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ELECTRICAL STIMULATION OF DENERVATED CANINE SKELETAL MUSCLE USING IMPLANTED ELECTRODES AND PULSE GENERATOR

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the Degree of Master of Science

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PREFACE

The experiments leading to this thesis were performed in the University Surgical Clinic of the Montreal General Hospital under the direction of Dr. H.B. Williams, director of Plastic and Reconstructive Surgery, McGill University. This research project was made possible by funding from Medtronic Inc., Minneapolis, and the Medical Research Council of Canada, and by the generous sponsorship of the surgical residency program of Memorial University of Newfoundland.

The results from this study were presented at the annual meetings of the Quebec Association of Plastic Surgeons in February 1989 and the Canadian Society of Plastic Surgeons held in Edmonton in June 1989. This work has also been accepted for presentation at the annual meeting of the American Society for Reconstructive Microsurgery which will take place in Seattle, Washington in September 1989.

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Dr. Ray C.-J. Chiu, Research Director of the University Surgical Clinic, is acknowledged for his valuable academic guidance. It was a pleasure to collaborate with my colleagues, Dr. Koichi Nemoto, who led me through the first steps of learning microsurgery, and with Dr. Dan Durand, my research associate. Melanie Gemeinhardt and Norman Comtois are appreciated for their dedicated involvement as research assistants, and in particular, for their excellent care of the animals.

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Finally, I owe a debt of gratitude to my family and friends for their support and love during this period of intense work.

ABSTRACT

Following nerve injury and repair, functional and esthetic results are often poor due to denervation changes in the muscle. We hypothesize that continuous electrical stimulation (ES) of denervated muscle using implanted electrodes and a pulse generator lessens the effects of denervation and helps to preserve muscle integrity. Ten dogs underwent sciatic nerve severance, common peroneal nerve repair, and implantation of stimulating devices. In the denervated-stimulated group, DS (n=5), the tibialis cranialis muscle was treated with ES. Group D, (n=5), served as the unstimulated denervated control. The contralateral normal muscle of all animals, N (n=10), provided a third group for independent t-test analysis of data. The normal muscle also served as an intrinsic control (n=5) for both D and DS in paired t-test data analysis. The muscle was assessed structurally and functionally 10 weeks post-operatively. The proportion of muscle weight preserved was significantly greater in the stimulated muscle than in the untreated denervated muscle ($p<0.01$). There was evidence of less atrophy and degeneration in DS seen in both light and electron microscopy. Significantly larger type 1 ($p<0.0001$) and type 2 ($p<0.005$) fiber cross-sectional areas were found in DS than in D. The type 1 fiber in group DS was hypertrophied compared to that in group N ($p<0.04$). The proportion of type 2 to type 1 muscle fibers did not differ significantly between the three groups at 10 weeks. When an additional group of animals (n=4) was studied histochemically 16 weeks following denervation (unstimulated) and compared to the 10 week denervated group, a significant decrease in the percentage of type 2 fibers was found ($p<0.003$). Dependent t test analysis using the normal contralateral muscle groups as the control showed that twitch contraction time was maintained within normal limits by stimulation whereas in the unstimulated group prolongation of twitch time occurred ($p<0.004$). The electrical resistance property of the stimulated muscle was also preserved when compared to the normal control ($p=0.7$) but did differ between D and N ($p<0.04$). Although trends in the data suggested beneficial functional effects of ES, statistical analysis failed to support differences between the stimulated and unstimulated groups in functional testing. The incidence of complications related to the implanted devices was low. In conclusion, this study indicated some structural and functional preservation of denervated muscle treated with ES using a newly developed implantable system. Recommendations for further research are presented.

RÉSUMÉ

Les changements de dénervation survenant dans le muscle après une lésion traumatique du nerf suivie de sa réparation entraînent souvent une atteinte fonctionnelle et esthétique. Or, notre hypothèse est qu'une stimulation électrique continuelle du muscle dénervé par l'implantation d'électrodes et d'un stimulateur réduira les conséquences de la lésion et préservera l'intégrité du muscle. Dix chiens ont subi une section du nerf sciatique grand, la réparation du nerf sciatique poplité externe et l'implantation des dispositifs de stimulation. Dans le groupe dénervé-stimulé, DS (n=5), le muscle jambier antérieur fut traité par des stimulations électriques; le groupe dénervé, D, (n=5), servit de groupe témoin non traité. Le muscle contralatéral normal de tous les animaux, N (n=10), constitua un troisième groupe satisfaisant afin de produire une analyse indépendante des données à l'aide du test de Student. Le muscle normal servit aussi de témoin intrinsèque (n=5) pour les groupes D et DS lors de l'analyse dépendante des données basée sur le test de Student. Après 10 semaines, le muscle fut évalué sur le plan de la structure et de la fonction. La proportion de la masse musculaire preservée s'est avéré significativement plus grande à l'égard du muscle dénervé-stimulé au muscle dénervé non stimulé ($p<0.01$). Nous avons observé, tant au microscope optique qu'électronique, moins d'atrophie et de dégénérescence musculaire dans le groupe DS. En coupe transversale, le muscle stimulé montra d'une manière significative des fibres de type 1 ($p<0.0001$) et de type II ($p<0.005$) de plus grande surface que celles du groupe D. Comparées à celles du groupe N, les fibres de type 1 du groupe DS étaient hypertrophiées ($p<0.04$). Avec la stimulation, le temps de contraction musculaire a été maintenu dans les limites de la normale, alors que le groupe non stimulé a montré un prolongement du temps de contraction ($p<0.004$). La propriété de résistance électrique du muscle stimulé était aussi préservée lorsque comparée au groupe normal de contrôle ($p=0.7$) mais différait entre D et N ($p<0.04$). Malgré que les tendances en ce domaine suggèrent des effets bénéfiques du point de vue fonctionnel pour l'ES, l'analyse statistique n'a pu démontrer une différence entre les groupes stimulé et non stimulé dans les tests fontionnels. L'incidence de complications relié aux implants était faible. En conclusion, cette étude a démontré qu'on pouvait préserver certaines qualités structurelles et fonctionnelles dans le muscle dénervé traité par ES en utilisant un système d'implant récemment mis au point. Des recommandations pouvant orienter la recherche sont proposées.

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CHAPTER I

INTRODUCTION

STATEMENT OF THE CLINICAL PROBLEM

Following motor nerve injury, debilitating functional and esthetic deficits may result despite present-day microsurgical reconstruction and physiotherapy. These deficits are related to the complex changes in the muscle which accompany a prolonged period of denervation and inactivity.

In clinical practice the mechanisms of nerve injury resulting in denervation are multiple. For example, trauma may result in injury to the brachial plexus; intraoperative damage to the facial nerve may occur during tumor resection; and proximal ulnar nerve, radial nerve, or common peroneal nerve injuries may arise from laceration, fracture, dislocation, or manipulation. In the central nervous system, injury from a cerebrovascular accident may manifest as hemiplegia. In this situation, the insult to the brain may partially resolve and a potential for the hemiplegia to improve exists. In the microsurgical transfer of free muscle flaps with neural supply, the changes of denervation which occur during a long period of nerve regeneration may adversely affect the functional result.

In all of the above clinical scenarios prevention of or reduction of the changes of denervation in muscle represents an important therapeutic goal. The preservation of the end organ during the period of reinnervation provides the focus for the present investigation.

EXPERIMENTAL GOAL AND HYPOTHESIS

The goal of the present research is to attempt to preserve *muscle integrity* following peripheral nerve injury using continuous electrical stimulation (ES) of the denervated muscle, provided by the implantable stimulating system designed in this laboratory.

Muscle integrity implies structural, physiological, biochemical, mechanical, and electrical competence of the skeletal muscle. When a muscle is denervated all of these components are altered. It is hypothesized that electrical stimulation will maintain denervated muscle in a more normal state during the period of nerve regeneration when compared to untreated denervated muscle. This study also examines the efficacy and safety of the proposed implantable stimulating system.

The integrity of normal muscle depends on the interplay of two key factors: *contractile activity* and the *neurotrophic influences* to which the muscle is exposed (Gutmann, 1976; Pette, 1986; Nix, 1986). Functional and structural discontinuity of a peripheral nerve effectively eliminates both of these factors. The body of literature (which is reviewed here) on the use of electrical stimulation of denervated muscle offers support for the hypothesis that contractile activity in the absence of neurotrophic influences is of benefit to the muscle. The production of contractile activity using ES can be conceptualized as an attempt to mimic the neural effect related to the activity transmitted by the motoneuron to the muscle fiber.

LITERATURE REVIEW

INTRODUCTION

The following literature review first presents the structural, biochemical, physiological, mechanical, and electrical changes of denervated mammalian skeletal muscle. This forms a foundation for the proceeding review of the effects of electrotherapy and artificially produced contractile activity on denervated muscle. Traditionally, in the literature, evaluation of the effects of electrical stimulation is accomplished by comparing denervation changes in the stimulated versus the unstimulated denervated muscle. In the present literature review emphasis is also placed on recognizing both the diversity in experimental design and the important relationship between design and experimental outcome.

DENERVATION CHANGES IN MUSCLE (Table 1)

Structural Changes

The structural changes of denervation were the first to be observed experimentally and to be documented in the literature. Reid, in 1841, noted that 7 weeks following sciatectomy in a rabbit the denervated muscle weight was approximately 50% that of the normal. He described the gross appearance of the affected muscle as being "evidently much smaller, paler, and softer than the corresponding muscles of the opposite leg" and using the microscopy available at the time wrote that the muscle fibers were "considerably smaller, had a somewhat shrivelled appearance, and the longitudinal and transverse striae were much less distinct than in the muscles of the leg". He attributed the observed atrophy and degeneration to "imperfect nutrition, consequent upon the state of inaction into which they are thrown".

Since Reid's time the structural changes have been further elaborated and can be summarized as follow: the reduced muscle weight and circumference of atrophy; the loss of myofilaments of early degeneration; the loss of striation and the appearance of central nuclei, vacuoles and lysosomes of late degeneration; and the proliferation of connective tissue with fatty infiltration after several months (Davis, 1984).

TABLE 1

Changes in Denervated Mammalian Skeletal Muscle

Type of Change	Changes of Denervation
Structural	Atrophy Degeneration Fibrosis
Biochemical	Fibre type change Decreased activities of glycolytic and oxidative enzymes, acetylcholinesterase Increased activity of acid proteinases Increased prostanoids
Physiological	Decreased resting membrane potential Increase in transmembrane resistance Cessation of release of ACh Supersensitivity to ACh Decreased activity of AChE Fibrillations
Mechanical/ Electrical	Increase in muscle chronaxy and altered strength-duration relationship. Decreased tetanic tension. Increased isometric twitch contraction time and half relaxation time.

(Adapted from Davis, 1983.)

The mechanism of degeneration may involve prolonged intramuscular vascular stasis and, as was hypothesized by Reid, an impaired nutritional state of the muscle. Vasconstrictor paralysis and loss of muscle-pump action leading to stasis, congestion and thrombosis could reduce muscle perfusion resulting in degenerative changes (Sunderland, 1978). Of real concern to the clinician is the end result of these structural changes - a permanent progressive muscular atrophy and contracture with loss of elasticity and connective tissue hyperplasia (Pinelli, 1978).

Ultrastructural changes in denervated muscle have been extensively studied. Typically, surrounding pleomorphic nuclei there are collections of glycogen granules, some lipid bodies and disorganized myofibrils. Some myofilaments disappear altogether and those remaining are often randomly distributed and have an abnormal sarcomere pattern. The Z-lines are thickened and distorted. The sarcoplasmic reticulum may be distended. The number, size and shape of the mitochondria may be altered. Loose folds of basement membrane project from some atrophying muscle fibers and sometimes only empty basement membrane tubes remain, the muscle fiber having completely degenerated (Mair & Tomé, 1972). Several of these ultrastructural alterations can be quantitatively measured (Engel & Stonnington, 1974) and may provide objective aseessment of the extent of the disease process.

Biochemical Changes

Skeletal muscle fibers can be divided into two basic groups, type 1 and type 2, based on distinguishing biochemical features. Quantitative differences between the fiber types are listed in Table 2 and provide a background against which biochemical changes in the experimental muscle can be assessed.

Using histochemical staining techniques which differentiate muscle fiber types, some researchers report that with denervation, type 2 fibers (fast twitch, glycolytic metabolism) atrophy to a greater extent than do type 1 fibers (slow twitch, oxidative metabolism) (Karpati, 1969; Pachter, 1982; Goldberg, 1972). Within two to three days of denervation a decrease in activities of glycolytic and oxidative enzymes occurs. These metabolic enzymatic changes are linked to the fiber type changes which occur with denervation, namely, a relative increase in type 1 fibers.

TABLE 2.

Quantitative Differences Between Type I and Type II Muscle Fibers

	Type I	Type II
Physiology: twitch and fatigue	Slow	Rapid
Vascular supply	More (red)	Less (white)
Intermyofibrillar sarcoplasm	More	Less
Mitochondria	Many	Fewer
Concentration of oxidative enzymes	High	Low
Glycogen content	Low	High
concentration of glycolytic enzymes	Low	High
concentration of cytochrome	High	Low
Content of neutral lipids	High	Low
Myofibrillar ATPase activity with acid preincubation	High	Low
ATPase activity with alkaline preincubation	Low	High

(From Sarnai, H.B.; p.8, 1983.)

Richter (1982) demonstrated that the rabbit peroneus longus muscle, a predominantly type 2 glycolytic muscle which normally has only a few scattered type 1 fibers, failed to regain the normal fiber population six months after nerve suture despite good functional return. Acetylcholinesterase activity, particularly the endplate form, decreases very rapidly and after two weeks may be absent. The overall cholinesterase activity may be as low as 15% of normal levels (Guth et al., 1964; Hall, 1973; Vigny et al., 1970). Acid proteinases, which participate in the degradation of proteins within lysosomes show a marked increase in activity two weeks after denervation (Maskrey et al., 1983). Jaweed (1982), using radioimmunoassay, found an increase in the prostanoids thromboxane B2 and 6-keto-PGF1 in denervated rat gastrocnemius.

Physiological Changes

The earliest detectable physiological change after denervation is a decrease in the resting membrane potential. This begins as early as six hours post-injury and within a week falls from the normal -80 mV to about -65 mV. This change is maintained at that level (Deshpande et al., 1976; Albuquerque et al., 1978). The transmembrane resistance increases with denervation (Ware et al., 1954; Albuquerque et al., 1970; Guth et al., 1981).

Also associated with denervation is an immediate cessation of evoked release of acetylcholine (ACh) from the nerve terminal. In the rat it has been noted that spontaneous release of ACh, which results in normal miniature endplate potentials, can be detected for up to 24 hours following denervation (Albuquerque et al., 1970).

ACh hypersensitivity develops during the first few weeks after denervation This change follows the so-called *Law of Denervation Supersensitivity* (Cannon & Rosenblueth, 1949) whereby a denervated structure (which could be skeletal muscle, smooth muscle, or gland) becomes hypersensitive to the agents that normally activate it. ACh supersensitivity can be attributed to formation of extra-junctional ACh receptors. Normally, ACh receptors are present only in the endplate region of the sarcolemma. With denervation they become incorporated into the sarcolemma along the entire length of the muscle fiber. Contractions are thus easily elicited using low concentrations of ACh because the whole surface of the fiber is sensitive (Jones & Vrobova, 1974; Thesleff, 1974; Axelsson & Thesleff, 1959).

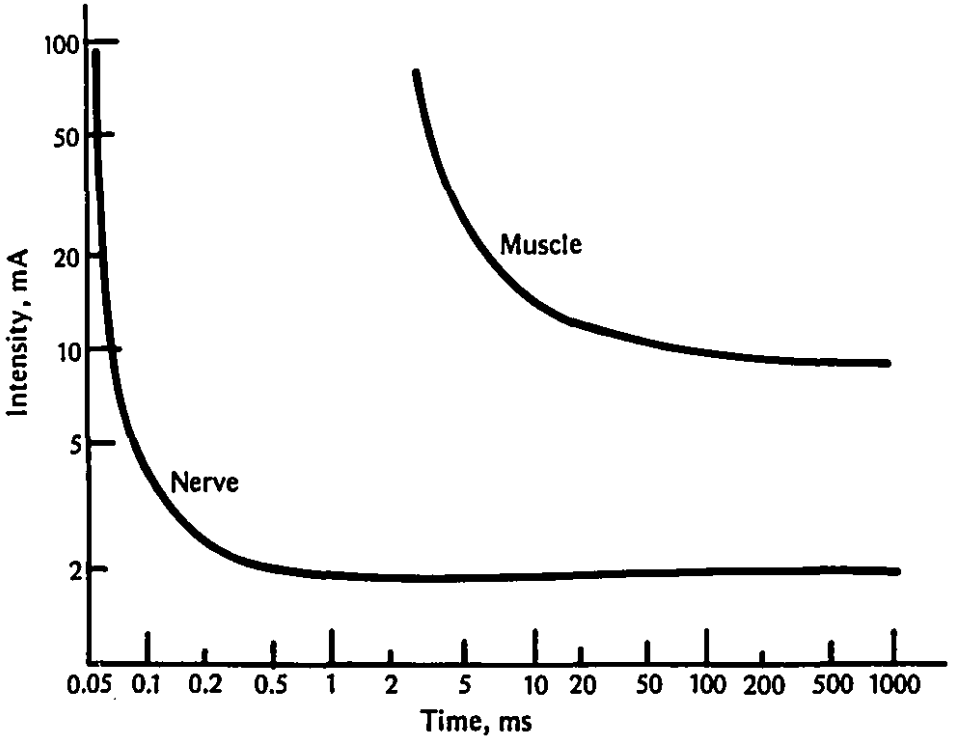
Fibrillation is another physiological marker of muscle denervation. Denervation fibrillation involves spontaneous, uncoordinated contractions of muscle fibers, which are detectable using electromyography. Fibrillation is seen two to three days following the nerve insult (Hughes, 1974). Measuring the frequency of fibrillation potentials has been used to quantify the degree of denervation and reinnervation and the effects of electrical stimulation experimentally (Herbison et al., 1983).

Mechanical and Electrical Changes

A distinctive pattern of deviation from normal contractile properties in denervated muscle has been reported (Kotsias & Muchnik, 1987). These changes may be related to the contractile speed, strength, or fatiguability of the muscle. With denervation one typically sees a prolongation of the twitch contraction time (i.e. the time to peak tension) and the half-relaxation time. Because of this sluggish response, stimuli of lower than normal frequencies can fuse these contractions into tetany. Another significant effect of denervation is the reduction in tetanic tension. This decrease becomes more apparent at high rates of stimulation: for example, 100 to 300 Hz (Kotsias & Muchnik, 1987). Fatiguability, which refers to the decrease in force of a muscle which contracts repetitively or has a sustained tetanic contraction, is also adversely affected by denervation. Nix (1986) found that fatiguing the normal rabbit extensor digitorum longus muscle resulted in a 35% loss of force output over 3 minutes. Denervated muscle showed a force decline of 63%.

Strength-duration testing is another method used by physiologists and physiotherapists to assess a muscle's mechanical and electrical properties. It is based on the knowledge that production of a muscle contraction using ES depends on the innervation of the muscle and the relationship between the current or voltage amplitude and the pulse duration (PD). In general, if a voltage or current is decreased, the PD will need to be increased in order to produce a contraction of the same magnitude. In Figure 1 strength-duration curves are shown for indirect stimulation of normal muscle (via its nerve) and for direct stimulation of normal muscle. The nerve stimulation curve lies to the left of the indirect muscle stimulation curve indicating the lower amplitude and PD requirements of indirect stimulation of normal muscle.

The strength-duration curve is used in the clinical setting as an adjunct to electromyography to assess the contractility and excitability of muscle which has suffered insult to its innervation. Figure 2 illustrates the relation between normally innervated, partially denervated and completely denervated muscle. When assessing muscle using the strength-duration curve two measurements are of value - the rheobase and the chronaxy. Rheobase refers to twice the threshold voltage required to produce a contraction and chronaxy refers to the pulse duration required for production of a contraction at the rheobase. Alterations in the chronaxy and strength-duration relationship occur with denervation. Since the late 1800's, it has been known that denervated muscle loses its ability to respond to short-duration electrical pulses (i.e. 50 μs-1 ms). To a long-duration pulse (i.e. 1 ms-200 ms) it will contract, although less briskly (Speilholz, 1987). Kosman (1947) found that electrically treated denervated muscle required lower PD and current to produce a contraction than did untreated denervated muscle.



Strength/duration curves for nerve and muscle.

FIGURE 1. Strength-duration curves of normal muscle obtained by indirect stimulation (via the nerve) and by direct muscle stimulation.

(From Nelson, R.M. & Currier, D.P., p. 84, 1987.)

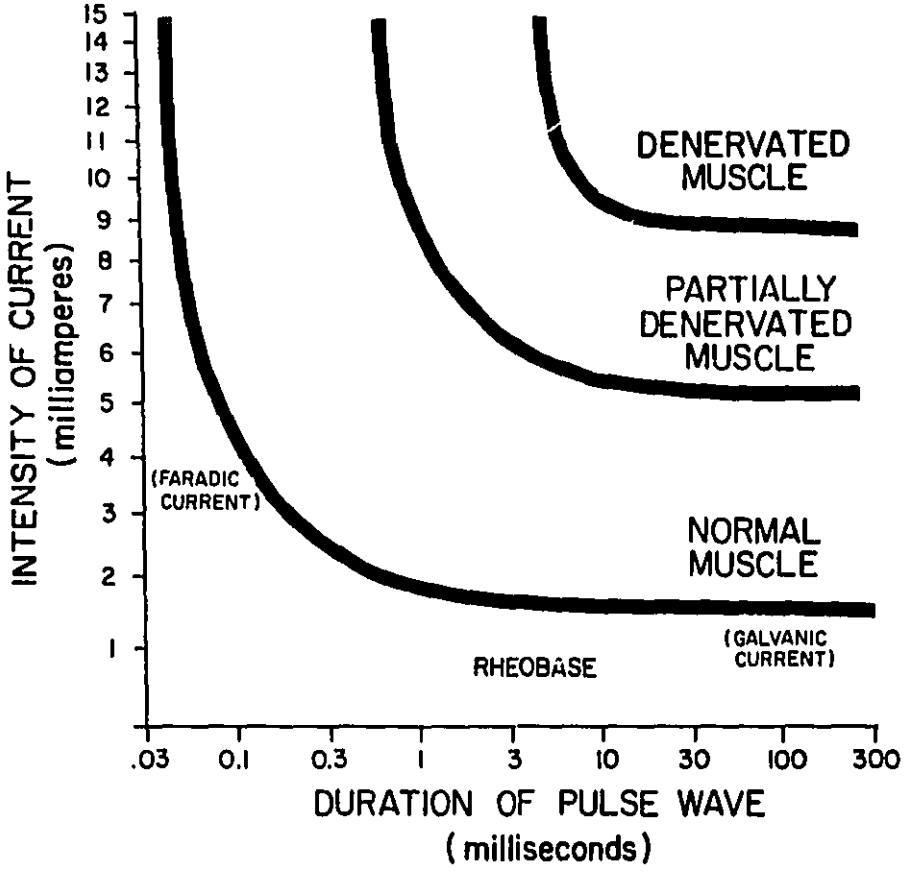


FIGURE 2. Strength-duration curves for normal, partially denervated, and denervated muscle.

(From Schwartz, S.I., p. 1850, 1984.)

THE EFFECTS OF ELECTRICAL STIMULATION

HISTORICAL REVIEW

The earliest recognition of the effects of electric current on living organisms can be traced to the ancient Egyptians and Greeks, several centuries B.C. These people noted the ability of certain fish to stun other creatures into immobility (Figure 3). Fishing scenes on reliefs from Pharaonic Egypt depict these fish which are comprised of disparate species: eels, rays, stargazers, and shad. The Greeks designated the electric fish by the collective term "narke", or numbness-producing, from which the word "narcosis" originates.

FIGURE 3. First illustration of the application of electricity to the human body. *(From Hortus sanitatis. Strassburgh, 1497. Photographed from Still, D., 1974.)*

The first known commentaries on the medicinal utility of the live fish can be found in the *Compositiones medicae*, circa A.D. 50, written by physician Scribonius Largus. He was a proponent of the use of electric rays (torpedos) (Figure 4) for the management of gout and headache:

> For any type of gout a live black torpedo should, when the pain begins, be placed under the feet. The patient must stand on a moist shore washed by the sea and he should stay like this until his whole foot and leg up to the knee is numb. This takes away present pain and prevents pain from coming on if it has not already arisen. Headache even if it is chronic and unbearable is taken away and remedied forever by a live black torpedo placed on the spot which is in pain, until the pain ceases. *(Kellaway, 1946; in Electrical Cardiac stimulation, p. 19; Schecter, D.C.; Medtronic, Inc. 1983).*

FIGURE 4. Electric ray or "torpedo" such as that used by physician Scribonius Largus.

In the sixteenth and seventeenth centuries the groundwork was laid for the production and leashing of man-made electricity with the invention of crude electrostatic induction machines. Then in the 1740's the Leyden jar, a device that would create and conserve large quantities of electricity, was created by von Kleistand and van Musschenbroek. After this time the medicinal uses to which electricity was put were numerous. Charlatanry abounded. Of the nonthoracic disorders the main uses concerned neuralgic, myalgic, and psychiatric derangements. Kratzenstein reputedly freed, by sparks from an electrostatic machine, a chronically contracted l le finger of a patient, who was then able to play the harpsichord again. Jallabert was th to underscore that individual muscles could be made to react to electrostimulation. Duchenne de Boulogne, also an important early scientist in the study of the effect of electricity on muscle, is shown testing the muscles of facial expression using electric current in Figure 5. Benjamin Franklin, in 1758, warned that the shocks used in muscle electrostimulation of paralytic patients were too painful and were of dubious value (Schecter, 1983).

One hundred years later Reid performed his landmark controlled study of denervated rabbit muscle exercised daily by a weak galvanic battery, showing that ES is effective in maintaining contractility and bulk of denervated muscle (Reid, 1841). This experiment set the stage for the present day approach to investigation of the effects of stimulation on denervated muscle using controlled animal studies.

Duchenne du Boulogne (1806-1875)

FIGURE 5. Early investigation of the facial muscles using electric current.
(From Rothbottom, M. & Susskind, C., p. 71, 1984.)

CURRENT LITERATURE

The question, "Is electrical stimulation beneficial to denervated muscle?" remains controversial despite the growing body of literature on the subject. The ameliorative, deleterious, and equivocal effects of this intervention will be reviewed here.

Muscle Atrophy

The diminution of muscle atrophy (as reflected by whole muscle weight) following denervation is one of the more frequently documented beneficial effects of ES. This observation was first reported by Reid in 1841. Interest was then rekindled a century later with Gutmann's confirmation of the value of ES in delaying and decreasing the loss of muscle bulk in a denervated rabbit model (Gutmann, 1942). This has since been supported by many of his successors (Jaweed et al., 1982; Harada, 1983; Herbison et al., 1983; Cole & Gardiner, 1984; Kanaya, 1985; Nemoto et al., 1988). Other investigators have avoided measuring muscle weight claiming "it is not a good estimate of the condition and state of the contractile properties of the muscle" (Nix, 1982). Furthermore, weight may be confounded by factors such as proliferation of connective tissue, edema, and possible differences in the amounts of muscle removed for weighing (Speilholz, 1987).

The measurement of muscle fiber diameter after histochemical staining is an alternative and possibly more accurate method of assessing atrophy. It's accuracy depends on studying representative samples of the muscle. Using morphometric analysis many studies show that ES of denervated muscle results in greater fiber cross-sectional area than in the unstimulated control group (Pachter et al., 1982; Martin, 1983; Kanaya, 1985; Hennig & Lomo, 1987). Nix (1988) found that ES resulted in larger fiber diameter only if the muscle was treated with isometric contractions. Change in fiber size was also found to be related to the stimulus frequency: type 1 fibers atrophied less than type 2 fibers with a stimulus of 10 Hz (Pachter et al., 1982; Harada, 1983) and high frequency stimulation suppressed enlargement of type 2 fibers (Harada, 1983). In contrast, Girlanda and colleagues (1982) found that a high frequency pattern of stimulation antagonized the reduction of type 2b fibers in rabbit fast muscle but actually accentuated the diameter of type 1 fibers. Contrary to other findings, his treatment regimen *increased* the weight loss of the denervated slow soleus muscle!

Degeneration and Fibrosis

Assessment of structural changes such as fibrosis in stimulated denervated muscle is more difficult because it is done subjectively, often by the principal investigator. Nevertheless, the observation that ES results in muscle with less connective tissue than the unstimulated control must be recognized (Gutmann & Guttman, 1942; Nemoto et al., 1988). This finding may depend on the length of time the muscle has been denervated since other studies fail to support this finding in the early post-denervation period (Harada, 1983). Still other investigators provide evidence for possible damaging effects of ES. Hennig & Lomo (1987) attributed fiber damage to excessive direct stimulation and Nix (1987) attributed permanent contractures to a tetanic pattern of stimulation. Schimrigk and associates (1977) reported that electrically stimulated muscles showed fewer central nuclei and a greater number of necrotic single fibers.

Unfortunately the published literature to date contains no evaluation of the ultrastructural differences between stimulated and unstimulated denervated muscle. This may provide an objective assessment of structural changes in the future.

Acetylcholine Sensitivity and Fibrillation Potentials

Contractile activity produced by direct stimulation of denervated muscle is able to maintain the extrajunctional region of the muscle fiber surface in a normal state with respect to both ACh sensitivity and to the ability to accept new innervation. However, even a short period (2 days) of inactivity allows the expression of the full post-denervation effect (Lomo & Slater, 1978). Herbison (1983), in his rat crush-denervation model, failed to find a difference in ACh sensitivity between stimulated and unstimulated muscle. He did however conclude that ES reduced the number of fibrillations of the denervated soleus muscle.

Other Biochemical Changes

Muscle activity independent of neural influences appears to play an essential role in the control of several enzymes including acetylcholinesterase (AChE) and oxidative enzymes. Direct stimulation of crush denervated rat muscle resulted in increased activity of specific forms of AChE, depending on the type of muscle and the stimulus pattern (Lomo et al., 1984).

Electrical stimulation of denervated guinea pig soleus muscle prevented the decreases in oxidative enzymes normally seen with denervation (Nemeth, 1982). David (1983) found

that electrotherapy restored the utilization of carbohydrates through the hexose mono- and diphosphate pathways and oxidative metabolism. An increase in prostaglandins thromboxane B2 and 6-keto-PGF1 was found following stimulation of denervated rat gastrocnemius.

The phenomenon of fiber type transformation and associated change in contractile properties in denervated stimulated muscle will be reviewed in the section Stimulating Parameters - stimulus frequency.

Reinnervation

The work of Sebille (1980) supports the hypothesis that there is a positive effect of ES of denervated muscle on the course of reinnervation and acceleration of axonal growth after peripheral nerve lesion. Schimrigk (1977), however, raised the suspicion that ES has a retarding effect on regeneration of muscle fibers. He studied reinnervating crush-denervated quadriceps femoris muscle of rats 7 weeks following nerve injury. In the untreated group the growth of the muscle fiber diameter was faster than in the treated group. Other investigators have found no difference in reinnervation between stimulated and unstimulated animals when assessed with electromyography (Girlanda et al., 1982; Nemoto et al., 1988).

Electrical Properties

According to Westgaard (1975), direct ES after denervation normalizes the resting membrane potential and passive electrical properties of denervated muscle membrane. It restores the electrical parameters including specific membrane resistance, specific membrane capacitance, and transition frequency. Westgaard (1975) concludes that ES appears to compensate fully for the absence of neural influences with respect to membrane properties.

SURVEY OF RESEARCH METHODOLOGY

The evaluation of the literature has become a complex task due to the diversity in research design. The components which vary so widely from study to study in the field of stimulation of denervated muscle merit description. These include the animal species used, the muscle selected for study, the method of nerve injury and denervation, the degree of denervation, and the stimulating protocols used. Illustrative experiments are summarized in Table 3 and provide a focus for discussion.

Species and Muscle Studied

The three species commonly studied when testing the effect of ES on denervated muscle in order of decreasing use are rats, rabbits, and dogs. The guinea pig was used in one study (Nemeth, 1982). Rats and rabbits have been the primary experimental animal because they are relatively inexpensive and easy to house.

Technically, small animals provide advantages when attempting to electrically stimulate their muscles. The comparatively smaller muscle size permits circumferential or anterior-posterior placement of wire or other slender electrodes around the muscle (Westgaard, 1975; Nemeth, 1982) thus enabling a more complete stimulation of the muscle. However, the difficulty in applying small animal technology to larger species makes the extrapolation of their results to the human clinical situation less feasible. In larger species such as the dog (and the human) the method of stimulating with wire electrodes placed circumferentially or on the posterior and anterior aspects of the muscle may not be technically feasible. Firstly, the exposure and dissection required for this technique make it more surgically invasive. Secondly, the surface area of a wire electrode would not be adequate for contact with and depolarization of larger muscles. A larger electrode would be necessary to encircle the muscle and make adequate contact with the muscle surface for depolarization to occur. This increase in electrode size would in turn result in a decrease in current density thus necessitating a higher initial voltage or current output and a larger power source. This additional demand for voltage would make a larger battery necessary and would make implantation more awkward. Electrodes with large enough surface area for depolarization, implanted on the muscle surface through small skin incisions (Nemoto et al., 1988) rather than circumferentially, would be more clinically feasible.

TABLE 3 - Survey of Research Methodology

Study	Investigator	Species	Muscle	Denervation Method	Electrode	Stimulation Method	Stimulating Parameters
1	Gutmann, 1942	rabbit	ant. tibial, peroneus long.	peroneal n. crushed or cut & repaired	skin	immediate, transcutaneous, intermittent	4-6 mA, 120 v battery, 15-20 min/24 hr 500-600 contractions/24 hrs
2	Westgaard, 1975	rat	soleus	sciatic n. resected	platinum wire	delayed 4 days, implanted continuous	2-3 x threshold v, 10 Hz, 2-2.5 ms PD, on 8 s, off 12 s, x 2 weeks
3	Gilliatt, 1978	baboon	4th lumbricals	ulnar n. compression	needle	delayed 21 days, subcutaneous, intermittent	150 v, 5/20 Hz, 5 ms PD, 500 impulses/24 hrs x 6 days
4	Nemeth, 1982	guinea pig	soleus	tibial n. resected	steel wire	immediate, implanted, continuous	3-8 v, 10 Hz, 0.8 ms PD, on 1 s, off 1 s, 8-9 hr/24 hr, x 4 weeks
5	David, 1983	dog	gastrocnemius	sciatic n. cut	skin	immediate, transcutaneous, intermittent	10 v, 100 ms PD, 2 Hz, 30 min/24 hrs, x 2 or 4 weeks
6	Martin, 1983	rabbit	laryngeal	recurrent laryngeal n. cut	skin	immediate, transcutaneous, intermittent	2.5 mA, 10/50/100 ms PD, 10 min OD/BID x 2 or 4 weeks
7	Jayasree, 1984	dog	gastrocnemius	sciatic n. resected	skin	immediate, transcutaneous, intermittent	10 v, 2 Hz, 100 ms PD, 30 min/24 hrs x 15 days
8	Cole, 1984	rat	gastrocnemius	sciatic n. crush	disc Ag-Ag chloride	immediate, transcutaneous, intermittent	20 Hz, 25 ms PD, 5 s on, 1 s off x 1 min, TID, 30 contractions/24 hrs
9	Nemoto, 1986 (unpublished)	rabbit	rectus femoris	femoral n. cut & repaired	steel leaf	immediate implanted continuous	4 v, 36 Hz, 210 us PD, on 0.1 s, off 0.4 s, x 6-8 weeks
10	Valencic, 1986	human	ant. tibial	variable levels of sciatic & peroneal n. injury	skin	delayed 62+ days, transcutaneous, intermittent	30 mA, 10-50 ms PD, 20 min BID 5 days/week x 3 weeks
11	Nix, 1987	rabbit	ext. digit. long.	peroneal n. resected	steel wire	delayed 2 days, implanted intermittent	1 Hz, 7 ms PD/40 Hz, 1 ms PD, isometric, 20 min/24 hrs
12	Gemeinhardt, 1989 (unpublished)	dog	ant. tibial	sciatic n. cut, proximal c. peroneal n. repaired	carbon-silicone	immediate, implanted, continuous	10.5 v, 85 Hz, 450 us PD, on 1.5 s, off 24 s, x 10 weeks

The resistivity of skeletal muscle in different species varies considerably. It has been measured in the human, and several animals including the dog, pig, rabbit, guinea pig, and frog (Geddes & Baker, 1967). Part of the variability in resistivity would appear to be related to the muscle fibers which resemble bundles of poorly-conducting tubes filled with electrolytes. These "tubes" may be more conductive in one species than another. The current pathway through the specimen and orientation of the fibers (which may be species-dependent or technique-dependent) also influence the resistivity of the muscle.

Apart from the species variability in the literature, there is also diversity among the muscles selected for denervation and electrotherapy. The muscles studied in the rat include the slow soleus (Westgaard, 1975; Lomo et al., 1980; Herbison et al.; Herbison et al., 1986; Al-Amood, 1986), the fast muscles: gastrocnemius (Jaweed et al., 1982); extensor digitorum longus (Pachter, 1982; Kotsias, 1987); anterior crural group (Harada, 1983); and quadriceps femoris (Schimrigk, 1977). When both fast and slow muscles were studied simultaneously in an experiment the slow soleus and the fast extensor digitorum longus were consistently selected as a pair (Lomo et al., 1984; Hennig & Lomo, 1987; Kotsias & Muchnik, 1987).

Rabbit denervation-stimulation experiments, in addition to studying the anterior crural muscles (Gutmann & Guttman, 1942) and the extensor digitorum longus (Nix, 1987) have made use of an array of other denervation models. Martin (1983) examined the effect of ES on the laryngeal muscles including the vocalis and the posterior cricoarytenoid following unilateral paralysis of the recurrent laryngeal nerve. An experiment in functional ES of denervated rabbit facial muscles was performed by Tobey in 1978. Nemoto (1988) and others (D. Durand, personal communication) have applied electrotherapy to the denervated fast rectus femoris muscle.

In the canine studies the anatomical models used to date have all involved transection of the sciatic nerve and stimulation of either the gastrocnemius (David et al., 1983; Jayasree, et al. 1984) or the anterior crural muscles (Nemoto et al., 1988).

Primate studies are rare and include the human clinical work by Valencic et al. (1986) in which adults with completely denervated tibialis anterior muscle were treated with transcutaneous ES. The treatment was delayed and intermittent yet in all patients the level of dorsiflexion due to electrical stimulation increased after the treatment program. The only other primate study in the literature to date is an uncontrolled investigation using baboons

whose fourth lumbrical muscles were denervated by compression of the ulnar nerve through forearm compression and then treated with ES (Gilliatt et al., 1978). Stimulation was started 21 days following denervation and continued for 6 days. Each baboon had a different stimulation regimen. The author concluded that muscular activity was able to influence extrajunctional muscle properties in the baboon. Interpretation of these conclusions are questionable in view of the weak study design.

Method and Degree of Denervation

Evaluation of the literature and comparison of experimental results also depends on homogeneity in the method of nerve injury and in the extent to which the muscle is denervated when stimulated and finally studied. This diversity is illustrated by representative examples in Table 3.

Experimental nerve injuries range from crushing (Gutmann & Guttman, 1942; Girlanda et al., 1982; Jaweed, 1982; Nix, 1982; Harada, 1983; Herbison et al., 1973; Herbison et al., 1983; Cole & Gardiner, 1984) to freezing (Sebille & Bondoux-Jahan, 1980) to external compression (Gilliatt et al., 1978). The most common form of nerve injury however has been transection with or without resection of a part of the nerve (Lomo et al., 1978, 1980, & 1984; Kanaya, 1985; Al-mood et al., 1986; Nix, 1986 & 1987; Martin, 1983; Jayasree, 1984; Westgaard, 1975; Nemeth, 1982). In some studies, the nerve was severed and then microsurgically repaired (Gutmann & Guttman, 1942; Nemoto et al., 1986) with the purpose of better simulating the clinical situation.

The extent of axonal regeneration would be expected to be greater in a crush, freeze, or compression injury after a given period of healing than in a nerve section injury. One woud expect the process of reinnervation to be more advanced in muscle with the severed nerve ends approximated than left apart. Furthermore, the rate of denervation changes and nerve regrowth is species-dependent. Animals having a shorter life-span and higher metabolic rate, such as rats and rabbits, have a faster rate of muscle atrophy following denervation (Knowlton & Hines, 1936) and a faster rate of axonal regeneration than animals with a longer life-span. Thus the canine model of denervation would more closely resemble the human situation than the rat or rabbit.

The degree of denervation may thus vary from study to study. This has implications for the ability of the muscle to respond to ES and for the stimulating parameters required by a muscle. Figure 2 illustrates this concept well: partially denervated muscle requires a lower stimulating current or voltage and pulse duration than completely denervated muscle to produce the same contraction. It is important to recall also that the mechanism of excitation is different in the completely denervated muscle from muscle having functional neuromuscular junctions.

Method of Stimulation

The methodology of electrical stimulation of denervated muscle encompasses many components, all of which should be defined in order to permit valid assessment of the experiment and its outcome.

Stimulating Apparatus

The stimulating apparatus, namely the electrodes and power source vary considerably from study to study. They carry with them inherent problems which need to be addressed if technology for human application is to be developed. These issues are frequently ignored in animal research.

Stimulating electrodes may be applied externally or may be internally implanted. They may be composed of a number of different materials such as steel (Nemoto et al., 1988; Nix, 1987), platinum (Westgaard, 1975), or silicone and carbon, as in the present study. The electrodes can be assessed in terms of impedance properties, durability, production of local tissue damage, electrode encapsulation, and migration. The latter items apply more to indwelling electrodes than to external electrodes. One investigator succinctly voiced the difficulties encountered with implanted electrodes (in rats) when he wrote, "Implanting an electrode that will stimulate the denervated muscle but will not obstruct the contraction, stretch the muscle, damage adjacent muscles, or cause internal infection proved difficult." (Pachter, 1982).

The current delivered to the denervated muscle may be monophasic (in which there is a net charge accumulation) or biphasic (in which the net charge per cycle is zero). Unfortunately, the type of current and rationale for choosing it over another current type is not often described in the literature. It is important to include this type of information for a number of reasons. Mortimer and Peckham (1973) emphasized differences in tissue

reaction produced by monophasic and biphasic current pulses. Histological examination showed a larger damaged area with monophasic current. In a more recent study he found that the increased tissue damage with monophasic current resulted not only from a higher local pH but also from an increased rate of tissue pH change (Mortimer, 1981). Eichorn (1984) emphasized that bidirectional current stimulation decreases the rate of fatigue to the alternating stimulation of varying parts of the muscle and to the charge balance which does not change the ionic density in the membrane area.

Temporal Aspects of Stimulation

The temporal relation of the nerve injury and its subsequent treatment merits recognition and discussion in any study on the effects of ES on denervated muscle. In the vast majority of experiments stimulation is initiated immediately after nerve injury. Only a few studies exist in which treatment was started after a period of delay. A rationale for the delay is not always given.

Delayed versus immediate stimulation

In the clinical study by Valencic (1986), stimulation of the anterior tibial muscle was begun 62 or more days after nerve injury. Positive remedial effects were reported in all patients despite the delay in initiation of treatment. The reason for delay was not given. One could surmise that the patients' post-traumatic condition did not permit immediate therapy with ES. This factor may need to be considered when planning treatment with ES in the clinical setting. Nix (1987) delayed treatment of denervated rabbit muscle for 2 days: no reason was specified. In Westgaard's rat experiment stimulation was postponed until the fifth day post sciatic nerve resection. He was able to show that delayed ES could restore the electrical properies of denervated muscle. In the primate study evaluating extrajunctional acetylcholine sensitivity (Gilliatt et al., 1978), stimulation of the denervated lumbrical muscles was started after a 21 day delay and reduction in sensitivity was reported.

A few studies have attempted to evaluate the effects of immediate versus delayed ES or continuous versus intermittent treatment. Fischer found that the best results in terms of decrease in atrophy were obtained when treatment was started immediately after denervation (Fischer, 1939). Direct stimulation of muscle from the time of denervation was found to prevent the formation of functional neuromuscular junctions and extrajunctional ACh sensitivity. When stimulation was delayed 2 days after denervation this extrajunctional sensitivity developed (Lomo & Slater, 1978). Direct stimulation of rat

muscle started 32 months after denervation was shown to be virtually as effective as self-reinnervation in the reduction of atrophy and in the maintenance of speed and strength of contraction (Hennig & Lomo, 1987).

Continuous versus intermittent stimulation

Can one assume that stimulation continued 24 hours per day will result in superior muscle preservation than stimulation administered for short periods several times a day for only part of the week? Intuitively, more stimulation would be better. However, Kosman (1947) showed that brief periods of ES (30 seconds) were as effective as longer periods (15 minutes) in the retardation of denervation atrophy in the rat. This remains an area of controversy and in an attempt to quantify the treatment given, some investigators (Gutmann & Guttman, 1942; Cole & Gardiner, 1984) have tabulated the number of muscle contractions produced in a 24 hour period. The reporting of the total number of contractions in a given period of time in all studies using ES would facilitate the interpretation of experimental results from a particular stimulation regimen.

Stimulating Parameters

In the literature there is considerable variation among the stimulating parameters used in studies on the effects of ES on denervated skeletal muscle. The principal stimulating parameters include current or voltage intensity, pulse duration, pulse frequency, time ON, time OFF, duration and frequency of intermittent treatments, and time the stimulation regimen is continued. Figure 6 clarifies the relationship of these stimulating parameters and presents the commonly used nomenclature. The stimulation parameters need also to be examined in light of the type of muscle contraction desired experimentally i.e. twitch versus tetanic and isotonic versus isometric.

Stimulus intensity

The voltage or current used by the investigator depends on the impedance of the system, the strength-duration relationship i.e. the pulse duration used, the strength of contraction desired for experimental purposes and the strength of contraction tolerated by the experimental subject.

Herbison (1983), in his study of ES applied to crush-denervated rat muscle, used a current of 4 mA and found a retardation of atrophy inferior to that found in Lomo's rat experiment (1978) where a 25 mA current was used. Gutmann and Guttman (1942) demonstrated that ES applied to the skin maintained the fiber size at the surface of the denervated muscle but the deeper fibers lost as much bulk as the unstimulated-control muscle. A degenerated and denervated muscle can only contract if its whole length is permeated by the electrical field between bipolar electrodes (Eichhorn et al., 1984). In the situation where stimulation of a bulky muscle or an entire muscle group is being attempted (increased electrical resistance) a higher stimulus intensity may be required for the current to reach deeper fibers.

Stimulation Parameters for ES

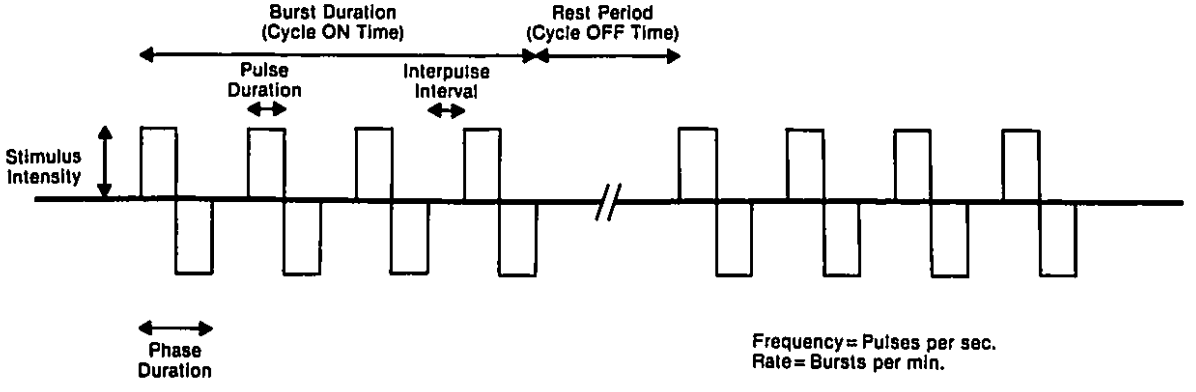


FIGURE 6

However this may result in muscle close to the electrode being exposed to excessively high current density and suffering damage, as Hennig's study emphasized (Hennig & Lomo, 1987).

As previously mentioned in this review, the current or voltage amplitude required to produce an adequate contraction in electrotherapy is also intimately related to the pulse duration. If the pulse duration is increased the stimulus intensity required to produce a contraction of equal force will be decreased. The studies presented in Table 3 demonstrate the range of pulse duration used to experimentally stimulate denervated muscle. The shortest duration listed here is 210 μs used in a project carried out by Dr. K. Nemoto in this laboratory involving the stimulation of denervated rabbit rectus femoris with implanted electrodes. The longest pulse duration listed in Table 3 is 100 ms.

Contraction produced

The strength of contraction is determined by the condition of the muscle, the stimulus intensity, pulse duration, and pulse frequency. What constitutes an ideal muscle contraction for the prevention of atrophy has not been clearly elucidated in the literature. Most researchers in the field strive to produce a contraction which is visible in the form of joint or extremity movement (isotonic contraction) and easily palpable. Herbison (1983), in response to other investigators' claims that ES impairs reinnervation (Lomo & Slater, 1978), adds that excessive muscle activity "may" cause local trauma to the newly forming neuro-muscular junction.

Few studies employ isometric conditions. Nix (1987) examined the effect of isometric ES on denervated rabbit extensor digitorum longus muscle. The hind paw was confined in a plaster cast. He subjected one group to low frequency stimulation (10 Hz) and the other to a tetanic frequency. The tetanic isometric group showed permanent contractures at 4 weeks which he attributed to microtrauma whereas the low frequency groups showed relative mechanical and histological integrity. It was the combination of tetany and rigid isometric confinement of the limb which produced these deleterious effects. In another study, short-term isometric stimulation was superior to isotonic therapy (both at 10 Hz) in retarding atrophy and decreasing twitch time (Nix, 1988). Goldspink (1978) originally attributed similar superior results to counteraction by immobilization of muscle in the stretched position. Wearmacher and associates (1945) also showed that ES was most effective when applied under conditions allowing maximal physiological stretch. This manoeuvre may slow down the breakdown of muscle proteins and enzymes.

Stimulus frequency

The stimulus frequency is one of the most crucial factors affecting the outcome of the stimulated denervated muscle. The foundations for understanding the influence of impulse frequency on muscle were laid by researchers who performed cross-innervation studies (Buller et al., 1960; Close, 1969). They established that neural influences largely determine the differentiation of mammalian skeletal muscle into fast or slow types. Using this basic concept researchers then applied electrical stimulation of various frequencies to normal muscle (Salmons & Vrobova; Salmons & Sreter, 1976; Pette, 1976, 1986). Using chronic nerve stimulation they showed that the pattern of nerve impulse activity is a major factor in regulating the muscle's characteristics. The expression "plasticity of muscle" was coined to refer to the capacity of skeletal muscle to undergo transformation from one muscle fiber type to another depending on the stimulus frequency applied. The above investigators showed that an almost complete transformation of fast- into slow-twitch muscle can be achieved in the rabbit by long-term indirect low frequency (10 Hz) stimulation. The transformation process is evident by structural, functional, and molecular changes that affect the three main functional systems of the muscle fiber, i.e. energy metabolism, sarcoplasmic reticulum and contractile apparatus. The basic mechanism appears to be a switch in gene expression (Pette, 1984). This phenomenon has been confirmed in other models including the chicken latissimus dorsi (Khaskiye et al., 1987) and in the cardiomyoplasty research employing latissimus dorsi muscles of sheep and goats (Chachques et al., 1988). In all these experiments innervated muscles were used. To distinguish between transformations in the muscle caused by changes in muscle activity per se or by changes in flow of trophic substances resulting from altered nerve activity, attention was then directed towards denervation-stimulation studies. Denervation precluded attributing fiber type transformation to neural influences.

Experiments examining the effects of stimulus frequency on denervated muscle have rather consistently shown the following: transformation of muscle fiber type occurs with heterologous stimulating frequencies and maintenance of fiber type occurs with homologous frequencies (Lomo et al.,1980; Hennig & Lomo, 1987). In simpler terms, fast muscle will become slow if treated with a low frequency impulse (Nix, 1982, 1986) and slow muscle will become fast if treated with a fast frequency. Slow and fast fibers retain their characteristics when exposed to stimulus frequencies which resemble their intrinsic motoneuron firing patterns (Hennig & Lomo, 1987). Denervated muscle will also become strong or weak, fatigue resistant or easily fatiguable depending on the particular pattern of stimulation (Lomo et al., 1980; Nix, 1988)

CHAPTER II

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Ten adult dogs weighing between 25 and 30 kg were utilized in this study. The animals were divided into two groups: a denervated unstimulated group, D (n=5), and a denervated stimulated group, DS (n=5). A third group, N (n=10), was comprised of all the contralateral normal limbs of the D and DS groups pooled together. These normal muscles also served as the intrinsic control (n=5) for both groups D and DS.

The acute experiment was performed 10 weeks following the chronic denervation procedure. This time period was selected because previous studies in this laboratory, including electromyography, showed a lack of significant reinnervation of the anterior crural muscles of the dog. Ten weeks was also, from earlier work, felt to be an adequate time period for detectable changes of denervation to take place.

An additional group of 4 animals was studied histologically and histochemically following a 16 week denervation period without stimulation. This group underwent the same chronic procedure as the other groups. The results obtained were compared to those of the 10 week denervation group.

CHRONIC PROCEDURE

Preparation

The animals were premedicated using a mixture of meperidine HCl 80 mg/cc and acetylpromazine maleate 4 mg/cc (1 cc/25 kg) intramuscular one hour preoperatively. They were then anaesthetized with intravenous sodium pentobarbital 30 mg/kg and intubated. Mechanical ventilation was not required. Anaesthesia was maintained during the procedure with periodic infusion of sodium pentobarbital. Cefazolin sodium 500 mg IV was given prophylactically 30 minutes prior to surgery.

The animal was placed in the lateral recumbency position with its right hind limb exposed. The shaved limb was aseptically prepared with poviodine and draped.

The operative procedure consisted of two steps: the first, implantation of the stimulating devices and the second, nerve transection and repair. Implantation was performed first to minimize operative time involving the foreign material. Although denervation was performed only on the right side implantation of electrodes was done bilaterally to permit study of both sides in a comparable manner. The stimulating devices consisted of a Medtronic Model 7420 Bipolar ITREL Implantable Pulse Generator (IPG) and two electrodes (Medtronic, Inc., Minneapolis) (Figure 7). A more detailed description of these materials will be presented in the section entitled Electrical Stimulation.

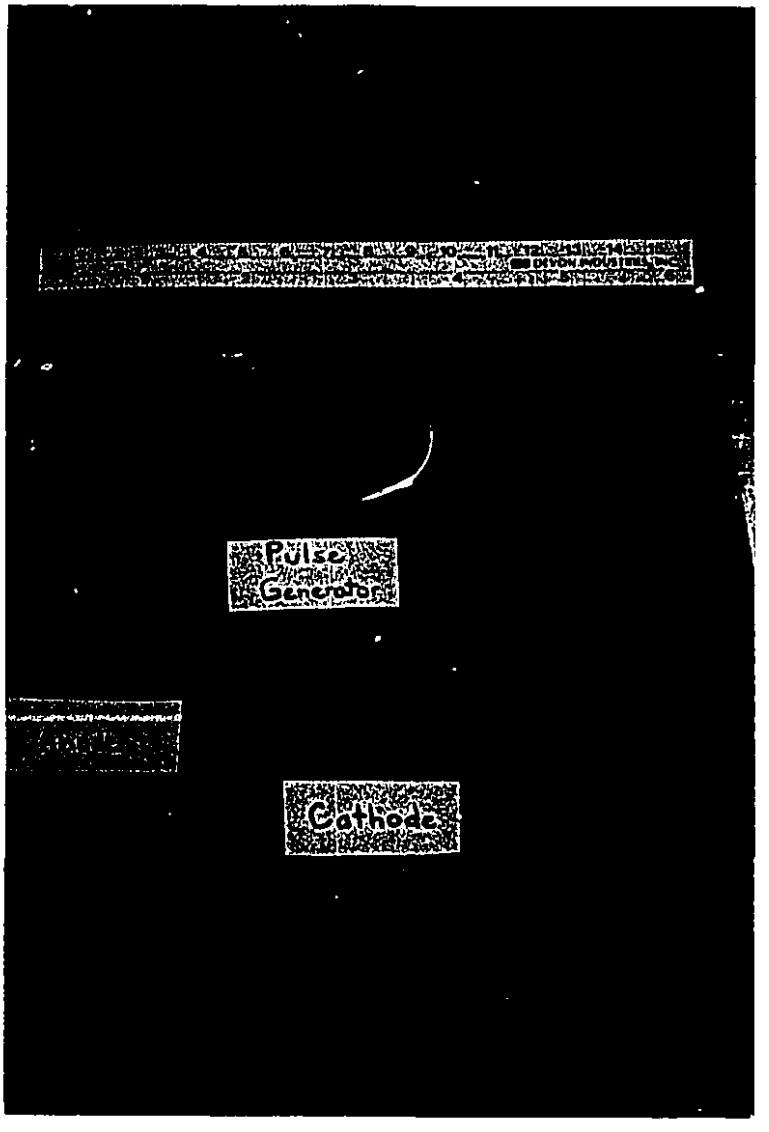


FIGURE 7. Implantable Stimulating Devices.

Implantation of Stimulating Devices

The operative plan was first drawn on the skin indicating the positions of the incisions, implantations, and the course of the sciatic and common peroneal nerves (Figure 8).

FIGURE 8. Preoperative markings and identification of structures. 8 cm (indicates distance from fibular head X where the sciatic nerve is transected and the common peroneal nerve repaired); PL (M. peroneus longus); TC (M. tibialis cranialis).

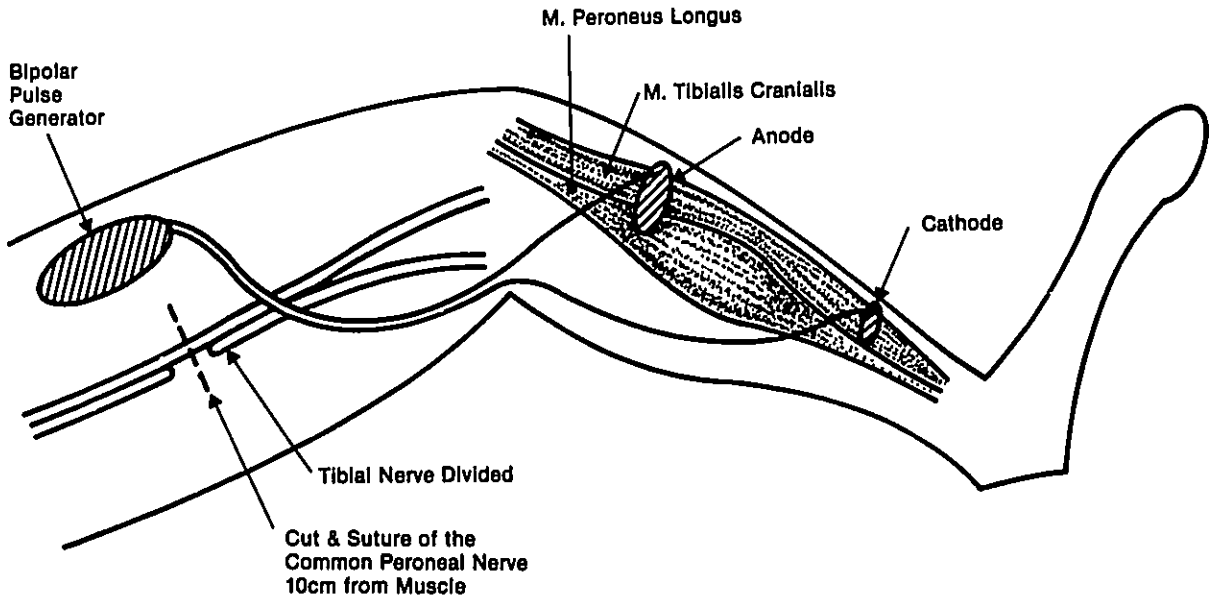


FIGURE 9. Schematic diagram indicating anatomic structures and operative plan.

Two 1.5 cm longitudinal skin incisions were made proximally and distally over the tibialis cranialis muscle. The incisions were deepened through the fascial layers, including the epimysium, to expose the muscle proper. A hemostat was gently inserted anteriorly and laterally to develop a pocket for the electrode. Next, incisions were made in the mid - anteromedial thigh for implantation of the IPG in the DS group or as a repository for the lead ends in the D and N groups. A pocket was created with blunt dissection.

The leads were tunnelled subcutaneously from their respective implantation sites on the muscle up to the thigh pocket using a hemostat passed down from the thigh incision. The electrodes were inserted into their pocket - the anode proximally and the cathode distally - and the incisions were closed in layers. In all groups, after implantation of the electrodes correct placement and functioning was ensured by connecting the leads to an IPG and observing muscle contraction. In the DS group the IPG was then left in situ and in the D and N groups it was removed following testing.

Nerve Division and Repair (Figures 10, 11, & 12)

An incision in the mid-lateral thigh over the course of the sciatic nerve was made. This incision was deepened by muscle splitting between the rectus femoris and semitendinosus muscles. Once exposed the sciatic nerve was sharply transected 8 cm proximal to the fibular head. Since the common peroneal nerve contacts the anterior crural muscles approximately 2 cm distal to the fibular head the total distance of nerve injury was considered to be 10 cm.

Two cm segments of the tibial branch and of the cutaneous nerve were resected to eliminate action of the antagonistic muscles and to prevent neurotization of the denervated tibialis cranialis muscle. The common peroneal branch was repaired microsurgically using an epineurial repair with 9-0 Ethilon suture (Ethicon, Canada). The incision was closed.

FIGURE 10. Sciatic nerve with common peroneal (forcep), tibial, and cutaneous branches separated.

FIGURE 11. Common peroneal nerve divided;
tibial nerve divided and ligated (suture).

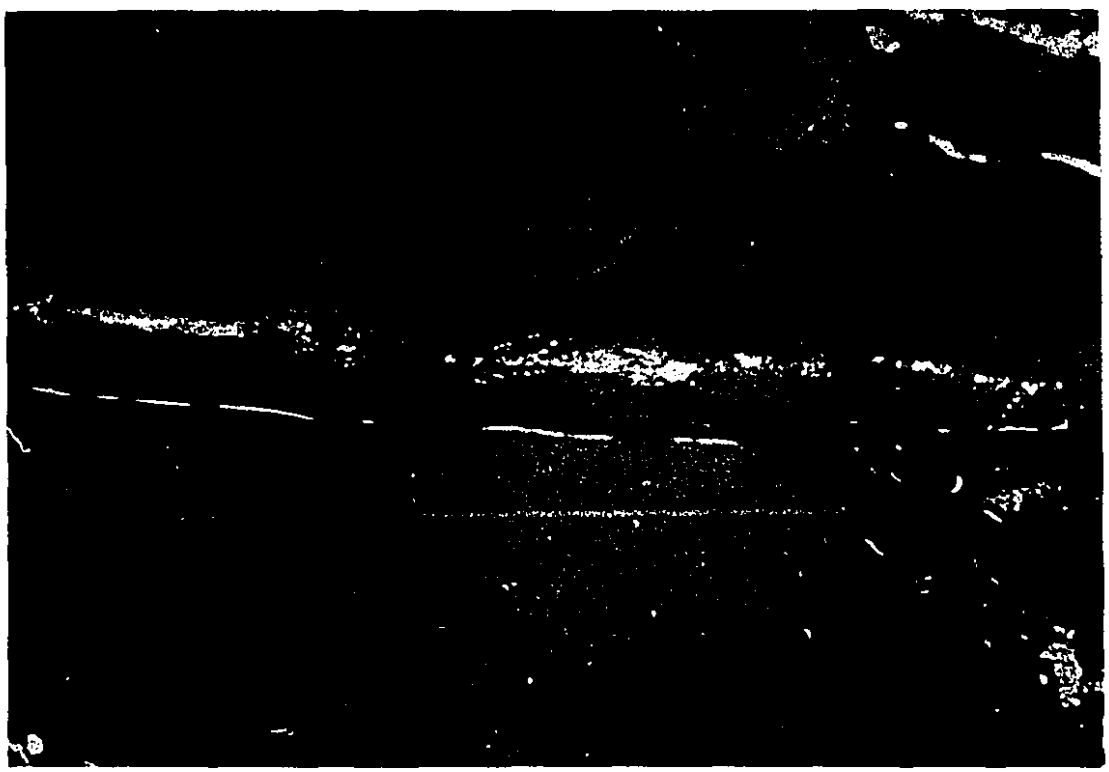


FIGURE 12. Common peroneal nerve repaired microsurgically.

Post-Operative Care

To avoid trauma to the denervated hind paw each animal was fitted with a Hexcelite NS splint. (This is a semi-rigid material produced by Hexcel Medical, U.S.A., which becomes malleable when immersed in hot water. It is commonly used by occupational therapists.) The splint maintained the paw in the normal weight-bearing position and helped to prevent "knuckling" (foot-drop) with its associated joint and soft tissue trauma. An Elizabethan collar was also applied to discourage removal of sutures and splints by the animal.

ELECTRICAL STIMULATION

Stimulating Devices

The stimulating electrodes were prepared for this experiment from flexible wire leads insulated with silicone tubing introduced at one end into a flexible carbon-silicone electrode. The electrode was insulated on its superior surface with silicone. The cathode (negative electrode) was made to measure 2.5 cm in length and the anode 4.0 cm in length. The proximal end of the lead terminated in a metallic hub for insertion into and contact with the IPG.

The ITREL IPG is a constant voltage stimulator providing a biphasic current. It is programmable through the skin using the Medtronic Model 7431 ITREL Portable Programmer (Figure 13) and has the parameter capabilities shown in Table 4.

Table 4 ITREL® IPGs
Electrical and Operating Characteristics

Programmable Parameters	Description*
Pulse Amplitude (Peak Voltage)	0 to 10.5 volts in 0.25 volt increments.
Rate	2, 5, 10, 15, 20, 25, 30, 33, 36, 43, 50, 65, 85, 130 Hz.
Pulse Width	60, 90, 120, 150, 180, 210, 270, 330, 400, 450 μsec.
Cycle ON Time	0.1, 1.5, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 50, 64 sec.
Cycle ON Time with SoftStart™ Ramp	8, 10, 15, 20, 25, 30, 35, 40, 50, 64 sec. Digital ramp provides approximately 1 volt per second increase from zero to the selected output pulse amplitude.
Cycle OFF Time	0.4, 1, 2, 4, 6, 10, 24, 40, sec. or 1, 2, 4, 8, 10, 12, 15, 17 min.

*All values are nominal.

FIGURE 13. ITREL Portable Programmer.

Stimulation Protocol

Electrical stimulation was started immediately following surgery in the DS group. Initial stimulating parameters (Table 5) were selected on the basis of production of a visible and palpable tetanic contraction of the tibialis cranialis muscle resulting in dorsiflexion of the paw. Within 72 hours of severing the common peroneal nerve Wallerian degeneration was complete and the muscle was no longer able to contract with the initial parameters. It was then necessary to reprogram the IPG. The highest voltage - 10.5 v - and the longest pulse duration - 450 μs - obtainable from the IPG were required to produce a palpable contraction in this now completely denervated muscle. Fluid collection in the early post-operative period and scar formation later between the electrode and the muscle may have also caused increased impedance necessitating higher parameters. The frequency was also increased because this was noted on visual inspection and palpation to produce a stronger contraction. The stimulation was continued 24 hours per day for 10 weeks producing 3388 tetanic contractions daily.

TABLE 5 - Stimulating Parameters

	Inital	**72 hrs post-op**
Voltage	4.5 v	10.5 v
Pulse width	210 μs	450 μs
Frequency	36 Hz	85 Hz
Time on/off	1.5 s/24 s	1.5 s/24 s

ACUTE EXPERIMENT

The acute experiment was performed 10 weeks post-operatively. The animal was anaesthetized in the manner described for the chronic procedure. Reinnervation was examined using electromyography (EMG) and nerve histology. The muscle was studied bilaterally using the following methods: strength-duration testing, assessment of muscle/electrode resistance, force-frequency testing, evaluation of contractile properties, measurement of wet muscle weight and electrode scar thickness, histology, histochemistry, and electron microscopy.

Assessment of Reinnervation

Electromyography

This test was performed with percutaneous insertion of a stainless steel needle electrode into the tibial cranial muscle between the two implanted electrodes and percutaneous needle stimulation of the common peroneal nerve at the fibular head. Supramaximal voltage was applied to the nerve and muscle action potentials (MAP) were recorded.

Serial Nerve Sections

A 9 cm length of common peroneal nerve from a denervated and unstimulated limb was dissected out and fixed in glutaraldehyde. Sections 2 mm thick were then cut every centimeter from the normal nerve proximal to the repair site to 8 cm distal to the repair site. These nerve sections were stained with osmium tetroxide to define the myelin sheaths. This examination provided qualitative information regarding the nerve regeneration.

Functional Assessment of Muscle

Strength-Duration Testing

This technique was used to test the hypothesis that stimulated denervated muscle has a strength-duration curve that more closely resembles that of normal muscle than does unstimulated denervated muscle.

The proximal ends of the implanted leads were exposed in the thigh and connected to the Neuroton 626 stimulator (Siemens, West Germany). This apparatus allows one to elicit a muscle contraction while manipulating the pulse duration and current (Figure 14).

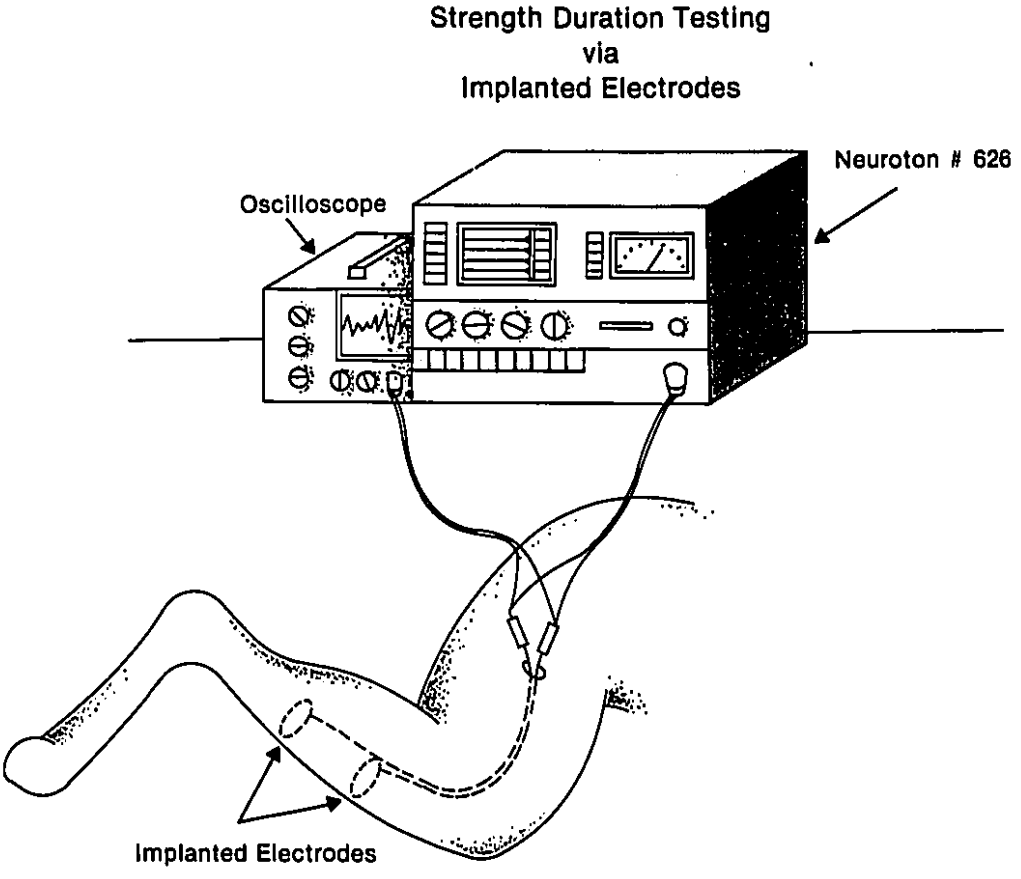


FIGURE 14

With the PD set at 200 msec the current was increased until a weak dorsiflexion of the paw was produced through direct stimulation of the tibialis cranialis muscle. Having obtained these calibration parameters the animal was then prepared for isometric strength-duration testing, measurement of speed-related contractile properties, and muscle force-frequency testing.

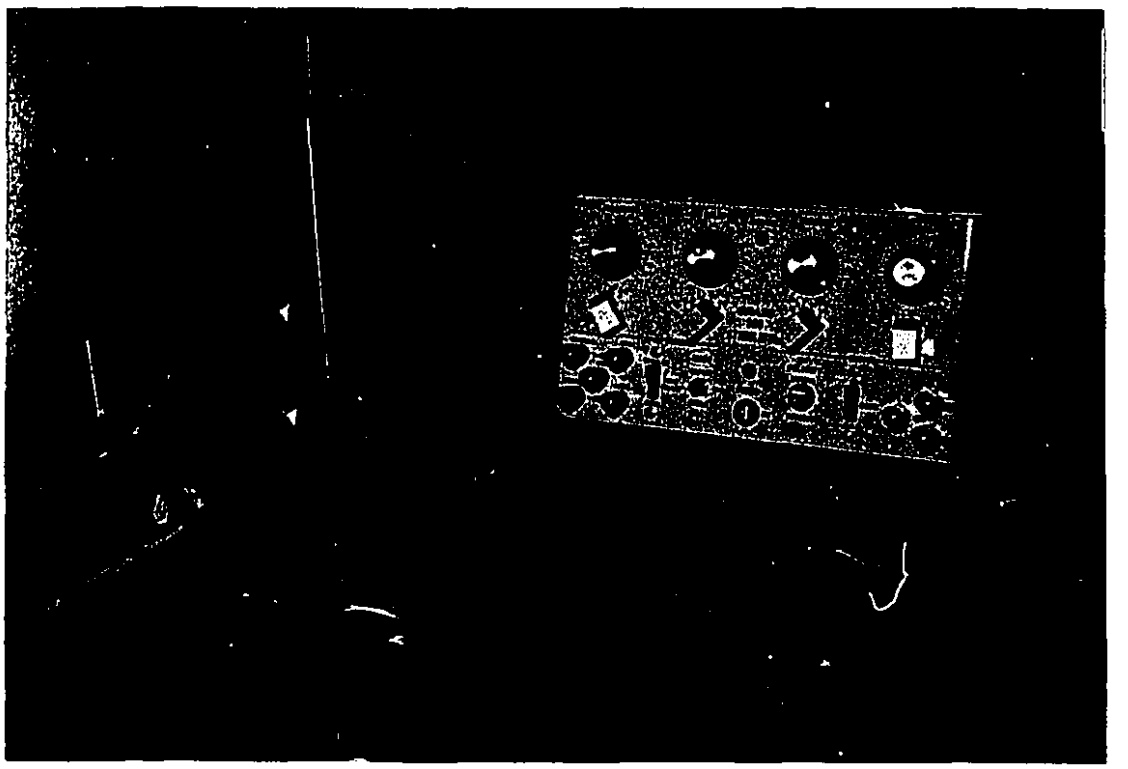


FIGURE 15. Strain-gauge apparatus used in functional testing of denervated muscle.

An anterior longitudinal incision was made over the distal part of the tibialis cranialis muscle and its tendon. The tendon was exposed and dissected free. The knee joint was fixed by pinning The protruding pin was rigidly fixed to the operating table with a clamp and bar. The paw was also fixed to a bar. The tendon ends were severed and secured onto one end of a small lightweight turnbuckle (Figure 15). The other end of the turnbuckle was attached to the force-displacement transducer (Grass FT 03C).

Electrode/Muscle Resistance

The muscles now attached to the strain gauge apparatus were again stimulated using the Neuroton and the calibration current and pulse duration. The force produced was measured on the polygraph (Grass model 7B). This calibration force provided the standard for each subsequent contraction during the strength-duration test, i.e. current was adjusted to produce this force at each PD. Current readings were taken at PD settings of 200, 100, 50, 21, 12, 6, 2, 0.5, 0.45, and 0.2 msec. The corresponding voltages were read from an oscilloscope (Figure 14). An alternate method of deriving voltages corresponding to the current from the Neuroton involved placing a 10 ohm resistor in series with the electrical circuit (Figure 16). Using Ohm's Law, R=V/I, the voltage V_1 was calculated. With information on both current and voltage it was then possible to calculate the resistance of the electrode/muscle configuration. This was defined as the total resistance R_T and was comprised of the electrode/tissue resistance R_L and the resistance of the surrounding scar and interposed muscle R_S.

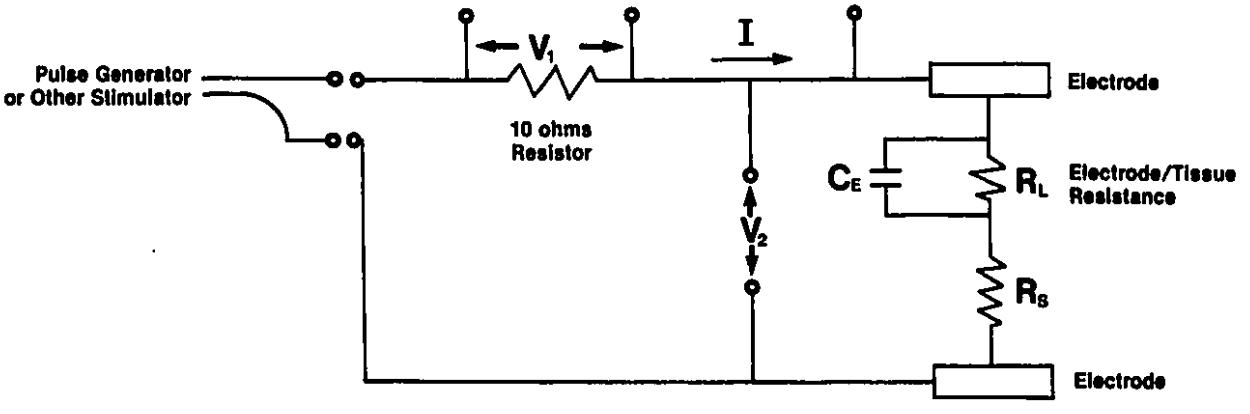


FIGURE 16. Bioelectric circuit for study of the electrode/muscle configuration.

Assessment of Contractile Properties

The contractile properties examined in this study included twitch contraction time and one-half relaxation time. These are speed-related properties which show a slowing with denervation. Force-frequency testing was also performed. This test evaluates the relationship between the frequency of electrical impulses applied to the muscle and the force the muscle is able to generate at each frequency. In normal muscle there is a relationship of increasing force with increasing frequency until maximal force is reached, after which an increase in frequency results in lower force output (Kosman, 1947). From this examination the maximal force produced at a tetanic frequency is obtained. A muscle fatigue test was also performed.

Technique

Following strength-duration testing, with the tendon still attached to the strain-gauge apparatus, the above tests were performed. The externalized lead ends were connected to the Grass stimulator SD9. The tests were done initially with the pulse duration set at 0.2 ms followed by a retest at 2 ms. The impulse used was biphasic and the voltage twice the contraction threshold (2T).

Threshold and Optimization of Load

With the load on the muscle set at approximately 100 gm and the frequency set at 1 Hz the applied voltage was gradually increased until the contraction threshold voltage (T) was reached. Threshold voltages were found for pulse durations of 0.2 ms and 2 ms. Threshold was indicated by the first twitch seen on the polygraph. Next, using the turnbuckle, the load was increased by increments of 100 gm. One Hz twitches were measured at each load and the load corresponding to the strongest twitch was found. All further testing was done using this optimal load.

Speed-Related Contractile Properties

For the analysis of speed-related contractile properties twitches were produced using 2T, 1 Hz, and 0.2 ms and recorded with a polygraph paper speed of 100 mm/s. Twitch time was measured from the time of onset of the twitch to the time at which the force was maximal. One-half relaxation time was measured from the twitch peak to its return to the baseline.

Force-Frequency Testing

Force-frequency testing was accomplished by stimulating the muscle directly through the implanted electrodes using the following frequency regimen: 0.5, 1, 5, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, and 50 Hz. The test was done with a pulse duration of 0.2 ms and the corresponding 2T followed by a retest using 2 ms and the corresponding 2T. Polygraph paper speed was 10 mm/s. Fusion frequency was defined as the tetanic frequency at which twitches were no longer visible. Maximal tetanic frequency corresponded to the frequency at which the greatest force was produced during tetany.

Fatigue Testing

The fatiguability of the tibialis cranialis muscle was assessed by subjecting it to a 10 minute period of continuous activity using a 1 Hz impulse of 2T with a pulse duration of 2 ms. Initial force and force after 10 minutes of stimulation were measured.

Structural Assessment

Wet Muscle Weight

The tibialis cranialis muscles were dissected out en bloc and weighed after the electrodes and surrounding connective tissue were removed.

Measurement of Electrode Scar

The connective tissue encapsulating the electrode (and separating the electrode from the muscle) was removed, stained with Mallory trichrome, and measured for thickness.

Technique of Muscle Biopsy

Following strength-duration testing and assessment of contractile properies, biopsies of muscle in the normal, denervated, and denervated-stimulated groups were obtained for histology, histochemistry, and electron microscopy.

It was necessary to define precisely the location of the biopsy because within a given muscle the fiber composition may change from area to area. An assumption was made that within the same species the fiber composition would be consistent in a precisely defined part of the muscle. The speciments were taken from the superficial position of the muscle at the junction between the proximal and middle thirds, where the muscle had its greatest cross-sectional thickness. This corresponded to the area midway between the two electrodes. Prior to taking the biopsies the epimysium was carefully stripped away.

Histology

Cross-sectional biopsies were preserved in formalin and later stained with hematoxylin-eosin (H&E) and Masson trichrome stains. The H&E stain was used to define the muscle fiber morphology (fiber shape, size, density of fibers, position and number of nuclei, etc.). The trichrome, with its pale blue staining of connective tissue, was intended to highlight any differences in interfascicular fibrosis between the three experimental groups.

Histochemistry

This technique was used to differentiate type 1 from type 2 muscle fibers and thereby permit a fiber composition analysis and inter-group comparison. Also, morphometric analysis of the area of type 2 fibers was facilitated using ATPase staining.

ATPase staining was performed according to the method by Dubrovitz and Brooke (1973) on cross-sections of tibialis cranialis muscle. The specimens, were snap frozen in isopentane cooled to -180° C by immersion in liquid nitrogen. They were stored at -80° C until processing. Ten micron thick sections were stained with ATPase at pH 4.3 and 10.4.

Electron Microscopy

Examination of the muscle ultrastructure was performed using electron microscopy (EM). Longitudinal sections 4 mm long and 2 mm thick were fixed in 4% formalin-1% glutaraldehyde in phosphate buffer, postfixed in osmium tetroxide, dehydrated in graded alcohols and embedded in Epon 812 (JBS-CHEM, Quebec, Canada). One micron thick sections were stained with Toluidine blue and examined by light microscopy. Sections were cut with a Reichert ultramicrotome (Austria), stained with uranyl acetate and lead citrate and examined with a Philips 201 electron microscope (Philips Electronic Instruments, Mount Vernon, New York).

Morphometric Analysis and Fiber Typing

This evaluation was performed by the same technician for all specimens to ensure consistency in the technique. Muscle specimens stained with ATPase (pH 10.4 or 4.3) were studied. Each slide was examined under a Leitz Dialux 20 microscope (magnification factor X25), mounted with a Hitachi CCTV camera (X30). The magnified image was presented on a video screen and all assessments were performed on this image. Data was processed by a Videoplan computer system (Carl Zeiss Inc., West Germany) using the morphometry software package.

Three random representative fields were selected from each slide and all type 1 and type 2 fibers in these fields were measured using a perimeter sketching technique employing the video pen and magnetized tablet. The computer program tabulated the mean fiber areas with corresponding standard deviations. Type 1 and type 2 fiber counts for each specimen were also given and with this data the proportion of type 1 to type 2 fibers was obtained.

DATA ANALYSIS

Data were analysed using the Macintosh computer and the software program MYSTAT. The paired Student's t-test was performed on the data from the denervated and denervated-stimulated groups and their respective intrinsic controls (normal muscle). Unpaired t-tests were performed to assess differences between the denervated, denervated-stimulated, and pooled normal groups. All results are expressed as the mean ± the standard deviation. The limit set for significance was $p<0.05$.

CHAPTER III

RESULTS

COMPLICATIONS

All experimental animals were assessed for complications related to the implanted devices (Table 6). There was no incidence of persistent inflammation (erythema, edema, seroma), infection of the implants, or dehiscence of the suture line with extrusion of the implants. (The two infections indicated were attributed to the animals biting out sutures and disrupting the incision). There were however three mechanical failures including two broken electrodes and one depleted battery. The break occurred at the insertion of the lead into the silicone-carbon electrode. These electrodes were removed through small incisions over the original scars after being detached from the IPG in the thigh.. New electrodes were inserted and the animals had no further problems. The depleted battery (which was recycled from previous experiments) was also uneventfully replaced.

TABLE 6 - Complications of Implanted Devices

Complications	Incidence (of 10 animals)	Comments
Persistent inflammatory reaction	0	Post-operative edema resolved spontaneously within several weeks .
Infection at implant sites	? 2	Both of these infections resulted from animals pulling out their sutures. No other infections were seen.
Extrusion of implants	0	
Electrode breakage	2	In both cases the broken cathode was removed and replaced without problem.
Battery depletion	1	This reused battery was replaced without problem.

ASSESSMENT OF REINNERVATION

Electromyography

In both the denervated and denervated-stimulated groups muscle action potentials were obtainable in only two of five animals. The remaining animals failed to respond. The recordings were compatible with very early reinnervation of the muscle in amplitude (Table 7), latency, and shape. With the exception of one animal in the stimulated group with a substantially higher amplitude of 3 mv and shorter latency of 12.1 ms, the other animals showed very low amplitudes (measured in μv) and more prolonged latency periods. The potentials were typically widely spread and polyphasic.

These EMG results indicate that reinnervation of neuromuscular units proceeds at a variable rate from animal to animal. This may be attributed to technical variations in nerve repair and to the individual rate of repair of each animal. At 10 weeks post nerve repair the majority of tibialis cranialis muscles can still be considered "completely denervated" on the basis of EMG. In some cases signs of early reinnervation are present.

TABLE 7 - Muscle Action Potentials

MAP (μv) Denervated n=5	MAP (μv) Denerv-Stim n=5	MAP (mv) Normal n=5 *
nil	125	11
510	nil	21
300	3000	11
nil	nil	9
nil	nil	16

**randomly selected from the group of 10*

Muscle action potentials (MAP) of normal denervated, and denervated-stimulated tibialis cranialis muscle 10 weeks following proximal nerve division and repair.

Serial Nerve Sections

Sections of the common peroneal nerve were taken at 1 cm intervals (Figures 17a-c) from the normal nerve proximal to the repair site to 8 cm distal to the repair. In these sections there is evidence of axonal regeneration and remyelination even in the most distal section. The axons are smaller and reduced in number when compared qualitatively to the normal. This exercise provides evidence of nerve regeneration and hence, early reinnervation in an animal which failed to produce a muscle action potential during electromyography.

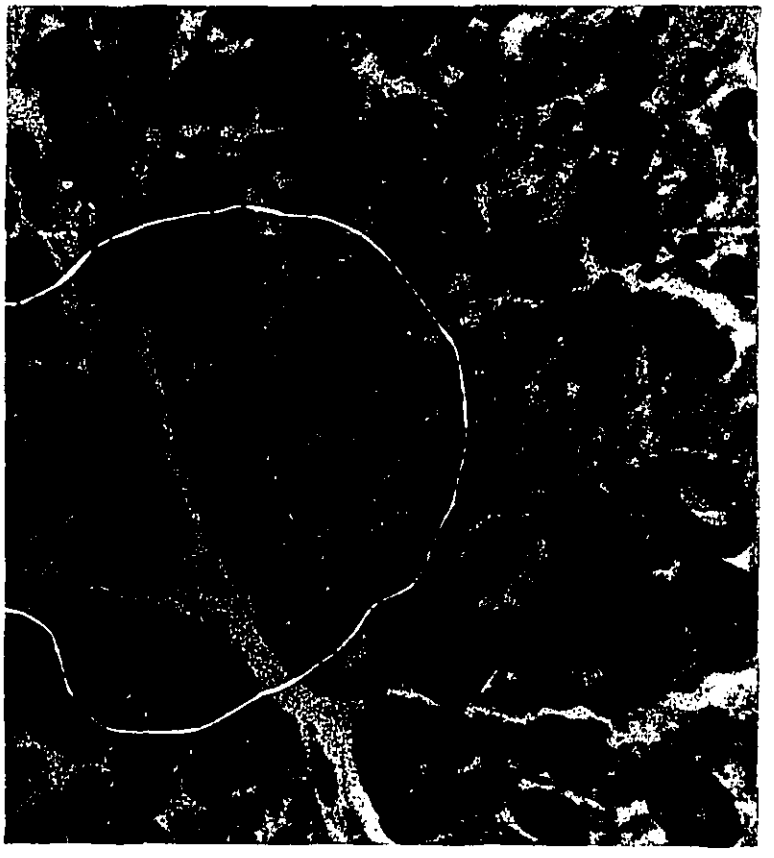


FIGURE 17a. Osmium tetroxide staining of normal common peroneal nerve proximal to the site of division and repair.

FIGURE 17b. Common peroneal nerve 1 cm distal to repair site (9 cm from muscle), 10 weeks following repair.

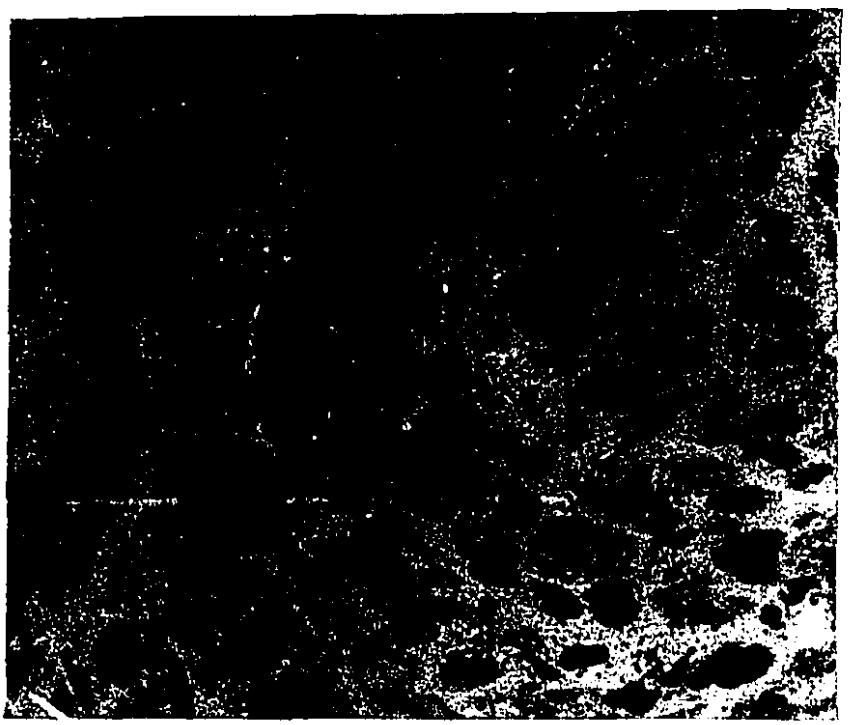


FIGURE 17c. Common peroneal nerve 7 cm distal to repair site (3 cm from muscle), 10 weeks following repair.
Note the poor axonal formation and lack of myelination.

FUNCTIONAL ASSESSMENT OF MUSCLE

Strength-Duration Testing

Representative strength-duration curves from the normal, denervated, and denervated-stimulated groups are shown in Figure 18. The curves indicate that both of the operated groups differ significantly from the normal in their pulse duration/stimulus intensity relationship. Both groups required higher current settings to produce a muscle contraction at each given PD setting. These curves are thus "shifted to the right" when compared to the normal. Both the operated groups were significantly different from their intrinsic controls in threshold current and in chronaxy when analysed with Student's paired t-test (Table 8). However, no differences were found between denervated and denervated-stimulated thresholds and chronaxie when the unpaired t-test was applied.

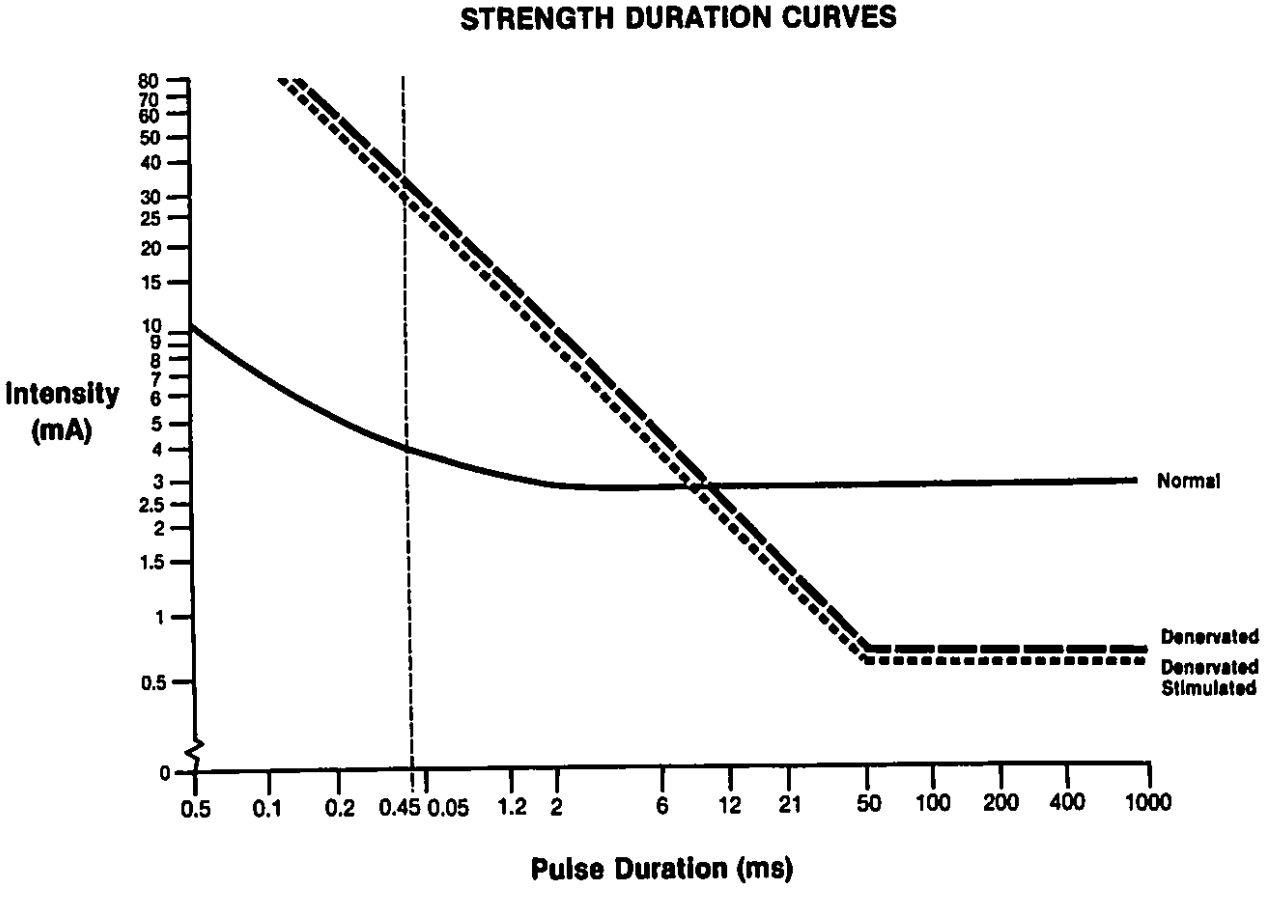


FIGURE 18

Contractile Properties (Tables 8 & 9)

Twitch Contraction Time

The tibialis cranialis muscle was assessed for twitch contraction time and one-half relaxation time. The contraction time of the denervated muscle was found to be significantly slower when compared to its normal control *($p<0.017$)* (Figure 19) and when compared to the pooled normal group using the unpaired t-test *($p<0.004$)*. No differences were seen between the denervated-stimulated group and its intrinsic control (Figure 19), nor between the denervated and denervated-stimulated groups with independent t-test analysis (Table 9).

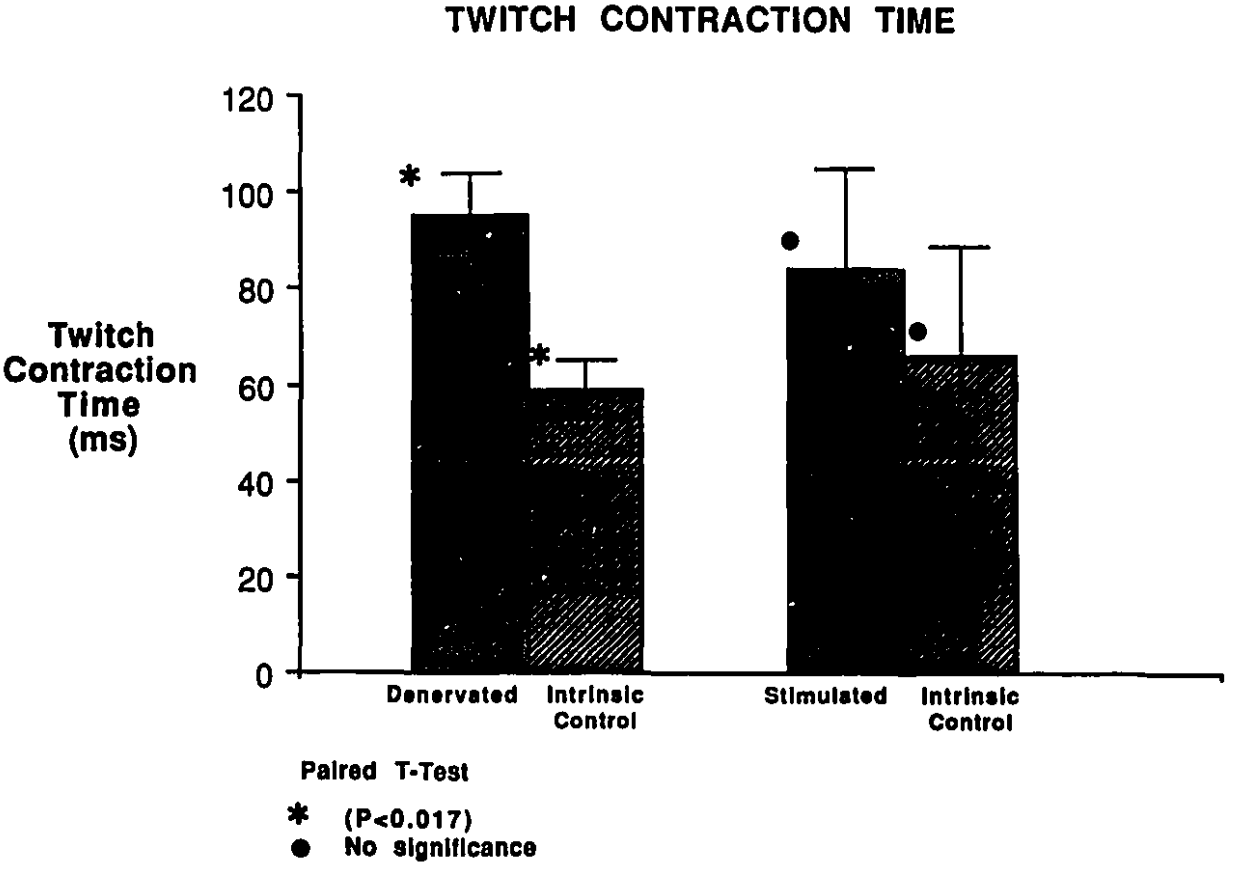


FIGURE 19

Table 8 - Functional Parameters: Paired T-Tests

PARAMETER	Denervated	Normal	Denerv-Stim	Normal
	n=5		n=5	
Threshold (mAmp)	1.07 ± 0.66	4.28 ± 1.78	0.89 ± 0.34	2.57 ± 1.16
	p<0.008		p<0.001	
	n=5		n=5	
Chronaxy (ms)	32 ± 22	0.2 ± 0.1	30 ± 12	0.2 ± 0.1
	p<0.03		p<0.001	
	n=5		n=5	
Twitch T (ms)	95 ± 9	59 ± 6	84 ± 21	66 ± 23
	p<0.02		Difference not significant	
	n=5		n=5	
Half Relax T (ms)	240 ± 87	101 ± 34	255 ± 34	144 ± 48
	p<0.03		Difference not significant	

TABLE 9 - Twitch Contraction Time

Group	N	Twitch time (ms)
Normal (pooled)	10	63 ± 16*
Denervated	5	95 ± 9*
Denerv-Stim	5	84 ± 21

*Unpaired t-test: *p<0.004*

One-Half Relaxation Time

When analyzed with paired t-tests, the mean one-half relaxation time of the denervated muscle (240 ms) was considerably prolonged when compared to its normal control (101 ms) *($p<0.03$)*. The mean relaxation time of the stimulated muscle (255 ms) was not significantly prolonged when compared to its intrinsic normal group (144 ms) *($p=0.05$)* (Table 8). However, when independent t-test analysis was performed using the pooled N group, significant differences were found between N and both experimental groups *($p<0.04$)*. Difference in the one-half relaxation time was not seen between the denervated and denervated-stimulated groups when the independent t-test was applied *($p=0.1$)* (Figure 20).

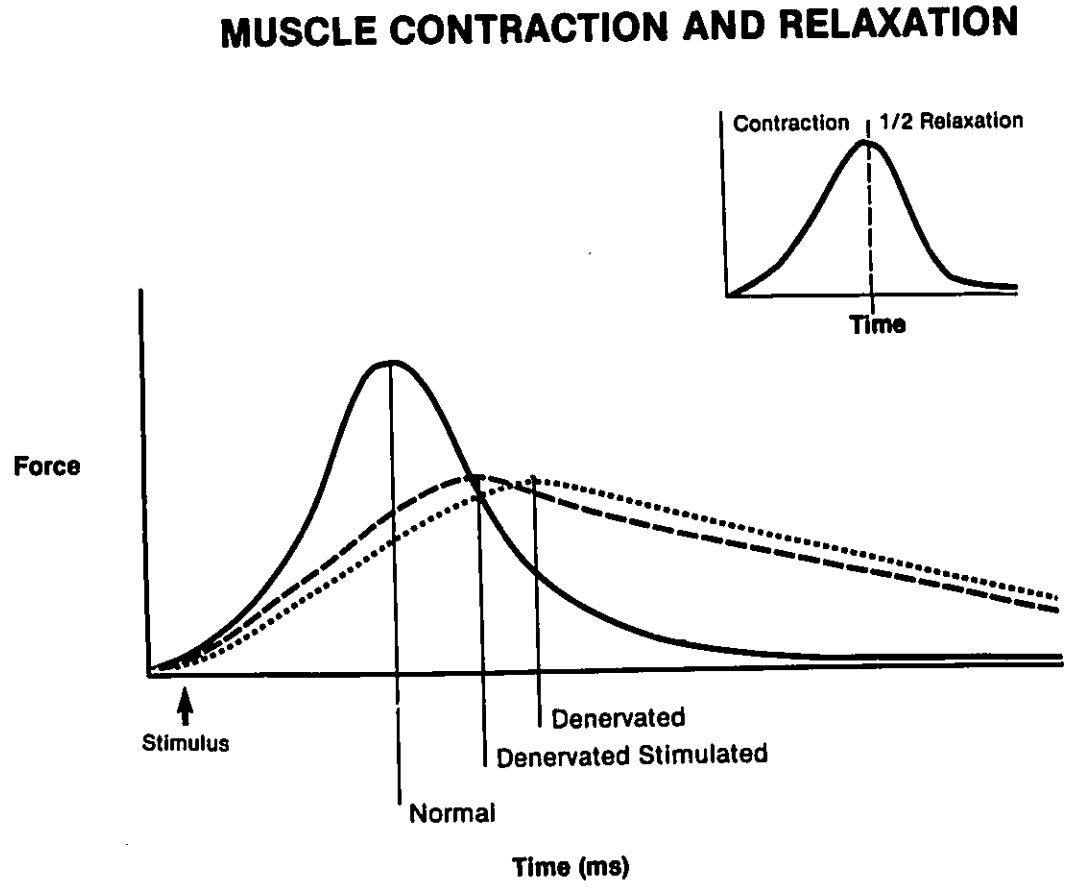


FIGURE 20

Maximum Force Frequency and Tetanizing Frequency

The mean frequencies at which maximum force and tetanus occurred were calculated for the denervated, denervated-stimulated, and normal control groups. No differences were found between the denervated and denervated-stimulated values when analyzed using independent t-tests. The only significant differences found were between the tetanizing frequencies of both operated groups and their intrinsic controls (Table 10).

TABLE 10 - Frequency Testing

	Denervated	Normal	Denerv-Stim	Normal
N	5	5	5	5
FREQUENCY (Hz) (maximum force)	19 ± 5	26 ± 9	25 ± 9	29 ± 14
FREQUENCY (Hz) (tetanizing)	7 ± 2	10 ± 2	5 ± 0	9 ± 0
		*p<0.03		*p<0.006

**Significant results obtained with paired t-test.*

Results of paired t-test analysis of the mean frequencies at which maximum force and tetanus were obtained.

Maximum Tetanic Force

The traditional method of assessing muscle force using the strain gauge apparatus and indirect stimulation of the muscle via the regenerating nerve was not possible in this experiment since at 10 weeks post nerve repair the nerve had not regenerated sufficiently to conduct the stimulus. The direct stimulation method via the implanted electrodes was attempted but did not yield useable data. For this method to succeed a *supramaximal stimulus* must be applied so that the amount of current or voltage and the degree of recruitment of muscle fibers is not a confounding factor in assessing force production. The denervated and denervated-stimulated muscle had high thresholds for contraction which prevented the application of a supramaximal stimulus. The maximum voltage available from the Grass stimulator was 100 v. In many cases, the supramaximal voltage far exceeded 100 v. Furthermore, it was feared that voltages in this range would damage the tissue and invalidate histological examination.

Muscle/Electrode Resistance

The resistances of the muscle/electrode configuration at pulse durations from 0.2 ms to 6 ms were obtained using the apparatus described under Methods. A regression of Resistance on Natural Log (LN) Pulse Duration was performed separately for each animal and the fitted slope of this regression was analyzed statistically. Mean slopes were obtained for each experimental group and compared using independent t-tests (Figure 21). Results indicate that the increase in Resistance with increase in LN Pulse Duration is significantly greater in the denervated group than in the pooled normal group *($p<0.04$)*. Neither the differences between the stimulated and normal groups *($p=0.7$)*, nor between the denervated and stimulated groups *($p<0.06$)*, were statistically significant (Table 11).

Summary of Functional Results

The twitch contraction time and electrical resistance property in the stimulated muscle remained within normal limits when compared to the contralateral normal muscle whereas the unstimulated group differed statistically from its normal control in these parameters. Statistical analysis failed to show significant differences between the stimulated and unstimulated groups in the functional tests including strength duration, threshold and chronaxy, twitch contraction and relaxation time, and maximal and tetanizing frequency.

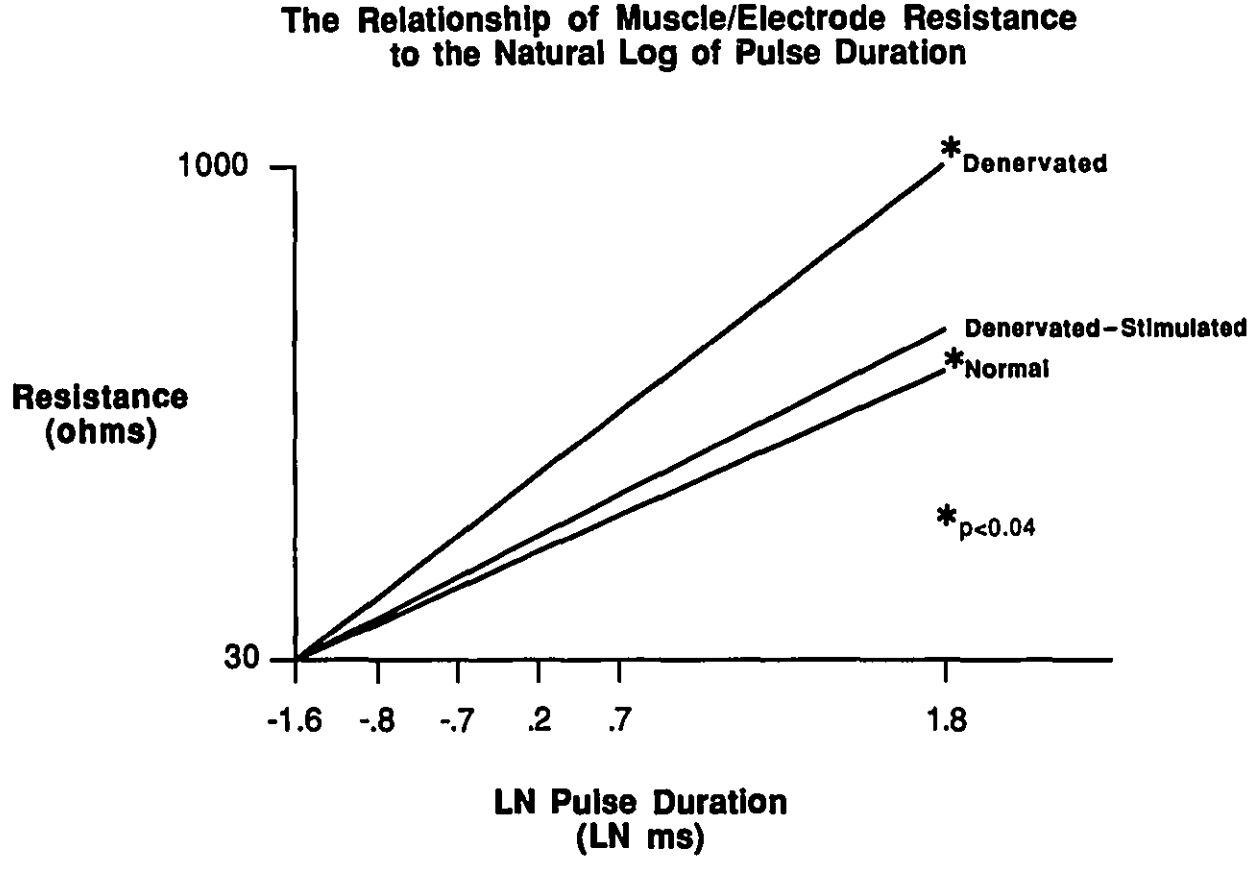

The Relationship of Muscle/Electrode Resistance
to the Natural Log of Pulse Duration
1000
30
Resistance
(ohms)
*Denervated
Denervated–Stimulated
*Normal
*p<0.04
-1.6
-.8
-.7
.2
.7
1.8
LN Pulse Duration
(LN ms)

FIGURE 21

TABLE 11 - Slope of Resistance Versus LN Pulse Duration

GROUP	N	SLOPE (ohms/LN ms)
Normal	9 †	5.6 ± 1.5 *
Denervated	5	7.8 ± 2.3 *
Denerv-Stim	5	5.8 ± 0.5

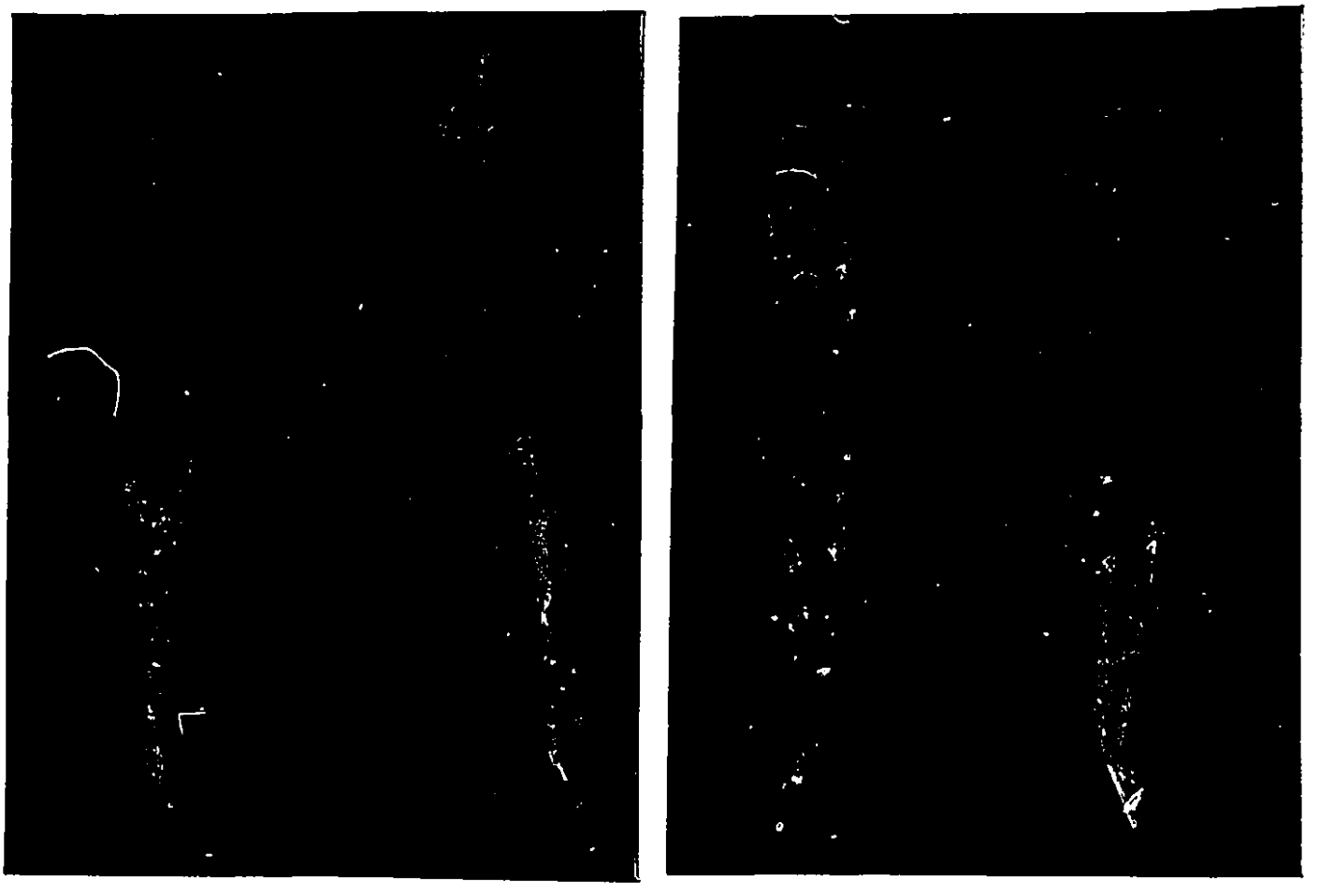
†Group is incomplete due to technical difficulty.

*Independent t-test: * $p<0.04$*

STRUCTURAL ASSESSMENT

Wet Muscle Weight

Muscle weights were expressed as a percentage of the contralateral normal tibialis cranialis muscle. The mean weight for the stimulated muscle (49% of normal) was significantly greater than that of the denervated muscle (41% of normal) *($p<0.01$)*.



Normal Unstimulated Stimulated Normal

FIGURE 22. Gross comparison of muscle bulk in treated and untreated muscle.

Electrode Scar

The layer of scar tissue which formed between the electrode and the underlying muscle measured approximately 0.4 mm (Figure 23). It appeared grossly as a thin white semi-lucent membrane which held the electrode in position on the muscle surface (Figure 24).

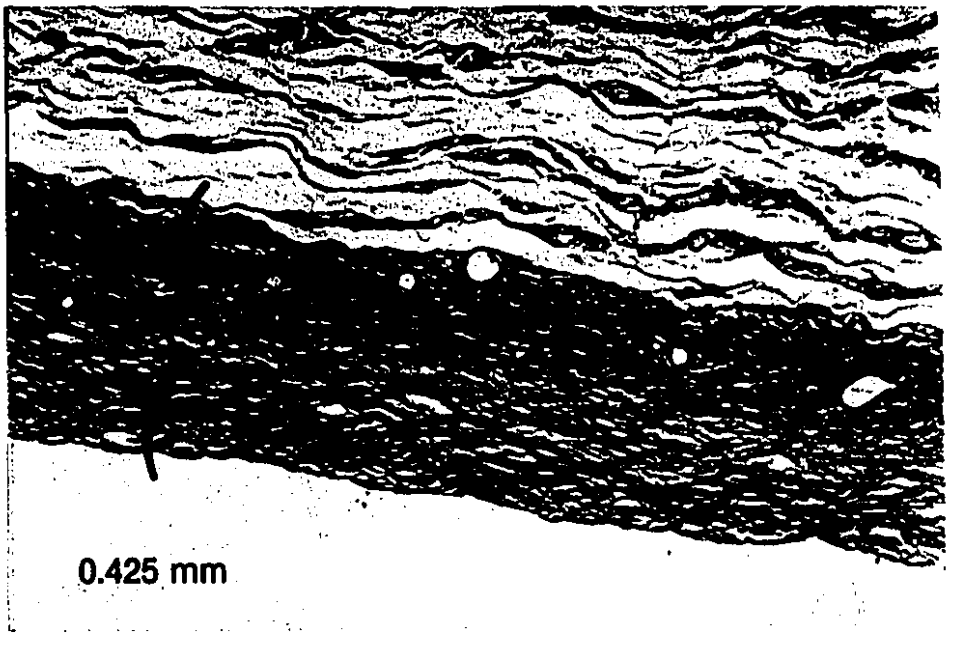


FIGURE 23. Cross-section of the scar encapsulating the electrode.

FIGURE 24. Electrodes exposed 10 weeks following implantation showing thin surrounding membrane.

Light Microscopy

Light microscopy was performed on tissue stained with hematoxylin-eosin, trichrome, and ATPase. Figures 25 and 27 show the normal, denervated and denervated-stimulated muscles juxtaposed and emphasize the histological differences between these groups 10 weeks following nerve transection and repair. In these photographs one can observe the pleomorphism and striking atrophy of the denervated muscle. In distinct contrast, the treated muscle fibers appear hypertrophied when compared to the normal, and are uniform in shape and size. The trichrome stain (Figure 25), which stains connective tissue blue, suggests an increase in interfascicular fibrosis in the denervated muscle. This change is time-related as indicated by Figure 26, which represents muscle completely denervated and untreated for 16 weeks. Here the increase in fibrosis is more prominent.

Histochemistry

Histochemistry of the muscle using ATPase staining at pH 4.3 and 10.4 provided baseline information about the normal canine tibialis cranialis muscle and permitted study of the two operated groups in terms of fiber type proportion, type 1 fiber area, and type 2 fiber area (Table 12). The normal proportion of type 2 fiber in this particular muscle and species, obtained in this study, is 75 ± 6% (i.e. Mean ± SD). After 10 weeks this proportion is unchanged in both the denervated and denervated-stimulated groups (Figures 27 & 28). Again, this change would appear to be time-related since muscle denervated for 16 weeks shows a decrease in the proportion of type 2 fibers from 75 to 51% (Figures 29 & 30) (Table 13).

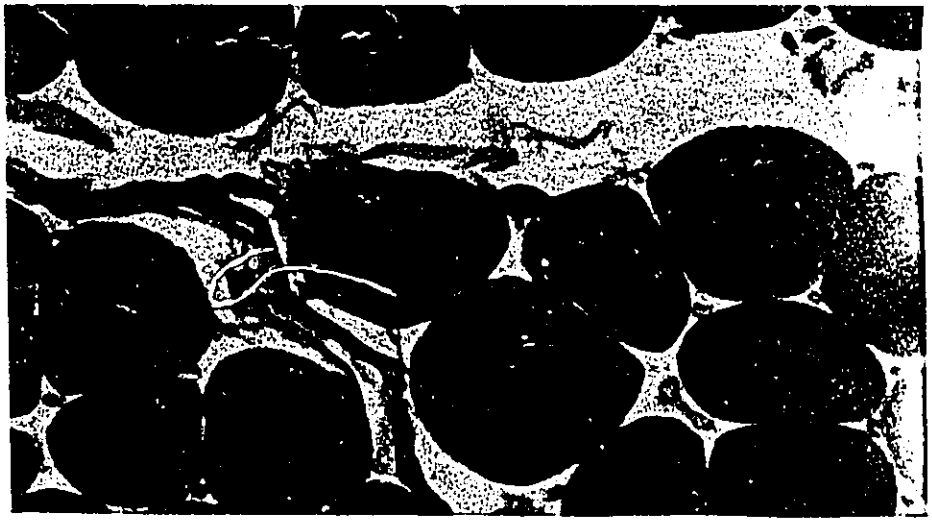
Analysis of the cross-sectional area of type 1 and type 2 fibers in the three groups substantiated the finding that fiber size is maintained in the stimulated muscle (Figures 31 & 32). The mean type 1 fiber area in the stimulated group (2742 μm^2) is significantly greater than the normal fiber area (2120 μm^2) *($p<0.038$)* and than the denervated fiber area (736 μm^2) *($p<0.0001$)*. The mean type 2 fiber area in the stimulated group (3060 μm^2) was considerably larger than its unstimulated counterpart (831 μm^2) *($p<0.005$)* but not significantly different from the normal muscle (2403 μm^2). The denervated fiber area in both fiber types was severely atrophied when compared to the normal tissue *($p<0.0001$)*.

Table 12 - Muscle Fiber Characteristics 10 Weeks Post Denervation

Group	N	Type 1 Fiber Area (μm^2)	Type 2 Fiber Area (μm^2)	Proportion of Type 1 (%)	Proportion of Type 2 (%)
Normal	6*	2120 ± 318	2403 ± 419	25 ± 6	75 ± 6
Denerv-stim	5	2742 ± 523	3060 ± 1457	22 ± 10	78 ± 11
Denervated	5	736 ± 225	831 ± 342	26 ± 4	74 ± 4

* pooled normal group incomplete due to technical problems

Normal



Denervated-
Stimulated

Denervated

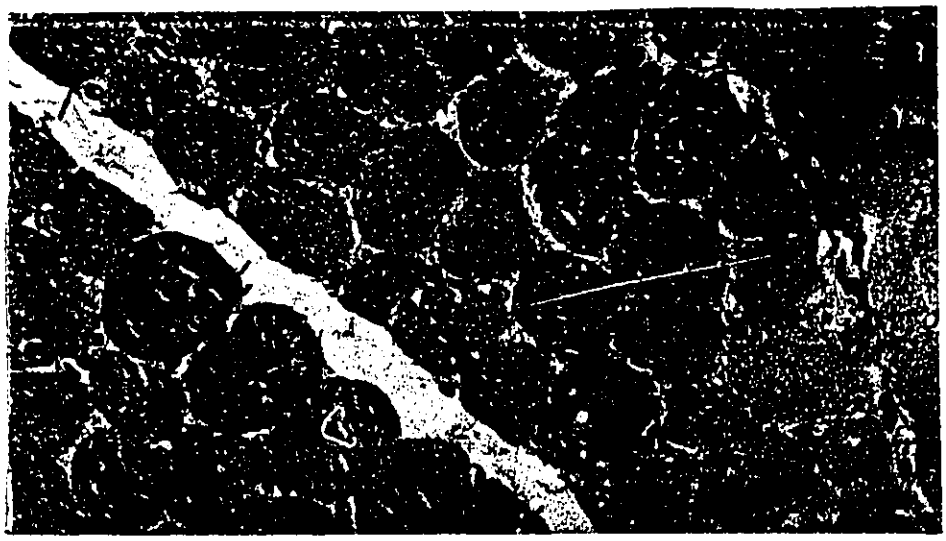


FIGURE 25. M. tibialis cranialis 10 weeks post nerve repair. Masson-trichrome stain. (X 260).

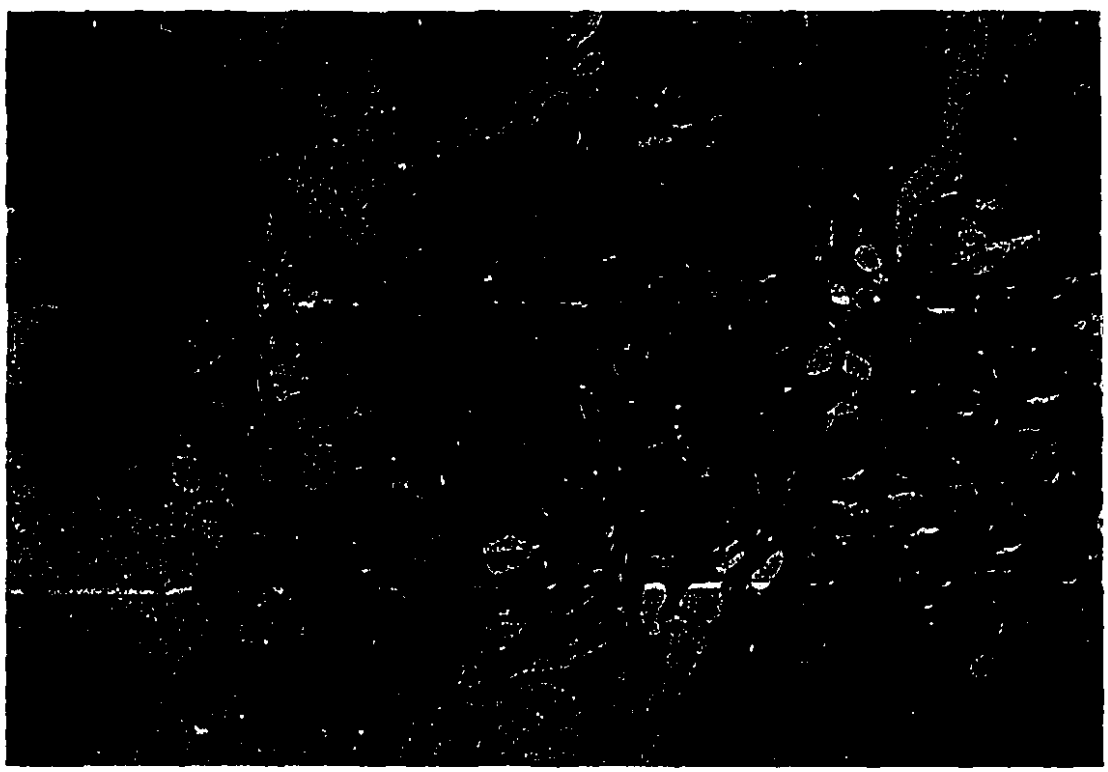
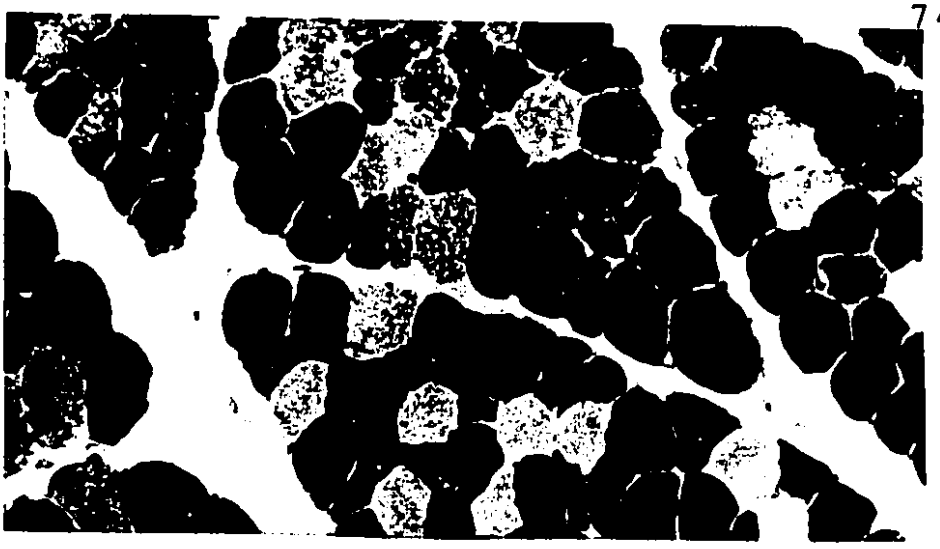
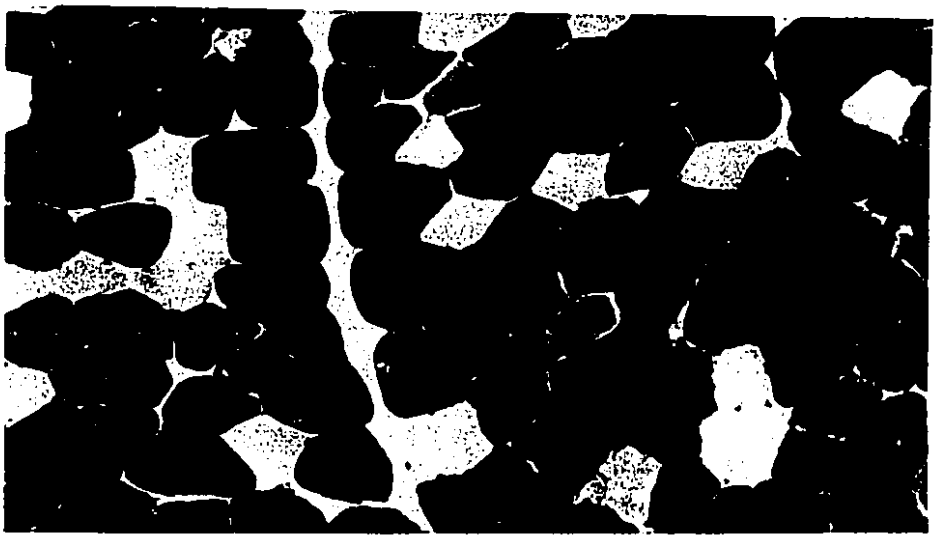


FIGURE 26. Untreated M. tibialis cranialis 16 weeks post nerve repair. Masson-trichrome stain. Note the prominence of connective tissue (stained blue). (X 260).

Normal



Denervated-
Stimulated



Denervated

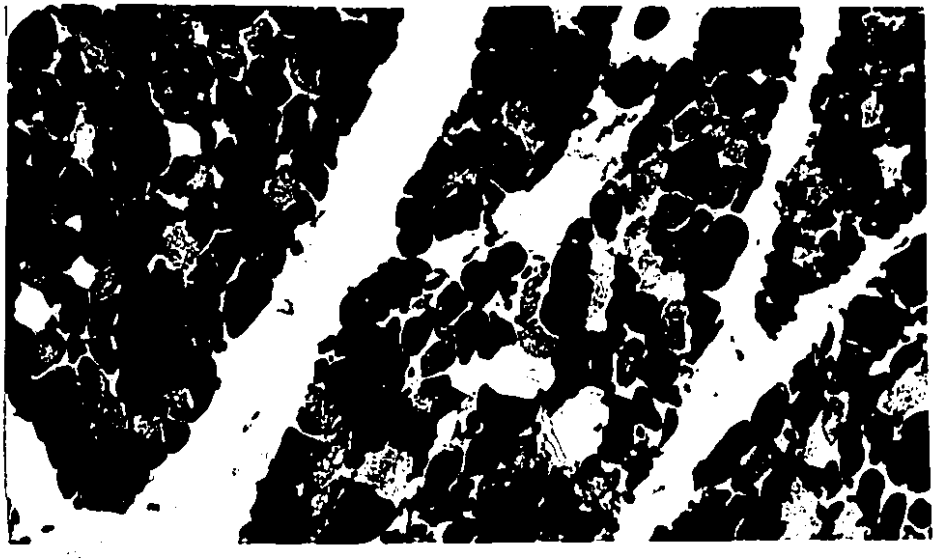
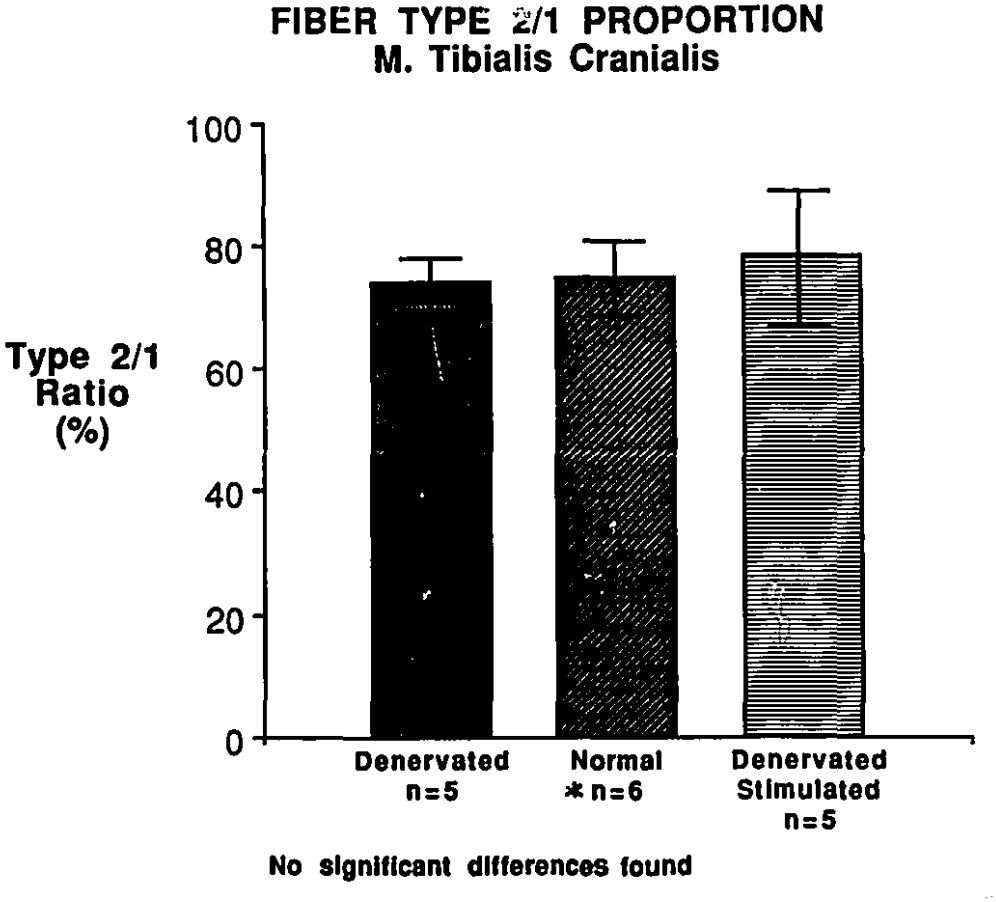


FIGURE 27. M. tibialis cranialis 10 weeks post nerve repair. ATPase stain (pH 10.4): light fibers (type 1), dark fibers (type 2). (X 260).



*Pooled Normal group incomplete due to technical problems.

FIGURE 28

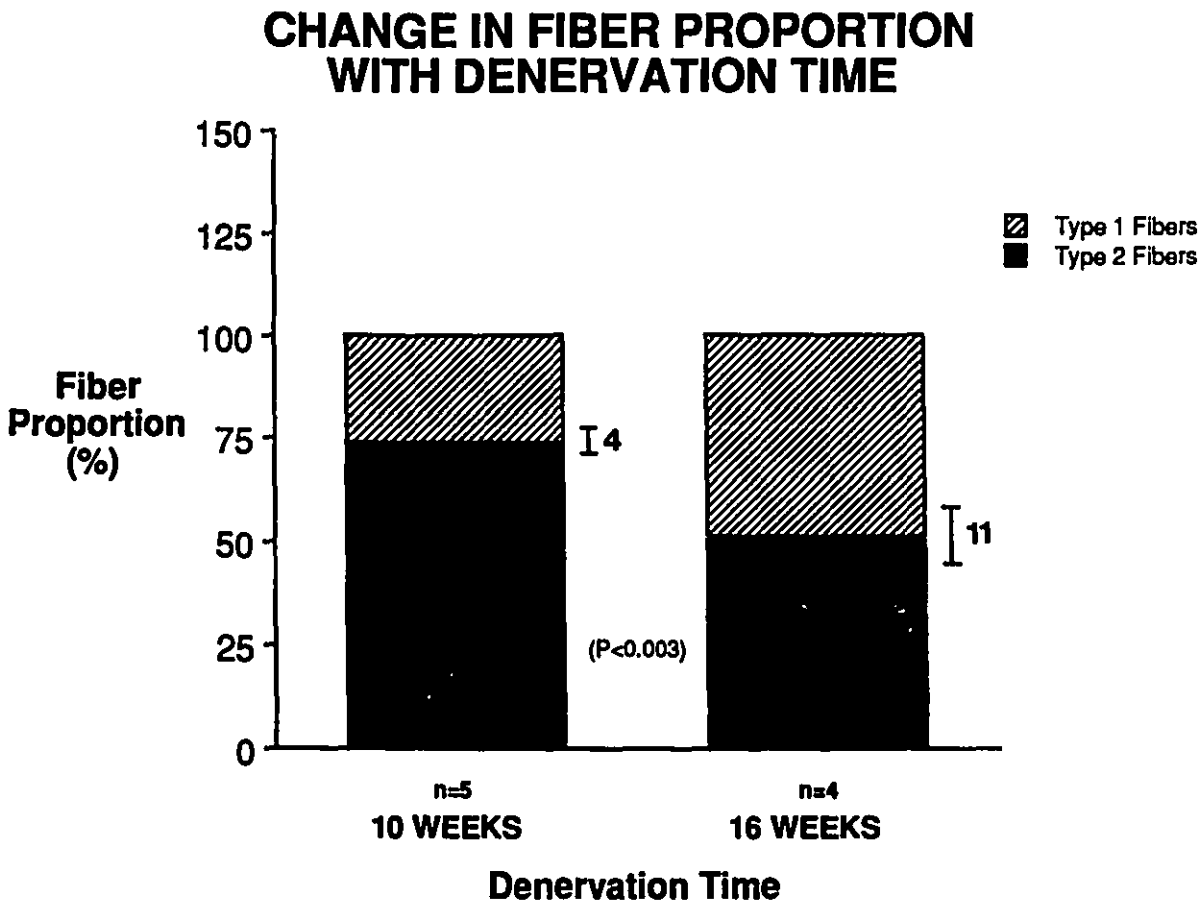

CHANGE IN FIBER PROPORTION
WITH DENERVATION TIME
Fiber
Proportion
(%)
150
125
100
75
50
25
0
Type 1 Fibers
Type 2 Fibers
4
11
(P<0.003)
n=5
10 WEEKS
n=4
16 WEEKS
Denervation Time

FIGURE 29

FIGURE 30. M. tibialis cranialis 16 weeks following denervation, showing a decreased type 2 (darkly stained) and increased type 1 fiber proportion. ATPase stain (pH 10.4). (X 260).

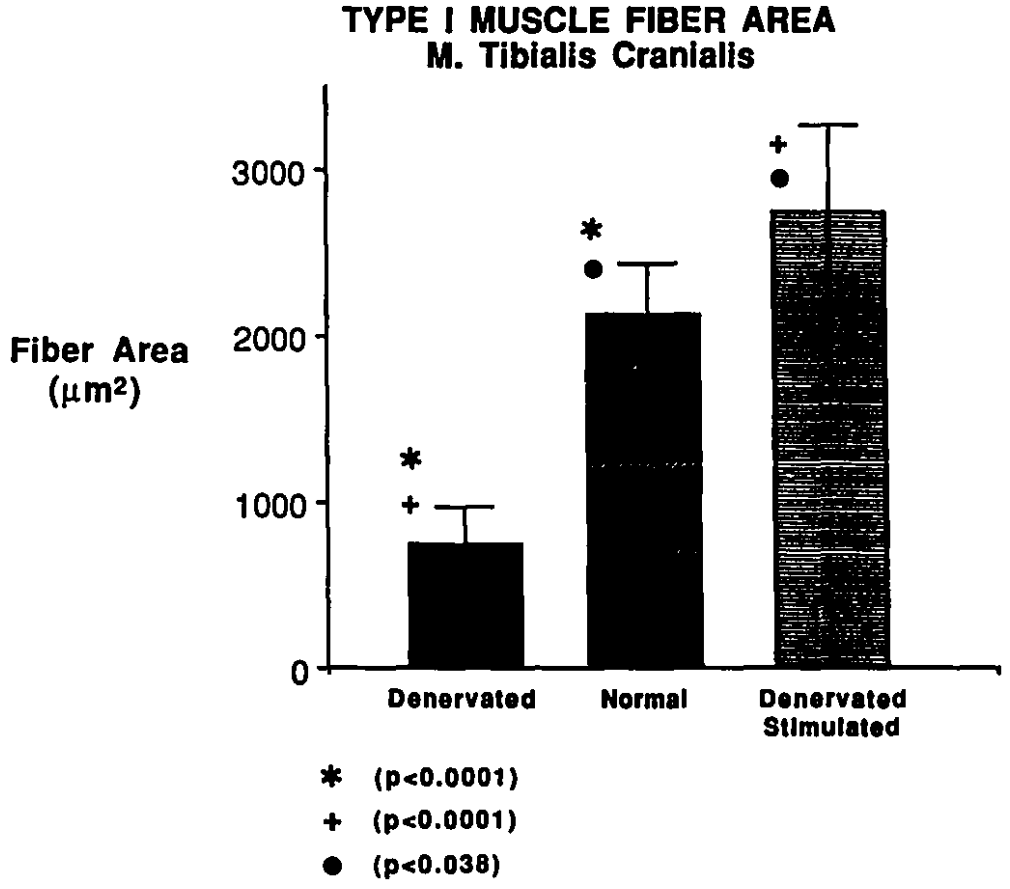
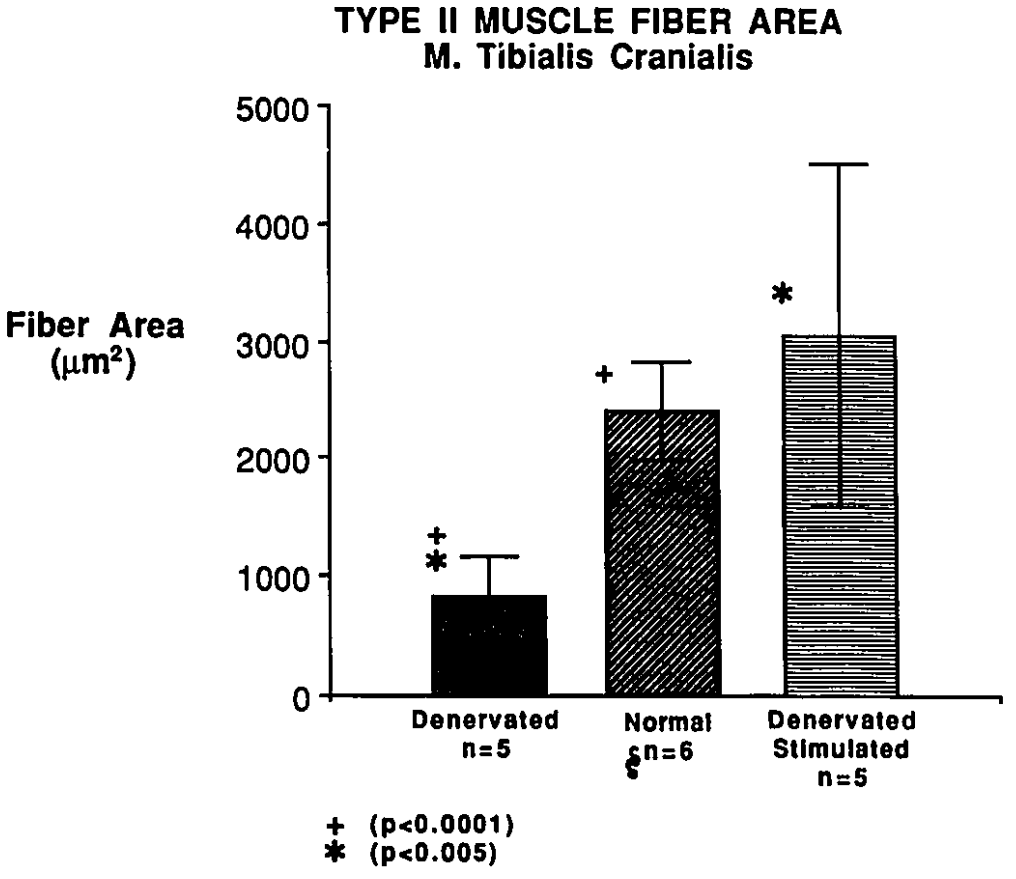

TYPE I MUSCLE FIBER AREA
M. Tibialis Cranialis
Fiber Area
(μm²)
3000
2000
1000
0
Denervated
Normal
Denervated
Stimulated
* (p<0.0001)
+ (p<0.0001)
● (p<0.038)

FIGURE 31

TABLE 13 - Muscle Fiber Composition
at 10 and 16 Weeks Post Denervation

TIME DENERVATED (no stimulation)	N	% TYPE 2 FIBERS (X ± SD)
10 weeks	5	75 ± 4 *
16 weeks	4	51 ± 11 *

*Independent t-test: *p<0.003*



§Group is incomplete due to inconsistencies in histochemistry.

+* Results of independent t-test.

FIGURE 32

Electron Microscopy

Electron microscopy provided evidence that stimulation has an ameliorative effect on the ultrastructure of denervated muscle. In the denervated muscle, in particular, the abnormal sarcotubular dilatations (D) are large and numerous and there is extensive myofilamentous disruption (M). Also of note is the undulating sarcoplasmic membrane (S) due to contracture and loss of contractile proteins. The translocation of the mitochondria and glycogen granules from the periphery to the center of the myofibril is further evidence of denervation, as is the increase in size of the ribosome. (Figures 33 & 34).

The electron micrograph of normal muscle, in contrast, shows myofilamentous continuity, peripheral mitochondria, a smooth sarcoplasmic membrane, and no dilatations (Figures 35).

In distinct contrast to the untreated denervated tissue, these abnormalities are less severe in the stimulated muscle. For example, one does see some myofilamentous discontinuity and dilatations but they appear less prominent (Figures 36 & 37). Abnormally located glycogen granule deposits (G) are also seen, but to a lesser degree than in the untreated denervated specimen. These findings, not documented previously in the literature, are supported by ultrastructural examination of the denervated-stimulated rabbit rectus femoris muscle (personal communication, D. Durand).

FIGURE 33. Electron micrograph of denervated M. tibialis cranialis. Arrows indicate the undulating sarcoplasmic membrane (S) and sarcotubular dilatations (D). (X 9821).

FIGURE 34. Electron micrograph of denervated M. tibialis cranialis. Arrows indicate myofilamentous disruptions (M) and abnormally located collections of glycogen granules (G). (X 36,306).

FIGURE 35. Electron micrograph of normal M. tibialis cranialis. Peripheral mitochondria (P) and a smooth sarcoplasmic membrane (M) are indicated. (X 22,694).

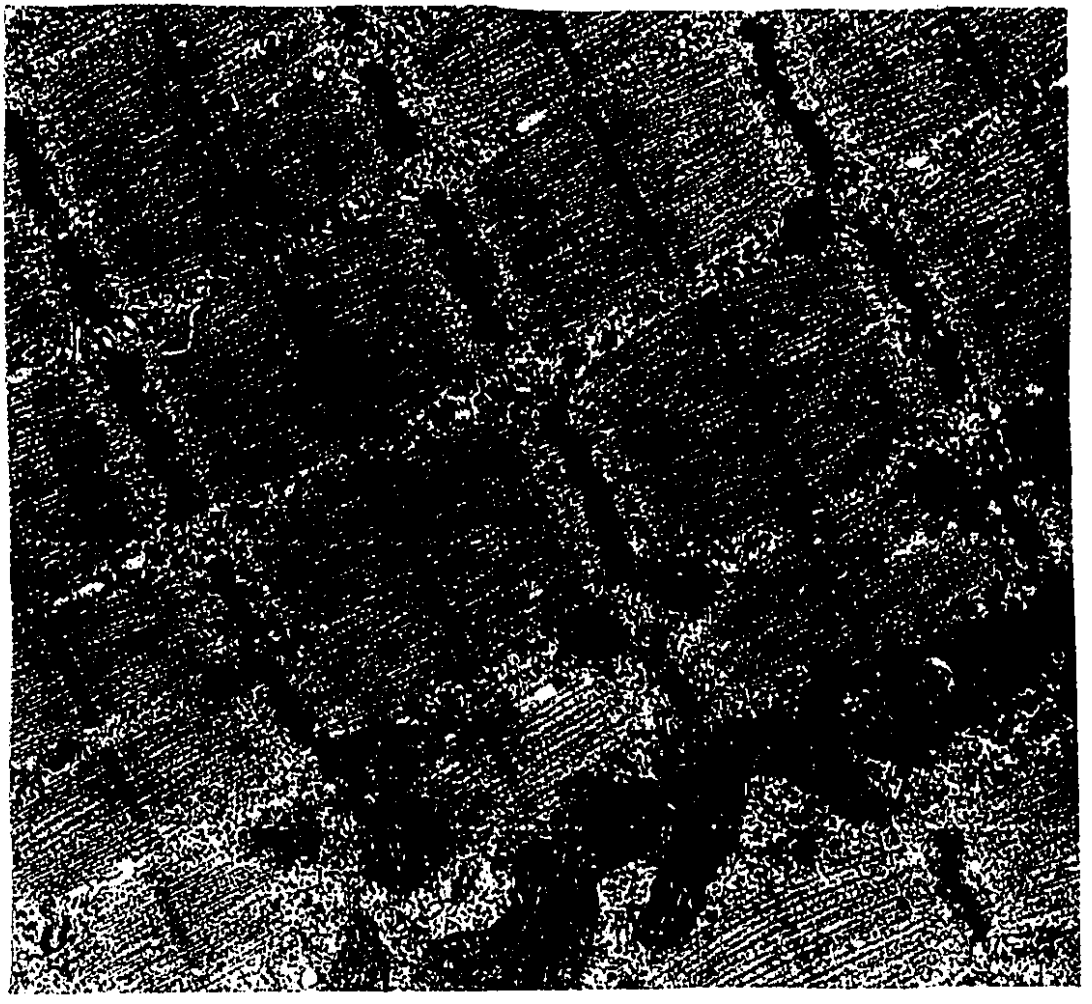


FIGURE 36. Electron micrograph of denervated-stimulated M. tibialis cranialis. Note relative absence of dilatations when compared to denervated muscle. Abnormally located glycogen granules are indicated (G). (X 30,215).

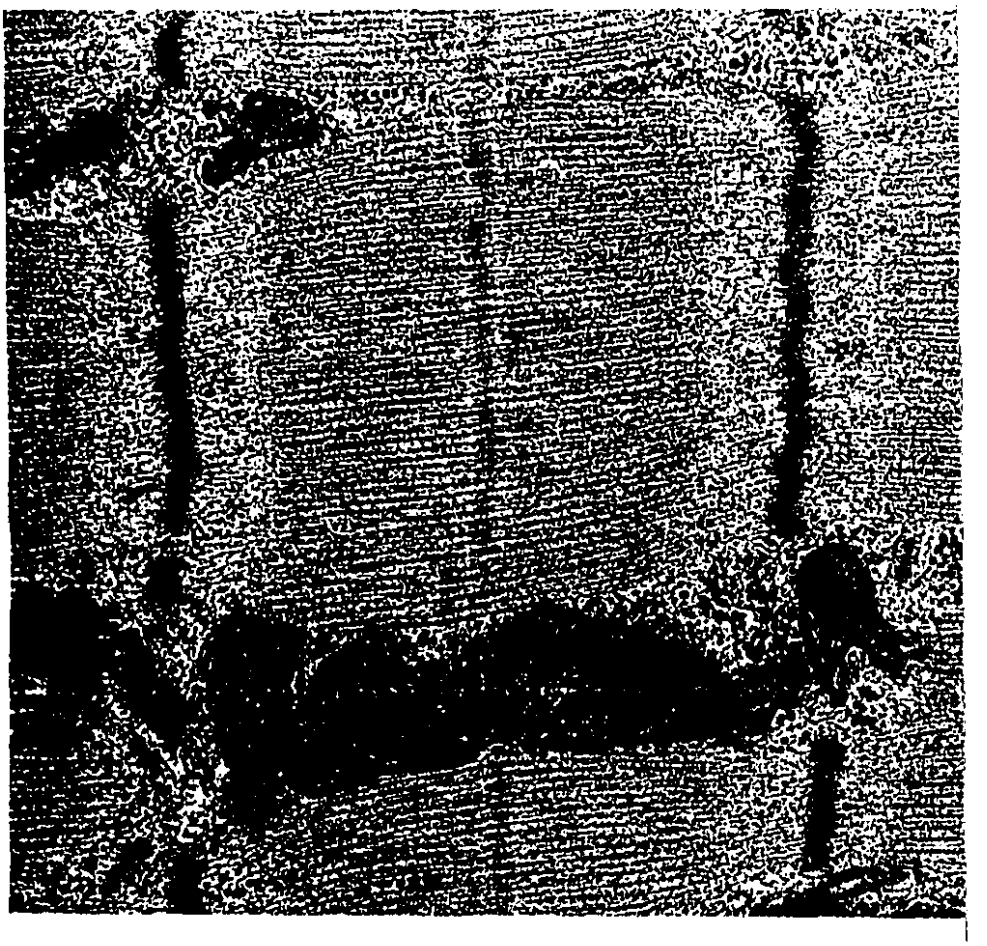


FIGURE 37. Electron micrograph of denervated-stimulated M. tibialis cranialis. Note relative lack of myofilamentous disruption compared to that in Figure 34. (X 33,574).

CHAPTER IV

DISCUSSION

EVOLUTION OF THE EXPERIMENTAL MODEL

The experimental model, materials, and method described in this thesis evolved over a period of several years and underwent a number of crucial changes. The canine model was modified from a distal nerve injury/repair model to one having a proximal injury. This change allowed for a longer period of complete denervation and was expected to more closely resemble the clinical problem of prolonged denervation. In the distal nerve injury models previously studied in our laboratory (Figures 38 & 39), the complete denervation period was short and substantial reinnervation had occurred by the time final assessment of the muscle was done. One drawback of the present "modified" experiment, however, was the difficulty encountered with muscle force testing employing the conventional method. Future studies using the proximal nerve injury model will require the development of an appropriate testing method.

The development of electrodes has been vital to the success of this implantable stimulating system. The original electrode used in our research, a rigid metal device (Figure 40), was handed down from the cardiac stimulation research. Its high incidence of infection, inflammatory reaction, and extrusion led to the development of the biocompatible silicone-carbon electrode used presently. The very low morbidity associated with this new electrode in this canine model provides optimism for its safe use in the human.

Innovation in the assessment of the denervated-stimulated muscle was also introduced into this study. Ultrastructural comparison of denervated and denervated-stimulated muscle using electron microscopy has not been previously reported. The observations described in this experiment could be further analyzed using objective quantitative morphometric analysis similar to that employed by Engel and Stonnington (1972). Measurement of resistance through the tissue between the cathode and anode was also novel in this field of research. This method may be applied to the study of bioelectric properties in the human clinical setting.

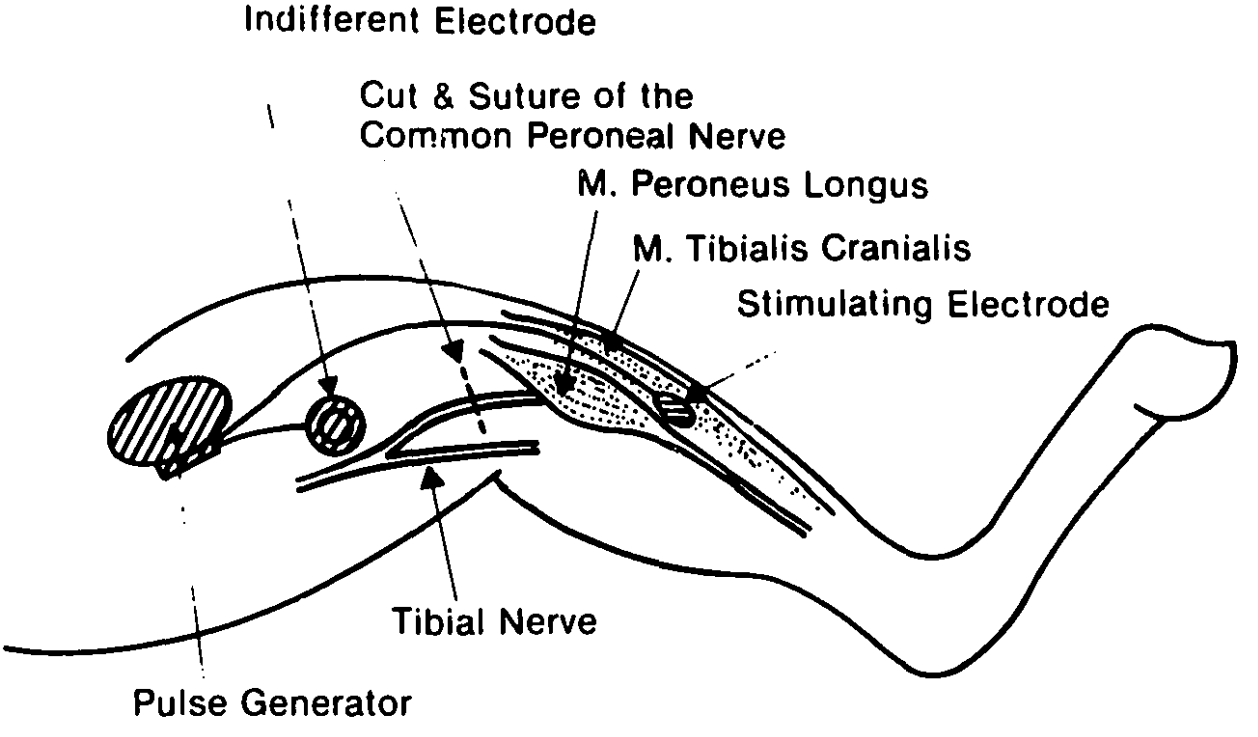


FIGURE 38. Canine distal nerve injury model. The common peroneal nerve was transected and repaired 4 cm from the muscle.

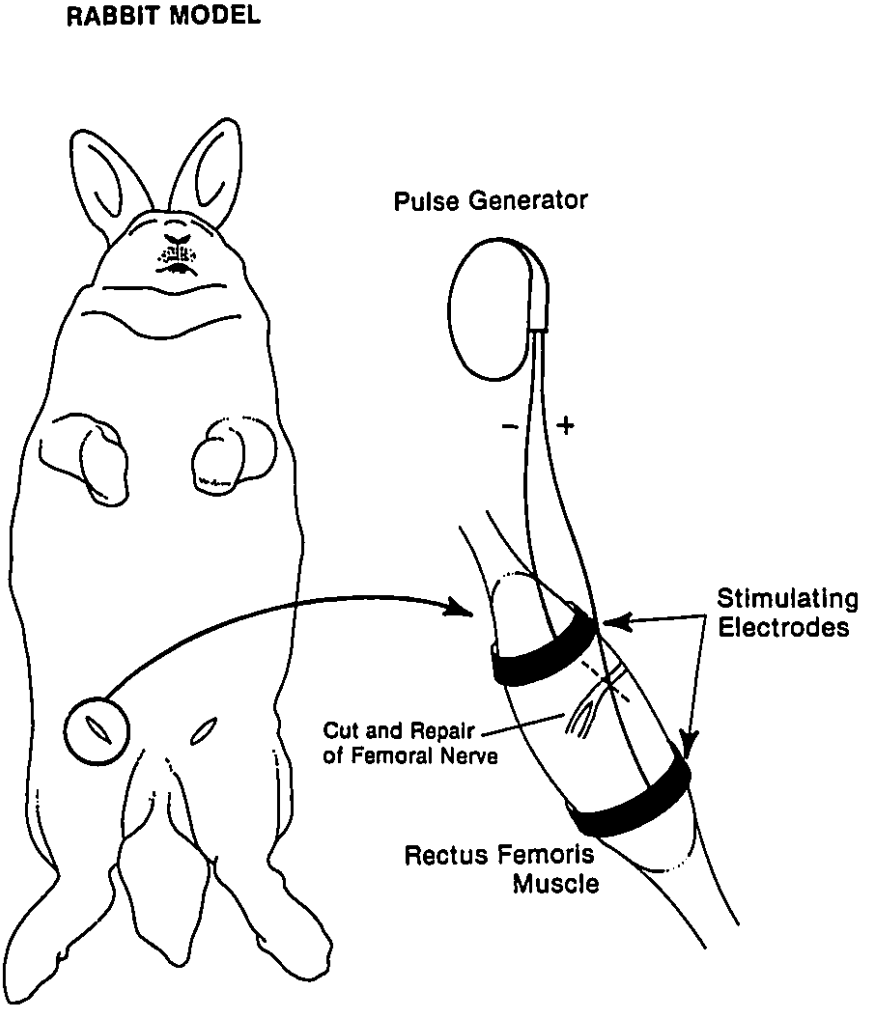


FIGURE 39. Rabbit distal nerve injury model. The femoral nerve was transected and repaired 2-3 cm from the target muscle.

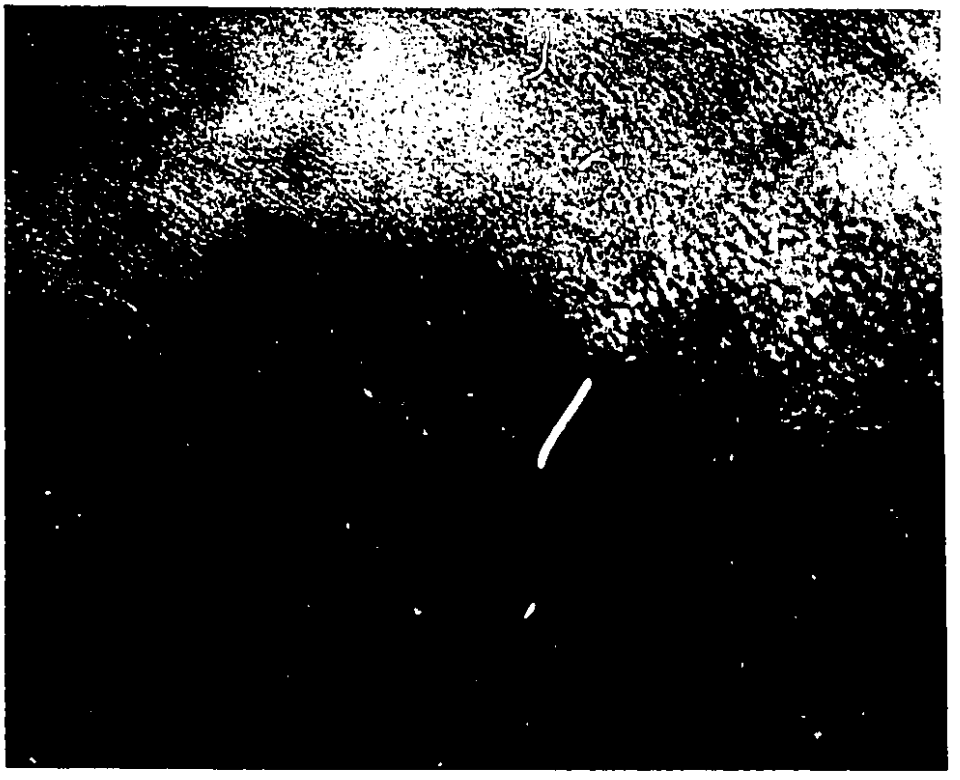


FIGURE 40. Infected metal electrode extruding from the insertion site.

SUMMARY AND INTERPRETATION OF RESULTS

The results of this experiment which support the body of literature describing the beneficial effects of ES on denervated muscle are substantial. The data reported in the present study indicate a relative maintenance of muscle function, in the form of twitch contraction time. Structurally, ES had positive effects on the quantitative preservation of muscle, seen both grossly and microscopically. On the ultrastructural level there was further qualitative evidence for the maintenance of denervated muscle using artificially induced contractile activity. The result of normal muscle/electrode resistance in the denervated-stimulated muscle offered another clinically relevant suggestion of the beneficial influence of this therapy on denervated muscle.

Not all examinations, however, substantiated the ameliorative effects of electrotherapy. The tetanizing frequencies of both denervated and treated muscle showed the decrease that is typical of denervated muscle, as described by Kosman in 1947. Also, assessment of the one-half relaxation time failed to support a more normal functional capacity of the stimulated muscle. Limited sample size and large deviations may have played an important rôle in some results.

The positive results merit critical interpretation. It is important to keep in perspective that this treatment is not a panacea. The structural and contractile changes of denervation are not *prevented.* Atrophy *does* occur. Ultrastructural abnormalities *are* apparent. There *is* slowing of the relaxation property of the muscle. However, electrotherapy and muscle contraction produced in the absence of the intact nerve appear to decrease and perhaps retard these changes of denervation.

Fiber Size and Muscle Weight

The fiber size analysis should be interpreted with caution. Muscle fibers from the biopsy region showed maintenance of the cross-sectional area and even hypertrophy compared to normal muscle, whereas the total weight of the muscle indicated atrophy compared to normal muscle. This discrepancy could be attributed to highly effective stimulation of the superficial layers from which the specimens were taken and to less effective stimulation in the deeper layers. Further investigation is required to clarify this. Another factor contributing to the discrepancy may be the size of the stimulated muscle: the tibialis cranialis muscle of the dog is large and bulky compared to other muscles treated in this field of research and as a result, the superficial muscle electrodes, although easy to

implant, may not have permitted stimulation of the entire muscle. Circumferentially placed electrodes may have been more effective although technically more difficult to implant.

Influence of Denervation and Stimulation on Fiber Type

The results of this investigation confirm that the transformation of a fast muscle composed of a high proportion of type 2 fibers to a muscle with a relatively high proportion of type 1 fibers occurs with denervation. This change was not evident at 10 weeks following nerve transection but was highly significant at 16 weeks.

Muscle fiber typing also indicated that this regimen of electrical stimulation, (i.e. high frequency stimulation, 85 Hz, of a fast muscle), did not result in a conversion of the fiber type. This is compatible with previous research showing that homologous stimulating frequencies maintain muscle characteristics whereas heterologous patterns produce fiber type changes (Lomo et al., 1980; Hennig & Lomo, 1987). It is however in contrast to the recent findings of Nemoto et al. (1988). In this canine study, the proportion of type 2 muscle fibers in the 8 week denervated tibialis cranialis muscle *decreased* while being stimulated with a frequency of 36 Hz. It is likely that this "intermediate" frequency did not mimic closely enough the intrinsic firing frequency. When selecting a stimulating frequency it is important to keep in mind the therapeutic goal. In the case of denervated muscle, maintenance of the characteristics normal for that muscle would be an appropriate goal. Muscle conversion may be desirable in other situations. For example, an athlete may benefit from the development of stronger, more fatigue-resistant slow muscle. Conditioning of the fast latissimus dorsi muscle into a slow, strong, fatigue-resistant muscle suitable for synchronized pumping with the heart is the goal in cardiomyoplastic research and surgery .

Effect of Electrotherapy on Regeneration and Reinnervation

It is not possible to make inferences about the effect of electrotherapy on the rate of nerve regeneration or muscle reinnervation from the results of the present study. Future studies may focus on this interesting topic.

Electrical Resistance

The decreased electrical resistance of the stimulated muscle when compared to the untreated denervated muscle is at present open to conjecture. A likely explanation of this important finding is that the muscle activity of the stimulated muscle encouraged homeostasis of fluids within the muscle. It promoted maintenance of a normal blood suppy and venous and lymphatic drainage. Hence the normal muscle and stimulated muscle had similar electrical conductivity. In the denervated, flaccid muscle the blood supply was likely impaired, thus decreasing the flow of current through it. The observation of a "pale" muscle could be objectively assessed in the future using quantitative flow studies, such as the radioactive microsphere technique.

CLINICAL APPLICATION MODELS

An attempt is made here at applying the concepts of electrotherapy of denervated muscle learned in this animal model to the human clinical situation. Three hypothetical models for stimulation using a long-term implantable system are proposed (Figures 41 to 43). In these models, multiple implantable electrodes are used to stimulate the major muscles affected by a nerve injury. Unipolar rather than bipolar IPGs are employed. In the unipolar IPG the metal casing acts as the anode. Only the stimulating electrode (cathode) inserts into the IPG.

The clinical application models follow several principles: firstly, the IPG is implanted in a cosmetically acceptable area. For example, in the facial model it may be hidden in the retroauricular area. A location having adequate skin laxity is selected. Joints are avoided. When possible, the unipolar IPG is placed in an area which facilitates current flow from the stimulating electrode (cathode) through the denervated muscles back to the IPG (anode). This principle could, in particular, be effectively applied to the ulnar/median nerve and radial nerve injury models where the electrodes could be implanted on one aspect of the extremity and the IPG on the other.

When using multiple electrodes the IPG may not have the capacity to provide adequate current density to each electrode for effective muscle stimulation. Furthermore, the size and surface area differences of the electrodes may result in varying impedances, current densities and stimulus intensities. Implantation of more than one IPG may be required. A multi-channelled battery power source may need to be developed to provide modulation to each electrode.

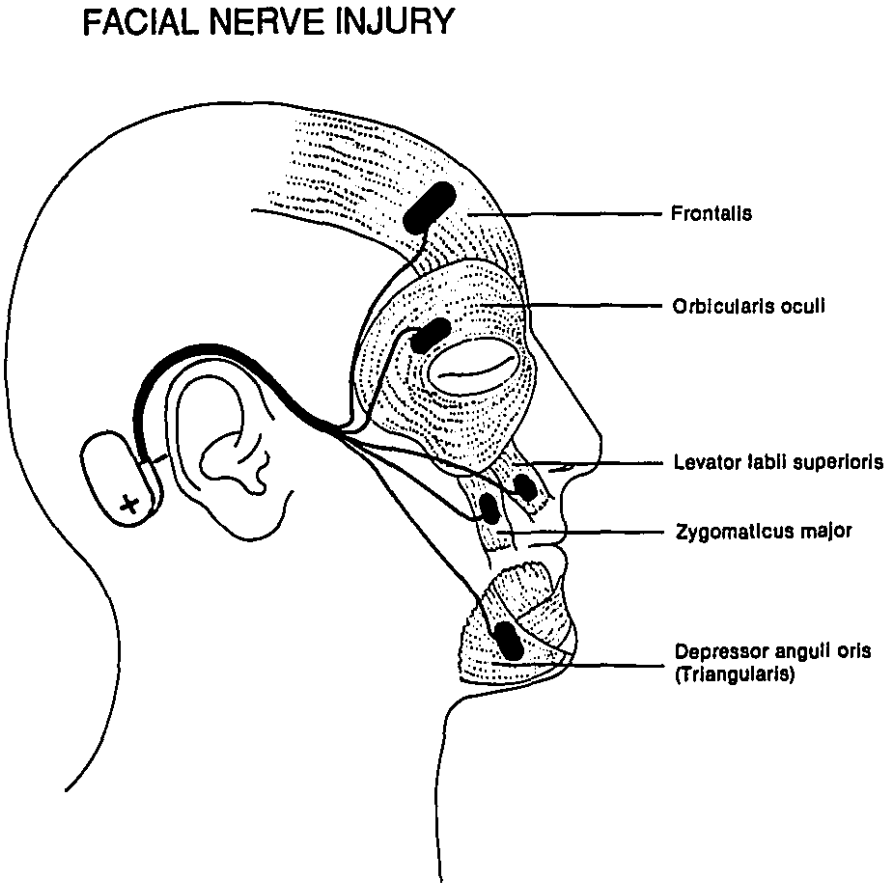


FIGURE 41. A hypothetical branched electrode applied to the muscles of facial expression.

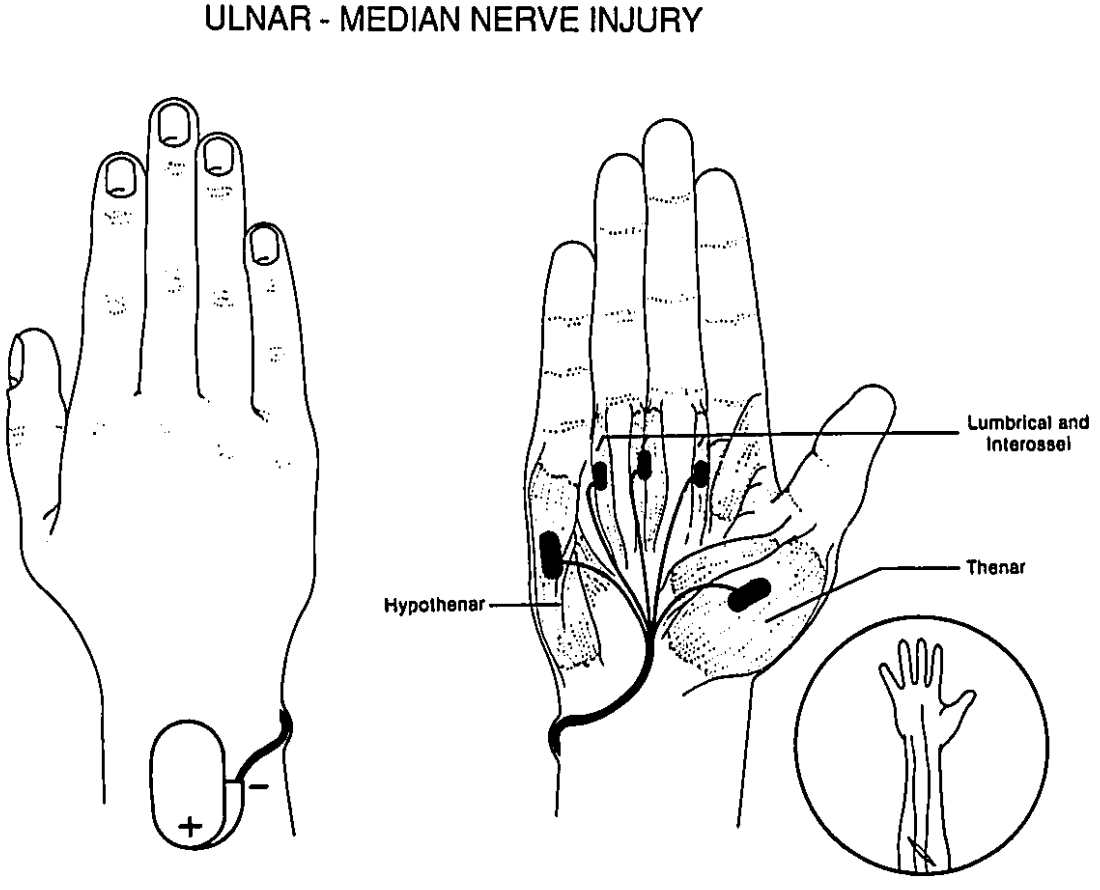


FIGURE 42. Electrodes are applied to the intrinsic, hypothenar, and thenar muscle groups following denervation.

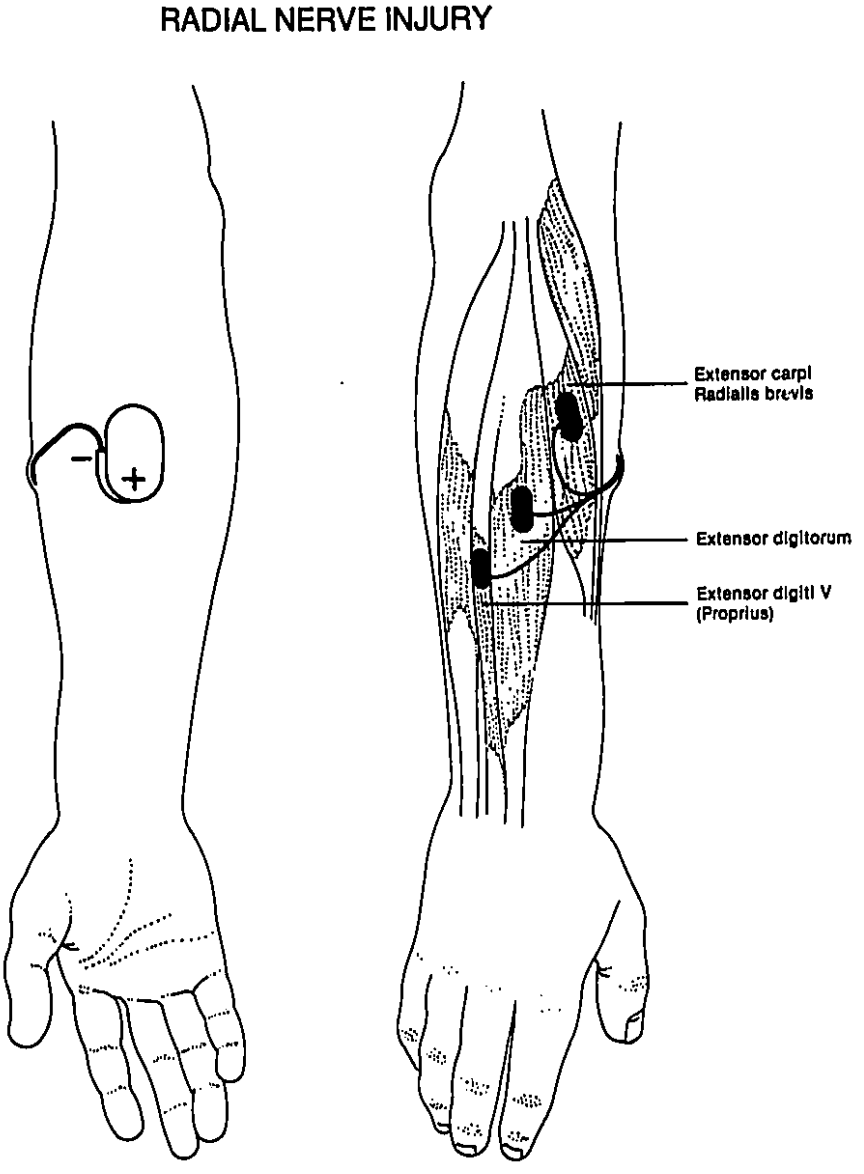


FIGURE 43. Hypothetical stimulation of the extensor muscles of the forearm using implanted electrodes.

FUTURE RESEARCH PERSPECTIVES

Future research, drawing on the experience of existing studies, will be essential for the advancement of this therapeutic modality. New investigation may take the form of development of different anatomic models in animal species, new methods of assessment of the effects of ES on the muscle, and refinements in the stimulating hardware. Specifically, a clearer understanding of the optimal parameters for stimulation is needed. The issue of tetanic versus twitch contractions and isometric versus isotonic contractions requires rigorous examination. Still to be addressed, also, is the effect of this stimulation modality on the production of discomfort or pain in the subject.

Refinement of and study of the properties of the stimulating hardware represents an important area of future concern. Technical improvement could prevent electrode breakages. Bioelectric properties of the electrodes could be further investigated. The carbon-silicone material of the electrode could be examined in terms of events such as lipid absorption and degradation. The longevity and stimulating parameters of the pulse generator could be augmented. The parameter most limiting the stimulation of denervated muscle is the pulse duration. The development of a pulse generator offering wider durations, (i.e. up to 10 ms), would be crucial to the delayed stimulation of denervated muscle.

Many interesting questions pertaining to the basic science of stimulation of denervated skeletal muscle could provide a focus for future research. There are questions relating to the effects of ES on muscle which had a prolonged period of denervation (i.e. 6 months). A prolonged denervation-stimulation study, with and without nerve repair, would offer valuable information on the interaction of ES and the regenerating nerve. We question, "Does early regeneration of the nerve (and thus availability of neurotrophic factors) render the muscle more receptive to the positive effects of ES?". Also, "What effect, if any, does ES have on the motor endplate?". This gap in our knowledge could be addressed using ultrastructural examination of the motor endplate. Another vital question posed is, "Are there beneficial effects of ES used on muscle which is already atrophied?".

Furthermore, we need to assess the effects of this system of stimulation on normal muscle. A normal-stimulated group would provide additional information.

Perhaps the most important next step in assessing the feasibility of further development of a clinically applicable system of implanted electrodes is a study comparing the relative benefits of the traditional method of external stimulation to the approach explored in the present study. No such investigation is documented in the literature to date. The application of external electrodes in physiotherapy departments and in home treatment programs (Figure 44) for the treatment of denervated muscle has apparent disadvantages including the infrequency of treatment and the patient's non-compliance.

A study designed to produce comparative data on the effects of external stimulation could be accomplished using the animal model presented here. A regimen of transcutaneous electrical stimulation representative of that used by physiotherapists would be administered. This group of animals would then be evaluated acutely in a manner identical to the implanted group and the results compared.

FIGURE 44. External stimulating device used by physiotherapists and in home treatment programs.

CONCLUSION

In this experiment we have seen that immediate continuous electrical stimulation of denervated canine muscle, using a newly designed fully implantable stimulating system, is an effective and safe method of reducing some of the deleterious effects of denervation. Data support the relative preservation of structural and functional integrity of the treated muscle, as shown by assessment of muscle weight, fiber area, ultrastructure, electrical properties, and twitch contraction time.

Much investigation is required to further clarify the physiological effects of electrical stimulation. In particular, a comparative study examining the relative benefits of an implantable system versus the traditional external method of stimulation is recommended.

Hypothetical models for the clinical use of implanted electrodes have been presented and provide a springboard for prospective innovation in the field of human electrotherapy of denervated muscle.

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