The tetanic tension and the tetanic tension normalized to muscle weight from Control (C), Reinnerrvated (R), and Non–reinnervated (NR) mouse SOL.

	Tetanic Tension (g)			Tetanic Tension/Muscle Weight (g/mg)		
Time[a]	C (n)	R (n)	NR (n)	C (n)	R (n)	NR (n)
3[b]	15.81±2.7[c] (6)	12.98±7.6 (8)		1.59±.21 (6)	1.07±.45 (8)	
6	19.46± 2.4 (6)	12.89±1.5* (5)	2.56±.64[+Δ] (2)	1.34±.19 (6)	1.00±.23* (5)	0.45±.07[+Δ] (2)
9	24.33±3.1 (6)	15.81±1.9* (3)	5.58±2.7[+Δ] (4)	1.75±.24 (6)	1.19±.30* (3)	0.70±.41[Δ] (4)
12	19.78±1.6 (6)	19.32±4.1 (5)	10.89±6.6 (2)	1.36±.15 (6)	1.04±.36 (5)	1.349±.63 (2)

a: weeks post–surgery
b: This data was not separable into reinnervated and non–reinnervated based on twitch and tetanic tensions.
c: All values are means±SD and the level of significant difference is p<0.05
*: A significant difference exists between the reinnervated and the control muscles
Δ: A significant difference exists between the non–reinnervated and the control muscles
+: A significant difference exists between the reinnervated and the non–reinnervated muscles

Finally, at 9 weeks no difference was seen between the control, reinnervated and non-reinnervated muscles in terms of the normalized tetanic tension.

Table 4 summarizes the absolute and normalized tetanic tensions for the SOL muscle. Three weeks following surgery, the denervated/devascularized muscles still produced 82% of the control twitch tension. However, the reinnervated and the non-reinnervated muscles produced significantly less tetanic tension than the controls at 6 and 9 weeks. In addition, at these 2 age groups, the reinnervated muscles produced significantly greater tetanic tension than the non-reinnervated muscles. Finally, at 12 weeks there was no difference in tetanic tension between the control and the operated muscles. In particular, the reinnervated muscles recovered up to 98% of the control tetanic tension. There was no significant difference in the normalized tetanic tension produced by SOL at 3 weeks post-surgery. At 6 weeks, the control muscles produced the greatest tetanic tension per muscle weight followed by the reinnervated muscles and finally the non-reinnervated muscles. The difference in the normalized tetanic tension produced between reinnervated and non-reinnervated muscles disappeared by 9 weeks post-denervation/devascularization; however, both muscle groups still produced significantly less tetanic tension per wet muscle weight than the age-matched controls. Finally, by 12 weeks there was no significant difference in this parameter between any of the three experimental groups.

A comparison of the changes in TTP and 1/2RT of control and denervated/devascularized EDL are shown in Fig 7. The 3 week denervated/devascularized EDL had a significantly slower TTP and 1/2RT than the age-matched control. The reinnervated EDL initially showed a slowing of the TTP and 1/2RT at 6 and 9 weeks post-denervation and devascularization. However, by 12 weeks, there was no significant

Fig 7. The time-to-peak twitch tensions and the half-relaxation times

of control, reinnervated, and non-reinnervated

mouse EDL muscles. (All values are means±SD).

EDL TTP
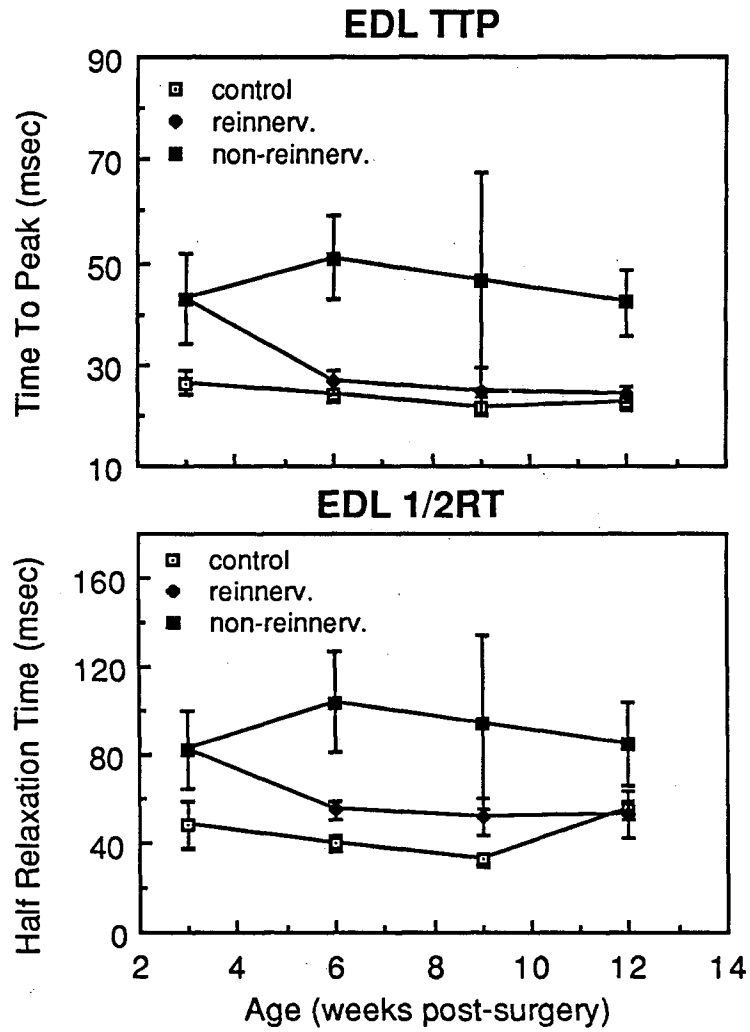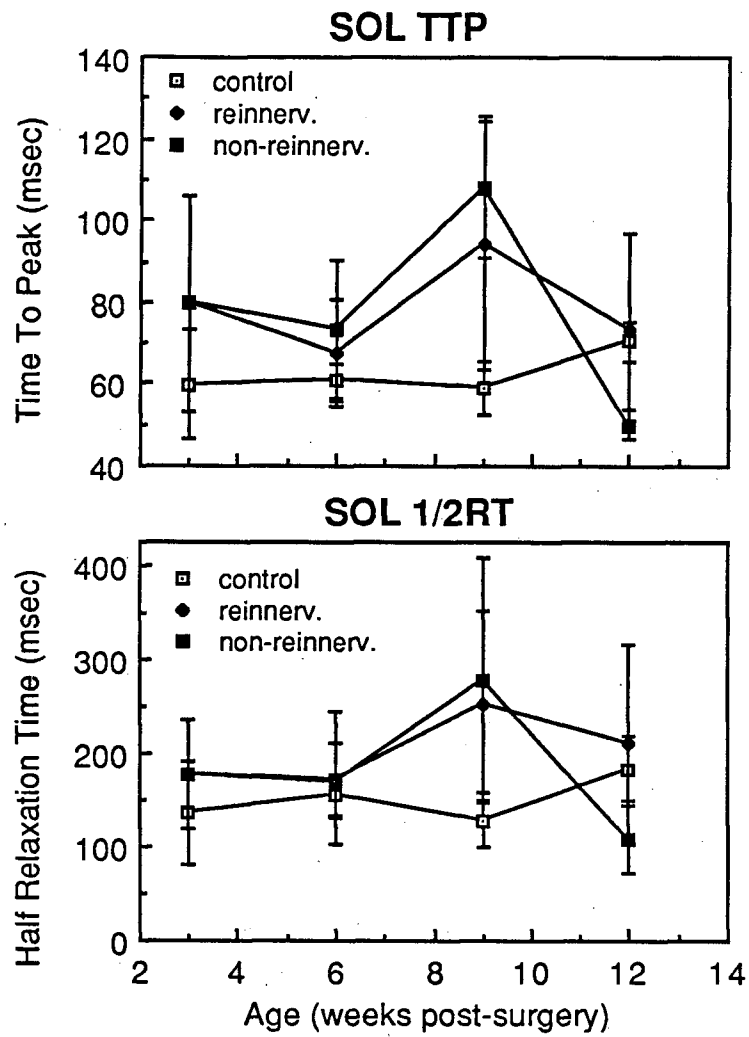
EDL 1/2RT

Fig 8. The time-to-peak twitch tension and the half-relaxation time
        of control, reinnervated, and non-reinnervated
        mouse SOL muscle. (All values are means±SD).

SOL TTP

SOL 1/2RT

difference observed in either of these parameters between the control and reinnervated muscles. The non-reinnervated muscles still exhibited a significant slowing of both TTP and 1/2RT up to 12 weeks. For the SOL muscle the TTP and 1/2RT were not significantly different from the controls at 3 and 6 weeks (Fig 8.). By 9 weeks the TTP of reinnervated and non-reinnervated SOL was slower than the controls with no difference existing between the former two. However, by 12 weeks post-surgery the TTP of the reinnervated and non-reinnervated muscles was not different from the controls. The 1/2RT followed the same pattern as for TTP ie. a slowing at 6 and 9 weeks. By 12 weeks however, the 1/2RT of both reinnervated and non-reinnervated SOL was not significantly different from control muscles.

Tables 5 and 6 are summaries of the mean values of the maximum velocity of unloaded shortening in denervated/devascularized and control EDL and SOL muscles respectively. Strikingly, Vo values for both SOL and EDL were not significantly different between the controls and the denervated/devascularized muscles at any age group. This was true for both the reinnervated and the non-reinnervated muscles.

Values of post-tetanic twitch potentiation, a characteristic typical of fast-twitch skeletal muscle but not slow-twitch muscle, are contained in Tables 7 and 8. The control EDL exhibits a 6% potentiation at 3 weeks and by 6, 9, and 12 weeks it has reached an average value of 20%. The reinnervated EDL followed a similar trend with no significant difference between it and the controls at any age group. The non-reinnervated EDL only showed a 5% potentiation at 6 weeks but by 9 and 12 weeks the difference between the non-reinnervated PTP and the control PTP had disappeared. The slow-twitch SOL does not normally exhibit any PTP.

Table 5: The maximum velocity of shortening in Control(C), Reinnervated (R)
and Non-reinnervated (NR) mouse EDL.

Maximum Velocity of Unloaded Shortening (Lo/sec)

Time[a]	C (n)	R (n)	NR (n)
3[b]	8.15±1.2[c] (5)	7.08±1.7 (6)	
6	6.26±1.9 (6)	5.16±.24 (2)	5.86±2.5 (4)
9	5.37±.91 (5)	5.16±1.4 (3)	3.06±1.3 (5)
12	7.57±3.0 (5)	9.10±1.7 (3)	10.5±11 (2)

a: weeks post-surgery
b: This data was not separable into reinnervated and non-reinnervated based on
     twitch and tetanic tensions.
c: All values are means±SD and the level of significant difference is $p < 0.05$

Table 6: The maximum velocity of shortening in Control(C), Reinnervated (R) and Non-reinnervated (NR) mouse SOL.

Maximum Velocity of Unloaded Shortening (Lo/sec)

Time[a]	C (n)	R (n)	NR (n)
3[b]	6.07±1.0[c] (6)	6.39±2.0 (8)	
6	4.95±1.2 (6)	7.86±3.6 (5)	5.73±1.6 (2)
9	5.82±2.4 (6)	10.6±7.7 (3)	2.73±.66 (3)
12	5.58±1.3 (5)	5.46±2.0 (5)	5.05±2.0 (2)

a: weeks post-surgery
b: These data were not separable into reinnervated and non-reinnervated based on twitch and tetanic tensions.
c: All values are means±SD and the level of significant difference is p<0.05

Table 7: The post-tetanic potentiation in Control (C), Reinnervated (R), and Non-reinnervated (NR) mouse EDL.

Post-Tetanic Potentiation

Time[a] (n)	C (n)	R (n)	NR (n)
3[b]	$1.06 \pm .07$[c] (6)	$1.03 \pm .05$ (6)	
6	$1.18 \pm .07$ (6)	$1.23 \pm .01$ (2)	$1.05 \pm .04$[*+] (4)
9	$1.20 \pm .09$ (6)	$1.19 \pm .04$ (3)	$1.04 \pm .14$ (6)
12	$1.21 \pm .05$ (5)	$1.14 \pm .09$ (3)	$1.08 \pm .08$ (4)

a: weeks post-surgery
b: These data were not separable into reinnervated and non-reinnervated based on twitch and tetanic tensions.
c: All values are means$\pm$SD and the level of significant difference is $p < 0.05$
*: A significant difference exists between the non-reinnervated and the control muscles
+: A significant difference exists between the reinnervated and the non-reinnervated muscles

Table 8: The post-tetanic potentiation in Control (C), Reinnervated (R), and Non-reinnervated (NR) mouse SOL.

Post-Tetanic Potentiation

Time[a] (n)	C (n)	R (n)	NR (n)
3[b]	$1.04 \pm .05$[c] (6)	0.99±.03 (8)	
6	0.97±.04 (6)	1.09±.23 (5)	1.02±.03 (2)
9	0.99±.02 (6)	0.98±.03 (3)	0.95±.04 (4)
12	0.98±.02 (6)	0.98±.03 (5)	1.12±.16 (2)

a: weeks post-surgery
b: This data was not separable into reinnervated and non-reinnervated based on twitch and tetanic tensions.
c: All values are means±SD and the level of significant difference is p<0.05

There was no significant difference between control, reinnervated and non-reinnervated SOL muscles in terms of their PTP at any of the time periods studied.

The fatigue patterns derived from pooled data of control and denervated and devascularized muscles are shown in Figs 9. and 10. The fatigue regime was analysed based on trends in fatigability. If a statistically significant difference persisted between any of the groups (control, reinnervated or non-reinnervated) i.e. beginning at approximately 40 s and continuing for the remainder of the stimulus, then this was included in the description of the fatigue data as a statistically significant difference.

At 3 weeks post-surgery, the denervated/devascularized EDL was significantly ($p < 0.05$ after 35 s) more fatigue-resistant than the age-matched controls. By 6 weeks, the reinnervated muscles more closely resemble the pattern of fatigue exhibited by the controls. However, the non-reinnervated muscles were significantly more fatigue-resistant than both the controls and the reinnervated muscles at this time. This pattern continued up to 12 week time period with the non-reinnervated muscles exhibiting greater fatigue-resistance than the control and the reinnervated muscles.

At 3 weeks, the operated SOL was significantly more fatigue-resistant than the control SOL. The reinnnervated 6 week SOL is significantly more fatigue resistant than the control; however, no difference existed between the non-reinnervated SOL in terms of their fatigue regime. The 9 week reinnervated and non-reinnervated muscles fatigue similarly although they were both more fatigue-resistant than the controls. Finally, by 12 weeks there was no significant difference in the fatigability of the controls and the reinnervated muscles. The non-reinnervated muscles fatigued similarly to the control muscle until the latter half of stimulation when

the non-reinnervated muscles were significantly more fatigue resistant than the controls.

The statistics used also accounted for any interaction between time and treatment. There was no interaction between time and treatment for any of the parameters studied i.e. the control muscles show a developmental increase in twitch and tetanic tensions and a basic maturity of the muscle (contractile times achieve those of a mature fast or slow-twitch skeletal muscle) as does the denervated/devascularized muscle; however, the latter muscles do not show an increase in these parameters above and beyond the control level.

Fig 9. The fatigue regime of control, denervated/devascularized,
        reinnervated, and non-reinnervated mouse EDL muscle. The
        values are expressed as a proportion of the initial Po value.

Fatigue:

EDL 3 weeks post-surgery
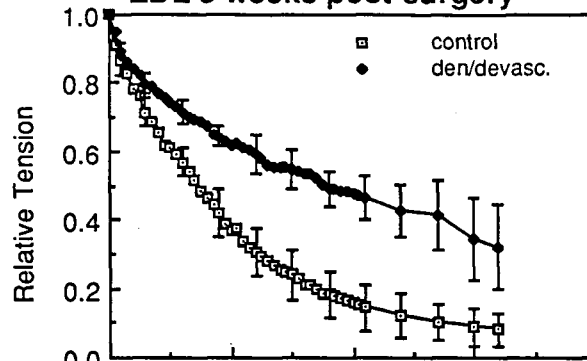
EDL 6 weeks post-surgery
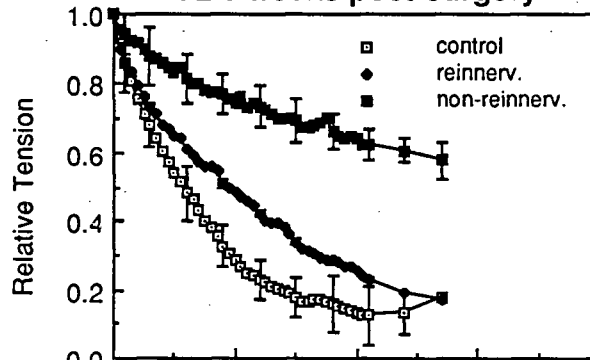
EDL 9 weeks post-surgery

EDL 12 weeks post-surgery
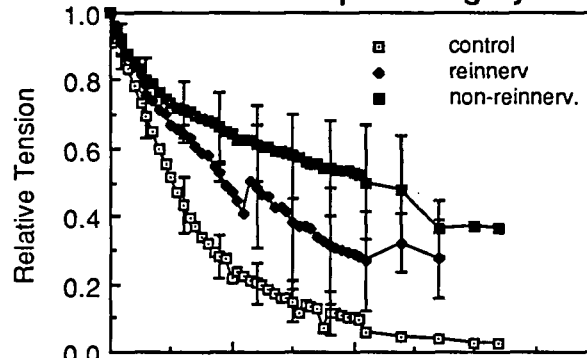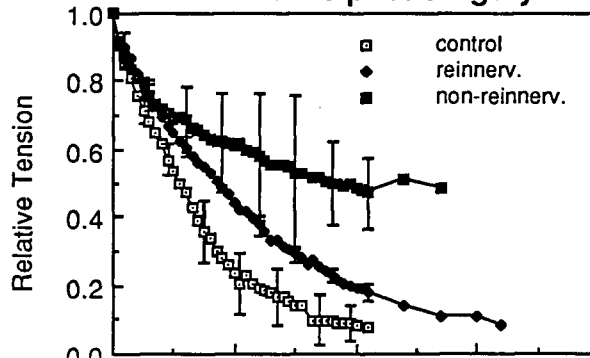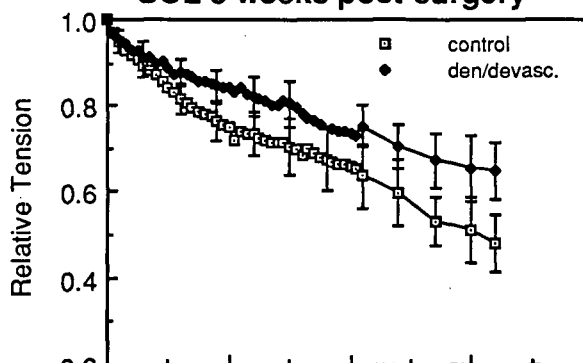
Fig 10. The fatigue pattern of control, denervated/devascularized,
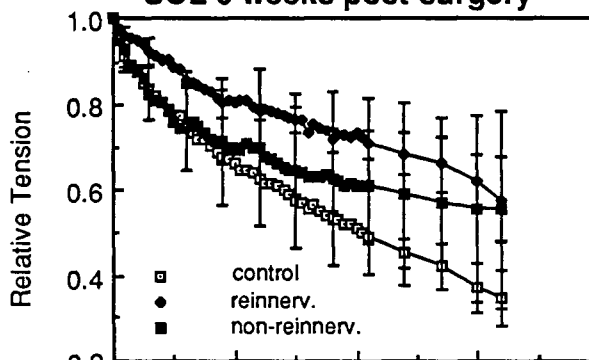reinnervated, and non-reinnervated mouse SOL muscle.

Fatigue:

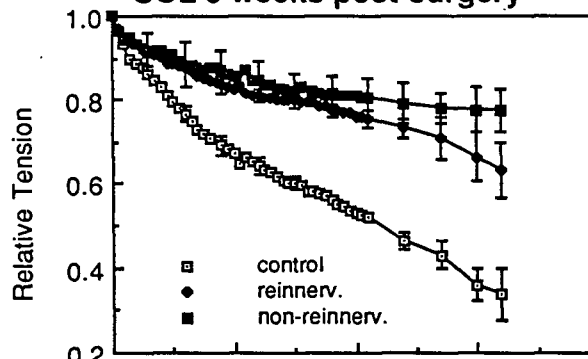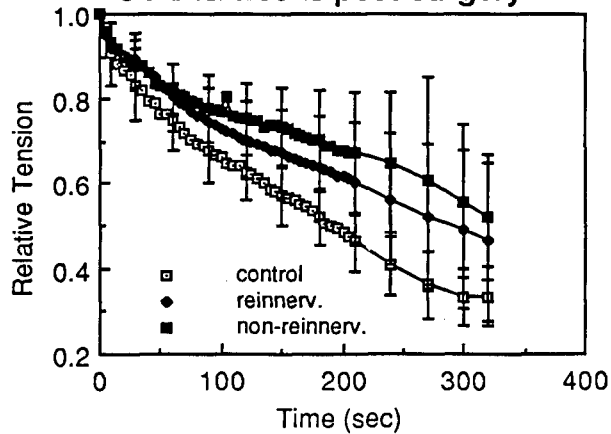## SOL 3 weeks post-surgery

SOL 6 weeks post-surgery

SOL 9 weeks post-surgery

SOL 12 weeks post-surgery

DISCUSSION

In this project a comparison was made of the the isometric contractile properties of the fast-twitch glycolytic EDL and the slow-twitch oxidative SOL muscles from normal and denervated/devascularized mice of the C57BL/6J strain. The surgical technique of denervation and devascularization brought the muscle to a very immature state, from which the regenerative responses of these muscles at 3, 6, 9, and 12 weeks post-surgery were studied.

During the recovery, or regenerative process, there was a strong relationship between the muscle weights and the absolute twitch and tetanic tensions. Furthermore, particularly with EDL, it was apparent that the larger muscles which produced the greater absolute twitch and tetanic tensions were the same muscles that had recovered their appropriate contraction times (TTP and 1/2RT) by 12 weeks post-surgery (reinnervated). Conversely, muscles with smaller muscle weights (non-reinnervated) produced less absolute twitch and tetanus tension and their TTP and 1/2RT were generally slower than the control muscles or the group of muscles which were designated as reinnervated. The early decline in twitch and tetanic tension coincided with similar reports by Webster and Bressler (1985) and by Carlson (1973). In addition, minced and subsequently grafted rat triceps surae and plantaris muscles revealed a 40% decline in tetanic tension (Bertrand, Plaghki, and Marechal, 1981) . The actin content of the muscles increased as the tension increased indicating that this reduced tension was due to a poor regeneration of the myofibrillar apparatus.

It is interesting to note the different regenerative responses of the SOL and the EDL in our study. The 3, 6, 9, and 12 week post-operative EDL muscles produced less twitch tension than the controls. The SOL showed a biphasic response in twitch tension. The 3 week denervated/devascularized SOL produced 93% of the control twitch tension before dropping to

approximately 75% and then rising again at 12 weeks to 107% of the control values. Millar and Das (1981) reported a biphasic response in twitch tension after orthotopic grafting of flexor digitorum superficialis rabbit muscle. They attributed the initial change which occurred between 1 and 2 weeks post-grafting to the acquisition of a new blood supply and the second change 1 or 2 months later to the reinnervation of the graft. Although we observed this biphasic response the timing of the rise in twitch tension differed from their study. It is agreed (Hansen-Smith, Carlson, and Irwin, (1980); Vrbova et al, 1978) that under optimal conditions revascularization takes approximately 7-10 days and reinnervation 14-21 days after injury to mouse muscle; therefore, the alterations which we saw at 3 and 12 weeks must be due to some other cause. Other transplant studies, including Carlson and Gutmann (1975), have attributed the initial rise in twitch tension to surviving muscle fibres while the recovery seen at 12 weeks may be accounted for by the maturation of the muscle. While the EDL exhibits a steady recovery in twitch tension with time post-injury it never reaches a level greater than 58% of the control value. A sharper rise was found in the tetanic tension of EDL although again the SOL was much more successful in its recovery (Tables 3 and 4).

The recovery of the tetanic tension of the reinnervated muscles by 12 weeks coincides with the work of Beranek et al (1957) who used adult mouse muscle and observed that following nerve crush the nerve regenerates. They found a complete recovery in all contractile properties. Faulkner, Niemeyer, Maxwell, and White (1980) working on adult cats found a 50% recovery of the tetanic tension 120 days after EDL transplantation with nerve anastomoses. The fatigability of these muscles only reached 40% of the controls and the muscle mass reached 80% of the controls; therefore, the decreased tetanic tension was accounted for by the lower muscle mass.

In our experiments, the muscle weights of the reinnervated muscles was comparable to that of the controls. If the content of connective tissue was quite high as observed in denervated muscles (Carlson, 1973; personal observation), the decreased absolute tetanic tension could be attributed to a genuine decrease in the number of muscle fibres per muscle. Lowrie et al (1987) speculated that a large part of the loss in muscle mass of fast-twitch muscle can be accounted for by muscle fibre atrophy after reinnervation. They suggest that an immature muscle is actually traumatized by the reinnervation by a mature fast nerve. This results in some muscle fibre death. This of course would not apply to SOL because of the slow discharge frequency of its nerve. Additionally, the possible increase in connective tissue makes the analysis of the normalized twitch and tetanic tensions very difficult to interpret. This parameter was decreased for the reinnervated and non-reinnervated EDL compared to controls at all ages, but it had recovered to control values by 12 weeks post-surgery in the SOL. Faulkner and Cote (1986) found that the normalized tetanic tension of grafted EDL decreased when compared to the control but when the tetanic tension was measured per square centimeter of viable muscle fibres then there was no difference between the grafts and the controls.

In addition to the reduced muscle mass accounting for the decreased tetanic tension, Faulkner and Cote (1986) reported deficits in single motor unit functions. Again, using grafts made with rat EDL, they found a small but significant decrease in the average tension developed of single motor units. This would imply that there is a genuine decrease in the ability of the muscle fibres to generate tension at this point in the regenerative response.

Carlson, Hnik, Tucek, Vejsada, Bader, and Faulkner, (1981) studied the problem of reinnervation and revascularization in muscle grafting.

They found that 80% of the original muscle fibres degenerated and postulated that the major limiting factor for regeneration of the muscle was the lack of successful reinnervation. The major blockade to reinnervation is the mass of connective tissue that infiltrates the injured site. He had evidence to support that revascularization, as long as it occurs, is not the limiting factor of the regeneration of muscle. This stems from the finding that in the initial phases of regeneration the myoblasts appear before the blood vessels, indicating that the initial phases of regeneration occur without a fully intact blood supply. Of course, to produce a mature muscle fibre both revascularization and reinnervation would seem mandatory.

The denervated/devascularized procedure used in the present series experiments was designed to maximize the chances for reinnervation. By severing the nerve and blood vessels at their entrance to the muscle, we expected the reinnervation and revascularization to take place quickly and easily since the distance for these processes to occur was minimized. The literature indicated that revascularization would occur within the first week post-surgery and reinnervation would soon follow. Subsequently, the myotubes would mature and eventually develop into a normal functioning muscle. Our results indicate that a complete recovery was not always achieved. The data seemed to fall into two groups for EDL and SOL. One group consisted of muscles successfully reinnervated and revascularized and another group where this apparently was not the case. For these latter muscles, it is possible that connective tissue prevented their reinnervation, revascularization, or both. The non-reinnervated muscles of EDL were quite clearly representative of a denervated muscle; however, the non-reinnervated SOL exhibited an eventual return of twitch and tetanic tensions to control values. In addition the TTP, 1/2RT, PTP, and fatigue regime suggested that the SOL resembled fast-twitch muscle in its

contractile properties. The failure of a complete return of the tension may be accounted for by an incomplete recovery of the muscle, resulting from its lack of reinnervation. The failure of the nerve to reinnervate the muscle may reflect a problem with the actual technique. When the thread was shimmied along the belly of the muscle, it was possible that the nerve was pulled away or pushed further from the site of entry into the muscle. This increased distance over which reinnervation must occur would slow the regenerative process of the muscle or completely prevent it.

The faster contractile speeds, the PTP and the increased fatigability of the non-reinnervated SOL indicate, moreover, that these muscles may have been reinnervated by a fast nerve, possibly a branch from the fast-twitch gastrocnemius. A similar sprouting by adjacent nerves in the region of the fast-twitch EDL muscle was not apparent.

One conclusion we can draw regarding the above factors, which heavily influence the regenerative ability of the muscles, is that we have 2 subgroups, reinnervated and non-reinnervated muscles.

It is apparent in our study that there was a difference in the regenerative capacity of EDL and SOL. This was noticable in both absolute twitch and tetanic tensions. The SOL recovered a larger percent of its twitch and tetanic tensions compared to EDL.

In the early phases of EDL and SOL regeneration, there was an initial slowing of the contraction times for the reinnervated and the non-reinnervated muscles. By 12 weeks, the contractile times of the reinnervated EDL had recovered to control values; however, the non-reinnervated muscles were still significantly slower contracting than their control or reinnervated counterparts.

The contractile times of SOL were more puzzling. There was no difference in the TTP and the 1/2RT between the denervated/devascularized muscles and the controls at 3 and 6 weeks (reinnervated and

non-reinnervated); however, by 9 weeks the reinnervated and the non-reinnervated muscles both contracted slower than the controls with no difference between the former two. Finally by 12 weeks, there was no significant difference in TTP and 1/2RT between the reinnervated, non-reinnervated and control muscles. Using EDL and SOL muscle grafts in one month old rats, Carlson and Gutmann (1975) also found a differential regenerative response of the EDL and SOL in terms of TTP and 1/2RT. Similar to our experiments, the EDL initially slowed and then sped up to reach control values by 60 days post-surgery. The grafted SOL also exhibited slower contractile speeds and never did reach control values. This was not the case in our study. The TTP and 1/2RT of reinnervated SOL were slower than the controls early on but by 12 weeks they had recovered.

The slowing and eventual return of the EDL contractile speeds to normal values was a pattern similar to that seen during embryological development. All early developing muscles which are slow contracting produce an embryonic form of the myosin heavy chain (MHC)(Close, 1964). The fast-twitch EDL, during the first 4 weeks of age, contains 4 myosin isozymes (f_1-f_4)(Rubenstein et al, 1983). The f_4 fraction and parts of f_2 and f_3 contain the embryonic MHC (eMHC). The rest of f_3, f_2, and all of f_1 represent isozymes with neonatal MHC. Between 10-15 days of age the adult fast isozymes FM1-FM3 appear. At this point, the developmental isozymes begin to disappear and the muscle becomes fast contracting. The slow-twitch SOL follows a similar course during embryonic and neonatal development; however, the loss of eMHC is much slower. Additionally, the SOL contains 2 isozymes, s1 and s2, which are precursors to the adult slow isozymes SM1 and SM2. By 15 days of age, the slow adult isozyme forms appear (Rubenstein et al,1983). It is apparent that the reinnervated and regenerated SOL and EDL recapitulate embryological development. Young EDL muscle produces a slower acting form

of myosin but with development, the mature fast form predominates as is manifested in our data by the gradual shortening of the contraction times. The reinnervated SOL, on the other hand, initially exhibits a shortened contraction time because of the embryonic form of MHC. However, with time the contractile speeds gradually slow. Unlike the EDL, the SOL contractile speeds are greatly affected by reinnervation (Rubenstein et al, 1983). The final return to the control values of TTP and 1/2RT indicates that the muscle has been appropriately reinnervated and therefore, the nerve has transformed the muscle to the normal SOL type. Others have reported similar findings. Strohman and Matsuda (1983), using cold-injured adult chicken muscle, followed the regeneration of the fast pectoralis major and the slow anterior latissimus dorsi. The initial muscle fibres contained the embryonic forms of MHC and the quantity of FLC3, characteristic of embryonic muscle, was greatly reduced. In addition, slow light chains and B-tropomyosin were expressed. By 3-4 weeks, the adult isoform pattern appeared. Marechal et al (1984) conducted a similar study on rat EDL and SOL with similar results. They studied the time course of development during regeneration as opposed to embryological development. They discovered that the time course of the early phase of regeneration that is characterized by the synthesis of embryonic and neonatal isomyosins closely parallels that of early development; however, the second stage, which is characterized by the synthesis of the adult forms of isomyosins, does not. It appears that this second stage takes longer in regenerating muscle.

The maximum velocity of unloaded shortening for EDL and SOL was unchanged throughout the regenerative response in the present study. Studies conducted by Faulkner and Cote (1986) and Faulkner et al (1980) agreed that after muscle grafting the Vo values returned to 100% of the control value. Unfortunately in neither of these studies did the

investigators report Vo during the early phases of regeneration. Close (1964) studied force-velocity properties of EDL and SOL from birth to 100 days. He discovered that at birth the force-velocity properties of the two muscles are similar. With aging the speed of shortening of EDL increases but that of SOL remains fairly constant. Our Vo data for EDL differs from that found by Close but one must realize that the immature muscles in Close's study had a mature nerve reinnervating them. This differs from embryological development where the nerve matures along with the muscle. Another possible reason for the discrepancy is the mode of data analysis. Our Vo was determined from the slope derived from change in length over change in time, whereas Close determined the speeds of shortening from a force-velocity relationship.

The 20% PTP for EDL by 12 weeks post-surgery was a further indication that some of the EDL muscles had reinnervated. On the other hand, a typical slow-twitch SOL should not show any PTP because the reinnervated SOL do not. However, the non-reinnervated SOL revealed post-tetanic tension which may be attributed to some of the slow SOL fibres being reinnervated by a fast nerve.

The fatigue data gathered for EDL and SOL were analyzed using ANOVA and t-tests. A significant difference was found if $p < 0.05$. We have interpreted the data with the understanding that we are looking for trends in the fatigability of the denervated/devascularized muscles. The fatigue pattern of the early regenerating EDL was similar to the early stages of development of muscle which were more fatigue-resistant than the controls (personal communication with D. Redenbach). By 6 weeks, the fatigue pattern of the reinnervated muscles resembled that of the controls. The reinnervated muscles remain slightly more fatigue-resistant up to 12 weeks. This may be accounted for by a greater capillary density and a greater oxidative capacity of the regenerated muscle fibres, as reported

by Faulkner and Cote (1986) in a study on rat EDL. The non-reinnervated muscles remain more fatigue-resistant than the controls and the reinnervated muscles which is consistent with denervated muscle (Davis et al, 1988).

The denervated/devascularized SOL is also more fatigue-resistant than the controls at 3 weeks; however, the fatigability pattern of SOL contrasts that of EDL between 6-12 weeks. By 12 weeks, there was no difference in the fatigability between the control, reinnervated, and non-reinnervated muscles unlike what we saw in the EDL. Two possibilities for this arise. One, which is supported by the PTP data, is that some of the slow SOL muscles have been reinnervated by a fast nerve and this would account for the increased fatigability of the older SOL muscles. Alternatively, the non-reinnervated muscles may not be completely non-reinnervated but rather, as suggested above, have delayed reinnervation.

It is apparent that under certain conditions and given the optimal chance for recovery, i.e. reinnervation and revascularization of both fast-twitch and slow-twitch skeletal muscle do occur. Moreover, this regeneration differs slightly between these two types of muscle. Both muscles go through stages similar to those during embryological development with the final product a fast-twitch or a slow-twitch skeletal muscle very similar to its age-matched control. Those fast-twitch EDL muscles that did not reinnervate maintain characteristics typical of denervated muscle. The non-reinnervated SOL muscles reveal a PTP and a fatigue pattern suggesting that it too may have been aberrantly reinnervated by the sprouting of a fast nerve.

Conclusions

The basis of this project was to investigate the usefulness of a new preparation i.e. denervated/devascularized slow and fast-twitch muscle, and to characterize the mechanical properties of these mouse muscles during regeneration. After such surgery, the physiological properties of the fast-twitch EDL and the slow-twitch SOL were statistically analysed and were found to recover in those muscles that had reinnervated. Another group of muscles designated as non-reinnervated did not recover to the same extent. In particular, the non-reinnervated SOL appeared to receive delayed reinnervation or they were reinnervated by a fast nerve. The results of this study were to provide a mouse model of regeneration; however, the difficulty with reinnervation of some of the muscles leads us to question its use as a mouse model of regeneration.

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APPENDICES

APPENDIX A-Control and Sham Experiments

A set of sham experiments was done on EDL and SOL muscles for comparison to the normal unoperated C57BL/6J mouse. This was to determine the effect, if any, of surgery on the contractile properties of the respective muscles.

The results of these experiments can be seen in Tables 9 (EDL) and 10 (SOL), and in Fig 11. Statistical analysis (t-test) determined that there was no difference in any of these parameters between the EDL and SOL and their respective controls.

DISCUSSION

This set of experiments was conducted to determine our set of controls. The technique used mimicked the technique used for the denervation/devascularization surgery but here the blood vessels and nerves were not severed. We hoped to determine what effect the anesthesia and the initial phases of surgery ie. opening the leg, cutting connective tissue planes, had on the contractile parameters of the EDL or SOL muscles.

The results consistently suggested that the sham operation had no effect on the contractile parameters of either the EDL or the SOL muscles. This is consistent with the results found by other members of our lab.

Table 9: The contractile properties and muscle weight of Control and Sham EDL Muscles

|	C–EDL (n)	Sham (n)
Twitch tension (g)	8.560 ± 1.2^{a} (6)	8.330 ± 1.1 (5)
Twitch tension/muscle weight (g/mg)	$0.698\pm.13$ (6)	$0.662\pm.05$ (5)
Tetanus tension (g)	36.56 ± 3.1 (6)	34.38 ± 2.7 (5)
Tetanus tension/muscle weight (g/mg)	$3.011\pm.63$ (6)	$2.742\pm.26$ (5)
Time –to–peak twitch (ms)	24.01 ± 1.4 (6)	24.17 ± 3.4 (5)
Half–relaxation time (ms)	40.06 ± 3.9 (6)	37.89 ± 5.3 (5)
Shortening velocity (Lo/s)	6.26 ± 1.9 (6)	$5.00\pm.89$ (5)
Twitch potentiation	$1.18\pm.07$ (6)	$1.24\pm.20$ (5)
EDL muscle weight (g)	12.48 ± 2.4 (6)	12.56 ± 1.1 (5)

a: All values are means$\pm$SD and the level of significant difference is $p<0.05$

Table 10: The contractile properties and muscle weight of Control and Sham SOL Muscles

	C–SOL (n)	Sham (n)
Twitch tension (g)	3.288±.53[a] (6)	3.197±.51 (5)
Twitch tension/muscle weight (g/mg)	0.246±.05 (6)	0.227±.04 (5)
Tetanus tension (g)	19.46±2.4 (6)	18.08±1.9 (5)
Tetanus tension/muscle weight (g/mg)	1.340±.19 (6)	1.412±.36 (5)
Time –to–peak twitch (ms)	60.62±4.1 (6)	67.48±11 (5)
Half–relaxation time (ms)	155.9±22 (6)	137.3±14 (5)
Shortening velocity (Lo/s)	4.95±1.2 (6)	5.40±1.3 (5)
Twitch potentiation	0.974±.04 (6)	1.01±.07 (5)
SOL muscle weigh (g)	14.57±.80 (6)	13.36±1.9 (5)

a: All values are means±SD and the level of significant difference is $p < 0.05$

Fig. 11. The fatigue regime for control and sham EDL and SOL.

Fatigue: Sham EDL

Fatigue: Sham SOL
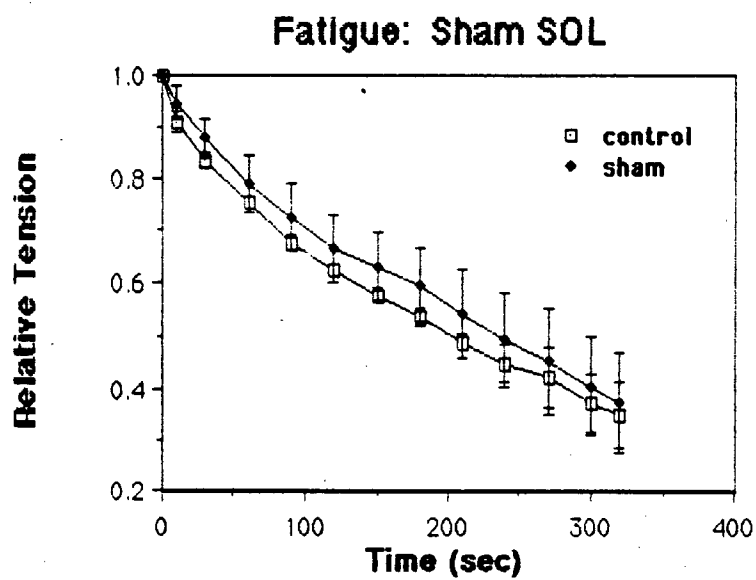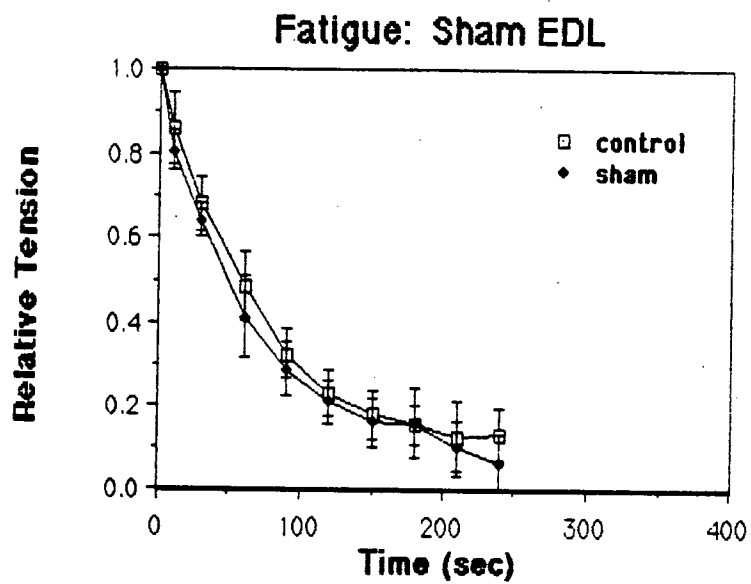
Appendix B—Sciatic Neurectomy

In order to assess the effects of denervation alone without devascularization, a group of experiments was carried out on which a sciatic neurectomy was performed on 5 mice at 4 weeks of age. The resulting data were then compared to the age-matched control data and to the data from the denervated/devascularized experiments. The parameters studied included absolute and normalized twitch and tetanic tensions, TTP, 1/2RT, Vo, PTP, and fatigue. A significance level of $p < 0.05$ was used in the t-tests.

A set of animals, in which a sciatic neurectomy was done at 4 weeks of age and studied at 12 weeks post-denervation produced muscle weights for both EDL and SOL that were significantly less than the controls and the reinnervated muscle weights, but they were not different from the non-reinnervated muscle weights ($p < 0.05$)

The twitch tension produced by the denervated EDL (Table 11) was significantly less than that produced by the controls; however, it was not different from that produced by the reinnervated muscles. On the other hand, the denervated EDL produced significantly greater tension than the non-reinnervated EDL. When the twitch tension is normalized to muscle weight no difference exists between the denervated and the control muscles. Moreover, the denervated EDL produced a significantly greater normalized twitch tension than both the reinnervated and the non-reinnervated muscles. In contrast, the absolute twitch tension produced by denervated SOL (Table 13) was not significantly different from the control, reinnervated or non-reinnervated muscle values; however, the normalized twitch tension for denervated SOL is significantly greater than the control, reinnervated and non-reinnervated values.

Table 11: The Absolute and Normalized Twitch and Tetanic Tensions of Control, Reinnervated, Non-reinnervated and Denervated EDL Muscles

	Pt (g) (n)	Pt/MW (g/mg) (n)	Po (g) (n)	Po/MW (g/mg) (n)
Control	10.12±1.0[a] (6)	0.784±.1 (6)	38.81±1.5 (6)	2.95±.26 (6)
Reinnervated	5.28±1.2 (3)	0.331±.09 (3)	25.00±5.0 (3)	1.54±.25 (3)
Non-reinnervated	1.55±1.1 (4)	0.287±.27 (4)	3.87±3.7 (4)	0.759±.93 (4)
Denervated	6.30±1.8$^{\square\alpha}$ (5)	0.704±.17$^{\dagger\alpha}$ (5)	25.14±11$^{\square\alpha}$ (5)	2.59±.74$^{\alpha}$ (5)

a: All values are means±SD and the level of significant difference is p<0.05
□: A significant difference exists between denervated and control muscles.
†: A significant difference exists between denervated and reinnervated muscles
α: A significant difference exists between denervated and non-reinnervated muscles

Table 12: The Remaining Contractile Properties and Muscle Weight of
Control, Reinnervated, Non-reinnervated and Denervated EDL Muscles

	TTP (ms) (n)	1/2RT (ms) (n)	Vo (Lo/sec) (n)	PTP (n)	Muscle Weight (g/mg) (n)
Control	22.42 ± 1.7^a (6)	54.71 ± 4.6 (6)	7.57 ± 3.0 (6)	$1.21 \pm .05$ (6)	13.00 ± 1.1 (6)
Reinnervated	24.23 ± 1.6 (3)	52.95 ± 11 (3)	9.10 ± 1.7 (3)	$1.14 \pm .09$ (3)	14.97 ± 2.3 (3)
Non-reinnervated	42.34 ± 6.5 (4)	84.52 ± 19 (4)	10.51 ± 11 (4)	$1.081 \pm .08$ (4)	6.325 ± 1.4 (4)
Denervated	$25.46 \pm 11^\alpha$ (5)	$41.46 \pm 15^\alpha$ (5)	$5.92 \pm 1.4^\dagger$ (5)	$1.116 \pm .10$ (5)	9.3 ± 2.9 (5)

a: All values are means$\pm$SD and the level of significant difference is $p<0.05$
$\dagger$: A significant difference exists between denervated and reinnervated muscles
α: A significant difference exists between denervated and non-reinnervated muscles

Table 13: The Absolute and Normalized Twitch and Tetanic Tensions of Control, Reinnervated, Non-reinnervated and Denervated SOL Muscles

	Pt (g) (n)	Pt/MW (g/mg) (n)	Po (g) (n)	Po/MW (g/mg) (n)
Control	3.44±.80[a] (6)	0.24±.07 (6)	19.78±1.6 (6)	1.36±.15 (6)
Reinnervated	3.68±1.1 (5)	0.19±.06 (5)	19.32±5.0 (5)	1.54±.25 (5)
Non-reinnervated	1.55±1.1 (2)	0.287±.27 (2)	3.87±3.7 (2)	0.759±.93 (2)
Denervated	6.30±1.8 (5)	0.704±.17□†α (5)	25.14±11 (5)	2.59±.74 (5)

a: All values are means±SD and the level of significant difference is p<0.05
□: A significant difference exists between denervated and control muscles
†: A significant difference exists between denervated and reinnervated muscles
α: A significant difference exists between denervated and non-reinnervated muscles

Table 14: The Remaining Contractile Properties and Muscle Weight of Control, Reinnervated, Non-reinnervated and Denervated SOL Muscles.

	TTP (ms) (n)	1/2 RT (ms) (n)	Vo (Lo/sec) (n)	PTP (n)	Muscle Weight (g/mg) (n)
Control	70.49 ± 5.1^a (6)	184.1 ± 35 (6)	5.58 ± 1.3 (6)	$0.980 \pm .02$ (6)	14.66 ± 1.5 (6)
Reinnervated	73.43 ± 23 (5)	211.7 ± 104 (5)	5.46 ± 2.0 (5)	$0.980 \pm .03$ (5)	19.54 ± 3.0 (5)
Non-reinnervated	50.07 ± 3.6 (2)	108.3 ± 37 (2)	5.05 ± 2.2 (2)	$1.120 \pm .16$ (2)	10.45 ± 9.8 (2)
Denervated	$42.23 \pm 18^{\square\dagger}$ (5)	$75.25 \pm 33^{\square\dagger}$ (5)	$8.24 \pm 2.1^{\square\dagger}$ (5)	$1.050 \pm .06^{\square\alpha}$ (5)	$10.38 \pm 3.7^{\square\dagger}$ (5)

a: All values are means±SD and the level of significant difference is $p < 0.05$
□: A significant difference exists between denervated and control muscles
α: A significant difference exists between denervated and non-reinnervated muscles

The denervated EDL produced less tetanic tension than the controls; however, it produced greater tetanic tension than the non-reinnervated muscles. In addition, there was no difference in the absolute tension produced by the denervated and reinnervated EDL. The normalized tetanic tension of denervated EDL did not differ from the control or reinnervated values but it was significantly greater than the non-reinnervated tetanic tension. The denervated SOL did not differ from the control or denervated/devascularized muscles in terms of absolute and normalized tetanic tension.

The TTP and 1/2RT of denervated EDL (Table 12) were not significantly different from the control or reinnervated muscles but they were significantly faster than the non-reinnervated muscles. The denervated SOL (Table 14) on the other hand was faster contracting than both the control and the reinnervated muscles but they were not different from the non-reinnervated SOL. Correlated with this is a faster Vo for denervated SOL versus control muscles. The maximum velocity of unloaded shortening for denervated EDL was slower than the reinnervated muscles but not significantly different from the control or non-reinnervated muscles.

As mentioned, the 12 week age-matched control EDL produces approximately a 20% potentiation. The denervated EDL produces a PTP which is not considered significantly different from the control, reinnervated and non-reinnervated PTP values. The denervated SOL produces a 5% potentiation which is uncharacteristic of a slow-twitch muscle including the control muscles in this experiment; however, there is no significant difference in PTP between the denervated SOL and the non-reinnervated SOL.
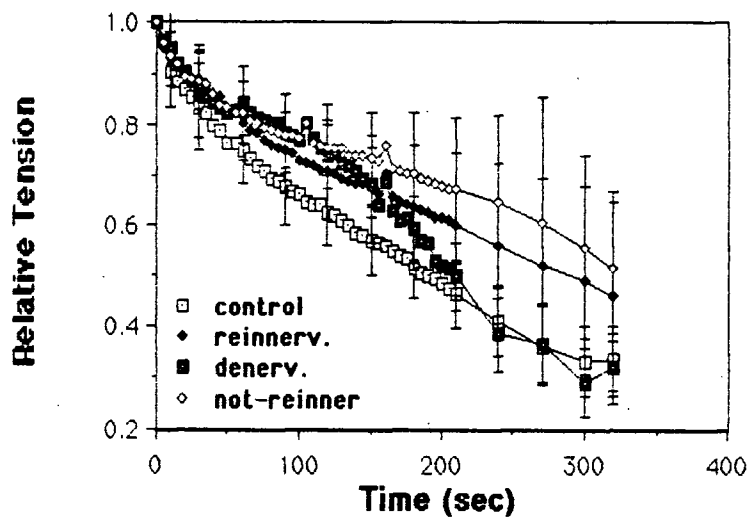
The fatigue pattern for the denervated EDL and SOL was also examined (Fig 12.). The denervated EDL was significantly more fatigue

Fig. 14. The fatigue regime for control, reinnervated,
non-reinnervated and denervated EDL AND SOL.

Fatigue: EDL Denervation
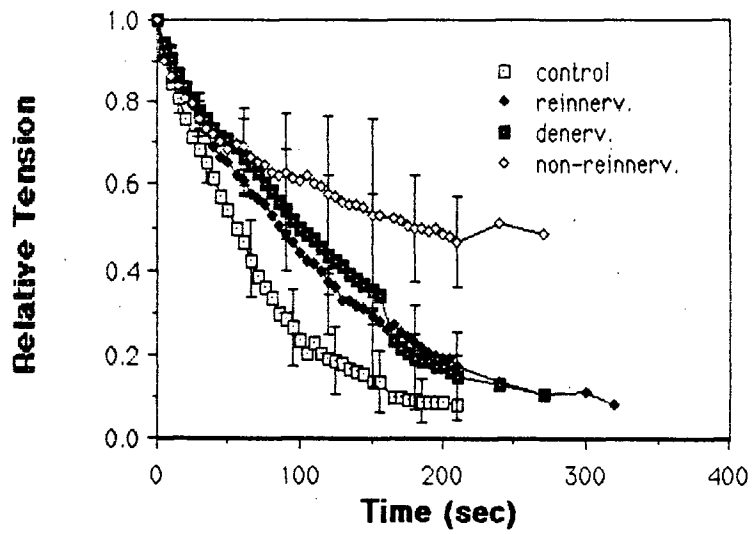
Fatigue: SOL Denervation

resistant than the controls. There was no difference between the sciatic neurectomy muscle fatigue pattern and the reinnervated fatigue pattern; however, the non-reinnervated muscles were more fatigue resistant than the denervated EDL. In contrast, the denervated SOL produced a fatigue pattern that was not significantly different from the controls, reinnervated, or non-reinnervated fatigue patterns at any point during the train of tetanic stimulation.

DISCUSSION

These experiments involved the study of the contractile parameters of denervated EDL and SOL. The muscles were denervated by sciatic neurectomy and then removed for physiological analysis 12 weeks later. The results were used to compare to the previous experiments involving denervation and devascularization of EDL and SOL muscles.

The denervated EDL closely resembled the control and the reinnervated EDL muscles of the previous study. The Po/MW, Pt/MW, 1/2RT, Vo, and PTP of the denervated EDL were not significantly different from the controls. Also, the Pt, Po, Po/MW, TTP, 1/2RT, PTP, and fatigue regime were all similar to the reinnervated EDL muscles. The only similarity between the denervated and the non-reinnervated muscles was the muscle weight. We have already discussed the resemblance of the non-reinnervated muscles to classically denervated muscles (Webster and Bressler, 1985). The denervated EDL in this study did not follow the pattern of a denervated muscle, rather it appeared to have become reinnervated. This could explain the similarity to the control and in particular to the reinnervated muscles.

The denervated SOL muscles appeared to be "truely" denervated. They resembled the non-reinnervated SOL muscles of the previous study in

terms of their muscle weights, Pt, Po, TTP, 1/2RT, PTP, and fatigue pattern. At 12 weeks post-denervation/devascularization, the reinnervated and non-reinnervated muscles closely resembled the control muscles but a difference was still seen among the control muscles, reinnervated muscles, and the non-reinnervated muscles with respect to muscle weight, TTP, 1/2RT, and PTP. It was these four parameters where the denervated SOL differed from the control and reinnervated muscles but resembled the non-reinnervated SOL. One problem with the interpretation of these data was that if we assumed that the non-reinnervated SOL had become reinnervated but by a fast nerve or they had acquired delayed innervation, then the TTP, 1/2RT, and PTP data are somewhat misleading. Consequently, we cannot be sure that the denervated SOL is actually completely denervated.

These experiments were designed to provide a basis for comparison of physiological responses between denervated/devascularized muscle and denervated muscle. The EDL and possibly the SOL appear to have reinnervated, thus excluding them from further analysis. For comparison, the denervation data from Finol et al (1981) can be used as they conducted their sciatic neurectomy experiments (specifically the lateral popliteal) on adult rats.