MUSCLE PRESERVATION

IN DENERVATION INJURY USING

CONTINUOUS IMPLANTABLE ELECTRICAL STIMULATION

by

Daniel Stewart Thomas, M.D.

Department of Surgery

McGill University, Montreal

A thesis submitted

to the Faculty of Graduate Studies and Research,

in partial fulfilment of the requirements for

the degree of Master of Science.

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ABSTRACT

Peripheral nerve injury results in a muscle fiber degeneration and fibrosis that will limit the functional return that one anticipates following a microsurgical nerve repair. The clinical use of electrical stimulation (ES) to induce muscular contraction in the denervation period to prevent muscle atrophy has been limited by its transcutaneous application.

Recently, a totally implantable ES method was developed by Nemoto, Williams, et al [1] and confirmed by Durand and Williams[2]. The major difference in their results was the sparing of Type II (fast) muscle fibers by ES in the latter experiment. The purpose of this project is to study the impact of ES in a more proximal nerve injury; to clarify the muscle fiber type effect of ES; and to assess the functional impact of ES on reinnervation.

Using a dog model, two experimental groups were defined. In group 1, the common peroneal nerve of the right hind limb was cut and repaired microsurgically 12 cm from the tibialis anterior muscle (fast muscle). Electrodes (applied to the muscle) and an Itrel pulse generator were implanted, and ES was delivered at 85 Hz, .45 msec pulse duration, 10.5 V, 1.5 sec every 24 sec (n=4). Group 2 was similar except there was no ES (n=5). After sixteen weeks, muscle weight, function, histochemistry, and microscopic morphology were assessed.

Comparing the experimental side to the normal contralateral muscle, ES versus no ES resulted in a preservation of muscle weight- 73% vs 39% ($p<.01$); twitch tension- 21% vs 7% ($p<.001$); nerve stimulated tetanic tension- 23% vs 7% ($p<.05$); direct muscle stimulated tetanic tension- 43% vs 11% ($p<.01$); Type I fiber area- 69% vs 46% ($p<.05$); and Type II fiber area of 63% vs 34% ($p<.05$). There was no statistically significant difference between the two groups in the fiber type proportions; both groups showed a relative fourfold decrease in the number of Type

II muscle fibers. The contraction times, one-half relaxation times, and unfused tetanic profiles also demonstrated a slowing of both muscle groups compared to the normal muscle.

The results of this project support the hypothesis that continuous, implantable ES delivered over and extended period of time has a positive functional and morphological impact on denervated muscle. The use of a fast frequency (85 Hz) stimulation regime did not, however, prevent the transformation of the fast tibialis anterior muscle into a slower type muscle. Also, a comparison of the direct muscle stimulated tetanic tension to nerve-stimulated tetanic tension reveals that reinnervation was not adversely effected by ES.

RESUMÉ

Les lésions des nerfs périphériques résultant de la dégénération et de la fibrose des fibres musculaires limitent le retour fonctionnel que l'on peut anticiper suivant la réparation du nerf par la microchirurgie. L'utilisation clinique de la stimulation électrique (SE) pour provoquer la contraction musculaire lors de la période de dénervation, pour prévenir l'atrophie, a été limitée par son application trans-cutanée.

Récemment, une méthode de SE implantée fut développée par Nemoto, Williams et al et confirmée par Durand et Williams. La différence majeur de leurs résultats fut la conservation du muscle de type II par la SE de la dernière expérience. Le but de ce projet est d'étudier l'impact de la SE pour une lésion a un nerf plus proximal; de clarifier l'effet de la SE du type musculaire; et d'évaluer l'impact fonctionnel de la SE sur la réinnervation.

En utilisant le modèle du chien, deux groupes expérimentaux furent définis. Dans le groupe 1, le nerf péroné de la main droite fut couper et réparer par microchirurgie 12 cm du muscle tibialis antérieur. Des électrodes (appliquer au muscle) et une batterie Itrel furent implantées, et la SE fut délivrée a 85 Hz, .45msec de duration, 10.5 de voltage, 1.5 secondes a toutes les 24 secondes (n = 4). Le groupe 2 fut similaire sans qu'aucune SE fut appliquée (n = 5). Après seize semaines, le poids, la fonction, l'histologie, et la morphologie microscopique du muscle furent évalués.

En comparant le coter expérimental au muscle controlatéral normal, la SE contre la non SE eu comme résultat la préservation du poids du muscle 73% contre 39% ($p<.01$); une tension contractée de 21% contre 7% ($p<.001$); la stimulation du nerf de tension tétanique de 23% contre 7% ($p<.05$); la stimulation directe du

muscle de tension tétanique de 43% contre 11% ($p<.01$); région de la fibre type I 69% contre 46% ($p<.05$); région de la fibre type II 63% contre 34% ($p<.05$). Il n'y a pas eu des différences statistiques significatives entre les deux groupes reliés a la proportion des types de fibres; les deux groupes démontrèrent une baisse relative quadruple dans le nombre des fibres musculaires de type II. Le temps de contraction, une demie le temps de relaxation, et le profil infusé tétanique démontrèrent aussi un ralentissement du muscle dans les deux groupes compares au muscle normal.

Les résultats de ce projet supportent l'hypothèse que la SE implantée de façon continue délivrée sur une période étendue de temps a un effet positif sur l'impact fonctionnel et morphologique des muscles dérivés. L'utilisation d'une fréquence rapide (85 Hz) de stimulation n'a cependant pas prévenue la transformation du muscle tibialis antérieur en un muscle de type plus lent. Aussi, une comparaison du muscle directement stimulé de tension tétanique au nerf stimulé de tension tétanique révèle que la réinnervation ne fut pas défavorablement affectée par la stimulation électrique.

PREFACE

The following work was made possible due to the effort and dedication of several individuals.

Firstly, I would like to thank Dr. H. Bruce Williams for the essential role he played in this project. His patience, insight, and commitment were invaluable. The opportunity to perform this work under his guidance was a privilege and a pleasure.

Also, I would like to thank Dr. Frank Campanile who worked on a parallel project in the research lab. His collaboration and friendship during every phase of this project was most appreciated.

Dr. John Lough played and essential role and it was through his efforts and experience that the pathological analysis was made possible.

Two laboratory assistants also deserve specific recognition. Norman Comtois ably assisted in the surgical procedures and the post operative care of the animals. Celine Lemay worked very hard in the microscopic processing of the surgical specimens. I thank them for their support.

Also, I would like to thank Dr. R. Chiu and his staff at the McGill University Surgical Research Center. Their organization and cooperation were essential to the completion of this project.

Finally, I would like to thank the Medtronics Corporation who supplied the electrodes, pulse generators as well as their time and expertise to this project.

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INTRODUCTION

Our ability to interact with our environment is determined by the nearly infinite complexity of our nervous system; from our most basic sensory perceptions to our most elaborate motor endeavors, the intricacy of our functional existence is boundless.

One of the important characteristics we possess is our capacity for motion, and this ability depends on a functioning neuromuscular interaction. In peripheral nerve injury, this system is specifically disrupted and may result in a permanent disability unless specific therapy is provided.

An important initial therapeutic measure is the repair of the injured nerve. With the development of microsurgical skills, the suturing of peripheral nerve lacerations has become widespread and has resulted in a substantial improvement in patient prognosis. Neuromuscular functional recovery is also dependent on the presence of viable muscle tissue. Denervation can cause irreversible structural damage to muscle such that even if normal neural regeneration occurs, there still may be no return of normal motor activity.

This research project and thesis are part of an ongoing effort in Dr. William's laboratory at the Montreal General Hospital in which muscle preservation in denervation injury is being assessed. While there are several different methods currently being used to treat this condition, we have focussed on the use of a totally implantable system. This system is designed to deliver electrical stimulation (ES) to the denervated muscle while the process of reinnervation is occurring. The overall goal of this collective effort is to add to the therapeutic armamentarium of health care professionals in preserving muscle form and function in peripheral nerve injury.

The specific goals of this project are to assess the ability of canine muscle to respond to ES over a prolonged period of time (four months); to measure the physiological, morphological and biochemical consequences of ES; and to determine if ES has any negative influence on neural regeneration.

To achieve these goals, we have used our implantable ES system and a newly developed force transducer platform which enables us to make accurate and reproducible in situ muscle force and velocity measurements. Our priority in data procurement has been the use of measureable physiological parameters of muscle form and function, and our conclusions are based on this quantitative data.

CLINICAL HISTORY OF NERVE REPAIR AND ELECTRICAL STIMULATION

While Hippocrates of Los is recognized as the most famous physician of the Classic Period, he failed to appreciate the difference between tendons and nerves (Figure 1). It was Clarissimus Galen (131-201 AD), a physician to the Roman Gladiators, who dissected the bodies of many apes (and two humans), who made this distinction (Figure 2). He is also credited with defining the dura and the pia mater, and describing the third and fourth ventricles, the hypophysis, and seven of the twelve cranial nerves.[3]

Figure 1: Hippocrates (460-377 BC)

Figure 2: Clarissimus Galen (130-200 AD)

Rhazes, an Arabic clinician of Bagdad in the ninth century, is credited with the first suture repair of a severed peripheral nerve though the results of his effort remain unknown (Figure 3). This approach to nerve injury was not, however, widely accepted. Ambroise Pare (1510-1590) wrote that a partially severed nerve should be totally divided to inhibit the likelihood of convulsions (Figure 4). Pare was not alone in his lack of understanding of the pathophysiology of nerve injuries. Virchow himself is quoted as saying "nerve gaps over 10 cm long may not completely regenerate, but in time, function return is sometimes unbelievable."[4]

Figure 3: Rhazes (841-926)

Figure 4: Ambroise Pare (1510-1590)

This hesitation to treat nerve injuries was not simply related to a lack of understanding of physiological principles. The main obstacle to the early surgeons was that of wound sepsis, with the gunshot wound being the worst offender. Firearms were introduced into Europe in the fourteenth century and their destructive force resulted in increased tissue damage and often fatal infectious complications. The use of these weapons in warfare was so common that by 1794 Hunter already observed that "Gunshot wounds are now becoming almost a distinct branch of surgery."[5] Thus, up until the mid nineteenth century, the usual approach to wounds with peripheral nerve injuries was to sacrifice the useless extremity.

The first significant advance in the total therapeutic approach to peripheral nerve injuries was that of Silas Weir Mitchell (1872).[6] Mitchell was a surgeon in the US Army during the Civil War stationed in the Philadelphia Hospital (Figures 5 and 6). The casualties were enormous and his writings dealt with the "vast collection of wounds and contusions of nerves, including all the rarest forms of nerve lesions of almost every great nerve in the human body."

He fully supported the direct repair of nerves with a properly applied end to end suture ligature made of fine wire or linen. He also noted that "nothing is more fatal to reunion of nerves than the formation in the wound of large amounts of matter".

In addition to Weir's understanding of neural healing, he also appreciated the important role the muscle played in the final result. He described the process of muscle atrophy including the muscle shortening and the blood supply diminution. To combat these problems, he succinctly stated:

> "we very early reached the conclusion that it was wiser in all cases, to apply to the muscle the stimulus of electricity rather than to leave it to itself... when once the nerve is repaired it finds the muscle in a far better condition to profit by this then could otherwise be the case."

Figure 5: Sketch of Civil War hospital ward

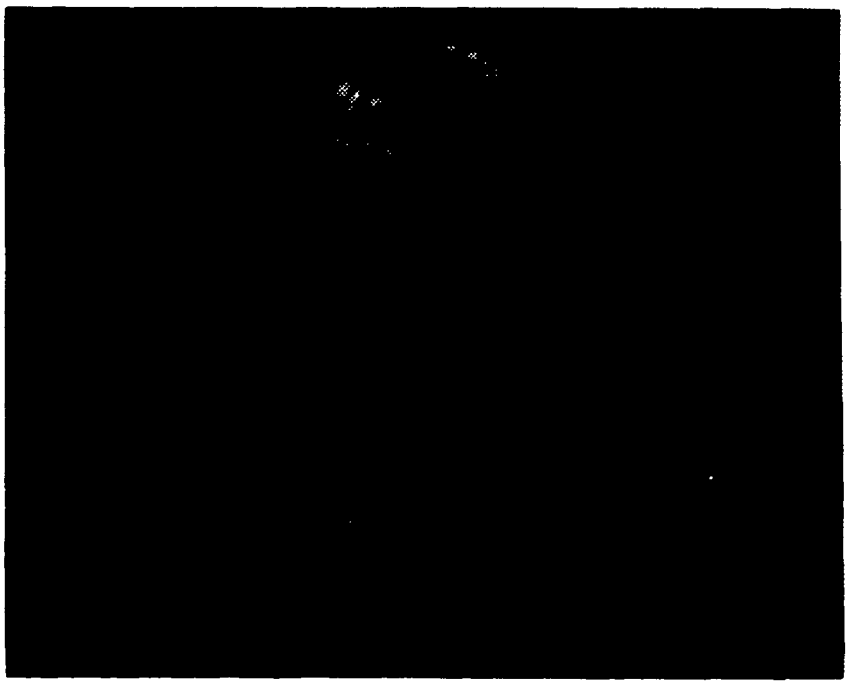


Figure 6: Photo of Silas Weir Mitchel (1829-1914)

The next great contributor to the field of nerve injuries was James Sherren (1908).[7] He also advocated the end to end suture of nerves (sterile catgut) but first he would trim the ends of the nerve transversely with a sharp scalpel; "Scissors should never be used for this purpose", he wrote, "their crushing action may prevent recovery." More important, perhaps, than his understanding of nerve repair was his clear recognition that the operation "is but one step in the treatment." This post operative care, he acknowledged, could be required for months or even years. Massage, movements, and electrical stimulation were part of this extensive care. He wrote "electrical treatment should be carried out whenever possible".

Robert Jones, a British surgeon in World War I, also realized the importance of post operative therapy in nerve injuries; he is credited the establishment of specific Orthopaedic Centres to handle the wounded soldiers. In this Centre, the peripheral nerve injury patients were segregated in a specific location and given complete physiotherapy and rehabilitation, including electrical stimulation therapy.[8]

With Carl Olaf Nylen's introduction of the microscope into clinical surgery in 1921, and Holmgren and Zeiss' design of the first binocular surgical microscope; the repair of severed nerve ends became more precise than ever. In addition, newer and finer needles and sutures were developed. The limiting factor in the success of peripheral nerve surgery became the availability and feasibility of the post operative care. Bristow, in reviewing the surgical experience of World War I and II, wrote in 1947 "the value of interrupted galvanism must be weighed against the economic factor - the loss of time and work during their attendance or in transit to hospital or clinic."[9] This remains the main deterrent to the widespread use of electrical stimulation in denervation injury, and the reason that in this project we are testing the feasibility of an implantable, autonomous ES system.

NEUROMUSCULAR FUNCTION

The interaction between nerves and muscles in the production of movement has long been an area of active speculation and research. In the nineteenth century, the scientists were in doubt as to where the property of contractility originated. The "Hallerians" maintained that the muscle was intrinsically capable of contraction while the "Neurologists" believed the contractile mechanism was confined to the nervous tissue.[10] Reid (1841) introduced the concept of chronic electrical stimulation (ES) of denervated muscle to address this question. He stimulated denervated frog muscle in his study and correctly concluded that while the action of voluntary contraction is dependent on an intact and functional nerve, the contractile mechanism itself was innate to the muscle. Because of this original work, and that of the aforementioned pioneers in wartime surgery, ES and its effects on neuromuscular activity continue to be focal points for researchers and clinicians. In the following section, the details of neuromuscular function will be outlined with reference to the researchers who have addressed the increasingly complex issues.

The Motor Unit and Muscle Fiber Types

Lidell and Sherrington first introduced the concept of the motor unit - the basic functional unit of the motor system.[11] It is comprised of the large anterior horn cell, its motor axon, and all the muscle fibers it innervates (Figure 7).

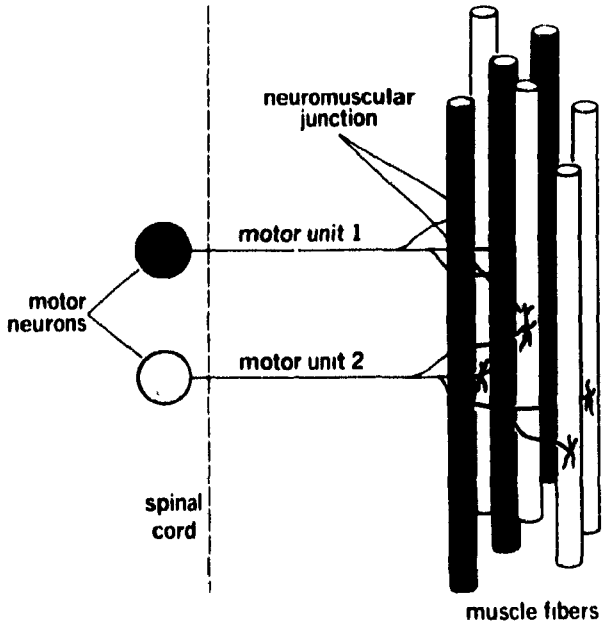


Figure 7: Schematic representation of the Motor Unit

The number of muscle fiber cells innervated by a given motoneuron is variable and depends on the function of the muscle. Neurons which control

muscles involved in fine movements tend to innervate only a few muscle fibers each. For instance, the extraocular muscles are believed to have a neuron to muscle fiber ratio as low as one to one. Larger and more powerful muscles, such as the glutei or quadriceps femoris muscles, have several hundred muscle fibers supplied by one neuron[12].

Apart from size, motor units also differ in mechanical, structural, and chemical properties; this is defined in terms of motor unit typing. Type 1 (type S, slow twitch) motor units are characterized mechanically by slow twitch contraction and one-half relaxation times; small twitch and tetanic force production; "sag" in non fused tetanic contractions; and a high resistance to fatigue (see later for formal definition of terms). Type 2 (type F, fast twitch) motor units are more rapid in their contraction and relaxation velocities; have a greater force production capacity; and do not sag in non-fused tetanic contractions. Type 2 fibers are variable in their resistance to fatigue and are thus further subclassified into fast, fatiguable and fast, fatigue-resistent motor units.[13] All the muscle fibers in a given motor unit are of the same type (Figure 8 and Figure 9).

Table 23–2 Characteristics of Predominant Motor Unit Types in Cat Medial Gastrocnemius Muscle

Motor Unit Type	S	FR	FF
Muscle fiber type	I	IIA	IIB
Muscle fiber size (cross-sectional area, μm^2)	1980	2370	5290
Tetanic tension (mN)	10	150	640
Twitch contraction time (ms)	50	25	24
Profile of unfused tetanus	nonsagging	sagging	sagging
Fatigue index*	1	>0.75	<0.25
Innervation ratio	550	500	700
Specific tension (N/cm^2)†	6	22	24

After Burke, R.E. *Handbk. Physiol.* Sec. 1, 2(1):345–422, 1981.
*Force after 2 min stimulation/initial force.
†Tetanic tension normalized to muscle fiber cross-sectional area.

Figure 8: Structural and Contractile Fiber Type Features

These broad, mechanical features used to classify fiber types are a reflection of their histological and chemical variation. For instance, type 2 muscle fibers have the greater cross sectional area and this would in part explain their increased force production. As well, they have a more abundant supply of glycolytic enzymes and fewer mitochondria, resulting in a reduced capacity for endurance work. The presence of a distinct "fast" myosin ATPase, which can be selectively stained for in light microscopical analysis, is a key contributory factor to the increased speed of type 2 fiber contraction and relaxation. Type 1 muscle

fibers are smaller, predominantly oxidative, and have a "slow" myosin ATPase which can also be selectively stained for.[14]

Table 23–1 Profiles of Predominant Muscle Fiber Types

Fiber Types	I	IIA	IIB
Histochemical features*			
Myofibril ATPase (pH 9 4)	Low	High	High
NADH dehydrogenase	High	Medium-high	Low
Succinic dehydrogenase	High	Medium-high	Low
Men-ɑ-GPD	Low	High	High
Glycogen	Low	High	High
Phosphorylase	Low	High	High
Neutral fat	High	Medium	Low
Biochemical features†			
AM ATPase, μmol/min/mg (pH 9 4)	0 04	0 16	0 27
Lactate dehydrogenase, μmol/min/g	105	220	450
Succinic dehydrogenase, μmol/min/g	2 0	2 5	0 7
Hexokinase, nmol/min/g	980	620	300
Myoglobin mg/g	1 4	1 4	0 3
Morphology*			
Diameter	Small	Medium	Large
Capillary supply	Rich	Rich	Sparse
Z lines	Wide	Narrow	Narrow
Mitochondria	Rich	Moderate	Sparse
SR and t-tubular system	Least	More	Most

After Burke, R E *Handbk Physiol* Sec 1, 2(1) 345 422, 1981
*Cat
†Guinea pig
Am actomyosin, Men-ɑ-GPD menadione linked ɑ glycerophosphate dehydrogenase, SR sarcoplasmic reticulum

Figure 9: Histological and Biochemical Fiber Type Properties

The muscle fibers (cells) are the functioning contractile components of specific muscles. Unlike other mammals, human muscles are all composed of

both type 1 and type 2 muscle fibers in nearly equal proportions. Some human muscles have an imbalance in the ratio (eg soleus is 60 - 80 % type 1) but this is the exception rather than the rule.[15]

Motor Unit Differentiation

While researchers may agree on the descriptive features that characterize muscle fiber types, the cause of this variation is a much debated topic. Specifically, the contribution that the neuron makes to motor unit variation and differentiation, as opposed to the role of muscle activity, is an area of active speculation.

It was Eccles et al. in 1958[16] who suggested that muscle fiber type is dictated by the neuron. Cross-innervation experiments demonstrated that when nerves to fast and slow muscles were cross transplanted, the muscle properties changed according to the new nervous supply. Davis and others believe that there are specific neurotrophic factors which are responsible for this transformation. Other explanations are, however, possible. Fast and slow motoneuron generate different patterns of impulses (Fishbach and Robbins,1969; Hennig and Lomo, 1985)[17], and Vrbova (1963) proposed that the impulse activity imposed on the muscle via the motor nerve (and not simply the neural presence) dictated the muscle fiber type.[18] Evidence supporting this comes from experiments in which fast muscles became slower when their nerves are stimulated chronically by a large number of stimuli at 10 Hz (slow pattern, Salmons and Vrbova, 1969; Pette et al, 1973)[19], and slow muscle became faster when stimulated by brief trains of stimuli at 100Hz (fast pattern, Smith, 1978; Hennig and Lomo, 1987). Hence, the frequency (measured in hertz) of stimulation, and not a specific neurotrophic substance, was postulated to be the key factor in determining muscle fiber type.

In the above experiments, however, the relative importance of impulse activity versus neurotrophic substances was not clarified because the muscles in all cases were subject to both activity and neural influences. To circumvent this problem researchers have performed experiments on denervated muscle in which the activity was artificially induced by exogenous electrical stimulation (Lomo, 1974,1988; Westgard,1980; Nix,1985; Gunderson,1987)[20]. This approach is similar to that of Reid in 1841.

The results of these experiments remain controversial. While researchers such as Lomo, Westgard and others, believe that muscle fiber type is mainly a function of the frequency of stimulation, be it neural or electrical in origin, others have different interpretations. For them, the total aggregate activity (the number of contractions per day) is believed to be the most important factor in determining the muscle fiber type.[21]

This issue will be discussed further in the "Muscle Preservation", "Stimulation Parameters", and "Discussion" portions of this presentation.

DENERVATION INJURY

Classification

Seddon has classified nerve injuries into three types based on the pathophysiology.[22] Neuronopraxis is a form of compression injury whereby there is no gross anatomical destruction of the nerve structure but the axons are unable to generate membrane potentials. The result is a temporary functional deficit and spontaneous recovery usually occurs within six weeks of the injury.

Axonotmesis occurs when there is both axonal and myelin loss, but the endoneurial connective tissue scaffolding of the nerve remains intact. Distal to the lesion, there is atrophy and destruction of the axons, but proximally the nervous tissue remains viable. This is referred to as wallerian degeneration as it was first described by Waller in 1852.[23] Spontaneous recovery is expected because the axons grow into the preserved distal endoneurial sheaths. No active therapeutic intervention is required.

In neurotmesis, the peripheral nerve is completely severed. If this is a proximal injury, recovery of normal muscle function will only occur if a surgical reapproximation is performed. This is the type of injury that was studied in this work.

Nerve Repair

The goal of nerve repair is to construct a juncture across which regenerating axons can advance. This can be done in any of three ways. In the whole nerve repair, the external epineurium is approximated (Fig 10A); in the grouped fascicular repair, the internal epineurium is sutured (Fig 10C) .[24] Also, the individual fascicles could be separately approximated (Fig 10B).

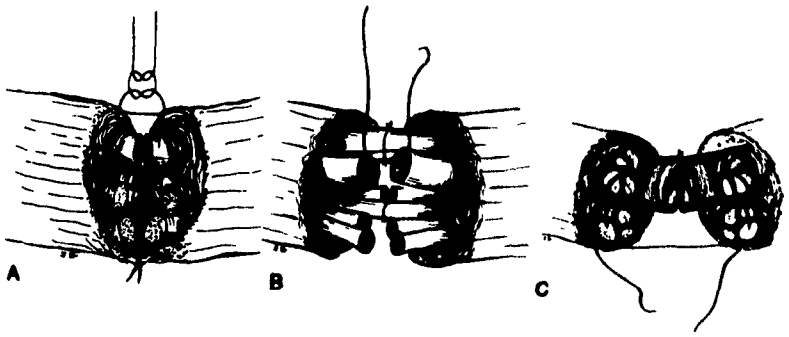


Figure 10: Nerve Repair Techniques

While there are researchers investigating the feasibility of fibrin glue and Lasers for neural anastomosis, the current clinical golden standard is the nerve suture technique.

Nerve repairs should occur at the earliest possible time following injury. When the nerve has been sharply transected in a clean fashion, this can be done immediately and is referred to as a primary nerve repair. If contamination is suspected, the wound can be cleaned and debrided and nerve repair can most safely be done three to four days after the injury; this is termed a delayed primary closure. There are circumstances, such as when significant crushing or stretching has occurred, when it is best to clean, repair, and stabilize the surrounding tissue and return in four to six weeks for the nerve repair; this is termed a secondary nerve repair.[25] In this experiment, a primary, whole nerve repair was performed.

Nerve Regeneration

In axonotmesis or repaired neurotmesis injuries, neural regeneration will occur in a proximal to distal direction. The axons will require about three weeks to cross the suture line and thereafter will grow at a rate of about 1 mm per day.[26]

In the normal situation, muscle fibers of a given motor unit have a scattered distribution within the muscle; following neuronal injury, each axon regenerates to a specific region and thus the muscle fibers of a motor unit are all adjacent to each other. This is referred to as type grouping.[27]

Regenerating axons are most apt to establish synapses at the site of the original end plate. Innervated and functionally active muscles do not tend to accept further innervation; that is, each muscle fiber is contacted by only one axonal terminal.[28]

Nerve Regeneration and Electrical Stimulation

Therapeutic electrical stimulation (ES) in denervation injury results in an active muscular contraction in the absence of any neuronal input. An important physiological and clinical question is whether this exogenous stimulation interferes with the reestablishment of functional synaptic contacts. There is no general consensus on this point.

The following researchers are among those who emphasize the negative impact of ES on neural regeneration. Frank noted that decreased neuronal activity was more conducive to an increase in the axon terminal arborization size and the number of synaptic connections.[29] Cohan observed that low frequency ES reversibly arrests the growth of neurite in isolated neuron cultures.[30]

Schimrigk[31] and Michel[32] are two others who support the idea that reinnervation and subsequent muscle recovery may be delayed by ES.

Alternatively, Jansen et al have demonstrated that ES which completely blocked the development of denervation induced ACh hypersensitivity, impaired foreign, but not self, reinnervation.[33] Hoffman showed by histologic techniques that after partial denervation, ES of intact neurons accelerated the growth of nerve sprouts from intact axons.[34] Herbison performed rat muscle denervation experiments and showed ES was found to neither enhance or impair reinnervation.[35]

To study the effect of ES on reinnervation, we performed a quantitative analysis of force production in the stimulated and the nonstimulated groups.

CHARACTERISTICS OF DENERVATED MUSCLE

Denervated muscle is substantially different from normal muscle. Clinically, one can readily observe the loss of voluntary and reflex activity. This is followed by a progressive muscle atrophy over the ensuing weeks and months. These obvious consequences of denervation are only a small reflection of the profound morphological, biochemical, physiological and mechanical transformation that is occurring.

Muscle Morphology

The most obvious morphological alteration in denervated muscle is its diminution in size. This muscle fiber atrophy is caused, in part, by the loss of its myofilamentous elements (actin and myosin). This can be quantified in the experimental setting by using either muscle weight or, preferably, microscopic muscle fiber area measurements.

Sunderland reviewed the literature on this subject and he concluded that a denervated muscle would lose about thirty percent of its initial weight in the first month, 50 to 60 percent by the end of the second month, and then a plateau loss of 60 to 80 percent would be reached by four months.[36]

Microscopic muscle fiber area measurements parallel these weight changes, and one observes a decreased muscle fiber area and a proliferation of interfascicular connective tissue as the denervation period progresses. The cross sectional view demonstrates small fibers that are acutely angular in shape rather than the normal polygonal configuration.[37] In the final stages of degeneration, all the contractile myofibrils may be lost and only tubes of endomysial connective tissue, containing degenerating chains of nuclei, remain. Such fibers are present in human muscle after one year of denervation.[38] Some contend that there is a

preferential atrophy of the Type II muscle fibers in this setting, but this is not a universally accepted concept.[39]

On electron microscopy, mean fiber area decreases (by 80% over three weeks in rats). Mitochondria become smaller in size over time; and instead of maintaining their normal transverse orientation, they reposition in a longitudinal direction. The sarcotubular components become increasingly irregular in distribution and relatively more abundant.[40] Also, the motor endplate flattens out; and the postjunctional folds decrease in amount and size, and become more irregular in appearance.

It was Hines and Knowlton who first correctly observed that the degenerative muscular changes in denervation injury are species specific. High metabolic rate, short lifespan species (eg rats) show more rapid atrophy than larger animals and humans.[41]

Muscle Biochemistry

Denervated muscle is known to have a decreased rate of DNA and protein synthesis as well as an increased rate of protein degradation.[42] This results in reduction in the muscle's structural protein content and a decrease in the activity of the glycolytic and oxidative enzymes.

In addition there is a proliferation of extrajunctional ACh receptors. This results in a muscle hypersensitivity, an effect first seen by Brown in 1937 when muscle contractures were observed in chronically denervated muscles in response to extremely small doses of ACh.[43] In normally innervated muscles these receptors are only localized in the endplate region. In developing and denervated muscles, however, we see this diffuse scattering of these receptors over the entire surface of the muscle membrane.[44] As well, there is a rapid loss of

acetylcholinesterase in the motor endplate region. This enzyme is responsible for the degradation of the acetylcholine neurotransmitter.[45]

Muscle Physiology

The earliest detectable change after denervation, first identified by Albuquerque in 1971, is a fall in the resting membrane potential.[46] The cause of this unknown, though it occurs approximately six hours after denervation and prior to any quantifiable reduction in neurotransmitter release.

In addition to this early membrane alteration in denervation, the contractile properties of the muscle are also abruptly transformed. Specifically, a prolongation of the action potential develops. This results in an increased intrafibrillar calcium which is presumably responsible for the prolongation of the twitch contractions observed in denervated muscle. The calcium uptake by the sarcoplasmic reticulum also decreases with denervation and contributes to the prolonged contraction and relaxation times.[47]

The development of fibrillation potentials can also be observed, usually within two or three days of denervation. These are spontaneous uncoordinated rhythmic twitchings of the muscle fibers that are only apparent if the muscle is visibly exposed or if EMG electrodes are applied (Figure 11). Early investigators thought these fibrillations caused muscle fatigue and facilitated the atrophy process; this assumption has subsequently proven to be false.[48]

Muscle Mechanics

As a consequence of the aforementioned morphological, biochemical, and physiological alterations, the mechanical, contractile properties of denervated muscle change as well.

In lieu of the muscle atrophy, it is not surprising that the forces of the twitch and tetanic tensions decrease in denervated muscle. Also, the contraction and relaxation phases of the twitches become slower; some believe that this only occurs in the Type II muscle fibers while others claim that the same holds true for the Type I fibers as well.[49] Muscle atrophy, force, and fiber type preservation are all specifically addressed in this project.

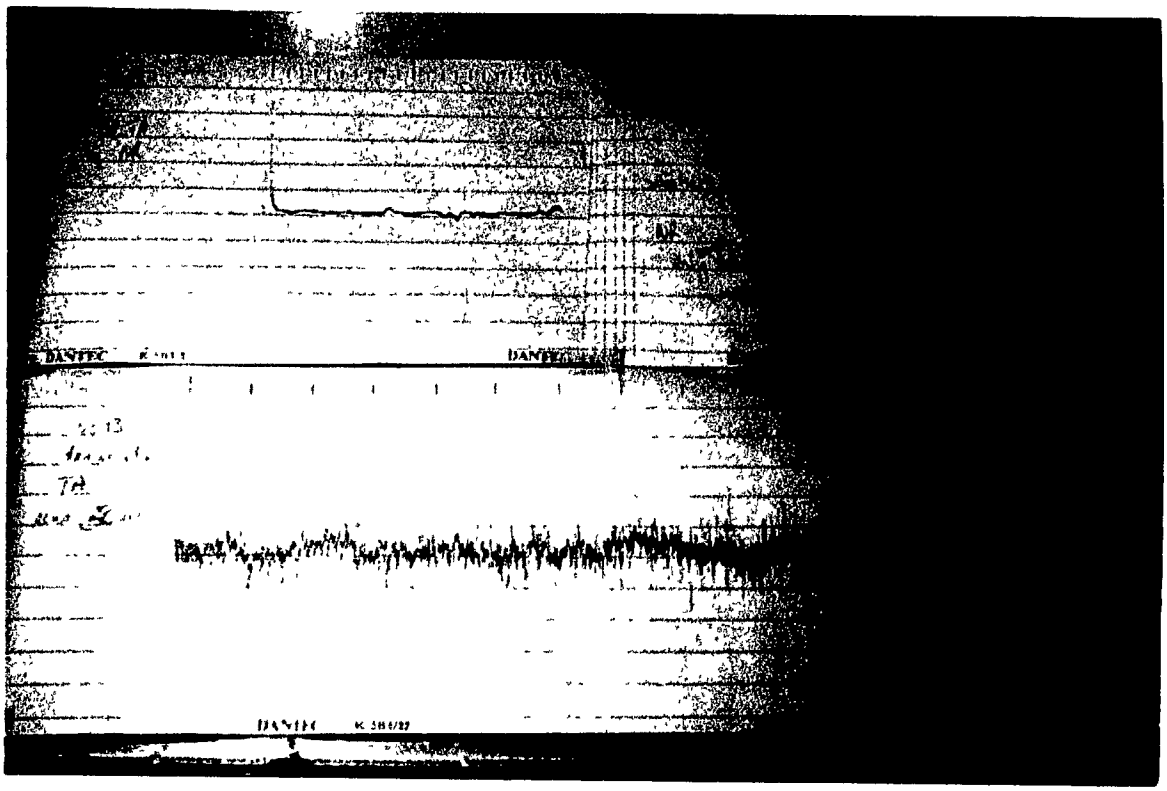


Figure 11: Lower tracing shows denervation fibrillation potentials

MUSCLE PRESERVATION

Even when reinnervation does occur following nerve injury, the muscle will be downgraded to a decreased functional capacity if too much time has elapsed. To combat this problem, and to further the understanding of neuromuscular interaction, researchers have been interested in the feasibility and dynamics of muscle preservation. The two major consequences of denervation are the absence of any neurotrophic influence and the absence of muscle activity; both play a role in muscle preservation.

Neurotrophic Substances

The concept of a neural influence on muscle integrity, which is activity-independent, was suggested by Tower in 1935. She observed that the atrophy in paralyzed muscles in paraplegic muscles (neural contact still established) was not as great as the atrophy in denervated muscles.[50]

This neurotrophic element was best defined by Gutmann (1976) as "long-term maintenance regulations not mediated by nerve impulses".[51] The evidence for its role in muscle preservation comes from both nerve stump and axoplasmic blockade studies.

Several investigators have established that the normal physiological consequences of denervation can be altered by the length of the nerve stump attached to the denervated muscle. Albuquerque showed that the normal drop in the resting membrane potential seen after denervation injury decreased more rapidly if the nerve was cut near to the muscle rather than far from it.[52] Luco first demonstrated that muscle fibrillations and ACh supersensitivity were also nerve stump length dependent.

The axoplasmic flow studies support the nerve stump studies in demonstrating the important neural role. The specific blockade of this neural transport mechanism (by giving vinblastine or colchicine) causes denervation type changes even though an action potential can still be propagated. In particular, one can observe Ach supersensitivity and a fall in the resting membrane potential.[53]

Another method of demonstrating the importance of neurotrophic substances was that of Davis who showed that by injecting nerve extract into denervated muscle, there was a relative sparing of muscle weight and total protein content. She noted that almost 50% of the type IIB fiber atrophy was prevented by this method.[54]

While the importance of neurotrophic substances in muscle preservation is well accepted, the exact compounds themselves have never been precisely identified. Also, a practical system of delivery of these substances to the denervated muscle has never been developed. For these reasons, the optimization of electrically induced muscle activity remains the most important tool in the experimental and clinical setting in muscle preservation.

Muscle Activity

The rationale for stimulating denervated muscles is obvious: an exercised muscle is stronger and healthier in appearance than an inactive one. Since the muscle cell is intrinsically capable of propagating an action potential and generating a contractile response, only the initiation of the action potential is lacking in denervated muscle. This can be provided by the use of direct electrical stimulation (ES) of the denervated muscle.

The use of ES for therapeutic purposes has a colorful and elaborate

history. Scribonius Largus described the use of the torpedo fish as a treatment for chronic headache and gout in 46 AD. With the construction of the electrostatic generator and the Leyden jar (electrical charge storage device), electrical technology gained a widespread distribution (Figure 12). At the height of its popularity, ES was used therapeutically for such varied problems as angina, epilepsy, kidney stones and hemiplegia. In fact, by 1900 most physicians had some form of ES apparatus as part of their therapeutic armamentarium.[55]

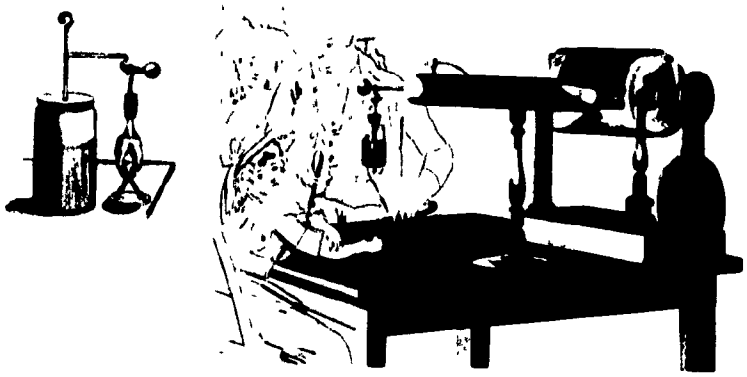


Figure 12: Leyden jar (left) and patient with denervated muscle being stimulated with the device

The first experimentation that addressed the relative merits of ES in denervated muscle were those of Reid in 1841. He wanted to establish if the deleterious effects of denervation were "dependant upon inaction, or upon any supposed nervous influence flowing along the nerves to the muscles". In his two month, denervated frog muscle experiment, he exercised one group of muscles with ES (galvanic, weak current) while the other group was not stimulated. He concluded that "the muscles of the exercised limb retained their original size and firmness, and contracted vigorously, while those of the quiescent limb had shrunk to at least one-half of their former bulk".[56]

These results were supported by Brown-Sequard (1853) who claimed that daily galvanic stimulation of various denervated mammalian and bird muscles produced a gradual but complete restoration of the bulk of the muscles.[57]

Further laboratory work was reported by Langly beginning in 1915. Working with denervated rabbit muscle, he demonstrated improved muscle weight and bulk with ES and a prevention of the development of muscle fibrillations. He incorrectly postulated that the muscle fibrillations were the cause of the muscle fatigue and the other deleterious effects of denervation.[58] It was Fischer (1939) who reemphasized the training effect of stimulation and dismissed the notion that fibrillation caused fatigue and was harmful. He was the first to demonstrate that muscle power was augmented by fatiguing exercise.[59]

Gutmann and Guttmann expanded the study of ES of denervated muscle by also assessing fiber diameters, connective tissue changes, and time to onset of recovery. Histologically, they found that the stimulated muscles were larger and contained less connective tissue; and that the rate of reinnervation (determined by the return of reflex or voluntary activity) was neither hastened or retarded by the treatment. Thus it was well demonstrated that muscle inactivity played an essential role in muscle atrophy, and adequate galvanic exercise could retard the

rate of this process.[60]

Gutmann and Guttmann also emphasized the fact that ES is not a uniform entity; there are several variables to control. In their study, treatment duration, number of treatments per day, and the interval between the injury and treatment implementation were studied. Among other things, they found that twenty to thirty minutes of treatment begun as early as possible was the optimal treatment regime.

The more recent studies on muscle preservation with ES have focused on the effects of altering the parameters of ES in order to optimize the potential gain. This will be discussed in the following section.

MUSCLE STIMULATION PARAMETERS

When stimulating normally innervated muscle with ES, it is the neurolemma of the axon, and not the sarcolemma of the muscle, which is being activated. In ES of denervated muscle, it is the sarcolemma alone which is being exited. The parameters in ES research have been extensively studied in both denervated and innervated conditions in order to define the influence that different activity patterns have on muscle physiology.

The frequency, on/off time, amplitude, pulse duration, waveform type, contraction type, electrode design, and the duration of therapy are the major electrical parameters of interest. In the following section, these parameters will be examined separately and their relation to internal and external stimulation techniques will be discussed.

Frequency

The frequency of a current is the number of waveforms per unit time; its unit of measurement is the Hertz (Hz) or pulses per second (pps). If the voltage is sufficient, a stimulus of 1 Hz will result in a muscle twitch. Increasing the frequency will result in a summation of the twitches and ultimately a smooth muscle contraction will occur (at 25 to 35 Hz).[61] Also, increasing the frequency will, to a point, decrease the voltage of current required to elicit a twitch contraction, and augment the power of a tetanic contraction.

In 1947 Kosman studied the tension developed by different frequencies using supramaximal stimulation (.5 Hz to 500 Hz). He concluded that the optimal value lies between 25 and 100 Hz; greater or lesser values exert progressively decreasing effects on tension development.[62]

According to many authors, though, stimulating muscle at 10 to 25 hertz is much different than stimulating muscle at 100 hertz. Hennig and Lomo measured the natural frequency of discharge of both fast and slow muscles in 1985.[63] They observed that slow muscles are stimulated by neurons with low native frequencies (15 hertz), while fast muscle motoneurons have a higher stimulation frequency rate (100 to 150 hertz). Using these measurements as their template, Staron (1987),Gundersen, and Lomo (1988)[64] all attempted to manipulate the fiber type composition of the rat soleus (slow) and extensor digitorum longus (fast) muscles. They found that denervated soleus muscle could be completely transformed to fast muscle by using high frequency stimulation; and the EDL could be partially transformed in the slow direction by using low frequency stimulation. Also, both muscles maintained their native fiber type characteristics if they were stimulated by their native frequency pattern after denervation.

Numerous previous studies have supported the dominant role of frequency in determining and maintaining the muscle fiber type. Fast-twitch muscles were seen to contract more slowly (longer contraction and half relaxation times) when stimulation was delivered at slow frequencies (5-15 HZ) in studies by Salmons and Vrbova (1969), Brown et al (1976), Pette and Vrbova (1985), and others. Likewise slow-twitch muscles became faster when stimulated at a fast frequency (100 Hz) in studies by Smith (1978) and Lomo (1985).[65]

These results, however, are not without their detractors. Al-Ahmood emphasizes the point that denervation alone will result in a slow to fast transformation in slow muscle (1987).[66] Also, there is now some evidence that supports the concept that it is the total aggregate activity, rather than the frequency at which it is delivered, that determines a muscle's contractile characteristics. Specifically, fast muscle will acquire slow features if it has an overall increase in contractile activity regardless of frequency.[67]

Thus, the role of frequency in determining fiber type is an unresolved issue yet its relevance is obvious: muscle preservation and muscle fiber type preservation are synonymous in the clinical setting. Choosing the optimal frequency and aggregate activity will best preserve the muscle's native fiber type and thus its force, speed, and endurance. This issue was addressed in this project by using a fast frequency (85 Hz) to stimulate the predominantly fast tibialis anterior muscle.

On/Off Time

The on/off time of a stimulation regime is the amount of time the muscle is being stimulated in relation to its rest period. The goal is to optimize exercise duration without inducing muscle fatigue.

In 1947 Kosman noted that "In the past several years experimental evidence has accumulated that the more frequently a denervated muscle is stimulated the better is the maintenance of its weight and strength."[68] This point was experimentally demonstrated by Baker with implanted electrodes and by Kots, using the Russian neuromuscular stimulator; a 1 to 5 on/off ratio was shown to be effective.[69] Baker supports the use of a 1 to 3 ratio but cautions that a more than 1 second on time is usually required to create the desired motor response. For instance, 4 seconds on to 12 seconds off would be appropriate.[70]

Davis was critical of the merits of ES for precisely this reason. She stated in 1983 "many of the ameliorative effects [of stimulation] were obtained only with amounts of stimulation which greatly exceeded those used clinically."[71] This sentiment is supported by Speilholtz who notes that the amount of stimulation delivered in most clinical studies still doesn't approach the level of exercise of a normally innervated muscle.[72]

Therefore, to reproduce the experimental benefits of ES in the clinical milieu, only an implantable system can provide the appropriate therapeutic on/off times. In this work, an implantable stimulator was used to deliver twenty four hour per day stimulation with an on/off time of 1.5/24 seconds.

Amplitude and Pulse Duration

In ES therapy, the goal is to obtain the optimal contraction with the least amount of energy possible. This is important in that it promotes patient comfort and reduces the power requirement of the battery device.

It has been determined that sine-waves are a less efficient way of stimulating excitable tissue than rectangular waves.[73] In order for a rectangular wave to activate muscular contraction, it must have an adequate amplitude and

pulse duration is the length of time permitted for current flow in one waveform.[74] Their physiological interrelationship was first described in terms of rheobase and chronaxie by Adrian in 1917.[75]

Rheobase is the lowest current intensity required to excite muscle or nervous tissue at an infinite pulse duration. The value of rheobase is about equal for nerve and muscle stimulation.

Chronaxie is the minimum pulse duration necessary to activate excitable tissue at twice the amplitude of rheobase. This value is very different for nerves and muscles; in fact, the pulse duration required to activate muscle directly is approximately two orders of magnitude greater than that used to activate muscle through intact nerve (Figure 12).[76] Over 50 years ago, Rushton demonstrated that a pulse duration of 100 msec or longer is required to activate the frog muscle membrane directly.[77] For humans, intact healthy nerve has a chronaxie of .03 msec while that of denervated muscle is 10 msec.[78]

Rheobase and chronaxie are not static properties of a muscle and it has been shown that the rheobase of a muscle decreases after denervation while the chronaxie increases from its normal value of 3 msec to 10 msec after 28 days.[79]

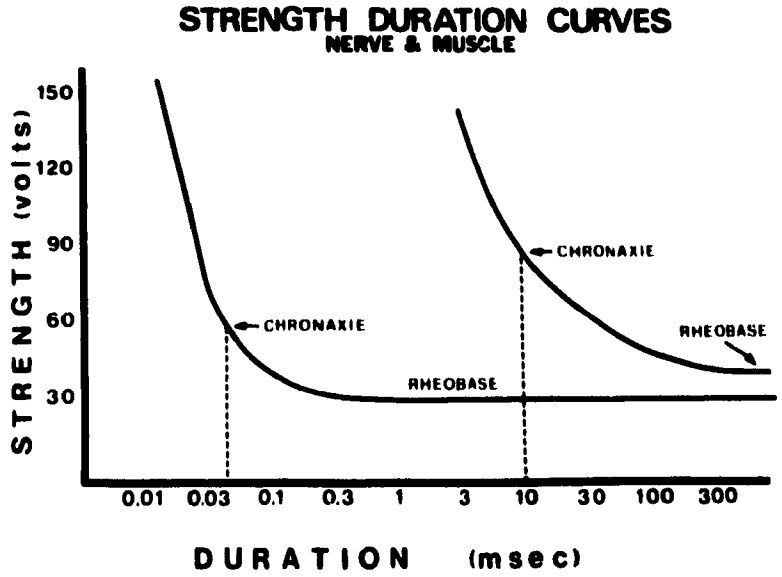


Figure 13: Muscle and Nerve Strength Duration Curves

The pulse duration will influence the voltage amplitude required to activate the nerve and muscle tissue. As pulse duration becomes shorter, the amplitude required to elicit a motor contraction becomes greater. This can be graphically depicted by the strength-duration curve in which the time (pulse duration) of the electrical stimulus is plotted against the amplitude of the stimulus required to produce a threshold response (muscle twitch).

In transcutaneous ES clinical trials, it has been shown that longer pulse durations (greater than .5 msec) are more uncomfortable than shorter ones. Too short a pulse duration, however, would necessitate a higher voltage amplitude and this too would be uncomfortable. Thus for both variables one is limited with transcutaneous ES.

ES applied directly to the muscle (implantable ES) requires a much lower stimulating voltage in order to achieve a contraction than transcutaneous ES.[80] This shifts the strength duration curve to a more energy efficient location and thus promotes patient comfort and reduces the battery requirement.

We utilized a battery source which was capable of delivering a maximal pulse amplitude of 10.5 Volts and a pulse duration of .45 msec.

Waveform

As was the case in the preceding description, the choice of waveform in ES of denervated muscle is determined by patient comfort and energy conservation.

Almost any waveform type can be created by the modern electrostatic generators. The three most common are the sine wave, (Figure 13A), the spike wave (Figure 13B), and the rectangular waveform (Figure 13C).

The sine wave and spike waves are not ideal for muscular stimulation because they allow no flexibility in the variation of pulse duration relative to the frequency; an adequate pulse duration is a critical factor in achieving muscular activation in denervated muscle. One would, therefore, require much more stimulus power to produce a given level of tension with non rectangular waveforms.[81]

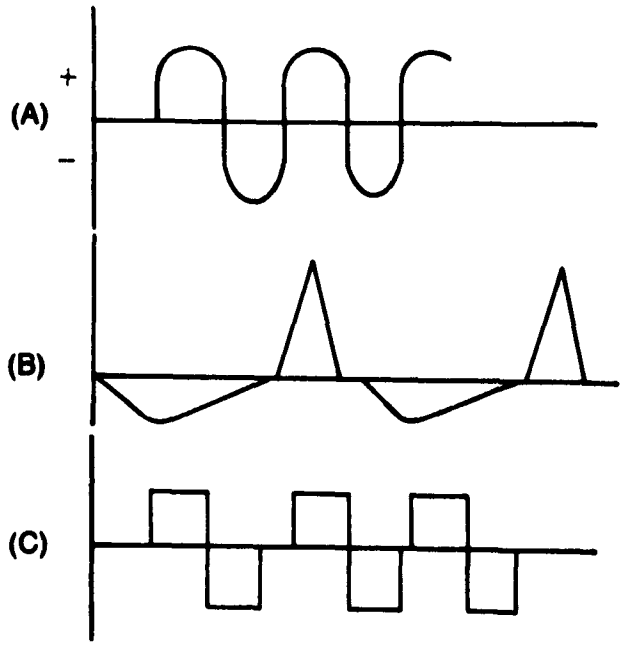


Figure 14: Sine wave (A), Spike wave (B), Rectangular wave (C)

Rectangular waveforms will result in muscular contraction with only moderate voltage outputs. They can be delivered in a monophasic or biphasic configuration. The monophasic form continually passes current in the same direction and has the disadvantage of causing polarization under the electrodes because of unequal ionic flow. Biphasic waveforms allows current flow in both directions during the respective alternating cycles, and thus both electrodes are activated in turn. This eliminates the polarization problem; additionally, this is reported by patients to be more comfortable than the monophasic option.

Another important consideration is defined by the Law of Dubois-Reymond which states that in order to electrically stimulate a nerve to contract, there must be a sudden variation in current flow. If the current amplitude has a

lesser rate of rise, the nerve will accommodate and require a greater voltage to reach threshold. Muscle fibers have far less of a tendency to accommodate in this fashion.[82] The rectangular waveform was used in this project.

Power Source

Electrical stimulators may provide constant voltage or constant current. The constant voltage source delivers the same wave shape and voltage amplitude regardless of changes in tissue or electrode impedance. If increased electrode impedance develops, the voltage would have to be adjusted upwards by the operator to accommodate for this decrease in effective current flow.

Constant current generators maintain the same current waveform regardless of the impedance. Thus, if increased tissue or electrode impedance developed, a greater power output would be automatically delivered. A voltage limitation setting is required to avoid excessive amplitudes which could cause tissue destruction.

According to Baker[83],there is no data to support a preference for one stimulus source over the other. The required intensity of the power output is mainly determined by the size of the muscle: the larger the muscle mass to be stimulated, the larger the electric field that is needed.[84] A constant voltage device (with the previously mentioned limits) was used for this research.

Electrode System

When deciding on the electrode system to use in ES, the variables of interest are electrode position (internal versus external) and size.

The externally located electrode would be the ideal form of stimulation

because it doesn't require any surgical intervention. Unfortunately, this type of stimulation has not gained therapeutic acceptance. The time course required for neural regeneration to occur in peripheral nerve injury can be from several months to over two years (depending on the site of injury), and daily visits to a treatment center for this length of time is simply not feasible. Also, even if such a program was embarked upon, the duration of treatment on any given day would be limited to thirty to sixty minutes at best. An internally implanted electrode and battery system would not be subject to this limitation.

Another factor which favors the internal delivery system over an external one is related to electrical charge. Charge is dissipated when delivered at a distance from its target. Therefore, a greater voltage and pulse duration would be required to stimulate muscle with external electrodes. Hultman estimated that the external voltage requirement is tenfold greater than the internal voltage requirement.[85] The fact that this increased charge is delivered directly to the skin in external stimulation, means that there would be increased patient discomfort as well.

Internal electrodes have the added advantage of specificity. For instance, while the small intrinsic muscles of the hand cannot be accurately stimulated externally, internal stimulation can precisely localize these muscles to ensure accurate stimulation.

Other variables of interest in electrode positioning relate to polarity and muscle topography. Pfleuger's Law, established in 1858, states that a healthy muscle would contract with less current if stimulated with the cathode rather than the anode.[86] Trontelj observed that there are discreet sites in muscle fibers where the threshold for spontaneous or externally induced depolarization is considerably lower.[87] Therefore electrode placement alone can significantly impact the power requirements and the quality of the muscular contraction.

Another factor that is important in choosing the electrode type is the electrode size. Whether an electrode is externally or internally located, the smaller the electrode size, the lower the output voltage required to elicit the excitatory response. This is caused by the increased pulse charge density that one delivers with the smaller electrode with any given voltage.[88] This poses a further restriction on external stimulation techniques because increased pulse charge density causes increased cutaneous pain sensation; thus, in order to achieve patient tolerance a larger, less specific electrode (with a larger power source) would be required.

For this study, completely implantable electrodes, placed at the most sensitive stimulating location, were utilized.

PURPOSE

The current research project was undertaken to further assess the impact of our original design, totally implantable, electrical stimulation system in denervation injury. A practical, implantable, and potentially clinically applicable system such as this has never been tested or implemented outside of our laboratory.

The significant differences between this project and the previous work done in our lab are:

1 The duration of ES in this project (four months) is significantly longer than any previous work.

2 The muscle force measurements were made using a newly designed force transducer platform. This device enabled us to make accurate, in situ force measurements without any aberrant motion artifacts.

3 The muscle was studied when reinnervation had occurred, and both nerve-stimulated and muscle-stimulated twitch and tetanic tensions were measured.

4 Twitch contraction time, one-half relaxation time, and unfused tetanic contractions (the measure for the sag phenomenon) were specifically measured in order to correlate these functional measurements of muscle fiber type with the microscopic ATPase fiber type data.

5 The effect of ES on reinnervation was functionally assessed by comparing nerve-stimulated tetanic tension to direct muscle-stimulated tetanic tension after pharmacological neuromuscular blockade.

HYPOTHESIS

1 Continuous implantable electrical stimulation is a beneficial adjunctive treatment in denervation injury when used over the extended period of four months.

2 This beneficial effect can be demonstrated by using morphological and functional measurements in reinnervated muscle.

3 The high frequency, continuous stimulation regime will result in a sparing of the muscle's fiber type, as shown by morphological (fiber type proportions and surface areas) and functional (contraction times, one-half relaxation times, unfused tetanic contractions) measurements.

4 Electrical stimulation that is started before, and carried into, the reinnervation period has no adverse effect on the reinnervation of the muscle.

MATERIALS AND METHODS

In this experiment the effects of continuous implantable ES on muscle preservation were studied using a dog model.

CHRONIC EXPERIMENT:

At the time of the initial surgical intervention the dogs, weighing between 28 to 35 kilograms, were assigned at random to one of two experimental groups .

Group 1 Right hindlimb common peroneal nerve transected and repaired primarily; pulse generator implanted and stimulating electrodes applied to the tibialis anterior (cranialis) muscle; ES activated. Electrodes applied to left hindlimb tibialis anterior but no nerve transection and no ES (four animals).

Group 2 as above except after the nerve transection and repair on the right side, no ES stimulation was given activated (five animals). See Figure 15.

Anatomy

The common peroneal nerve is one of the two terminal branches of the sciatic nerve in the dog hind limb. It divides into two terminal branches: the superficial and deep peroneal nerves. The entire motor supply of the tibialis anterior muscle is derived from the deep peroneal nerve. The distance between the origin of the common peroneal nerve and its most proximal point of contact with the tibialis anterior muscle is about 18 cm.

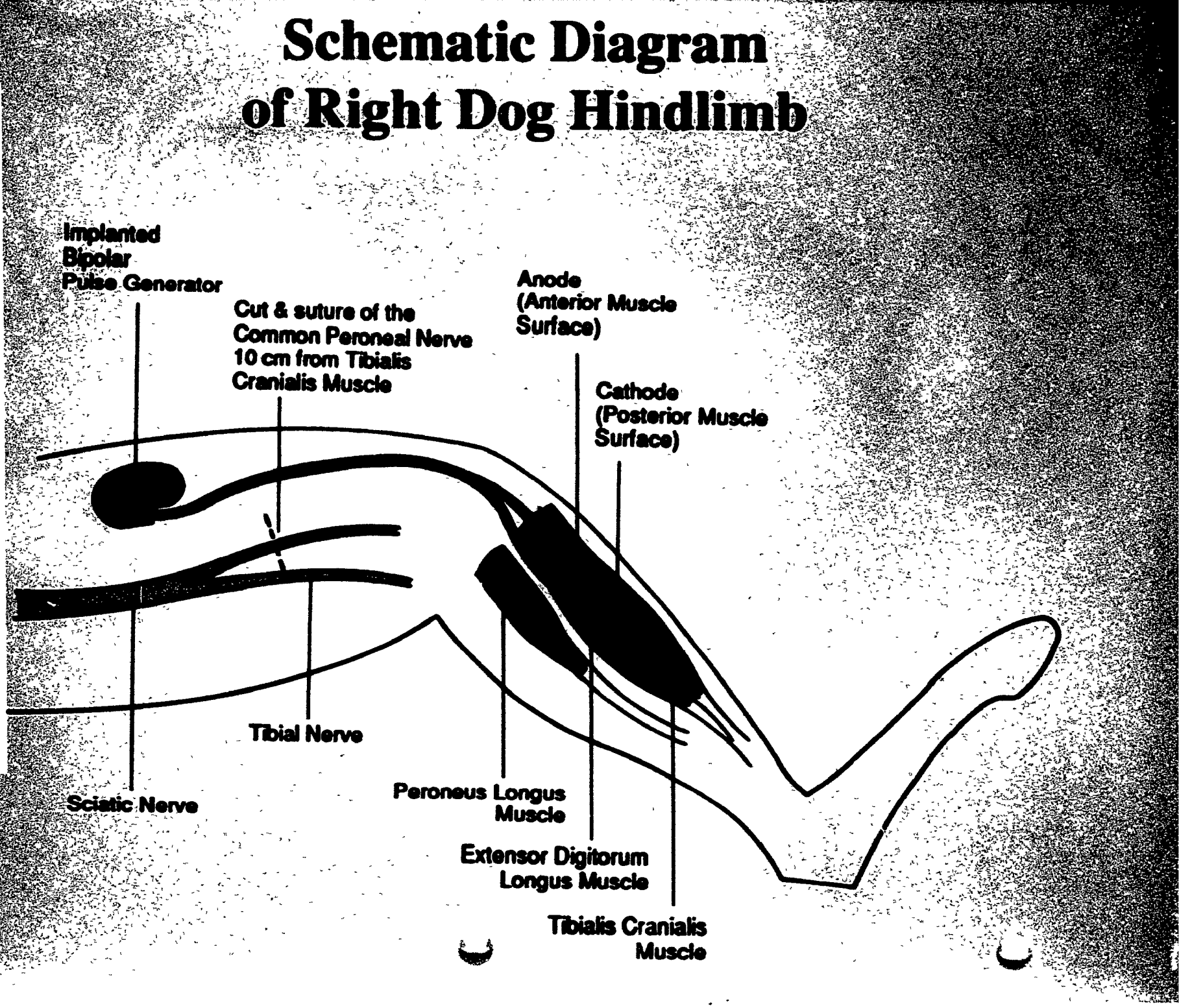


Figure 15: Schematic diagram of Anatomy and Experimental Design

The tibialis anterior (or tibialis cranialis) muscle is a superficial, strong, somewhat flattened muscle which arises from the cranial portion of the articular margin of the tibia, and from the laterally arched edge of the tibial crest. From this origin, which is about three cm wide, it passes over the craniomedial surface of the crus; near its distal third, it becomes a thin, flat tendon which inserts on the rudiment of metatarsal I and on the proximal end of metatarsal II). It functions as a rotator of the hindpaw in a lateral direction and an extensor of digit II.[89] See Figure 16.

Figure 16: Left hindlimb of dog with tibialis anterior muscle located anteriorly and extensor digitorum longus lying adjacent to it.

Equipment

The pulse generator used in this experiment was a Medtronic Model 7420 Bipolar ITREL Implantable Pulse Generator (IPG). This battery has two independent ports, and it is designed to deliver a constant voltage biphasic current. It is completely implantable and is programmable through the skin using the Medtronic Model 7431 ITREL Portable Programmer.

There were two electrodes (one for each port) used per muscle. Each electrode consisted of a distal carbon plate ending, and a proximal insulated wire region that could be plugged into the IPG. The plate region was silicone coated and flexible, measured one square cm, and was applied directly to the surface of the muscle. The wires were also flexible and silicone coated.

Surgical Procedure

All animals underwent the same initial surgical intervention. The operative site was shaved, 500 mg of cefazolin was given IV, general anaesthesia was induced with intravenous sodium pentobarbital (30 mg/kg), and intubation was performed.

A right thigh longitudinal incision was performed and dissection was carried down to the common peroneal nerve. Using the fibular head as the guide, the nerve was completely transected 8 cm away from this landmark (Figure 17). This corresponds to a distance of 12 cm from the most proximal entry point of the common peroneal nerve into the tibialis anterior muscle. A microscopic epineural repair was then immediately effected using an interrupted 10-O nonabsorbable suture. The overlying layers and skin were then closed in the usual fashion with an interrupted 2-O absorbable suture.

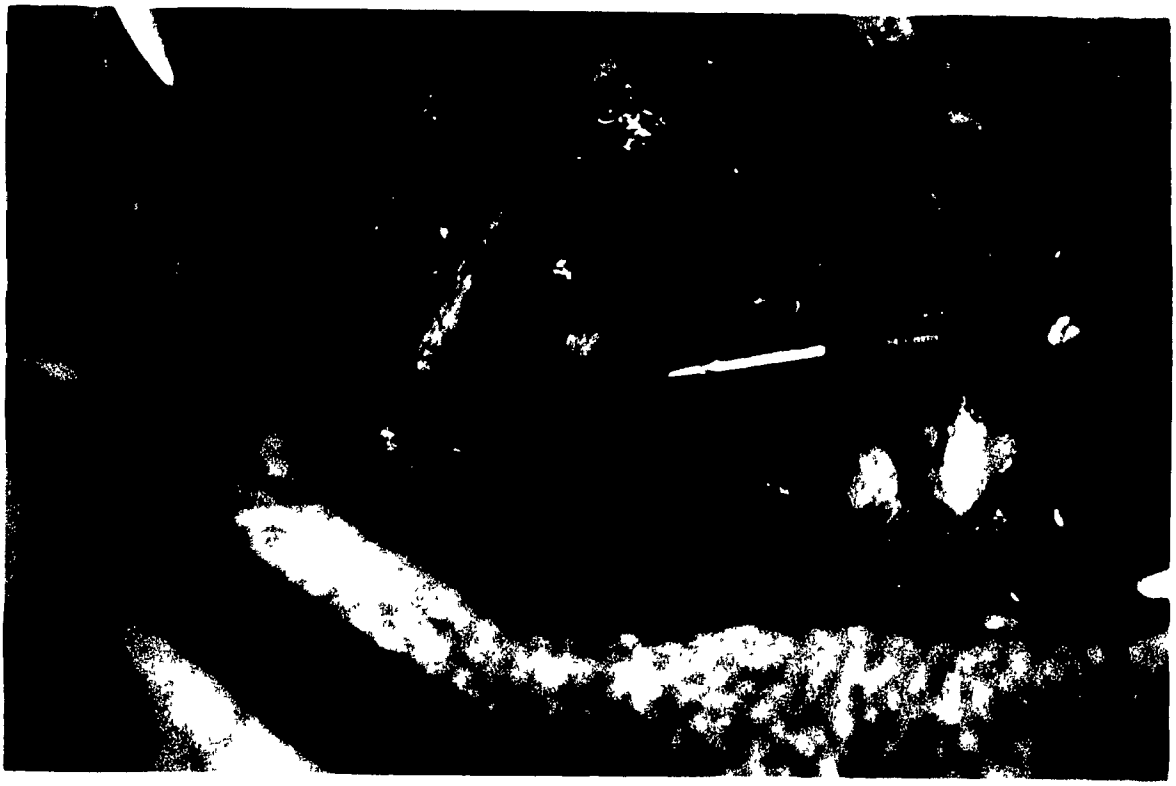


Figure 17: common peroneal nerve (smaller branch) and tibial nerve (larger branch being pointed at)

Next, the tibialis anterior muscle was exposed through a longitudinal, anterior leg incision. The electrodes were applied directly to the muscle. The distal electrode was applied to the posterior surface of the muscle (Figure 18); and the proximal electrode was applied to the anterior surface (Figure 19) being careful not to disrupt the posteriorly located neurovascular bundle. The electrodes were secured to the muscle with a 4-O prolene suture and the unattached ends were tunneled proximally.

Figure 18: Distal electrode placed posterior to the muscle

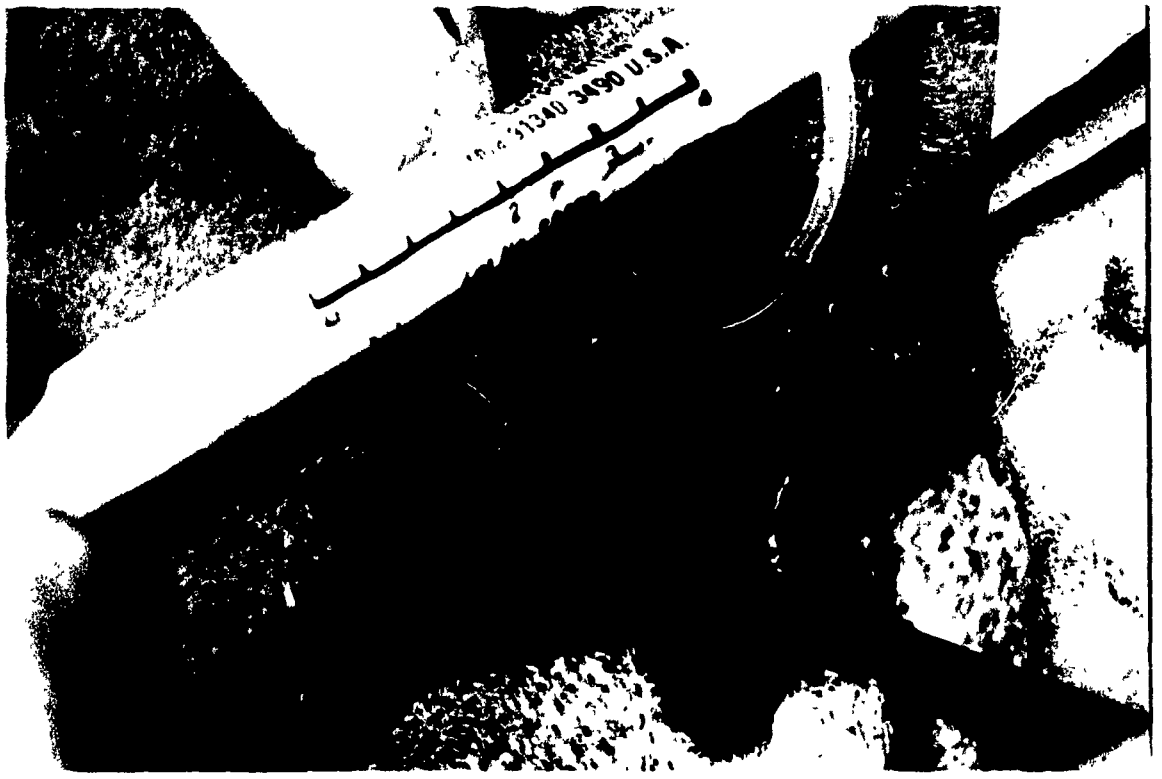


Figure 19: Proximal electrode then placed anteriorly

At the mid thigh location, a separate incision and subcutaneous pocket were created and the pulse generator was inserted; the proximal electrode ends were plugged in. The incisions were closed with an interrupted 2-O Vicryl suture (Figure 20).

Figure 20: Right hindlimb with electrodes in place on the muscle and the planned subcutaneous location of the leads and the power source. The second incision is at the nerve repair site.

The contralateral (left) posterior limb served as the intrinsic control. Therefore, the tibialis anterior muscle was similarly exposed, electrodes were applied, but there was no nerve injury or electrical stimulation on this side.

Group Assignment

At this point the dogs were randomly assigned to Group 1 (stimulated group) or Group 2 (non stimulated group). Group 1 received ES which caused a moderate muscle 1.5 second contraction every 24 seconds, twentyfour hours per day. The pulse frequency was 85 Hertz, the pulse duration was .45 milliseconds and the initial voltage delivered was 5.0 volts. Over the course of the next few days, because of Wallerian degeneration, the voltage was adjusted upwards, to a maximum of 10.5 volts, to maintain this contraction level.

For the next 16 weeks, the dogs were maintained in the above described condition with no restrictions on their normal activities. The electrodes and the pulse generator were well tolerated by the animals and at no time did they appear to cause any discomfort or infectious complications.

At 16 weeks, EMG neuromuscular testing was performed to verify reinnervation; the Acute Experiment was then performed.

ACUTE EXPERIMENT

The effects of ES on muscle form and function were assessed by measuring muscle weight; neurally induced twitch and tetanic tension; direct muscle stimulation induced tetanic tension; unfused tetanic tension profiles; contraction and one-half relaxation times; muscle endurance; and muscle fiber type areas and proportions. In both groups, these variables were studied by comparing the newly reinnervated right limb to the normal left limb.

Muscle Weight

The muscle weight was established at the end of the experiment by a careful bilateral excision of the tibialis anterior muscles from origin to insertion. These specimens, along with any biopsy specimens were weighed separately.

Muscle Function

The muscle force testing was done using a Grass FT O3C force transducer connected to a Grass model 7B polygraph machine. In this setup, the muscle is exposed, left in situ, and the distal tendon is divided and attached directly to the force transducer. The apparatus functions by transforming the muscle pull on the transducer into an electrical signal; this signal is then transformed and displayed as a pen deflection on graph paper. The magnitude of a contraction is proportional to the amplitude of the pen deflections. Also, since the graph paper is moving at a fixed rate, the velocity of contraction can also be determined.

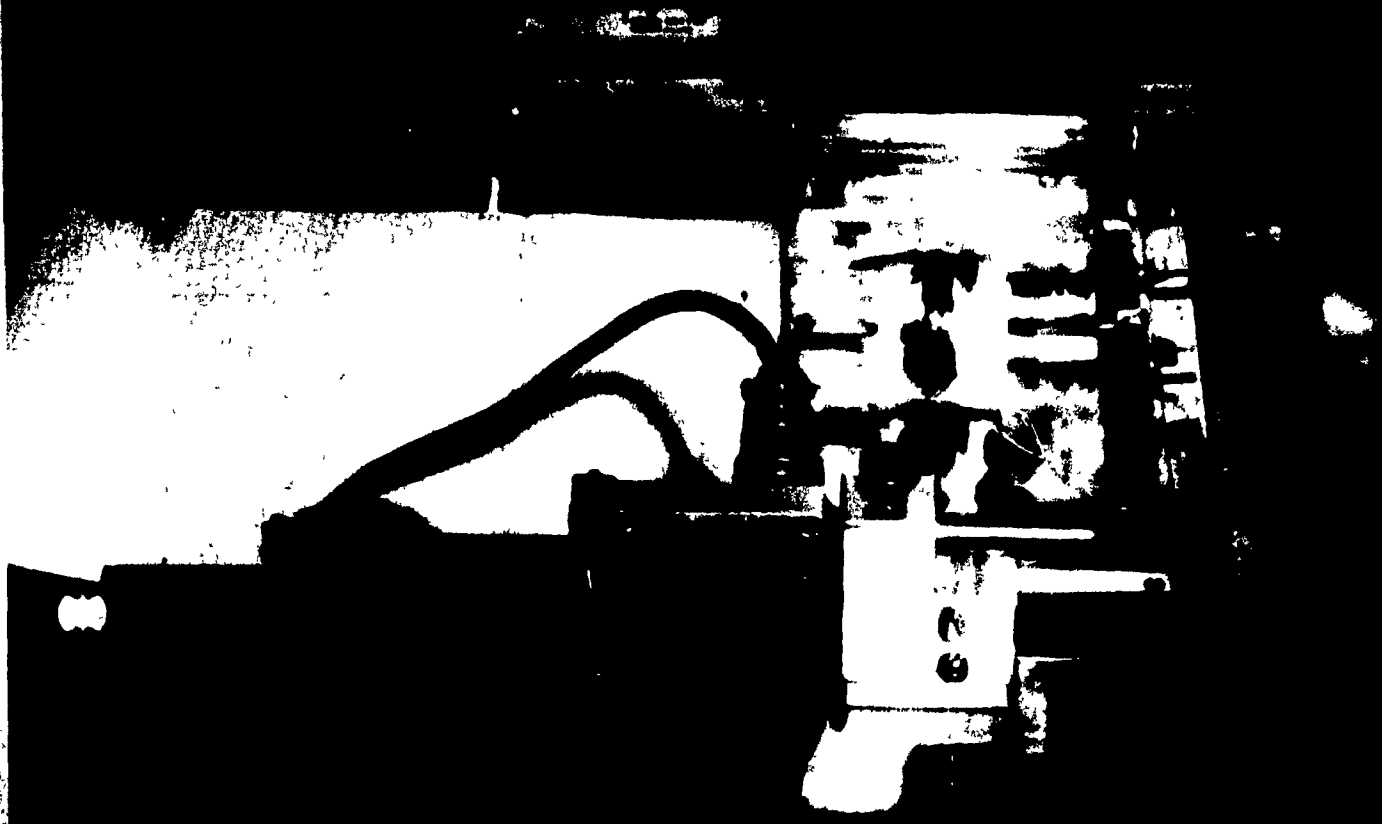
The main obstacle to obtaining reliable force measurements in the laboratory setting is that the contracting muscle inevitably disrupts the orientation of the force transducer. In fact, when a specific leg muscle contracts in response to stimulation, the entire leg will invariably move. This undesirable motion is

interpreted by the sensitive force transducer as additional force when in reality it is only artifact.

To circumvent this problem, we designed an original force transducer platform on which the force transducer and the involved leg could be jointly secured in position. The net effect was that even if the entire platform was voluntarily shaken during testing, this did not result in any additional or aberrant pen deflections. The commonly shared, secure reference point platform, to which both the limb and the force transducer were attached, eliminated motion artifact.

An additional innovation of our experiment relates to the manner in which the muscle was exposed prior to the force testing. Normally, force testing is performed by fully exposing the entire muscle and attaching it to the force transducer. This, unfortunately, results in muscle desiccation, altered temperature, and a potential disruption of the neurovascular supply. To prevent this, and to most closely maintain the muscle's normal anatomical orientation, we were careful to expose only the tendinous insertion and those points where the tibia was secured to the platform. The force measurements were, therefore, true, in situ values with no temperature, desiccation, or neurovascular compromising artifacts. See Figure 21. The common peroneal nerve was exposed through a separate incision and the Siemens Neuroton 626 Stimulator was used to excite the nerve for the muscle force testing.

Figure 21: Right hindlimb and the force transducer secured to the same platform. The tendon is attached to the force transducer; the polygraph machine (with its graph paper) is in the background.



The specific force measurements we tested are defined as follows:[90]

isometric twitch tension - that force produced by a muscle, held at a constant length, in response to a single neural impulse.

isometric tetanic tension - the maximal force produced by a muscle, held at a constant length, in response to repetitive neural stimulation.

direct muscle stimulation tetanic tension - the maximal force produced by a muscle, held at constant length, in response to directly applied muscle stimulation.

contraction time - the time from the end of the latent period to the peak of the isometric twitch response following a single neural impulse.

one-half relaxation time - the time for the decline of tension from the peak of the isometric twitch to one-half of the peak tension.

muscle endurance - a comparison of the initial force generated by a muscle to specific stimulation parameters, to that force generated after the repetitive delivery of the same parameters over a fixed period of time.

unfused tetanus profile - the profile of the tracing created when a low to mid frequency current is delivered to a muscle, via the nerve, in a continuous fashion. Fast muscle fibers show a "sag" (loss of amplitude) in the tracing under these circumstances while slow muscles do not.

All of these force values were obtained in the standard fashion by first establishing the optimal length (the length of the muscle at which the peak twitch tension, in excess of the initial tension, is maximal); and then determining the

optimal voltage (the voltage that induces the maximal twitch and tetanic tension and then doubling it).

Muscle Histochemistry and Histology:

After all the force and velocity measurements were made, we obtained the muscle biopsy specimens.

First, cross-sectional biopsies were obtained at fixed points for ATPase staining in order to differentiate between the type 1 and type 2 muscle fiber.

This was done by immediately freezing the specimens in isopentane (cooled to -180 degrees Celsius by liquid nitrogen) and storing them at -80 degrees Celsius. The specimens were then cut into 10 micrometer sections and stained according to the method of Durovitz and Brooke (1973).

A quantitative muscle fiber analysis could then be made. This was done by examining the specimens with the Lietz Dialux 20 microscope (X25 magnification) attached to a Hitachi CCTV camera (X25) which projected the image onto a video screen. By tracing the fibers and indicating their type, a Videoplan computer system software package could calculate the relative porportion of the two fiber types as well as their mean areas.

RESULTS

The following results represent the comparison of the Group 1 (stimulated) animals to the Group 2 (non stimulated) animals.

This is done by using percentages to relate the manipulated right limb to the contralateral control limb. The difference between the two sides (right vs left) for each parameter is statistically significant and these p values are not discussed.

The subject of the "STATISTICAL ANALYSIS" section is the comparison of Group 1 (stimulated) to Group 2 (non stimulated) animals. These p values are indicated.

MUSCLE WEIGHTS (grams)

Group 1

Dog #	Weight Right Limb	Weight Left Limb	% Preservation
A1	20.80	26.13	79.60
B1	19.82	38.43	51.57
C1	32.58	46.02	70.80
D1	27.40	30.25	90.58
Mean (SD)	25.15 (5.99)	35.21 (8.84)	73.14 (16.50)

Group 2

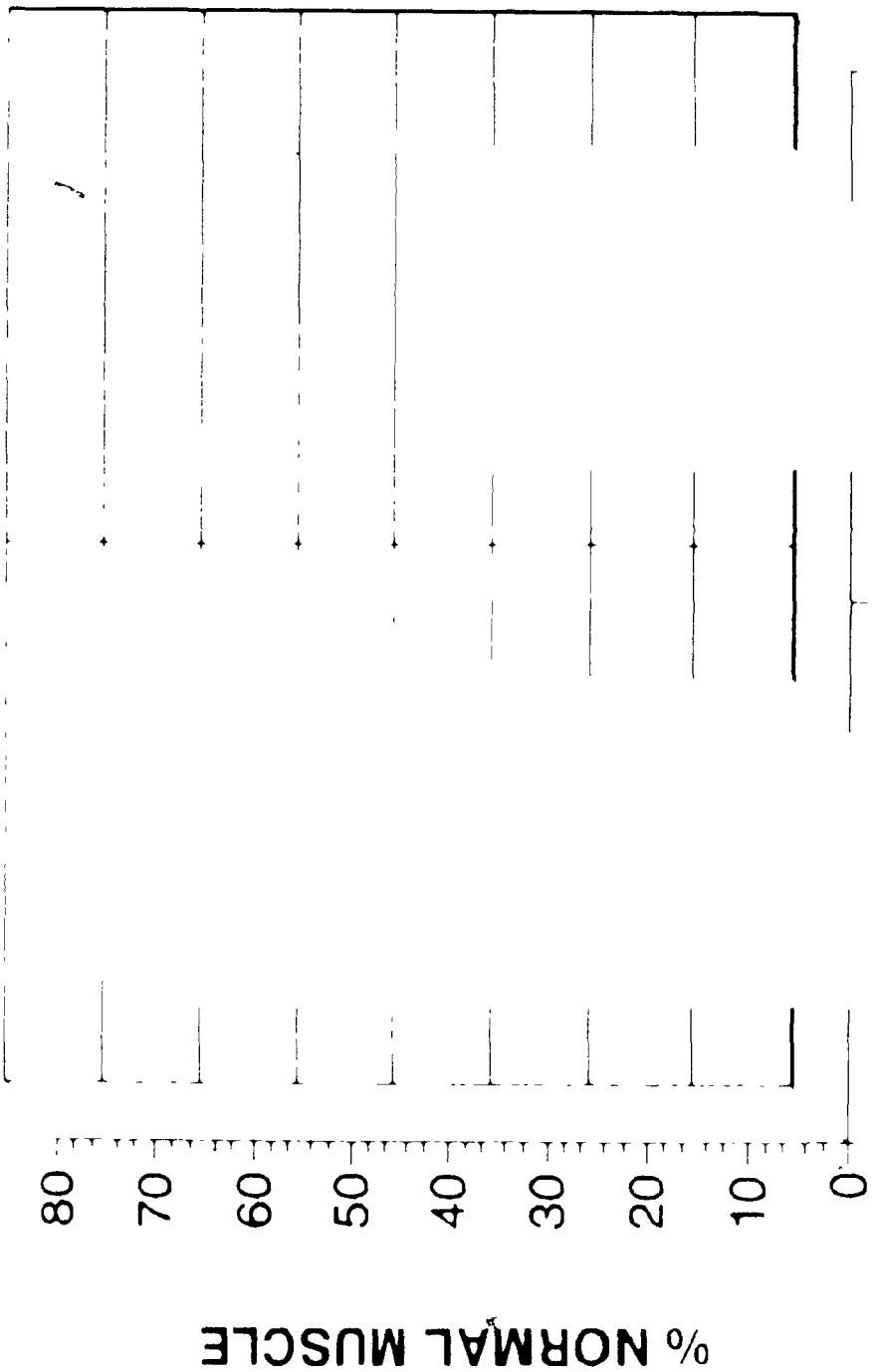
Dog #	Weight Right Limb	Weight Left Limb	% Preservation
A2	11.90	33.60	35.42
B2	14.75	26.74	55.16
C2	17.31	36.31	47.67
D2	11.05	47.99	23.03
E2	10.80	30.88	34.97
Mean (SD)	13.16 (2.80)	35.10 (8.02)	39.25 (12.45)

STATISTICAL ANALYSIS:

When the Mean % Preservation of Group 1 (73.14) is compared to that of Group 2 (39.25) using the student's t test, we find the results are statistically significant with $p<.02$.

These results indicate that ES resulted in a 73.14% preservation of muscle weight which is almost twice as great as the non stimulated group. See Figure 22

Figure 22



TWITCH TENSION (newtons)

Group 1

Dog #	Twitch Tension Right Limb	Twitch Tension Left Limb	% Preservation
A1	620	2800	22.14
B1	460	2750	16.73
C1	1860	8000	23.25
D1	440	2130	20.67
Mean (SEM)	845 (341)	3920 (1369)	20.70 (1.43)

Group 2

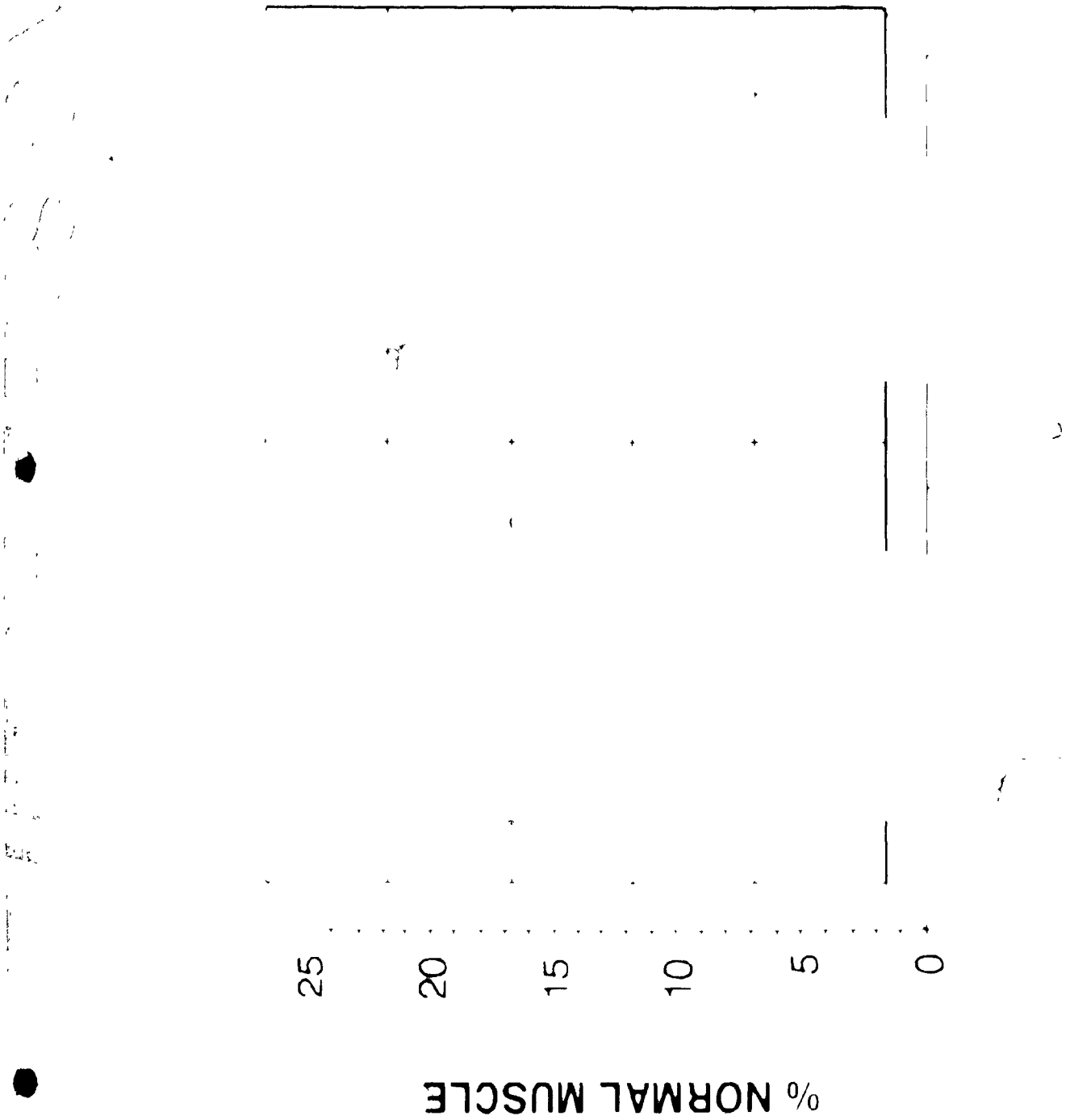
Dog #	Twitch Tension Right Limb	Twitch Tension Left Limb	% Preservation
A2	280	2580	10.85
B2	140	2190	6.39
C2	205	2420	8.47
D2	185	3630	5.10
E2	210	3650	5.75
Mean (SEM)	204 (22.66)	2894 (310)	7.31 (1.05)

STATISTICAL ANALYSIS:

When the Mean % Preservation of the Twitch Tension of Group 1 (20.70) is compared to that of Group 2 (7.31) using the student's t test we find that the results are statistically significant with $p<.001$.

The results indicate that ES resulted in an almost threefold greater twitch contraction force when compared to the non stimulated group. See Figure 23

Figure 23



TETANIC TENSION (newtons)

Group 1

Dog #	Tetanic Tension Right Limb	Tetanic Tension Left Limb	% Preservation
A1	2120	9400	22.55
B1	1130	10300	10.97
C1	6500	14480	44.89
D1	1430	10400	13.75
Mean (SEM)	2894 (1351)	11125 (1115)	23.04 (7.69)

Group 2

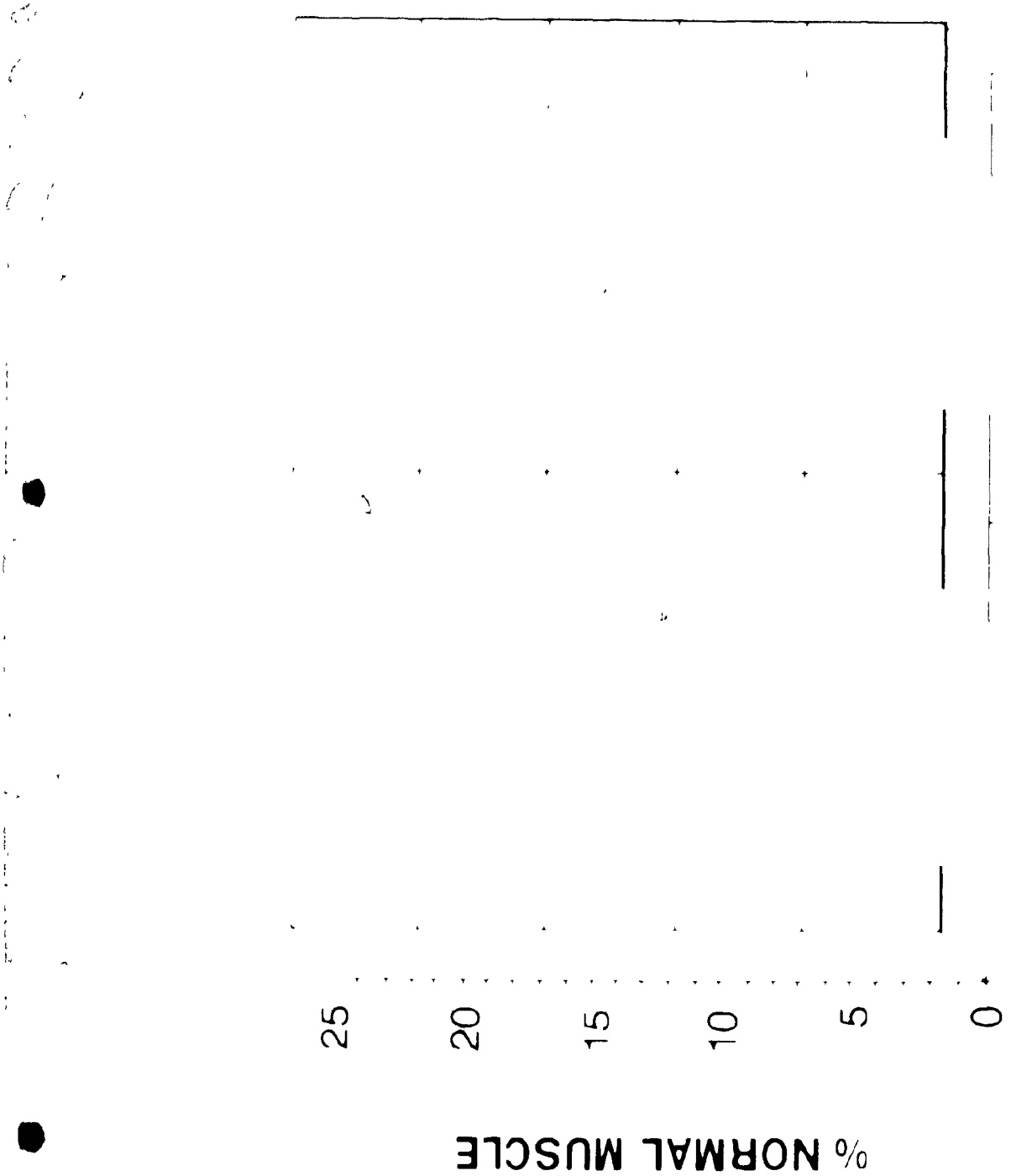
Dog #	Tetanic Tension Right Limb	Tetanic Tension Left Limb	% Preservation
A2	1410	13960	10.10
B2	540	9600	5.62
C2	720	9360	7.69
D2	510	13680	3.73
E2	800	14320	5.59
Mean (SEM)	796 (163)	12184 (1109)	6.55 (1.09)

STATISTICAL ANALYSIS:

When the Mean % Preservation of the Tetanic Tension of Group 1 (23.04) is compared to that of Group 2 (6.55) using the student's t test, the difference is statistically significant with $p<.05$.

These results indicate that ES resulted in a greater than threefold larger tetanic tension force compared to the non stimulated group. See Figure 24

Figure 24



DIRECT MUSCLE STIMULATION
TETANIC TENSION (newtons)

Group 1

Dog #	Tetanic Tension Right Limb	Tetanic Tension Left Limb	% Preservation
A1	3840	9400	40.85
B1	2820	10600	26.60
C1	8080	14400	56.11
D1	5030	10400	48.37
Mean (SEM)	4942 (1139)	11200 (1098)	42.98 (6.29)

Group 2

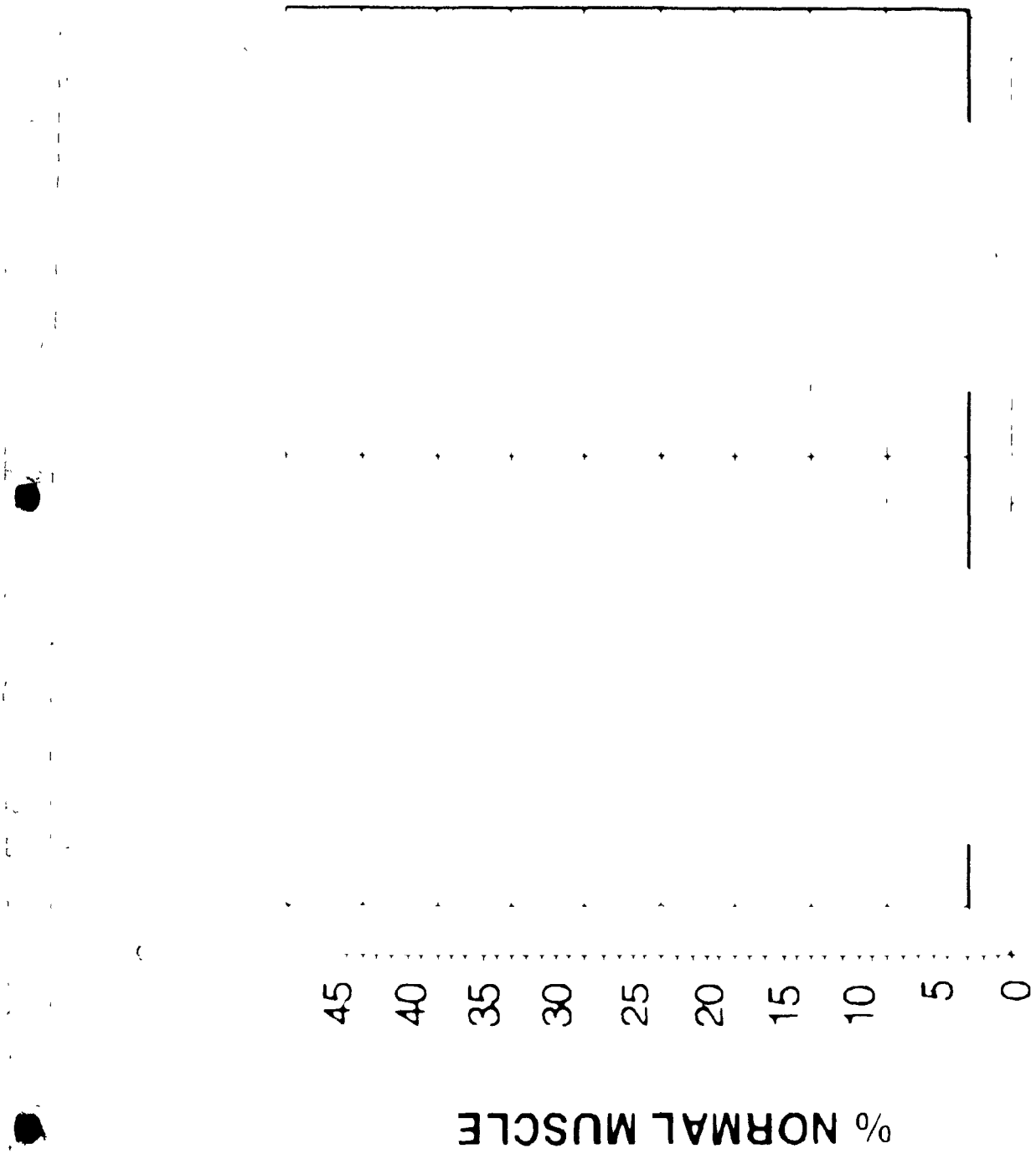
Dog #	Tetanic Tension Right Limb	Tetanic Tension Left Limb	% Preservation
A2	1600	13960	11.46
B2	780	9600	8.12
C2	1320	9360	14.10
D2	1200	13680	8.77
E2	1480	14320	10.34
Mean (SEM)	1276 (141)	12184 (1109)	10.56 (1.06)

STATISTICAL ANALYSIS:

In this tetanic tension trial, the muscle itself was stimulated with a directly applied electrode. The value of this measurement is that the tibialis anterior muscle in this experiment is a newly reinnervated muscle; that is, some of the viable muscle fibers are not as yet reinnervated. (See Discussion). Thus, in order to assess the total muscle potential force, the muscle (not the nerve) must be stimulated directly.

When the Mean % Preservation of the Tetanic Tension of Group 1 (42.98) is compared to that of Group 2 (10.56) using the student's t test, the difference is statistically significant with $p<.001$. These results indicate that ES resulted in a fourfold larger direct muscle stimulated tetanic force than the non stimulated controls. See Figure 25

Figure 25



MUSCLE ENDURANCE

Group 1

Dog #	Initial Force Right	Final Force Right	% Fatigue Right	Initial Force Left	Final Force Left	% Fatigue Left	Net * Musc. Edur.
A1	1310	960	73.28	6950	5360	77.12	95.02
B1	830	495	59.64	7720	6360	82.38	72.39
C1	4260	3760	88.26	11200	9200	82.14	107.4
D1	900	630	70.00	4470	3990	89.26	78.42
Mean (SD)			72.80 (11.8)			82.73 (4.99)	88.32 (15.9

* Net Musc. Edur. -Stands for Net Muscle Endurance and is a percentage value calculated by dividing the % fatigue Right value (experimental side) by the % fatigue left value (normal side).

Group 2

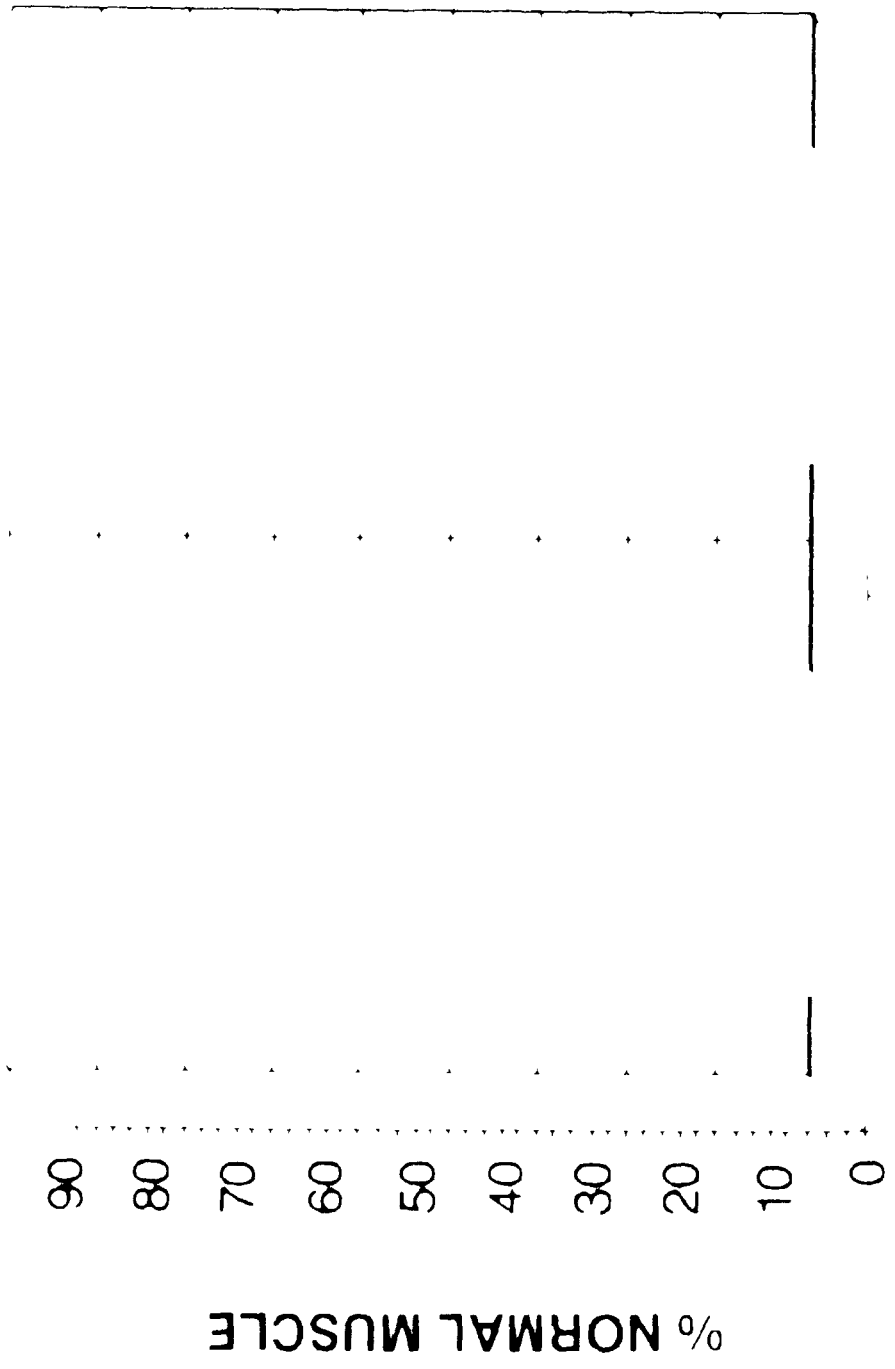
Dog #	initial force Right	final force Right	% fatigue	initial force Left	final force Left	% fatigue Left	Net Musc. Edur.
A2	715	275	38.46	9730	9290	95.48	40.28
B2	350	205	58.57	6840	6260	91.52	64.00
C2	435	195	44.83	7800	7200	92.31	48.56
D2	305	175	57.38	10600	7600	71.70	80.03
E2	400	260	65.00	9820	8620	87.78	74.05
Mean (SD)			52.85 (10.9)			87.76 (9.39)	61.38 (16.8

STATISTICAL ANALYSIS:

When the Net Muscle Endurance Mean of Group 1 (88.32 %) is compared to that of Group 2 (61.38) using the student's t test, the results are statistically significant with $p < .05$.

These results indicate that ES resulted in a muscle which, when stimulated repetitively to contract, was more resistent to fatigue. See Figure 26.

Figure 26



MUSCLE FIBER TYPE AREA (micrometers squared)

Group 1

Dog #	Type 1 Area Right	Type 1 Area Left	% Preserved Type 1	Type 2 Area Right	Type 2 Area Left	% Preserved Type 2
A1	1607	2645	60.76	1694	2949	57.44
B1	N/A	N/A	N/A	N/A	N/A	N/A
C1	1740	2444	71.19	2002	2940	68.10
D 1	1551	2022	76.71	1706	2657	64.21
Mean (SD)	1633 (97)	2370 (318)	69.55 (8.10)	1801 (174)	2849 (166)	63.25 (5.39)

Group 2

Dog #	Type 1 Area Right	Type 1 Area Left	% Preserved Type 1	Type 2 Area Right	Type 2 Area Left	% Preserved Type 2
A2	997	1527	65.29	1149	2012	57.11
B2	822	1883	43.65	515	2546	20.23
C2	791	2107	37.54	919	2522	36.44
D2	1133	2738	41.38	1164	3525	33.02
E2	1003	2439	41.12	697	3085	22.59
Mean (SD)	949 (142)	2139 (472)	45.80 (11.11)	889 (283)	2738 (581)	33.88 (14.67)

STATISTICAL ANALYSIS:

When the % Preserved Type 1 fiber area Mean of Group 1 (69.55) is compared to that of Group 2 (45.80) using the student's t test, the results are statistically significant with $p < .02$.

Also, when the % Preserved Type 2 fiber area Mean of Group 1 (63.25) is compared to that of Group 2 (33.80), the results are statistically significant with $p < .02$. Therefore, ES resulted in muscle fiber area preservation for both Type 1 and Type 2 muscle fibers (Fig. 27).

Fig 27

MUSCLE FIBER TYPE PROPORTIONS

Group 1

Dog #	Type 2 : Type 1 Fiber Number Ratio Right Limb	Type 2 : Type 1 Fiber Number Ratio Left Limb
A1	0.81 : 1.00	2.70 : 1.00
B1	N/A	N/A
C1	0.88 : 1.00	2.92 : 1.00
D1	0.96 : 1.00	4.36 : 1.00
Mean (SD)	0.88 : 1.00 (0.08)	3.33 : 1.00 (0.90)

Group 2

Dog #	Type 2 : Type 2 Fiber Number Ratio Right Limb	Type 2 : Type 1 Fiber Number Ratio Left Limb
A2	0.69 : 1.00	4.10 : 1.00
B2	0.84 : 1.00	4.17 : 1.00
C2	1.12 : 1.00	3.06 : 1.00
D2	0.64 : 1.00	3.68 : 1.00
E2	0.63 : 1.00	4.28 : 1.00
Mean +/- SD	0.78 : 1.00 (0.21)	3.86 : 1.00 (0.50)

STATISTICAL ANALYSIS:

When the Type 2 : Type 1 Fiber Number Ratio Mean for the Group 1 Right Limb (0.88) is compared to that of the Group 2 Right Limb (0.78), there is no statistically significant difference ($p > .05$).

These results indicate that ES did not result in a selective preservation of the normally occurring Type 2 muscle fiber predominance. In both the stimulated and non stimulated groups there was a relative increase in the Type 1 muscle fiber presence. See Figure 28

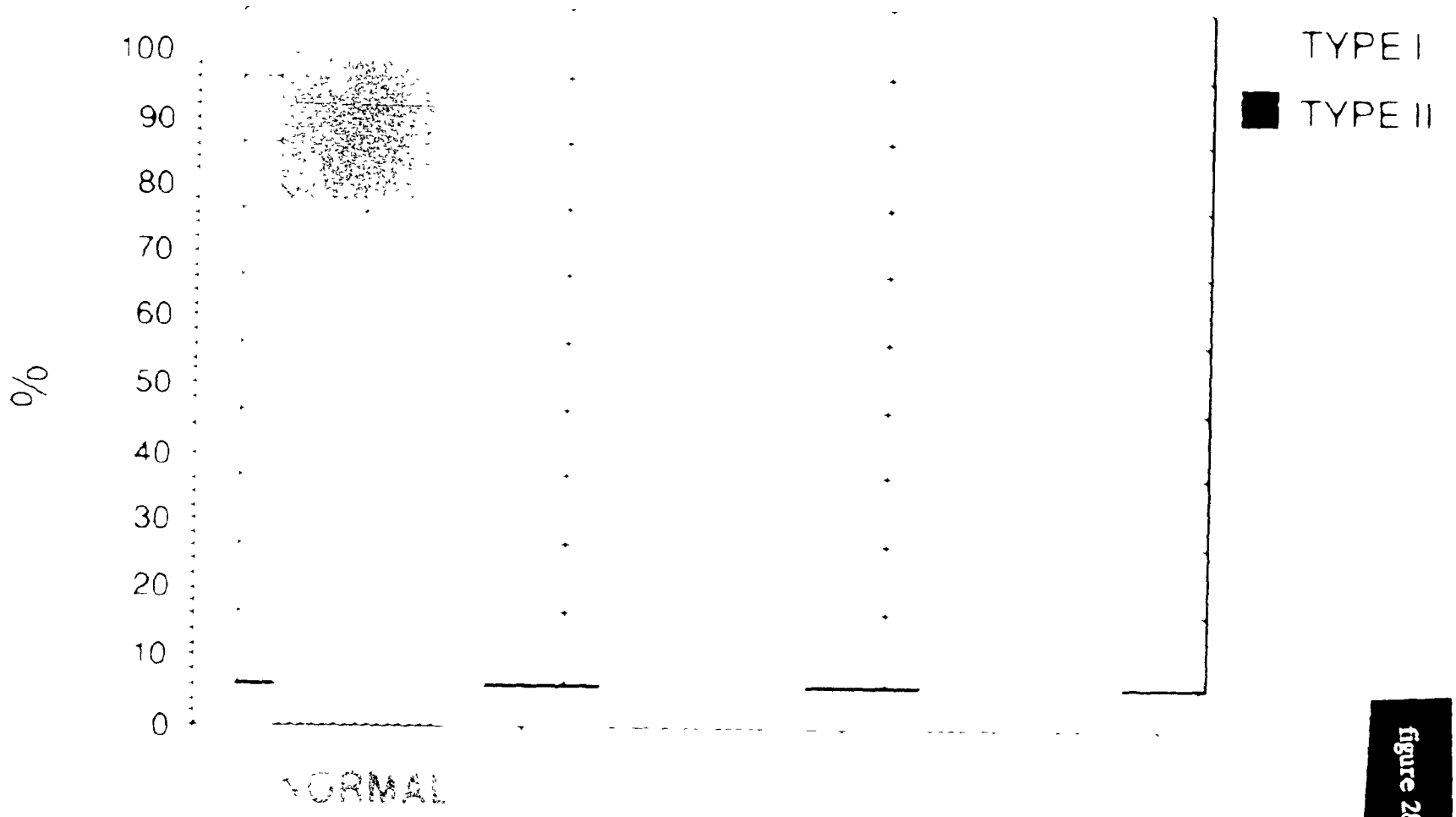


figure 28

CONTRACTION TIME
(milliseconds)

Group 1

Dog #	Contraction Time Right Limb	Contraction Time Left Limb	% Prolongation
A1	65	51	127.4
B1	76	63	121.6
C1	72	60	120.0
D1	75	52	144.2
Mean (SD)	72 (5.0)	56.5 (5.9)	128.1 (11.3)

Group 2

Dog #	Contraction Time Right Limb	Contraction Time Left Limb	% Prolongation
A2	65	44	147.7
B2	76	51	149.0
C2	64	51	125.5
D2	72	56	128.6
E2	62	50	124.0
Mean (SD)	67.8 (5.9)	50.4 (4.3)	135.0 (12.4)

STATISTICAL ANALYSIS:

When the % Prolongation Mean of Group 1 (128.1) is compared to that of Group 2 (135.0) using the student's t test, the results are not statistically significant ($p > .05$).

These results indicate that ES did not preserve the velocity of the muscle contraction; both the stimulated and non stimulated muscle groups had slower muscle contraction times compared to the normal controls.

These results are consistent with the muscle histology analysis which demonstrated a relative increase in the Type 1 (slow) muscle fiber number.

ONE-HALF RELAXATION TIME
(milliseconds)

Group 1

Dog #	Relaxation Time Right Limb	Relaxation Time Left Limb	% Prolongation
A1	78	46	169.6
B1	77	42	183.3
C1	63	58	108.6
D1	68	43	158.1
Mean (SD)	71.5 (7.2)	47.2 (7.4)	154.9 (32.5)

Group 2

Dog #	Relaxation Time Right Limb	Relaxation Time Left Limb	% Prolongation
A2	69	50	138.0
B2	79	51	154.9
C2	63	51	123.5
D2	72	54	133.3
E2	65	38	171.1
Mean (SD)	69.6 (6.3)	48.8 (6.2)	144.2 (18.8)

STATISTICAL ANALYSIS:

When the Mean % Prolongation Time of Group 1 (154.9) is compared to that of Group 2 (144.2) using the student's t test, the results are not statistically significant.

These results indicate that ES did not preserve muscle relaxation velocity; both the stimulated and non stimulated muscles were slower than the normal controls.

These results are consistent with the muscle histology analysis which demonstrated a relative increase in the Type 1 (slow) muscle fiber number; and the Contraction Time analysis which also showed a slowing of the muscle.

NERVE STIMULATED TETANIC TENSION (NSTT) VERSUS MUSCLE STIMULATED TETANIC TENSION (MSTT)

Group 1

Dog #	NSTT	MSTT	% GAIN
A1	2120	3840	181.1
B1	1130	2820	249.6
C1	6500	8080	124.3
D1	1430	5030	351.7
Mean (SD)			226.7 (97.8)

Group 2

Dog #	NSTT	MSTT	% GAIN
A2	1410	1600	113.5
B2	540	780	144.4
C2	720	1320	183.3
D2	510	1200	235.3
E2	800	1480	185.0
Mean (SD)			172.3 (46.1)

STATISTICAL ANALYSIS:

When the % GAIN mean of Group 1 (226.7) is compared to that of Group 2 (172.3) using the student's t test and the non parametric Mann-Whitney Rank Sum Test, the results are not statistically significant ($p > .06$).

These results indicate that ES did not selectively augment the difference between the muscle stimulated tetanic tension and the nerve stimulated tetanic tension in either group compared to the other. See Figure 29.

These results will be discussed with respect to reinnervation in the "Discussion" section of this paper. See Figure 29.

NERVE AND MUSCLE TETANIC TENSIONS

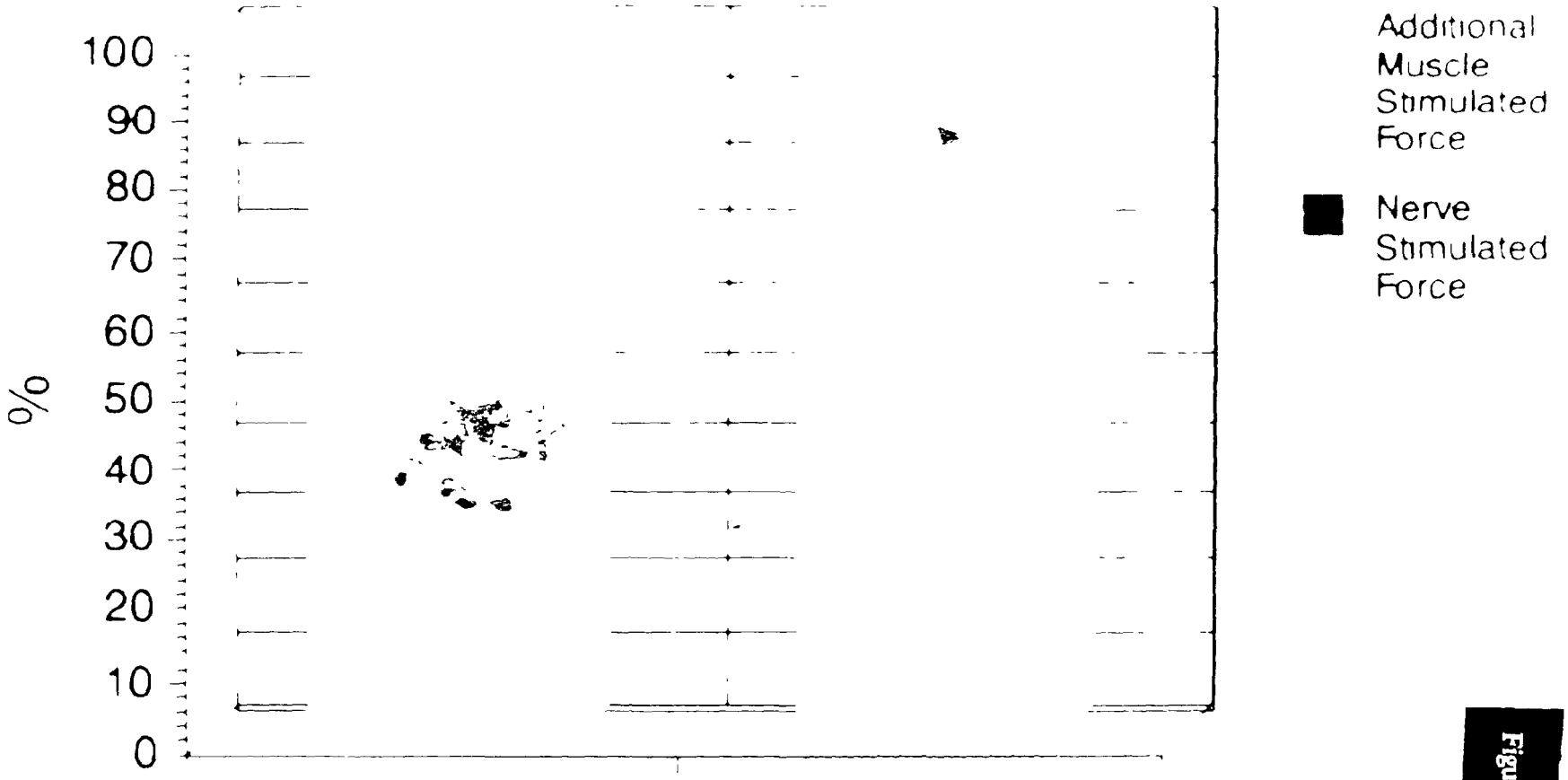


Figure 29

UNFUSED TETANUS PROFILE

When a low to mid frequency current is applied to a nerve in a continuous fashion, an unfused tetanic contraction is the result. An examination of this contraction profile is another means of assessing the muscle fiber type. Both the fast, fatiguable and the fast, fatigue-resistent muscle types demonstrate a sagging tracing under these circumstances. Slow muscle fibers do not sag.[91]

In this experiment, all the normal muscles tested (in both groups) showed sag when a fifteen hertz current was delivered in this fashion. This is consistent with the predominance of fast twitch (type 2) fibers seen in the ATPase measurements.

In both the stimulated (group 1) and nonstimulated (group 2) experimental muscles, there was no sag effect in any muscle tested. This is strong, supportive information which reaffirms the fact that ES, at the parameters settings we tested, did not preserve the tibialis anterior fast fiber type profile. Denervation, regardless of whether the muscle was stimulated or not, caused a transformation of the muscle towards that of a slow muscle fiber type predominance.

DISCUSSION

In this research project a continuous implantable ES system was tested using a dog model. As mentioned in the "Experimental Design and Hypothesis" section of this paper, there were four main goals in this project and these will be discussed in turn.

1 - Extended Period of Stimulation With an Implantable System

The benefits of ES in denervation injury have been debated in a general way because of the feasibility of daily treatments over an extended period of time. The cost of such a therapeutic modality for the patient and the health care institution makes its universal application impractical.

In addition, the detractors of ES correctly observe that the treatment voltages, frequencies and pulse durations used in the usual transcutaneous (external) delivery experimental studies are far greater than what humans would tolerate. In fact, during the 1972 Olympic games, rumors were spreading that the Russians were using ES as part of their training regime. This was reported on by Kotts, the main Russian researcher, in an exchange symposium at Concordia in 1977. According to him the major limiting factor in his efforts was pain.[92]

In this study, the feasibility and pain issues have been circumvented with the use of a totally implantable ES system. With this method, once the parameters were adjusted, twenty-four hour per day ES could be delivered without any assistance of a therapist. This would obviously be an advantage to both the treating institution and the individual patient.

The additional benefit of implantable technology is that lower voltages, frequencies and pulse durations are required to induce the required muscle

contraction. At no time in this experiment did the ES appear to cause any pain or discomfort to the experimental animals.

We observed that muscle could generate a good contraction even after extended periods of ES. In this study, the injury to the nerve was 12 cm from the muscle. Based on theoretical nerve growth rate estimates, actual histological sections of regenerating peripheral motoneuron, and EMG measurements; the experimental muscles were completely denervated for approximately 12 weeks. For the four ensuing weeks, actual muscle reinnervation was occurring (total = 16 weeks). Thus for three full months a completely denervated muscle was stimulated. In addition, a delay group was studied in which the total denervation time was up to 29 weeks, and even after this extended period, ES of the denervated muscle produced significant muscular contraction.

2 - Functional and Morphological Assessment of ES

The fact that ES was indeed beneficial in this study is demonstrated by a comparison between the ES group and the non stimulated group with functional and morphological measurements.

The functional measurements showed that with ES, the nerve stimulated twitch and tetanic tensions were threefold stronger than the unstimulated controls. When the neuromuscular junction was paralysed with pancuronium and the muscle was stimulated directly, the ES was four times stronger than the control.

Another measure of neuromuscular function is muscle endurance. In this experiment, endurance is measured by comparing the initial force generated by a muscle to that force attained after a set period of regular, intermittent fatiguing ES; the parameters we used for this assessment were 36 hertz, 1.5 sec on, 2.0 sec off, for five minutes. The ES group maintained almost ninety percent of its

original endurance compared to sixty-one percent in the non ES group.

These functional measurements of muscular contraction are perhaps the most important evidence in support of the beneficial impact that this form of ES provides. Morphological improvement with ES (see below) is useful adjunctive information but it is only significant if it correlates with improved muscle performance. Twitch tension, tetanic tension and muscle endurance are the physiologically defined measures of muscle function.

The morphological benefits of ES are best demonstrated by the quantitative structural measurements we studied.

When the muscle weights of the two groups were compared the ES group maintained seventy-three percent of its weight while the non ES group was less than forty percent of its original value. This result is significant because the difference in the weight preservation cannot be accounted for by an accumulation in fibrofatty connective tissue in the ES group. If this were the case, the ES muscles wouldn't have developed the improved muscle force parameters which we outlined earlier.

Further supportive evidence that the improved muscle weight was due to the actual presence of functional muscle tissue is the muscle fiber area data. Both the type 1 and type 2 muscle fiber areas, as measured by our quantitative morphometric analysis program, were significantly larger in the ES group compared to the non ES group.

3 - Electrical Stimulation Parameters and Fiber Type Preservation

In this study the ES parameters were chosen with the goal of preserving the muscle's native fiber type composition. To assess this, we first did ATPase

sections on normal tibialis anterior muscles. This analysis revealed that the muscle was a predominantly fast muscle with a type 2 : type 1 ratio of approximately 3.5 : 1.0.

Based on the previously listed reports in the literature that revealed that high frequency stimulation was capable of maintaining fast muscle fast, and also converting slow muscle in the fast direction (see Muscle Stimulation Parameters); we chose 85 hertz as our stimulation frequency.

To measure the effect of this stimulation frequency on muscle fiber type, we used both morphological and functional measurements.

Unexpectedly, no such Type 2 preferential preservation occurred. The fiber type area data demonstrated an equal preservation of the type 1 an type 2 fiber areas. Further, the fiber type proportion data indicated that the muscle was transformed from a predominantly fast muscle (type 2 : type 1 = 3.5 : 1.0) to a predominantly slow muscle in the ES group (0.88 : 1.0) and the non stimulated group (0.78 : 1.0). There was no significant difference between the ES and the non ES groups though both were significantly different from normal muscle in this regard.

This data, supporting a fiber type transformation with 85 hertz ES, was supported by our specific functional measurements of muscle fiber type. In particular, the contraction and one half relaxation times of the ES and non ES groups were equally and significantly prolonged when compared to the normal muscle indicating a slowing of the muscle. Also, an examination of the unfused tetanus profile revealed the sag effect (a characteristic feature of fast muscles) in only the normal control limbs. Both the ES and non ES groups demonstrated no sag reaffirming their slow type transformation.

There are two conclusions that may be drawn from this data. First, denervation alone is capable of transforming fast muscle into slower muscle. Second, high frequency ES alone is not sufficient to prevent this transformation.

As was mentioned earlier, several authors have argued that the total aggregate activity, and not the frequency, may be the critical factor in determining a stimulated muscles fiber type. Muscles that are stimulated to contract numerous times per day (in this experiment it was once every twenty four seconds) are, or would become, slow muscles regardless of the frequency (hertz) of the stimulating current.

While these results would seem to imply that the reports in the literature that demonstrate a fiber type preservation with high frequency stimulation are false, this is not necessarily the case. The more likely explanation is due to species difference. This was the only experiment to use the dog model; the other studies were mainly performed with the rat or mouse model, while others used the rabbit model.

4- ELECTRICAL STIMULATION AND REINNERVATION

There is controversy in the literature as to whether ES is a deterrent to reinnervation in peripheral nerve injury.

Lomo and Slater, using the rat model, hypothesized that since direct stimulation of denervated muscle prevented the development of Ach sensitivity; and that since the sensitivity to Ach and the ability to form new neuromuscular junctions appears at the same time, ES is actually a deterrent to new synapse formation.[93]

Cohan and Kater, using the snail model, demonstrated that neurite

elongation was reversibly stopped when action potentials were experimentally evoked.[94]

Also, Brown[95] and Michel[96] have separately demonstrated, in the mouse and rat model respectively, that in partial denervation injury neuronal sprouting is inhibited by ES.

On the other hand, other researchers have shown no such deleterious effects with ES. Jansen showed that ES impaired foreign but not self-reinnervation[97] while Herbison found that ES neither enhanced or retarded reinnervation.

While these studies measure specific biological parameters, no functional measurements are provided. An analysis of the results in this study demonstrates that no impairment of reinnervation occurred with ES.

This conclusion is based on the comparison of the nerve- stimulated tetanic tension to the direct muscle-stimulated tetanic tension in the ES versus the non ES group. Recall that the direct muscle-stimulated tetanic tension was measured after the neuromuscular junction was blocked with pancuronium and, therefore, no intramuscular nerve-induced muscular contractions were produced.

First, it should be noted that the muscles studied in this experiment were not as yet completely reinnervated. This is understandable because the reinnervation status of an entire muscle is not an all or none phenomenon; it is a gradual process that evolves over months once the initial neural contact is made. This was demonstrated in our results by the fact that the direct muscle- stimulated tetanic tension was greater than the neurally mediated tetanic tension in all the reinnervated muscles in the ES and the non ES group. Thus the increased tetanic contractions in the direct muscle-stimulation testing was secondary to the presence

of viable muscle fibers that responded to direct stimulation but were not, as yet, reinnervated and could not be exited by nerve stimulation. The normal control muscles showed equal values for their direct muscle stimulation and their nerve stimulation.

It is the percent increase in force gained by the direct stimulation which is the issue here. If ES simply strengthened the muscle but impeded reinnervation, the amount of additional force gained by direct muscle stimulation (when compared to neural stimulation) would be significantly higher than that gained in the non stimulated group. That is, the nonstimulated muscles would have a narrower gap in the muscle-stimulated versus the nerve-stimulated forces because reinnervation was not impeded by the electrical influence.

When the data is analyzed with either the student's t test or the Mann-Whitney rank sum test, there is no statistical difference in the amount of force gained by direct muscle stimulation. Both the ES and non ES groups were not fully reinnervated; both groups demonstrated increased force with direct stimulation measurements; and there was no statistically significant difference in the proportion of force gained between the two groups. Thus, there is no functional reason to suspect that reinnervation is impeded by the presence of electrically induced muscle activity.

While this project addressed four specific questions, there are numerous possible extensions to this work. The first would be to establish the ideal electrical parameters to maximize therapeutic gain. Related to this would be an analysis of what modifications of this system would be required to adapt the system to a clinical application. In particular, the development of a multiple lead output capability would be important. These factors are being actively investigated in our research facility.

CONCLUSION

Based on the data presented in this project, we conclude that:

1 Continuous implantable electrical stimulation is a beneficial adjunctive treatment in denervation injury in the dog model when used over the extended period of four months.

2 This beneficial effect is demonstrated by improved morphological and functional measurements in reinnervated muscle tissue.

3 A high frequency pattern of stimulation is not, by itself, able to maintain the fast nature of a native fast muscle. A high total aggregate activity or denervation alone will cause the slowing of a fast muscle.

4 Electrical stimulation that is started before and carried into the reinnervation period has no functionally measurable adverse effect on the reinnervation process.

1.Nemoto K, Williams HB, et al: The effects of electrical stimulation on denervated muscle using implantable electrodes. J. Reconstructive Microsurgery. 4:251-255, 1988

2.Durand D, Williams HB: The prevention of muscle atrophy after peripheral motor nerve repair with an implantable electrical system. Surg Forum 41:661-664, 1990.

3. Snyder CC: The History of Nerve Repair. Management of Peripheral Nerve Problems. Omer GE and Spinner M (ed). WB Saunder Company (1980).

4. Snyder CC: The history of Nerve Repair. Management of Peripheral Nerve Problems. Omer GE and Spinner M (ed). WB Saunders Company (1980).

5. Swan KG & Swan RC: Gunshot Wounds - Pathophysiology and Management. PSG Publishing Company, Inc (1980).

6. Mitchell SW: Injuries of Peripheral Nerves. JB Lippincott and Company (1872).

7. Sherren J: Injuries of Nerves and Their Treatment. James Nisbet and Company, Limited (1908).

8. Bristow WR: Robert Jones. The British Journal of Surgery. 34 (136); 133-143 (1947).

9. Bristow WR: The British Journal of Surgery. 34 (136); 133-143 (1947)

10. Reid J: On the Relation between Muscular Contractility and the Nervous System. Lond. Edin. Mon. J. Med. Sci. 1: 320-329 (1841)

11. Buchthal F, Schmalbruch H: Motor Unit of Mammalian Muscle. Physiological Reviews. Vol 60, No 1: 91-142, January (1980).

12. Sarnat HB: Muscle Pathology and Histochemistry. American Society of Clinical Pathologists. Chpt 1: 1-43 (1983).

13. Binder MD: Properties of Motor Units, in Patton HD, Fuchs AF, et al (eds): Textbook of Physiology. W.B. Sanders Company, 21st ed. p.514 (1989).

14. Rose SJ, Rothstein JM: Muscle Mutability, in Physical Therapy. Vol 62: 1773-1785 (1982).

15. Sarnat HB. Muscle Pathology and Histochemistry. American Society of Clinical Pathologists. Chapter 3: 35-43 (1983)

16. Eccles JC, Eccles RM, Lundberg A: The action potentials of the alpha motoneurone supplying fast and slow muscles. J. Physiol, 142: 275-291 (1958).

17. Gorza L, Gundersen K, Lomo T, et al: Slow to fast transformation of denervated soleus muscles by chronic high frequency stimulation in the rat. J Physiol, 402: 627-649 (1988)

18. Vrbova G: The effect of motoneurone activity on the speed of contraction of striated muscle. J Physiol, 169: 513-526 (1963)

19. Gorza L, Gundersen K, Lomo T: Slow to fast transformation of denervated soleus muscles by chronic high frequency stimulation. J Physiol, 402: 627-649 (1988).

20. Nix WA, Reichmann H, Schroeder MJ: Influence of Direct Low Frequency Stimulation on Contractile Properties of Denervated Fast-Twitch Rabbit Muscle. Pfluegers Arch 405: 141-147 (1985).

21. Al-Ahmood WS, Lewis DM: The role of frequency in the effects of long-term intermittent stimulation of denervated slow-twitch muscle of the rat. J Physiol 392: 377-395 (1987).

22. Seddon H: Surgical Disorders of the Peripheral Nerves. Edinburgh, Churchill Livingstone (1975).

23. Snyder CC: The History of Nerve Repair. Management of Peripheral Nerve Problems. Omer GE and Spinner M (ed). WB Saunders Company (1980).

24. McCarthy JG, Plastic Surgery. 1st ed. Chpt 109: p. 4764. WB Saunders Company (1990).

25. McCarthy JG, Plastic Surgery. 1st ed. Chpt 109: p. 4765. WB Saunders Company (1990).

26. Schwartz SI. The Principles and Practice of Surgery. 5th ed. Chpt 42: p. 1845. McGraw Hill Book Company (1989).

27. Sarnat HB: Muscle Pathology and Histochemistry. American Society of Clinical Pathologists. CHPT 3: 35-43 (1983).

28. Buchtal F, Schmalbruch H: Motor Unit Of Mammalian Muscle. Physiological Reviews, 60(1): p. 99 (1980).

29. Frank E: The influence of neuronal activity on patterns of synaptic connections. Trends Neurosci, 10: 188-190 (1987).

30. Cohan CS, Kater SB: Suppression of neurite elongation and growth cone motility by electrical activity. Science Wash. DC, 232: 1638-1640, 1986.

31. Schimrigk KJ, McLaughlen J, Gruniger W: The effect of electrical stimulation on the experimentally denervated rat muscle. Scand. J. Rehabil. Med. 9: 55-60 (1977).

32. Michel RN, Gardiner PF: Effect of previous overload on short-term neuromuscular responses to partial denervation. Can J Appl Sport Sci, 11: 30 (1986).

33. Jansen JK, Lomo T, et al: Hyperinnervation of skeletal muscle fibers: dependence on muscle activity, Science 181: 559-561 (1973).

34. Hoffman H: Acceleration and retardation of the process of axon sprouting in partially denervated muscles. Aust J Biol Med Sci 30: 541-566 (1952).

35. Herbison GJ, Jaweed MM, Ditunno JF: Acetylcholine sensitivity and fibrillation potentials in electrically stimulated crush-denervated rat skeletal muscle. Arch Phys Med Rehabil, 64: 217-220 (1983).

36. Sunderland S: Nerves and Nerve Injury, 2nd ed. Edinburgh, Churchill Livingston (1978).

37. Omer GE, Spinner M (ed). The Management of Peripheral Nerve Problems. WB Saunders Company (1980).

38. Davis HL: Is electrical stimulation beneficial to denervated muscles? A review of results from basic research. Physiotherapy Canada, 35,6: 306-312 (1983).

39. Jaweed MM, Herbison GJ, Ditunno JF: Dennervation and reinnervation of fast and slow muscles: A histochemical study in rats. J Histochem Cytochem 23: 808 (1975).

40. Engel AG: Morphological effects of denervation of muscle. A quantitative ultrastructural study. Annals New York Academy of Sciences 68-87 (1973).

41. Fischer E: The effect of faradic and galvanic stimulation upon the courseof atrophy in denervated skeletal muscles. Am J Physiol 127: 605-618 (1939).

42. Goldberg AL, Jablecki C, Li JB: Effects of use and disuse on amino acid transport and protein turnover in muscle. Ann N.Y. Accad of Sci. 190-201 (1974)

43. Jones R, Vrbova G: Two factors responsible for the development of denervation hypersensitivity. J Physiol 236: 517-538 (1974).

44. Hall ZW, Reiness CG: Electrical stimulation of denervated muscles reduces incorporation of methionine into the ACh receptor. Nature 268: 655-657 (1977).

45. Vigny M et al: The motor endplate specific form of acetylcholinesterase: Appearance during embryogenesis and reinnervation of rat muscle. J Neurochem 27: 1347-1353 (1976).

46. Albuquerque EX et al: Early membrane depolarization of the fast mammalian muscle after denervation. Pfluegers Arch 328: 36-50 (1971).

47. Finol HJ et al: The effects of denervation on contractile properties of rat skeletal muscle. J Physiol 319: 81-92 (1981).

48. Fischer E: The effect of faradic and galvanic stimulation upon the course of atrophy in denervated skeletal muscles. Am J of Physiol 127: 605-618) (1939).

49. Lewis DM: The effect of denervation on the mechanical and electrical responses of fast and slow mammalian twitch muscle. J Physiol 222: 51-75 (1972).

50. Tower SS: Atrophy and degeneration in skeletal muscle. Am J Anat 56: 1-43 (1935).

51. Guth L, Albuquerque EX: The neurotrophic regulation of resting membrane potential and extrajunctional acetylcholine sensitivity in mammalian skeletal muscle. Physiologia Bohemoslovaca 27: 401-412 (1978).

52. Albuquerque EX et al: Early membrane depolarization of the fast mammalian muscle after denervation. Pflugers Arch 328: 38-50 (1971).

53. Drachman DB: The role of acetylcholine as a neurotrophic transmitter. Ann New York Accad Sci 160-174 (1973).

54. Davis HL, Kiernan JA: Neurotrophic effects of sciatic nerve extract on denervated extensor digitorum longus muscle in the rat. Experimental Neurology 69: 124-134 (1980).

55. Baker LL: Neuromuscular electrical stimulation in the restoration of purposeful limb movements. Electrotherapy 25-48 chpt 2

56. Reid J: On the relation between muscular contractility and the nervous system. Lond Edinb Monogr J Med Sci 1: 320-329 (1841).

57. Brown-Sequard CE: Experimental researches applied to physiology and pathology. New York, Bailliere, 1853.

58. Langley JN, Kato T: The rate of loss of weight after nerve section with some observations on the effect of stimulation and other treatment. J Physiol 49: 432 (1915).

59. Fischer E: The effect of faradic and galvanic stimulation upon the course of atrophy in denervated skeletal muscles. Am J of Physiol 127: 605-618 (1939).

60. Gutmann E, Guttman L: Effect of galvanic exercise on denervated and reinnervated muscles in rabbit. J Neurol Neurosurg Psychiat 7: 7-17 (1944).

61. Newton R: Clinical Electrotherapy. High voltage pulsed galvanic stimulation. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

62. Kosman AJ et al: The comparative effectiveness of various electrical currents in preventing muscle atrophy in the rat. Arch of Physical Med. Jan: 7-17 (1947).

63. Hennig R, Lomo T: Firing patterns of motor units in normal rats. Nature 314: 164-166 (1985).

64. Gundersen K et al: Fibre types, calcium sequestering proteins and metabolic enzymes in denervated and chronically stimulated muscles of the rat. J Physiol 398: 177-189 (1988).

65. Gorza L et al: Slow to fast transformation of denervated soleus muscles by chronic high frequency stimulation in the rat. J Physiol 402: 627-649 (1988).

66. Al-Amood WS, Lewis DM: The role of frequency in the effects of longterm intermittent stimulation of denervated slow twitch muscle of the rat. J Physiol 392: 377-395 (1987).

67. Salmons S: Reanalysis: impulse activity and fiber type transformation: a reply. Muscle & Nerve Nov/Dec: 839-840 (1987).

68. Kosman AJ et al: The influence of duration and frequency of treatment in electrical stimulation of paralyzed muscle. Arch of Physical Med. Jan: 12-17 (1947).

69. Newton RA: Electrotherapeutic Treatment: Selecting appropriate waveform characteristics. JA Preston Corporation 1-50 (1984).

70. Lomo T, Rosenthal J: Control of Ach sensitivity by muscle activity in the rat. J Physiol 221: 493-513 (1972).

71. Davis HL: Is electrical stimulation beneficial to denervated muscles? A review of results from basic research. Physiotherapy Canada 35,6: 306-312 (1983).

72. Speilholtz N: Electrical stimulation of denervated muscle. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

73. Smith EM, Steinberger WW: Effects of stimulus parameter Variation on the recruitment in denervated rabbit muscle. Arch of Phys Med and Rehab, Oct 566-572 (1968).

74. Newton RA: Electrotherapeutic treatment: Selecting the appropriate waveform characteristics. JA Preston Corp. 1-50 (1984).

75. Stephens WGS: The assessment of muscle denervation by electrical stimulation. Physiotherapy 59(9): 292-294 (1973).

76. Cummings J: Electrical stimulation of Healthy Muscle. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

77. Rushton WAH: Identification of Lucas' alpha excitability. J Physiol 75: 445-470 (1932).

78. Cummings J: Electrical stimulation of healthy muscle. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

79. Stephens WGS: The assessment of muscle denervation by electrical stimulation. Physiotherapy 59(9): 292-294 (1973)

80. Hultman E et al: Evaluation of methods for electrical stimulation of human skeletal muscle in situ. Pflugers Arch 398: 139-141 (1983).

81. Smith EM, Steinberger WW: Effect of stimulus parameter variation on recruitment in denervated rabbit muscle. Arch Physical Med and Rehab. Oct 566-572 (1968).

82. Dubois-Reymond E. Unters chungenuber tierische elektrizitat. Virchows Arch 2: 258 (1845).

83. Alon G: Principles of electrical stimulation. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

84. Hultman E, et al: Evaluation of methods for electrical stimulation of human skeletal muscle in situ. Pflugers Arch 398: 139-141 (1983).

85. Alon G: Principles of electrical stimulation. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lang (1987).

86. Alon G: Principles of electrical stimulation. Clinical Electrotherapy. Nelson RM & Currier DP (ed). Appleton and Lange (1987).

87. Trontelj J, Stalberg E: Responses to electrical stimulation of denervated human muscle fibres recorded with single fibre EMG. J Neurol, Neurosurg, and Psych 46: 305-309 (1983).

88. Alon G: High voltage stimulation: Effects of electrode size on basic excitatory responses. Physical Therapy 65 (6): 890-895 (1985)

89. Miller ME: Anatomy of the Dog. WB Saunders Co. Philadelphia & London (1968).

90. Patton HD et al (ed). Neuromuscular transmission and Exitation Contraction coupling in skeletal muscle (chpt 6 & 7). Textbook of Physiology. 21st ed. WB Saunders Co (1989).

91. Patton HD et al (ed). Neuromuscular transmission and Exitation Contraction coupling in sleletal muscle. Textbook of Physiology. 21st ed. WB Saunders Co (1989).

92. Kotts YM: Exchange Symposium on electrical stimulation of denervated muscle. Concordia (1977).

93. Lomo T, Slater CR: Control of acetylcholine sensitivity and synapse formation by muscle activity. J Physiol 275: 391-402 (1978).

94. Cohan CS, Kater SB: Suppression of neurite elongation and growth cone motility by electrical stimulation. Science 232: 1638-1640 (1986).

95. Brown MC, Holland RL: A central role for denervated tissues in causing nerve sprouting. Nature 282: 724-728 (1979)

96. Michel RN, Gardiner PF: Effect of previous overload on short term neuromuscular responses to partial denervation. Can J Appl Sport Sci 11: 30 (1986).

97. Jansen JK, et al: Hyperinnervation of skeletal muscle fibers: dependence on muscle activity. Science 181: 559-561 (1973).