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# THE PREVENTION OF MUSCLE ATROPHY FOLLOWING PERIPHERAL NERVE REPAIR USING AN IMPLANTABLE ELECTRICAL SYSTEM



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## ABSTRACT

Despite the best of modern microsurgical skills, the functional result that is achieved following the repair of a severed peripheral motor nerve has usually been less than complete. In an attempt to reduce or prevent denervation atrophy we have devised a totally implantable system of electrical stimulation and have examined it's effect on the target muscle of a repaired peripheral nerve. In this study, twenty New Zealand white rabbits were divided into two groups: in one group the rectus femoris muscle is electrically stimulated after microsurgical repair of the femoral nerve, whereas the control group undergoes microsurgical repair of the femoral nerve only. Comparing the muscles to their contralateral counterparts at eight weeks post-op we found that electrical stimulation resulted in (a) the retention of 79+/-5(SEM)% of muscle bulk (compared to 42+/-3% in the non-stimulated group) $p<0.001$, (b) a maximum force of tetanic contraction averaging 41+/-5% of normal (compared to 19+/-4% in the non-stimulated group) $p<0.01$, (c) less myofiber atrophy, interfascicular fibrosis, and fatty infiltration, (d) significantly less myofilamentous disruption at the ultrastructural level, and (e) a preferential atrophy of type-II fibers ($p<0.001$) in the absence of electrical stimulation. There was no statistical difference between the two groups in the compound action potential waveform or amplitude of evoked contractions. In conclusion, these results suggest that the electrical stimulation of skeletal muscle will improve the ultimate functional result in patients undergoing peripheral motor nerve repair.

## RESUME

Malgré les meilleures techniques microchirurgicales modernes, les résultats fonctionnels atteint après réparation de nerf moteur peripherique laissent toujours a desirer. Dans le but d'ameliorer ces resultats, et d'empecher l'atrophie musculaire, on propose un systeme de stimulation electrique pour le muscle dénervé et dont le nerf a été coupé et réparé. Cette étude porte sur vingt lapins blancs, reparti en deux groupes: dans un, le muscle "grand femoral" est soumis a une stimulation électrique, et ce après avoir réparé le nerf femoral. Le groupe de controle etudié la reparation de ce nerf sans la stimulation du muscle. Huit semaines après chirurgie les muscles sont etudies en relation a (a) le poid et le contour du muscle, (b) les characteristiques electromyographiques, (c) la force tétanique maximale, (d) histologie a la lumiere et electronique, et (e) l'histochimie. En comparant ces muscle a leur voisin contralateral normal, on trouve que l'effect de stimulation electrique favorise (a) la conservation de 79±5(SEM)% de la masse musculaire (comparativement a 42±3% pour le groupe non-stimule) $p<0.001$, (b) une force de contraction maximale de 41±5% de la normale (comparativement a 19±4% pour le groupe non stimule) $p<0.01$, (c) moins d'atrophie, de fibrose, et d'infiltration graisseuse au microscope, (d) la conservation de l'ultrastructure, et (e) un atrophie de préférence des fibres du type II secondaire a l'absence de la stimulation electrique. Ces résultats nous laisse a penser que l'utilisation de la stimulation electrique des muscles dénervé ameliore le résultat fonctionel et éventuel chez les patients qui ont subi la transection d'un nerf moteur peripherique.

## DEDICATION

To my wife, Diane, and my family; whose unselfish love, patience, and encouragement, have allowed me to pursue my studies, and train for a career in plastic surgery.

# INTRODUCTION

The modern surgeon has the ability to repair severed peripheral nerves. The functional recovery which is achieved following the repair of a transected motor nerve, however, is usually less than complete. This has not been felt to be a function of the technical process of nerve coaptation, but rather represents a combination of disuse and denervation atrophy, with subsequent loss of integrity of the muscle of the motor unit during the period of axonal regeneration. The farther proximal the transection is from the muscle, the longer will be the period of time the muscle will undergo atrophy before it is reinnervated. If we are to reduce or prevent this atrophy, in an attempt to improve the functional result in such cases, the muscle of the affected motor unit must be provided with similar environmental and mechanical stimuli as are present in the normally innervated state. This thesis reviews the physiologic environment of the innervated as well as the denervated muscle, and explores the clinical effect of providing electrical stimulation to denervated muscle in an attempt to reduce this atrophy.

## Purpose of the Study

The purpose of the present study is to examine the effects of applying electrical stimulation to denervated muscle in an attempt to prevent or retard the deleterious functional and physiologic changes associated with denervation atrophy; and thus increase the functional recovery to those muscles whose motor nerve supply has been transected and subsequently repaired.

## Hypothesis

The application of electrical stimulation to denervated muscle will retard or prevent the deleterious functional and physiologic changes associated with denervation atrophy.

# HISTORICAL REVIEW

Electricity has carved it's niche in the annals of therapeutic medicine since the dawn of ancient times[8-10]. Perhaps the first therapeutic benefit of electricity was recorded by Scriboneus Largus in 46 AD during the reign of Emperor Tiberius Claudius Nero Caesar. According to legend, Anteros after being freed from slavery by the Emperor happened to step on a torpedo ray fish while walking on the beach. After the initial pain from the electrical discharge wore off, he noted that his chronic gouty pain was also gone. Thereafter, in the first genuine application of electricity in medicine, Scriboneus Largus, a noted physician of the times, began to prescribe live electric fish to be applied topically for not only the treatment of gout but also for such common ailments as headaches as well as rectal prolapse. The physiologic effects of these fish were also known to the Greek fisherman of the Mediterranean hundreds of years before the days of Anteros. They collectively called all of the electric fish narke; which in Greek means numbness producing -- from which the word narcosis originates. Electric fish continued to be prescribed until the middle ages when Otto Van Guericke, by constructing the first rotating frictional generator in 1660, introduced to the scientific community the notion of man-made electricity. The invention of man-made electricity popularized

electrical stimulation as a mode of treatment for a host of afflictions, a partial list of which is presented in Table 1. In 1745, Van Musschenbroek invented a device that would create and conserve large quantities of electricity; and it was this primordial capacitor -- the Leyden Jar -- which allowed man to harness and reproduce the jolting stimulus which had up until that time only been associated with the electric fish. Armed with the leyden jar and the electrostatic generator, self-proclaimed healers and electrotherapists set out to cure all types of ailments. The fact that electrical discharges could cause the spontaneous movement of the wasting limbs of quadri- and paraplegics allowed electrical stimulation to be seen as a therapeutic blessing.

**Table 1**

**ELECTRICAL TREATMENT**

| Indications | Contraindications |
|---|---|
| Frostbite | Pulmonary Tuberculosis |
| Gout | Menstruation |
| Hernias | Abdominal Pain |
| Dyspepsia | Haemorrhage |
| Ascites | |
| Amenorrhea | |
| Tumours | |
| Fever | |
| Tapeworm | |
| Sexual Impotence | |
| Anal Prolapse | |
| Epilepsy | |
| Migraine | |
| Melancholy | |

In fact, towards the mid 1700s the foremost indication for electrical therapy was generally considered to be paralysis of any kind. Such notable names as Luigi Galvani and John Wesley are among those having experimented at great length with electrical stimulation. However, towards the late 1700s the honeymoon with electrical stimulation was to end as practitioners and patients alike began to realize that this new form of therapy did not live up to it's over-exaggerated therapeutic expectations. Benjamin Franklin, who years earlier had reported remarkable cures with the use of electrical stimulation, summed up the scepticism of the times when he wrote: " Those appearances ( of contractions ) gave spirits to the patients and made them hope for a perfect cure...I never knew any advantage from electricity that was permanent".

The scientific foundation of electrotherapy had to wait another one hundred years for Duchenne de Boulogne. After graduating from medical school in Paris in 1831, he devoted his life to the definition of the action of specific muscles using electrical stimulation (figure 1). His favourite patients were those with facial nerve paralysis. With their help he could examine the effect of stimulation of specific facial muscles

**Figure -1**
Duchenne de Boulogne


**Figure - 2**

Injury to the ulnar nerve is shown by 'A'

(c)

unopposed by the distraction of antagonistic muscles. Duchenne de Boulogne also ascribed therapeutic benefits to the use of electrical stimulation of muscles. Figure 2 shows the site of injury--denoted by (A)--to the ulnar aspect of the forearm of one of his patients; with the resulting intrinsic minus deformity which is typical of ulnar motor nerve lesions. Through the use of electrical stimulation therapy, Duchenne de Boulogne treated the arm and claimed that eventually it was able to regain it's functional state (figure 2c). In the latter half of the 1800s, electrotherapy became popular once again. However, this time there were no exaggerated claims or

expectations of miraculous therapeutic benefits. Rather, specific motor points of muscles were mapped for the entire body and these were published in textbooks for teaching purposes. This approach was controlled as well as scientific. By referring to myographic charts, electrotherapists and students learned of, and treated, specific neuromuscular disorders in a controlled fashion. It is noteworthy that many of the principles of electrotherapy which evolved in this period remain in current practice by physiotherapists today.

# LITERATURE REVIEW

Although the use of electricity in medicine dates back thousands of years, it was Dr. John Reid, in 1841, by observing the histological and functional effects of the application of galvanic current to denervated frog and rabbit muscles, who first suggested that electrical stimulation may be beneficial in the treatment of muscles which have been deprived of their nerve supply[11]. Since this time the literature has abounded with reports on the use of electrical stimulation to denervated muscle. To date, however, conflicting conclusions with respect to the usefulness of electrical stimulation in such a setting has prevented it's wide acceptance as a preferred modality of treatment in cases of muscle denervation. At the present time we are presented with a host of reports in the literature proclaiming the beneficial effects of electrical stimulation[12-28]; while others suggest that electrical stimulation has no effect or may even be deleterious to the treatment of denervated muscles[29-38].

During World War I Langley and Kato compared three treatment modalities for denervated muscle that were used in those days to prevent or retard weight loss[39]. In the early 1900s it was believed that atrophy was primarily due to a lack of muscular activity. Langley's group used denervated muscle in the rabbit and compared the effects of (a)massage,

(b)electrical stimulation, and (c)passive range of movement on the prevention of muscle atrophy after denervation. It was concluded from their observations of these three groups, as well as combinations of these treatments, that "electrical stimulation with condenser shocks delays the atrophy, that rhythmic extension of the muscles has a similar but less effect, and that gentle massage for a like time has little, if any, effect"[39]. The fact that the muscle atrophy could not be completely prevented troubled Langley and he postulated that not enough electrical stimulation was applied to the muscle in order to prevent atrophy outright. Langley, in a follow-up study one year later, examined the effects of allowing a longer period of muscle stimulation per day. Two rabbits were subjected to two and one-half hours of muscle stimulation ( after denervation ) per day -- compared to a few minutes of stimulation per day in his previous study. A control rabbit was treated with passive range of motion exercises after it's neurectomy. The stimulated muscles were examined at twenty-one and twenty-three days post-operatively. Langley concluded that "neither electrical stimulation nor passive range of motion had any definite effect in preventing loss of weight" and thus "the atrophy of denervated muscle is not due to the absence of contraction"[40]. The latter of Langley's conclusions is supported by recent speculation and research implicating neurotrophic factors in addition to the occurrence of contractions as necessary for the maintenance of muscle

integrity[41].

Up until 1944, studies examining the effect of electrical stimulation on denervated muscle looked at only one aspect of denervation: atrophy as measured by muscle weight alone. In 1944, Guttman and Guttman undertook an ambitious study when they examined the effects of galvanic exercise on denervated and reinnervated muscles in the rabbit. Parameters that were studied included muscle weight, limb circumference, fibrotic changes, quality of contractions, and the presence or absence of fibrillations. Using galvanic current to produce supramaximal contractions, they found that atrophy could be slowed but not completely prevented[12]. Guttman and Guttman elicited supramaximal contractions in their experiment; and in-so-doing, employed a principle which was to be elucidated only years later--in 1951. This principle states that only those contractions which are vigorous enough to elicit 75% of the maximal tension of which the muscle is capable of generating will be sufficient to retard atrophy in denervated muscle, or promote hypertrophy in the normally innervated muscle[25,42].

In 1955 Wakim and Krusen examined the functional effect--rather than the anatomical or histological effects--of the application of electrical stimulation to denervated rat thigh muscles[26]. Treatment consisted of a square wave current of a duration of one millisecond, which was delivered at a frequency of sixteen impulses per second. This was sufficient

to produce a supramaximal contraction of the extremity. The duration of each period of stimulation varied, as did the number of times that it was delivered each day. The muscles were examined at one month post-op with respect to their ability to perform work. Wakim's group reported that, compared to controls, thirty minutes of electrical stimulation treatment delivered once daily was beneficial; that thirty minutes of stimulation delivered twice per day was better; and that five minute sessions delivered every half-hour over an eight hour day was as beneficial as longer periods of stimulation also delivered every half-hour. It was also reported that stimulated muscles, although stronger than non-stimulated muscle, could only produce 50% of the maximum power that could be generated by normal ( control ) muscles. They concluded by stating that "stimulated denervated muscle was more efficient than was non-stimulated denervated muscle; but neither was as efficient as intact muscle".

In more recent studies, Melichna and Guttman, in 1974, found that electrical stimulation partially prevented the loss of weight in the tibialis anterior muscle of the rat[43]. Once again, however, the prevention of atrophy could not be achieved. Harada and co-workers, in 1979, found that electrical stimulation retarded the atrophy of type-I (slow twitch) muscle fibers in the denervated extensor digitorum longus muscle of the rat[23]. Pachter, Eberstein, and Goodgold reported in 1982 that electrical stimulation suppressed the

atrophy of both type-I (slow twitch) and type-II (fast twitch) fibers in rat muscle after excision of a portion of the sciatic nerve. They reported, however, that this effect was greater with respect to type-II fibres when compared to type-I fibers[22]. The preferential responsiveness of type-II fibres to disuse, denervation, and electrical stimulation has been the subject of much investigation over the past twenty years. It has been reported that type-II fibres are more affected by both denervation[44] as well as disuse[7] compared to type-I fibres. This is to say that after denervation or, after a prolonged period of disuse, type-II fibres undergo atrophy earlier and to a greater extent than do slow twitch fibers[43,45,46]. Furthermore, type-II fibres are also more liable to undergo work hypertrophy than are type-I fibers[7].

Some of the most optimistic results on the effect of electrical stimulation to denervated muscle are noted when it's physiologic effects are examined. In fact, several groups of investigators have reported that electrical stimulation restored the normal resting potential and reduced the supersensitivity of the denervated muscle to acetylcholine[20,48-50]. In another study, which represents one of the only investigations of it's kind on primates, Gilliat, Westgaard, and Williams stimulated the intrinsic hand muscles of baboons--after denervation--with up to fifty-thousand impulses per day. Different patterns of stimulation were employed using a pulse width of one-half milliseconds, an

electric potential of one-hundred and fifty volts, and stimulation frequencies of either five or twenty hertz. Gilliat's group reported that electrical stimulation resulted in a decreased sensitivity of the denervated muscle to acetylcholine. They did not, however, address the investigation of any other parameters in this study. This is quite unfortunate owing to the particular applicability of this model to humans[47].

In 1988, Nemoto, Williams, et al published the first report examining the effects of electrical stimulation of denervated muscle following peripheral motor nerve repair. Both the pulse generator and the electrodes were implanted. The system was operational twenty-four hours per day and delivered four volts per pulse at a frequency of one-hundred and thirty pulses per second, with a pulse width of two-hundred and ten microseconds, a cycle on time of one and one-half seconds, and a cycle off time of sixty seconds. They reported that following transection and subsequent repair of the common peroneal nerve , four centimeters proximal to it's insertion into the peroneus longus muscle in the dog, the application of electrical stimulation was effective in decreasing muscle atrophy and in improving force when the muscle was examined and compared to controls at eight weeks post-op.

In contrast to the positive results presented above with respect to the use of electrical stimulation on denervated

muscle, the literature also contains publications which suggest that electrical stimulation has no effect or may even be deleterious in the treatment of denervated muscle[29-38]. In 1931, Cook and Gerard found that the electrical stimulation of the distal stumps of severed nerves hastened their degeneration[38]. Although this has no direct effect on the prevention or the retarding of muscle atrophy after denervation, it follows that if electrical stimulation discourages axonal sprouting, then the ultimate therapeutic goal--which is the independent functional recovery of the nerve-muscle (or motor) unit--will also be discouraged. Towards the end of that decade, in 1939, Chor, Cleveland, Davenport, et al reported that the electrical stimulation of denervated muscles in the monkey resulted in an increased amount of degenerative muscle fibres and fibrosis than was present in non-stimulated controls. According to this model, as intramuscular fibrosis would proceed, it would present a further impediment--in the form of a mechanical barrier--to the reinnervation of the remaining viable myofibers[31]. This work was supported by a study from Schmrigk and co-workers, who, in 1977 reported that electrical stimulation delayed the reinnervation of treated muscles[36]. In a controversial study in 1982, Girlanda et al directly opposed the positive results obtained by Harada's group three years earlier. Girlanda used a stimulation pattern for muscle which was being employed in clinical electrotherapy. This consisted of square wave current

four-hundred milliseconds in duration at a frequency of forty stimulations per minute and delivered via surface electrodes for five minutes per session. Treatments were carried out twice daily at a current sufficient to produce a supramaximal contraction. Girlanda's group studied rabbits fifty days after the denervation of the extensor digitorum longus and soleus muscles. They reported that electrical stimulation accelerated the atrophy of type-I fibres in both muscles and increased the weight loss of only the soleus muscle.This preferential weight loss is most likely due to the fact that the soleus muscle is composed of mostly type-I (slow twitch) fibres; and thus, the effect of atrophy was more pronounced in this muscle. Although their overall results were inconclusive, the authors pointed out that "the results raise further doubts about the clinical utility of electrotherapy" in the treatment of denervated muscle[37].

Drachman and Witzke, in 1972, reported that in the denervated muscle of rat diaphragms which were stimulated in a pattern mimicking normal activity, electrical stimulation had no effect in retarding atrophy[30]. This work, which suggests an equivocal effect of electrical stimulation on denervated muscle was supported one year later by Herbison, Teng, and Gordon in 1973. Herbison's group examined the effect of electrical stimulation on rat muscle which had been denervated by way of a crushing injury to the sciatic nerve. They reported no difference between the stimulated and control

groups with respect to muscle histology or muscle weight at six to seven weeks post reinnervation. However, a crushing injury and not a formal transection was used.

It is evident from reviewing the literature as well as the history of the electrical stimulation of muscle that the results obtained to date are both exciting as well as controversial. It is apparent from these investigations that, although many scientists would like to believe that electrical stimulation is a useful therapeutic tool, no definite conclusions can be made in the favour of or against the use of electrical stimulation to treat denervated muscle. In part this stems from a lack of meticulously designed and properly controlled scientific publications addressing this issue. Thus, more research remains to be done in order to convince the scientific community of the usefulness of electrostimulation in the treatment of denervated muscle.

# THE PHYSIOLOGIC ANATOMY OF SKELETAL MUSCLE

Since much of the research presented in this thesis deals with the detailed anatomy of skeletal muscle and how this changes in the state of denervation, it would only be appropriate to briefly review it's basic structure. All medically oriented scientists have at one point in time been exposed to the anatomy of skeletal muscle; however, due to the intricate semantics which are involved, the details of such anatomy are easily confused or forgotten. The proceeding section will first review the detailed physiologic anatomy of skeletal muscle in the normal (innervated) state, following which will be described what is currently understood and accepted regarding the physiology and anatomy of denervated skeletal muscle.

## INNERVATED MUSCLE

Skeletal muscle is composed of consolidated muscle fascicles, each of which is made up of discrete muscle cells known as muscle fibers (figure 3). Each muscle fiber is contained by it's own plasma membrane or sarcolemma. In most instances these fibers extend the entire length of the muscle and each is innervated by one nerve ending which is located near the mid-point of the fiber at a specialized region of the sarcolemma known as the motor end plate (figure 4). At the ends of the sarcolemma the muscle fibers fuse and become continuous with the tendons of the muscle.

Muscle cells (or myofibers) are composed of functional units known as myofibrils; and it is these which house the basic contractile elements of actin and myosin. The Z-membranes -- or Z-discs -- make up the cytoskeleton of the individual myofibrils and serve as anchoring points for the actin molecules. The Z-membrane also attaches the myofibrils to each other by passing continuously from one to the other across the muscle fiber.

When there is cross-bridging of actin and myosin molecules there is a resultant approximation of the Z-lines to one another (figure 5). This is the action which leads to muscle contraction. It is well accepted, therefore, that the area between successive Z-lines forms the basic contractile

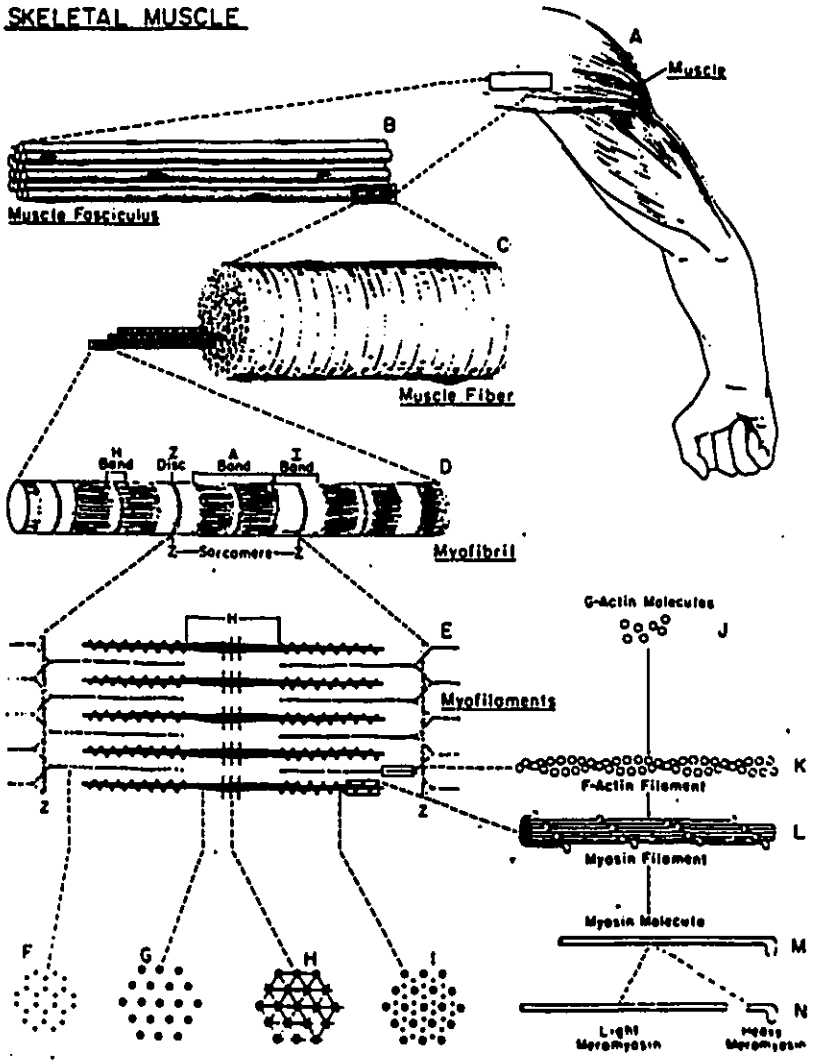



**Figure 3:** The anatomy of muscle (reproduced from Guyton AC: Textbook of Medical Physiology, 6th edition, WB Saunders Co., Philadelphia, 1981).

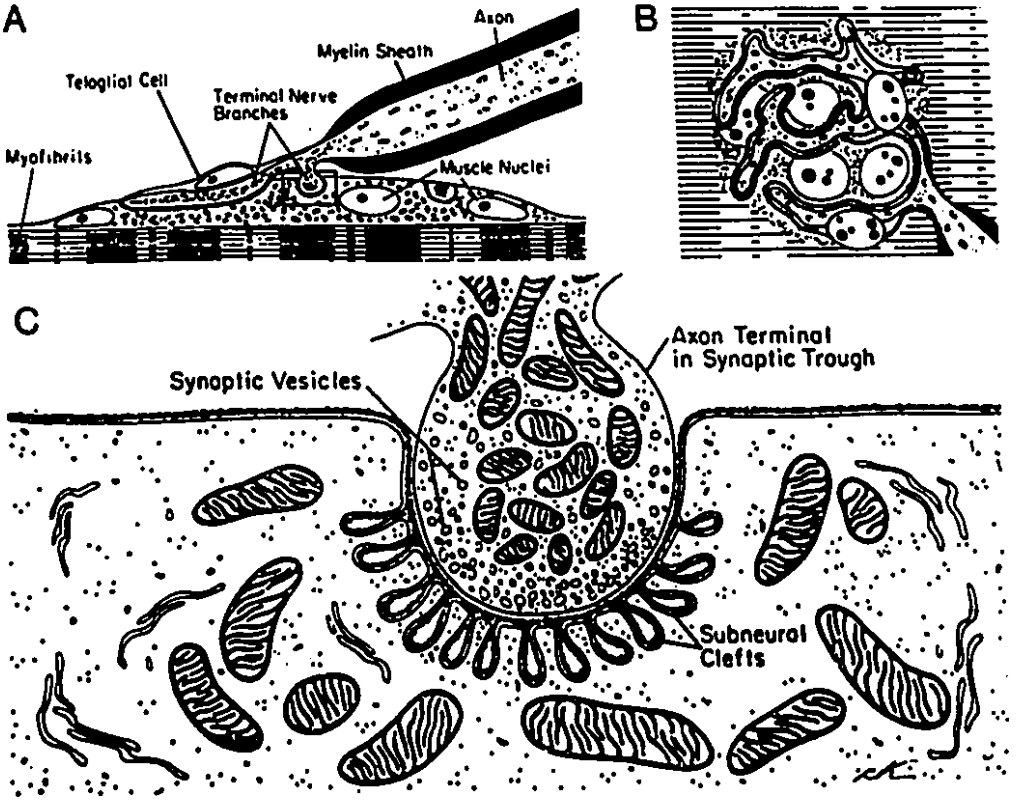



**Figure 4:** The anatomy of the motor end plate region (reproduced from Guyton AC: Textbook of Medical Physiology, 6th edition, WB Saunders Co., Philadelphia, 1981).

unit of skeletal muscle known as the sarcomere. At rest the sarcomere measures approximately two microns in width. The different regions of the sarcomere have been further subdivided into functional and microanatomical units (figure 5) which are described as follows:

**A Band:** Dark bands which contain the myosin filaments as well as the ends of the actin filaments where they overlap the myosin filaments.

**I Band:** Light bands which contain only actin filaments.

**Z-Lines:** These define the sarcomere and act as anchoring structures for the actin molecules.

The cross-bridging of actin and myosin molecules arises as a result of the flux of calcium ions from the terminal cisternae of the sarcoplasmic reticulum which surrounds each myofibril (figure 6). This release of $Ca^{+2}$ into the sarcoplasm around the myofibrils is strictly permissive for the cross-bridging of the myofilaments to occur. The energy required to effect the contraction is supplied from the high energy bonds of adenosine triphosphate (ATP) which is degraded to adenosine

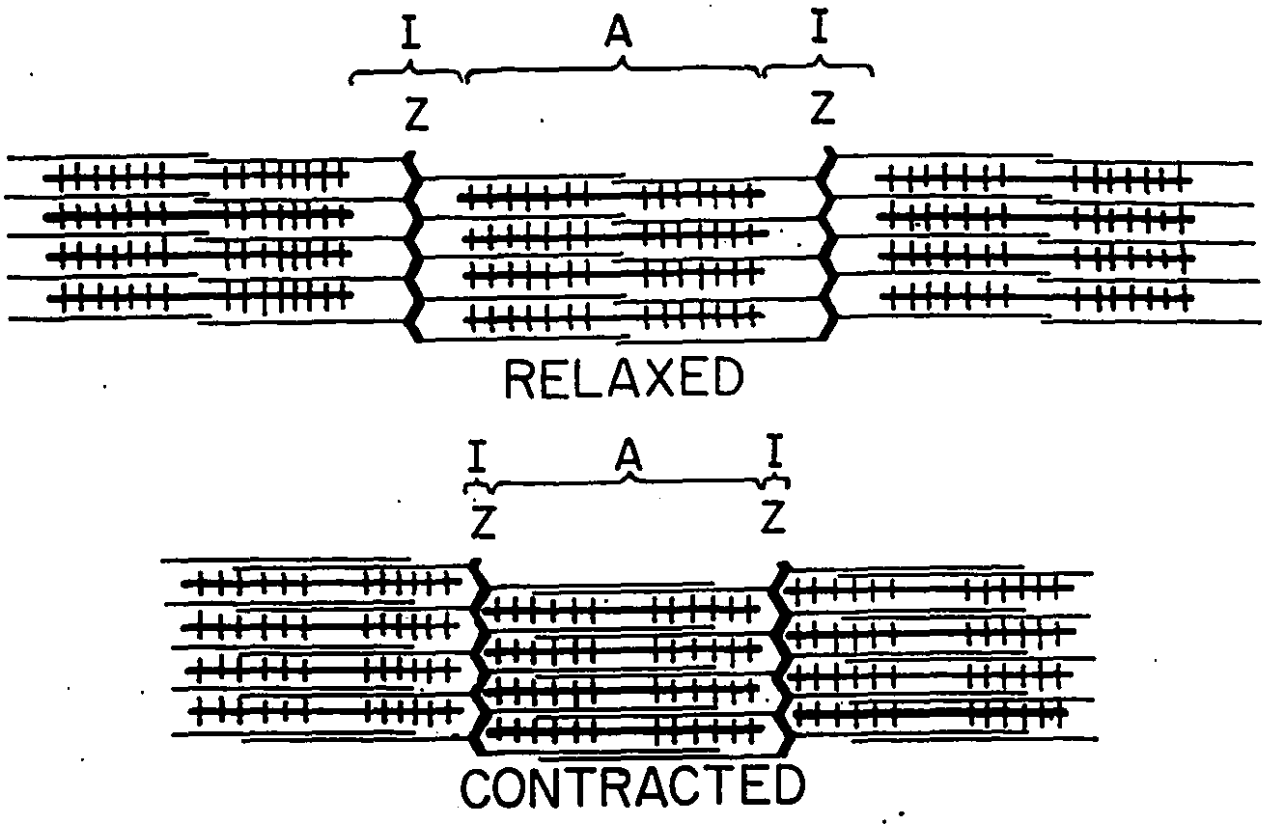



**Figure 5:** The anatomy and contraction of the myofilaments (reproduced from Guyton AC: Textbook of Medical Physiology, 6th edition, WB Saunders Co., Philadelphia, 1981).

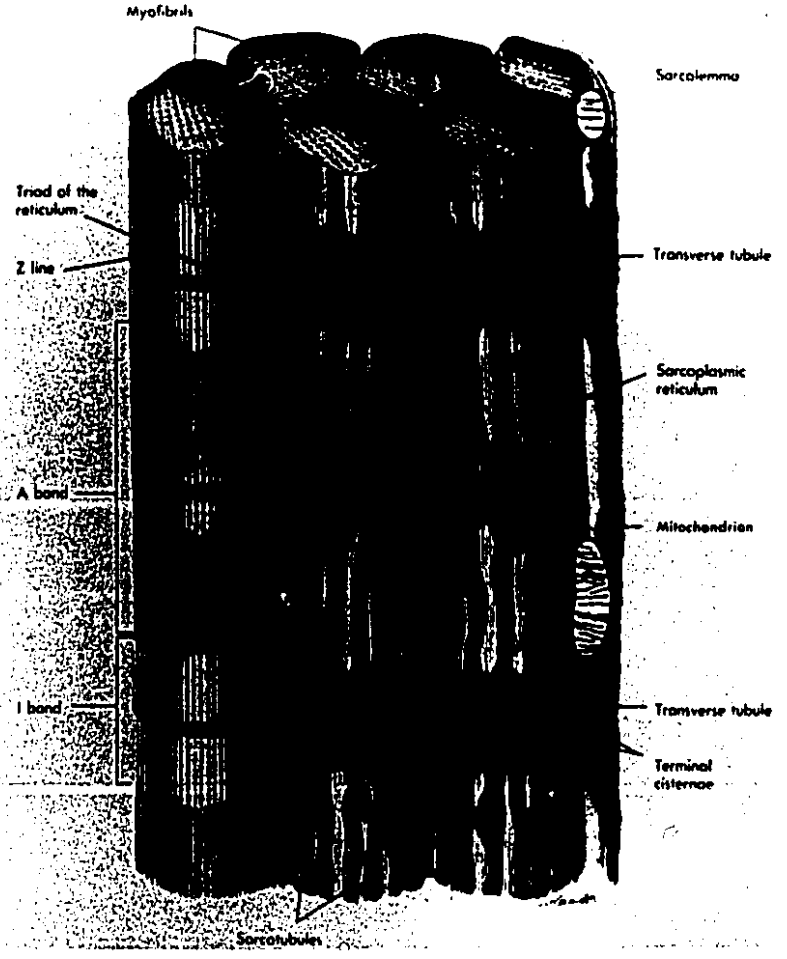



**Figure 6:** The anatomy of the t-tubules and the sarcoplasmic reticulum surrounding each myofibril in the muscle cell (reproduced from Guyton AC: Textbook of Medical Physiology, 6th edition, WB Saunders Co., Philadelphia, 1981).

diphosphate (ADP). The initial release of the calcium ions from the sacoplasmic reticulum is a result of the depolarization of the plasma membrane (in response to a muscle action potential) which is transmitted to the sarcoplasmic reticulum by way of transverse tubules. This t-tubular system is in fact an invagination of the sarcolemma into the cell and accompanies the cisternae of the sarcoplasmic reticulum in surrounding individual myofibrils (figure 6).

## Muscle Fiber Types

The fibers of a given muscle are not homogeneous in their physiologic characteristics and ability to do work over time[7]. Two basic subpopulations of muscle fibers are distinguished in humans: type I and type II. The relative distribution or ratio of type I to type II myofibers in a given muscle depends mainly on (a) the function of that muscle, and (b) the distribution of a given motor unit throughout the muscle. Although muscle fibers of a given motor unit overlap and interdigitate with those of adjacent motor units, it is important to stress that all muscle fibers of a given motor unit are of the same histochemical type[42].

The distinguishing features of the two main muscle fiber types is presented in table-2. If we were to describe the difference between type I and type II muscle fibers on a

fundamental level, we would describe type I as a slow twitch, fatigue resistant muscle fiber, whereas type II is a fast twitch , easily fatiguable muscle fiber. However, a more detailed analysis of their distinguishing features will help to understand the relative and quantitative distribution of myofiber types between specific muscle groups. It is important to note that neither fiber type has exclusive enzymes or metabolic products that are absent from the other, but rather their concentrations are different (table-2).

**Type I Muscle Fibers**

Type I muscle fibers have a relatively higher concentration and capacity for oxidative enzymatic activity such as that associated with the tricarboxylic acid cycle of Krebs and the electron transport chain. Structurally, these fibers are generally smaller, have more mitochondria, more sarcoplasm between myofibrils, and are surrounded by more blood capillaries than type II fibers[42]. They also have a larger amount of myoglobin present in their sarcoplasm than type II fibers. Functionally, what this means is that a muscle which is comprised of mostly type I fibers is more suited for prolonged work but has a slower rate of contraction than a muscle composed of mainly type II fibers. This slow muscle is

## Table 2

## Differences Between Type I & Type II Muscle Fibers

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

Table 2 reproduced from: Sarna HB: Muscle Pathology and Histochemistry. Chapter 1, American Society of Clinical Pathologists, Chicago, 1983.

frequently called a red muscle due to the reddish tint imparted to it by the myoglobin as well as the large amount of red blood cells present in the dense network of capillaries surrounding each myofibril. Two subtypes of type I muscle fibers are recognized; and these are differentiated by intermediate staining intensity with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR)[51].

## Type II Muscle Fibers

Type II muscle fibers, in contrast to type I myofibers, contain higher concentrations of glycogen and glycolytic enzymes such as those associated with the Embden-Meyerhof pathway. The difference in the relative concentrations of oxidative and glycolytic enzymes present in each myofiber population suggests that type I muscle fibers use mainly aerobic metabolism, whereas the type II muscle fibers rely mainly on anaerobic pathways. We make use of this basic principle in staining for and differentiating type I from type II muscle fibers histologically. The myofibrillar enzyme adenosine triphosphatase(ATPase) is unevenly distributed between the two histochemical fiber types such that the ATPase stain is able to yield excellent contrast between fiber types. Structurally, type II fibers have a much more extensive sarcoplasmic reticulum when compared to type I fibers; which

allows the rapid release and re-uptake of calcium ions. Functionally, we find that muscles which are comprised of mostly type II fibers have a more rapid rate of contraction but, due to their metabolism, they tend to fatigue more quickly than type I fibers. Three subtypes of type II fibers are recognized (IIa, IIb, IIc) and are differentiated by way of preincubation of muscle sections at various hydrogen ion concentrations before staining with ATPase.

## Distribution of Fiber Types

Based on the metabolic and physiologic characteristics between muscle fiber types, it is not surprising to find that some muscle in the human body exhibit disproportionate ratios of type I to type II fibers. In most instances these can be predicted from a knowledge of the function of a particular muscle. The most notable examples in this regard are the soleus, deltoid , rectus abdominis, and erector spinae muscles which are made up of anywhere from 60% to 80% type I fibers. On the other hand, the gastrocnemius and extra-ocular muscles are composed of mainly type II fibers.

## Fiber Type Transformation

This section on muscle physiology would not be complete without a word about the elastic nature of muscle fibers. As it was mentioned previously, the innervation of a muscle fiber is perhaps the most important factor determining the physiologic character of that fiber. The fact that this character is mutable and dependant on the source of innervation was well demonstrated in a study published as early as 1967[52]. In the cat, the soleus muscle is composed of mainly type I fibers, and the extensor digitorum of the hindleg, of type II fibers. When the nerve supply of each of theses muscles is crossed, the physiologic and histochemical properties of these two muscles becomes reversed: the soleus takes on properties of a type II muscle, and the extensor digitorum takes on the properties of a type I muscle. Since that time, the elastic nature of the muscle fiber has been confirmed by similar experiments[53];thus opening the door to a multitude of possible clinical applications. The transformation of innervated skeletal muscle into a fatigue resistant pedicle for use as a right ventricular assisting organ was pioneered by Dr. Stephenson's group at the University of Pennsylvania[54]. Similar work is presently being expanded and refined by Dr. Chiu's group at McGill University[55]. As an extension of this principle, it should not

be surprising to find that denervated muscle can be conditioned to become fast or slow, weak or strong, fatigue resistant or easily fatiguable, depending on the particular pattern of activity which is imposed on it.

## DENERVATED MUSCLE

Relatively little is known about the physiology of denervated muscle when compared to that of normal muscle. This particular fact cannot be underemphasized because even less is known about the physiology or the changes which occur in denervated, stimulated muscle. Thus, most of the work that is explored in this thesis deals with a relatively unexplored and poorly understood field of research. However, some facts relating to the physiology of denervated muscle have been elucidated and are currently accepted. These basic notions are described below; and constitute the infrastructure for all of the present-day research relating to denervated muscle. The changes that occur in denervated muscle are best described in terms of structural and physiologic changes.

### Structural Changes

The earliest structural change observed in skeletal muscle is the atrophy of muscle fibers. This atrophy--which manifests in the first three weeks of denervation--occurs as a result of the loss of myofilaments (actin and myosin)

without any gross cytoarchitectural changes being evident in the muscle fiber[58,59]. After three to four weeks of denervation, lysosomes, which are not normally present, appear between the myofibrils and further degrade the degenerating myofilaments, such that, after two months, most of the myofibrils have been lost[59].

Other structural changes that occur in response to denervation include the appearance of vacuoles, the loss of the special features of the motor endplate region, and the appearance of centrally located nuclei in the muscle fibers[45,57,58]. The sarcolemmal nuclei do not migrate internally from the periphery of the muscle cell--as is sometimes erroneously referred to--but, rather, the decrease in cytoplasmic mass, owing principally to a loss of myofibrils, generates the impression of both (i) a larger number, and (ii) more centrally located nuclei.

From a teleologic standpoint, the major impediment to the potential return of function of a muscle is the ultimate result of denervation: muscle fiber degeneration and fibrosis. It is theoretically conceivable that ultimately, myofibrillar degeneration will result in strings of endomysial collagen surrounding chains of degenerating nuclei. On the other hand, although it is atrophic, as long as a denervated muscle fiber retains it's basic cytoarchitecture, it is capable of undergoing hypertrophy once reinnervated. Thus, the potential return of function, and functional integrity, all comes down

to a question of time: how much time is allowed to pass before the structure of denervated muscle is irretrievably lost? The literature is presently unclear about the answer to this question. Some investigators report slow degeneration while others describe considerable degeneration rates[31,59-62]. The estimate is that irreversible degeneration of a muscle fiber occurs somewhere between one and three years following denervation.

## Physiologic Changes

Although denervation results in the loss of evoked release of acetylcholine (ACh) from nerve terminals, miniature endplate potentials have been recorded for up to twenty-four hours after denervation[63]. This is said to be the result of either (i) the emptying of ACh from the distal nerve terminals, or (ii) the degeneration of the nerve terminal.

The earliest physiologic detectable change is a decrease in the resting potential of the muscle cell (i.e. it becomes less negative), which is evident at six to eight hours after denervation[3]. The resting membrane potential then continues to fall from a value of -80mV to -65mV at approximately one week post denervation. As a result of this, there is a gradual increase in the transmembrane resistance over a similar time span[4,5].

In normal muscle, ACh receptors are located only at the specialized motor endplate region of the sarcolemma. However, in denervated muscle large numbers of new, extrajunctional ACh receptors are synthesized and become incorporated into the entire length of the fiber's membrane[63]. This occurs during the first few weeks of denervation. As a result, the myofiber becomes exquisitely responsive to low doses of ACh, and is termed ACH hypersensitive[48,64].

Fibrillation, which is apparent after two to three days of denervation, involves the spontaneous and uncoordinated rhythmic contraction of individual muscle fibers. It cannot be observed through the skin, but manifests as a continuous, fine rippling of the exposed surface of a denervated muscle. Fibrillation is thought to be the result of pacemaker activity that has developed in the region of the denervated endplate membrane[65,66]. The fibrillation is not the result of ACh hypersensitivity, as was once believed.

# MATERIALS AND METHODS

Twenty New Zealand white male rabbits each weighing between 2,8 and 3,2 kilograms were divided into two groups each comprised of ten animals(see Fig. 7). The animals in both groups were first sedated by an intramuscular injection of a suspension containing ketamine, xylazine, and acepromazine. After sedation, the rabbits were intubated with a non-cuffed neonatal endotracheal tube whose internal diameter measured 3,5 millimeters, and through which 1,5% halothane anaesthesia was induced and maintained for the duration of the procedure.

The operative procedure, which was performed under sterile conditions was similar, but differed between the control group (A) and the study group (B) in the following manner:

**Group A:** After the left femoral region had been exposed through a longitudinal groin incision, the femoral nerve was carefully dissected and it's distal branches were identified (see Fig. 8a). These are (a) the motor nerve to the rectus femoris muscle, (b) the saphenous nerve, and (c) the motor nerve to the gracilis muscle. The main trunk of the femoral nerve was transected at a point 3,5 centimeters from the insertion of it's branch into the rectus femoris muscle using a razor blade (see Fig. 8b). This was repaired with 10-0 (twenty-two micron thick) nylon suture using microsurgical technique (see Fig. 8c). Attention was then directed to the

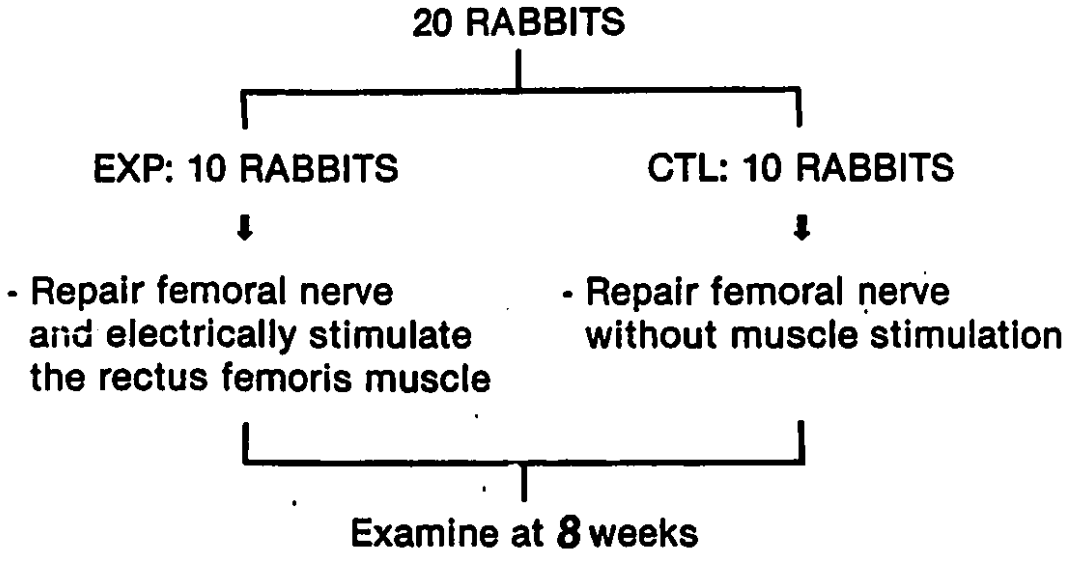
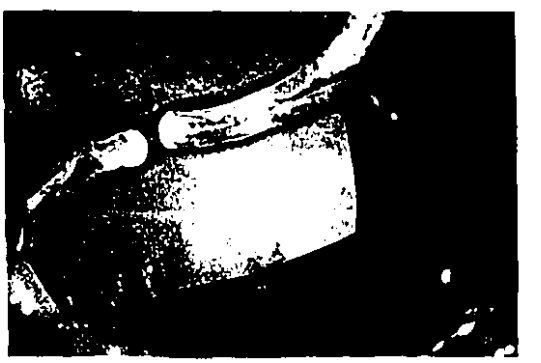



**Figure 7:** Schematic representation of the experimental design.

A

B



C

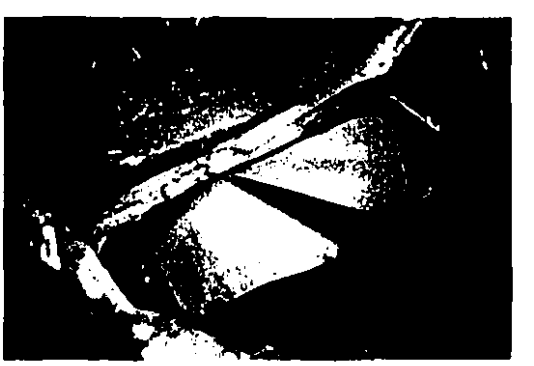


**Figure 8:** (a) The femoral nerve and it's three principal branches, (b) the main trunk has been transected, (c) the femoral nerve has been repaired by microsurgical technique.

anterior thigh where, through the same incision, the rectus femoris muscle was exposed and carbon matrix electrodes were circumferentially fastened about the proximal and distal belly of the muscle. The proximal portion of this electrode was tunnelled subcutaneously to the left flank of the animal. Following this, the wound was irrigated with normal saline solution and was closed in layers.

**Group B:** In this group of animals the same procedure was carried out as in group A but the proximal portion of the electrodes were connected to an **Itrel** stimulating device (medtronic model number 7420--see Fig. 9). Through a separate incision the appliance was placed into a subcutaneous pocket on the left flank of the animal and the proximal portion of the electrodes were connected to it in situ. In this manner the entire electrical system was implanted (Figure 10). Both wounds were irrigated and closed in layers.

Once implanted, the **Itrel** appliance is capable of offering a wide range of stimulation parameters which are controlled and determined by means of an external programmer. These parameters include:

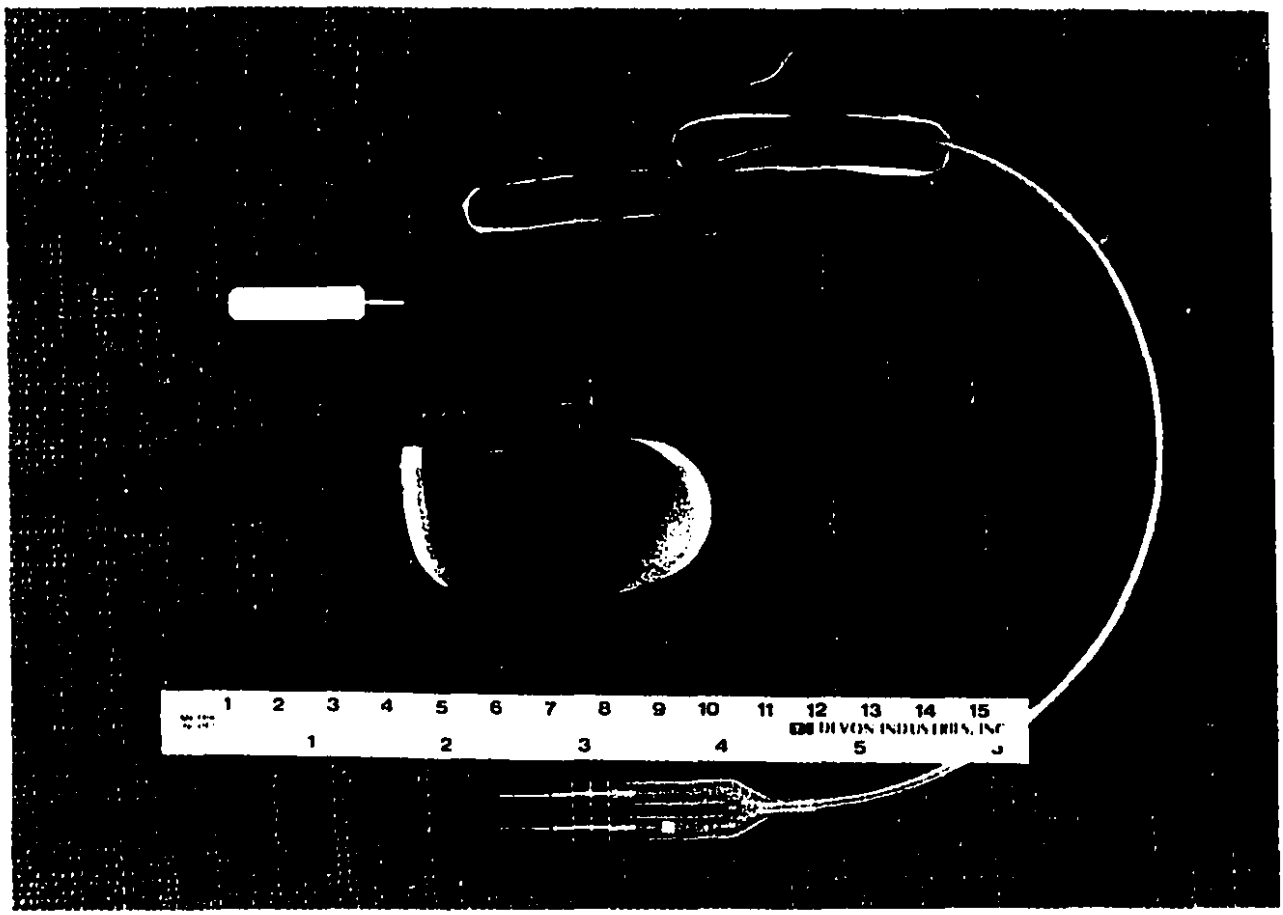



**Figure 9:** The Itrel stimulating device and carbon matrix-mesh electrode. Also shown is the tool used to secure the electrodes to the appliance.

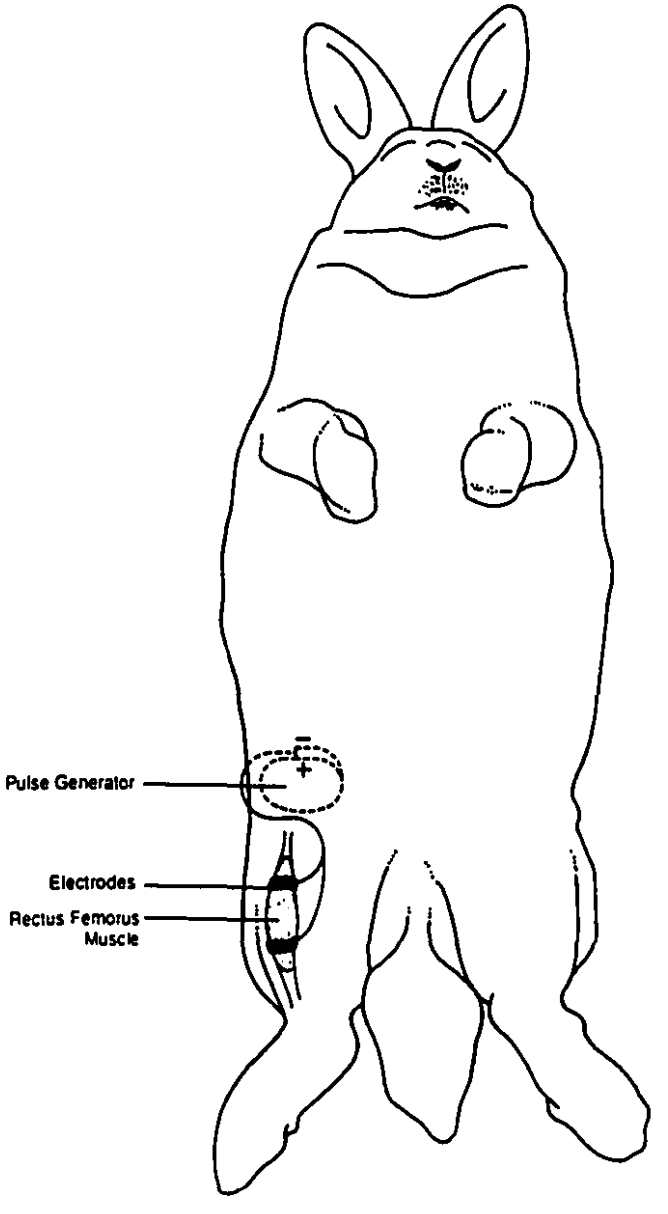



**Figure 10:** Schematic representation of the electrical stimulating system in-situ.

**The Amplitude:** This is the amount of electrical intensity delivered by a stimulating pulse, measured in volts.

**The Rate:** This is the number of times, in pulses per second (Hz), that a stimulating pulse is delivered.

**Pulse Width:** This is the measure, in microseconds, of the duration of a stimulating pulse.

**Cycle Time On:** In a cycling mode, this is the length of time of actual stimulation, i.e., how long the appliance is on.

**Cycle Time Off:** This is the length of time between stimulation periods, i.e., the time of the resting period.

The relationship of these parameters to one-another is depicted in figure 11. Using the electrical system described in this study, the animals in group-B had the rectus femoris muscle stimulated using the following parameters:

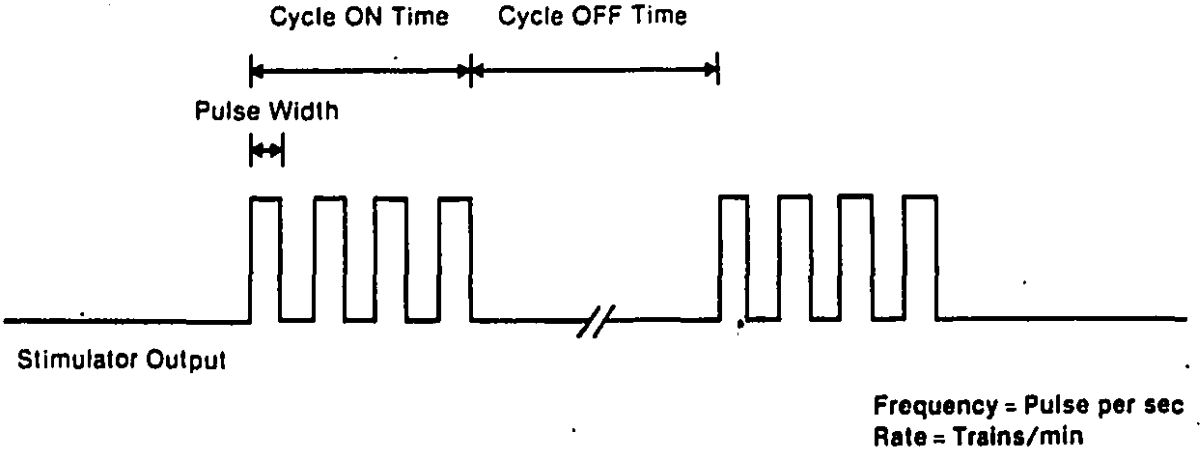



**Figure 11:** The relationship of the described stimulating parameters to one-another (reproduced from Nemoto K, Williams HB, et al: The effects of electrical stimulation on denervated muscle using implantable electrodes. J Reconstructive Microsurgery 4:251, 1988).

Amplitude: 4->6 volts

Rate: 36 pulses per second

Pulse Width: 210 microseconds

Cycle On: 0,1 seconds

Cycle Off: 4 seconds

It is of note that two values are cited for the amplitude. The reason for this being that once cycled stimulation is applied to the rectus femoris muscle (usually within three hours of denervation and nerve repair), the force of the evoked contraction decreased over the ensuing three days. In order to maintain the same force of contraction throughout the experiment, the amplitude of the delivered pulse had to be increased by 50% at three days post-op. At no other time did any of the other parameters or the amplitude have to be readjusted for the remainder of the experiment. The need to increase the amplitude of the stimulus in order to retain an effective contraction is most likely a reflection of the increase in transmembrane resistance and the fall of the resting membrane potential that is typical of muscle fibers in the early phase of denervation[1-5].

At six to eight weeks post-operatively the animals undergo an acute experiment. The nerve-muscle preparation of each animals's operated leg is examined according to the modes of evaluation listed below. The results obtained are then

compared to their contralateral counterparts.

1. Muscle Force Measurement:

This was assessed with the use of Grass instrumentation (force displacement transducer FT03C, FT10C, stimulator SD9, and polygraph 7B). With both heads of the rectus femoris muscle still attached to their origins, the distal quadriceps tendon was disinserted and was sutured to the transducer. The femoral nerve was stimulated at a point distal to the site of repair with twice the minimal voltage required to generate a twitch response. Stimulating frequencies were 1, 2, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, and 60 pulses per second and the pulse width was 1,0 milliseconds. The maximum force of the tetanic contraction was measured.

2. Electromyographic Studies:

Using the DISA electromyographic apparatus (model 1500 from DANTEC), evoked potentials were recorded with platinum needle electrodes. The stimulating electrode was inserted proximal to the site of nerve repair after the recording electrode had been placed within the substance of the rectus femoris muscle belly. The position of the recording electrode was verified by recording fibrillation activity as well as positive sharp waves from the muscle. These findings are characteristic

of denervated and recovering muscle[6]. Attention was directed to the analysis of the muscle action potential waveform and amplitude which was evoked with a sufficient stimulus to produce a supramaximal contraction.

### 3. Wet Muscle Weight:

Soon after the muscle is harvested, it is weighed on a Mettler electric scale (model P 120). The weight of the muscle is taken to represent the mass of the myofibers that are contained within it. Such being the case, the atrophy of the muscle fibers and hence of the entire muscle itself will be reflected as a decrease in it's wet weight.

### 4. Histologic Evaluation:

Samples of both stimulated and non-stimulated muscle are prepared for examination by light microscopy using hematoxylin and eosin (H & E) as well as Mallory trichrome stains. The H & E stain yields information about the muscle architecture, the presence or absence of fatty infiltration, and the ingrowth of fibrous tissue. With the use of a Leitz Dialux 20 microscope connected to a Carl Zeiss MOP-Videoplan measuring system, muscle fiber size as well as type can be assessed and quantified. The Mallory trichrome stain serves to improve the definition of the fibrous tissue network by staining

the collagen tissue blue.

5. Histochemical Determination:

Since animal and human skeletal muscle fibers are not homogenous in their physiologic or metabolic characteristics, the definition of the subpopulations of muscle fiber types present in the samples is necessary. This aids in evaluating what effect electrical stimulation has on the physiologic and metabolic properties of the muscle cell. These basic characteristics between type I (slow twitch) and type II (fast twitch) muscle fibers allows each population to be separately identified with histochemical stains[7]. For this purpose, staining for myofibrillar ATPase (adenosine triphosphatase) is useful since it yields very good contrast between fiber types. At a ph of 10,4, type II fibers stain darker than type I fibers; and this staining characteristic can be reversed using acidic preincubation at a ph of 4,3. Sections are taken from the middle third of each muscle specimen and the cross sectional area of individual fibers (i.e. individual muscle cells) of each population are measured and compared.

6. Electron Microscopy:

Three one centimeter fascicles were dissected from the central portion of each specimen and were fixed in a glutaraldehyde solution. For each specimen, transversely as well as longitudinally oriented blocks were embedded in Epon and were ultra-thin sectioned for electron microscopy. At least three different fibers were photographed from each block at magnifications ranging from 3000 to 39500 X. Each photomicrograph was quantitatively and qualitatively analyzed.

# RESULTS

Six different modes of evaluation were used to assess the effects of electrical stimulation on denervated muscle. Of these, four describe objective, and two are subjective modes of evaluation. In this section the results are presented in the order in which the experiments were conducted.

## 1. Electromyographic Studies:

This was the first test conducted at the time of the final evaluation of each nerve-muscle preparation. Each specimen was evaluated in the same manner; which is described in the section on materials and methods . The data collected was aimed at the analysis of the muscle action potential waveform and amplitude; which was evoked with a sufficient stimulus to produce a supramaximal contraction. The data describing the maximum evoked potentials (measured in millivolts) of each specimen in the stimulated group is presented in Table 3, and for the non-stimulated group in Table 4. It was found that the waveform was of the same morphology in all the specimens in both groups. The amplitude of the evoked potentials averaged 39.7 $\pm$ 16 (SEM)% of normal in the stimulated group, and 19 $\pm$ 4 (SEM)% of normal in the non-stimulated group. Analysis of the evoked potentials by the Student's t-test confirmed that the results between the two groups did not differ significantly (p=0.15).

## Table 3

### ELECTROMYOGRAPHY:STIMULATED GROUP

| Subject # | Maximum Evoked Potential (millivolts) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 2.8 | 3.5 | 80.0 |
| 2 | 0.5 | 5.0 | 10 |
| 3 | 0.8 | 29.0 | 2.8 |
| 4 | 4.0 | 24.0 | 16.7 |
| 5 | 10.0 | 13.0 | 76.9 |
| 6 | 12.8 | 24.6 | 52.0 |
| 7 | 9.2 | 18.0 | 49.7 |
| 8 | 6.6 | 22.5 | 29.4 |
| 9 | 7.6 | 17.4 | 43.4 |
| 10 | 8.8 | 24.2 | 36.3 |

## Table 4

### ELECTROMYOGRAPHY:NON-STIMULATED GROUP

| Subject # | Maximum Evoked Potential (millivolts) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 0.5 | 10.0 | 5.0 |
| 2 | 0.8 | 29.0 | 2.6 |
| 3 | 6.0 | 15.0 | 40.0 |
| 4 | 4.0 | 15.0 | 26.7 |
| 5 | 3.1 | 15.0 | 20.7 |
| 6 | 4.6 | 27.2 | 17.0 |
| 7 | 3.0 | 12.5 | 24.0 |
| 8 | 6.0 | 28.0 | 21.4 |
| 9 | 3.4 | 16.3 | 21.0 |
| 10 | 2.8 | 15.4 | 18.2 |

## 2. Muscle Force Determination:

As a functional evaluation of the effect of electrical stimulation on denervated muscle, the maximum force of tetanic contraction was evaluated. The maximum force that could be generated by the operated side was compared to that generated by the contralateral muscle of each animal. In this way the maximum force generated by each nerve-muscle preparation was expressed as a percentage of normal for purposes of analysis. The raw data for each specimen in the stimulated and the non-stimulated groups are presented in Table 5 and Table 6 respectively. Electrical stimulation resulted in a maximum force of tetanic contraction averaging 41 ± 5 (SEM)% of normal, whereas the non stimulated group generated a maximum force of tetanic contraction averaging 19 ± 4 (SEM)% of normal (figure 12). Analysis of the data using the Student's t-test shows this to be a significant difference $p < 0,01$.

## 3. Wet Muscle Weight:

The operated and non-operated rectus femoris muscles from each animal was weighed; and the value of the operated side was expressed as a percentage of it's contralateral rectus femoris muscle for purposes of analysis ( tables 7 and

Table 5

MUSCLE FORCE:STIMULATED GROUP

| Subject # | Maximum Force of Contraction (grams) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 3 | 650 | 58.5 |
| 2 | 210 | 630 | 33.7 |
| 3 | 240 | 650 | 36.9 |
| 4 | 400 | 750 | 53.3 |
| 5 | 260 | 800 | 32.5 |
| 6 | 100 | 340 | 29.4 |
| 7 | 230 | 610 | 37.3 |
| 8 | 325 | 650 | 49.9 |
| 9 | 315 | 715 | 44.1 |
| 10 | 180 | 565 | 31.5 |

Table 6

MUSCLE FORCE:NON-STIMULATED GROUP

| Subject # | Maximum Force of Contraction (grams) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 280 | 600 | 46.7 |
| 2 | 190 | 750 | 25.3 |
| 3 | 70 | 700 | 10.0 |
| 4 | 85 | 590 | 14.6 |
| 5 | 60 | 660 | 9.1 |
| 6 | 100 | 650 | 15.4 |
| 7 | 65 | 380 | 17.1 |
| 8 | 30 | 650 | 14,0 |
| 9 | 70 | 670 | 10.6 |
| 10 | 200 | 720 | 27.4 |

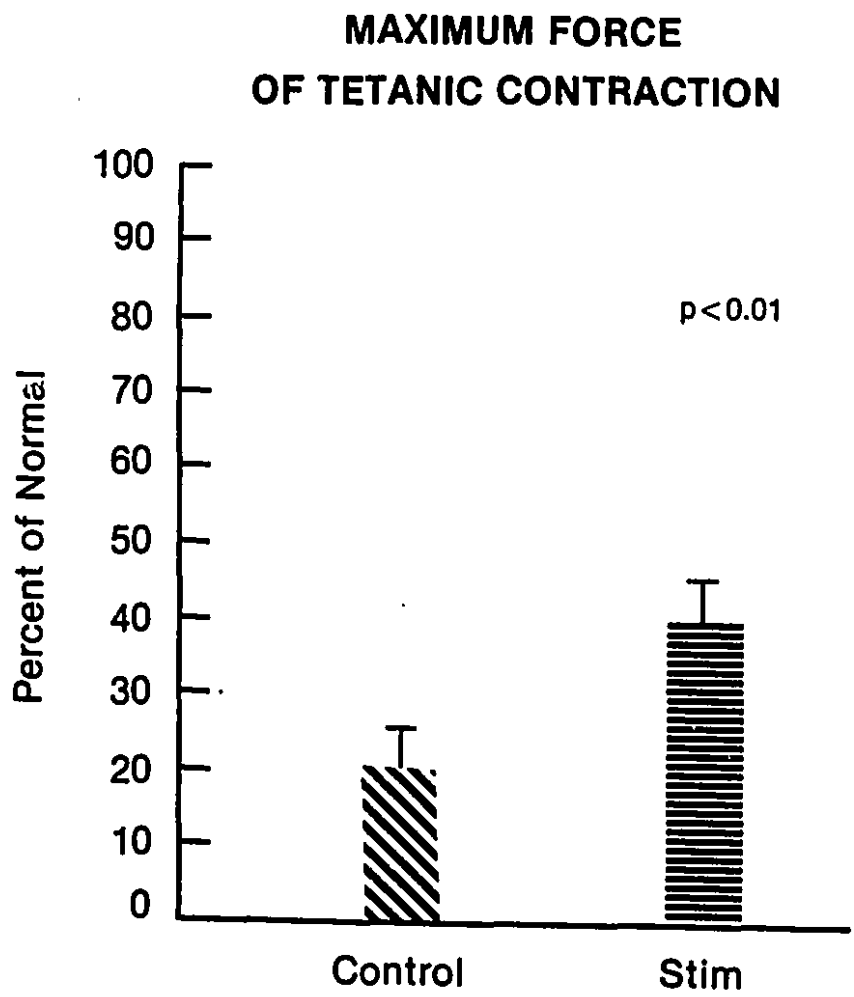



**Figure 12:** Electrical stimulation resulted in a maximum force of tetanic contraction averaging 41±5 (SEM)% of normal, compared to 19±4 (SEM)% in the non-stimulated group (p<0,01).

8. On gross examination the non-stimulated muscles appeared more lean than did the stimulated muscles who, themselves, were less bulky than a normal rectus femoris muscle (figure 13). Electrical stimulation resulted in the retention of 79 ± 5 (SEM)% of muscle mass, compared to the non-stimulated group which retained only 42 ± 3 (SEM)% of their original muscle mass (figure 14). This difference in muscle mass between the stimulated and non-stimulated groups was significant ($p<0,001$).

## 4. Histological Evaluation:

Examination of the histologic sections of normal, stimulated, and non-stimulated muscle revealed striking differences between the three groups (figure 15). The stimulated muscle retained a well organized architecture of it's myofibers within individual fascicles; but, however, it did show a moderate degree of interfascicular fibrosis when compared to normal muscle. Also, myofiber size did not vary significantly in the stimulated group, and was similar to that found in normal muscle.

Conversely, examination of the non-stimulated muscle shows severe interfascicular fibrosis, a large variability in the size of the myofibers with a marked tendency to atrophy within all fascicles, and, in addition to a

Table 7

MUSCLE WEIGHTS:STIMULATED GROUP

| Subject # | Wet Muscle Weight (grams) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 10.0 | 11.0 | 91.0 |
| 2 | 8.0 | 9.5 | 84.2 |
| 3 | 6.2 | 10.0 | 61.4 |
| 4 | 7.0 | 10.2 | 68.6 |
| 5 | 8.6 | 9.5 | 90.5 |
| 6 | 10.0 | 12.5 | 80.0 |
| 7 | 8.4 | 9.4 | 89.5 |
| 8 | 7.0 | 10.2 | 68.9 |
| 9 | 8.3 | 11.3 | 73.7 |
| 10 | 8.5 | 10.0 | 84.7 |

Table 8

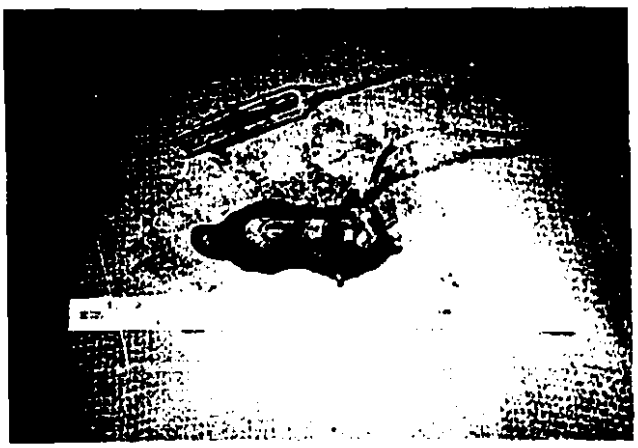
MUSCLE WEIGHTS:NON-STIMULATED GROUP

| Subject # | Wet Muscle Weight (grams) | | %(exp/ctl) |
|---|---|---|---|
| | experimental | control | |
| 1 | 3.9 | 10.2 | 38.1 |
| 2 | 4.1 | 10.1 | 40.4 |
| 3 | 4.5 | 12.0 | 37.5 |
| 4 | 3.0 | 9.0 | 33.3 |
| 5 | 4.0 | 9.5 | 42.1 |
| 6 | 4.6 | 10.5 | 43.8 |
| 7 | 4.9 | 12.2 | 40.6 |
| 8 | 6.6 | 10.1 | 65.3 |
| 9 | 4.4 | 9.6 | 45.8 |
| 10 | 4.5 | 11.4 | 39.4 |

# RECTUS FEMORIS

Normal

Stimulated



Non-stimulated

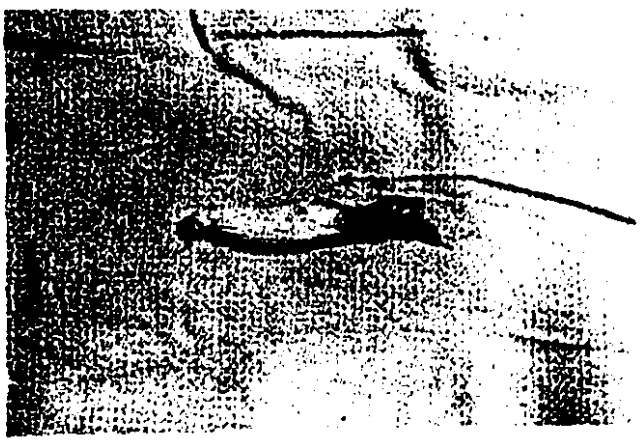


**Figure 13:** The rectus femoris muscle.

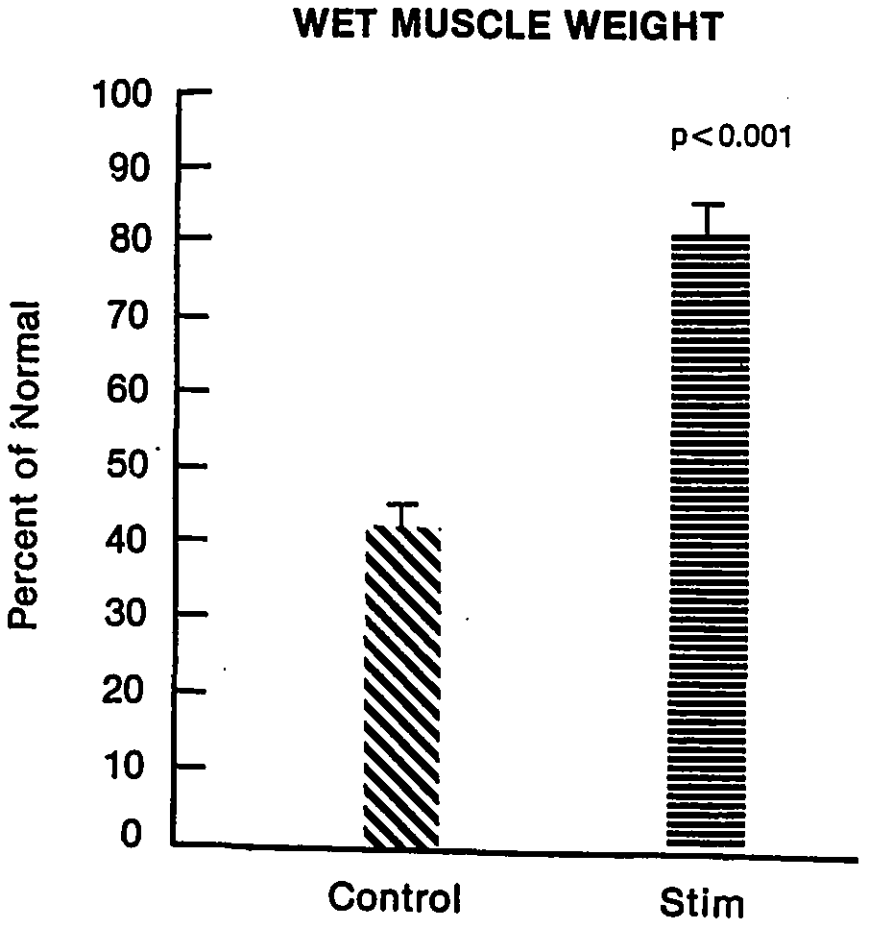
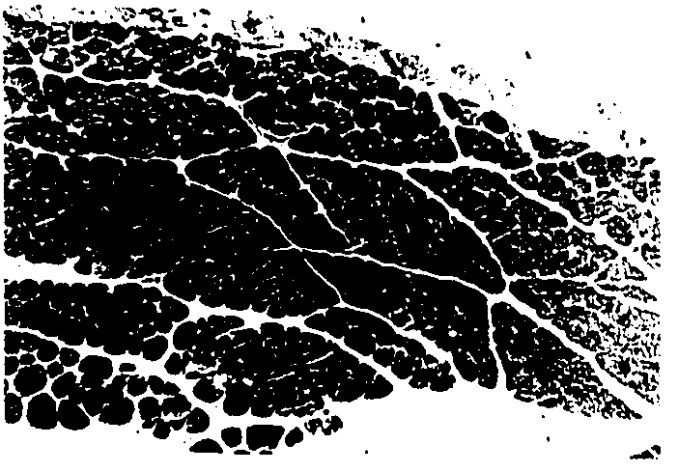



**Figure 14:** Electrical stimulation resulted in the retention of 79±5 (SEM)% of muscle bulk, compared to 42±3 (SEM)% in the non-stimulated group (p<0,001).

TRICHROME STAIN × 40

Normal



Stimulated

Non-stimulated

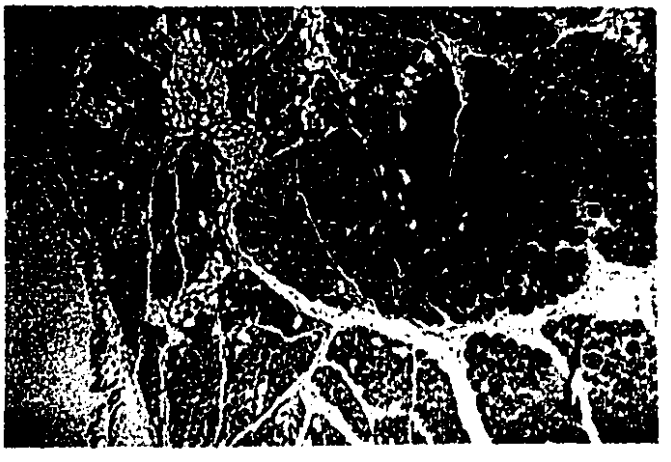


**Figure 15:** Trichrome stained sections of the three groups. Magnification X40.

significant disruption of the fascicular architecture, there was a tremendous degree of fatty infiltration of the muscle. In sum, it was noted that electrical stimulation resulted in less myofiber atrophy, less fatty infiltration, and less interfascicular fibrosis when compared to non-stimulated muscle (figure 15).

## 5. Histochemical Studies:

Each sample of stimulated and non-stimulated muscle was stained using ATPase in order to differentiate between type I and type II sub-populations of muscle fibers. Over one thousand readings of myofiber diameters was collected for the type I and type II fiber subpopulations of each muscle sample. The mean fiber diameters for both type I and type II cells for each sample are presented in table 9 for the stimulated muscles, and in table 10 for the non-stimulated muscles. As well, calculations of mean fiber diameters for type I and type II cells in normal muscle was carried out. In normal muscle type I myofibers averaged 2807 ± 274(SD) microns in diameter, whereas the type II myofibers averaged 3917 ± 661(SD) microns in diameter (Figure 16).

Figure 17 shows representative sections of normal, stimulated, and non-stimulated muscle which have been stained with ATPase in order to differentiate type I from type II

muscle fibers. In these sections type I fibers are stained black, and the type II fibers are light coloured. It is evident that in the normal state the rectus femoris is composed of mainly type II (fast twitch) fibers; as one would have predicted from knowledge of this muscle's function. Several aspects of the analysis of these fibers is quite worthy of note: the proportion of type I to type II muscle fibers is unchanged by either the application of electrical stimulation (at the parameters previously defined) or it's absence. Approximately ninety percent of the muscle fibers in both groups as well as in normal muscle was composed of type II fibers (figure 17). The apparent increase in the number of type I fibers seen in the non-stimulated group in figure 17 is but a reflection of the atrophy of type II fibers such that an absolute greater number of both types of muscle fibers was photographed in the grid shown. The proportion of type I to type II fibers, however, did not change.

The size of the type I muscle fibers did not significantly differ between the stimulated and non-stimulated muscles (or their normal counterparts) as found by a comparison of the population means by analysis of variance (figure 18). This is reflected in figure 17 where the dark type I fibers appear the same size in each of the samples shown.

Conversely, the size of the type II muscle fibers differed significantly between normal , stimulated ,

## Table 9

## MYOFIBER DIAMETERS: STIMULATED MUSCLE

| Subject # | Mean Area Type I ± SD(u) | Mean Area Type II ±SD(u) |
|---|---|---|
| 1 | 1649 ± 367 | 2886 ± 779 |
| 2 | 4093 ± 2503 | 2566 ± 1037 |
| 3 | 2997 ± 599 | 3209 ± 660 |
| 4 | 3336 ± 202 | 2397 ± 512 |
| 5 | 2544 ± 767 | 3010 ± 759 |
| 6 | 2259 ± 1028 | 2261 ± 350 |
| 7 | 3567 ± 854 | 2767 ± 526 |
| 8 | 3243 ± 514 | 3159 ± 483 |
| 9 | 2490 ± 921 | 2573 ± 537 |
| 10 | 2592 ± 615 | 2752 ± 614 |

## Table 10

### MYOFIBER DIAMETERS: NON-STIMULATED MUSCLE

| Subject # | Mean Area Type I ± SD(u) | Mean Area Type II ± SD(u) |
|---|---|---|
| 1 | 3609 ± 752 | 1916 ± 768 |
| 2 | 2375 ± 509 | 1524 ± 606 |
| 3 | 3688 ± 1304 | 1246 ± 413 |
| 4 | 3119 ± 940 | 975 ± 338 |
| 5 | 3613 ± 942 | 1036 ± 351 |
| 6 | 3565 ± 726 | 1123 ± 417 |
| 7 | 3172 ± 652 | 1452 ± 537 |
| 8 | 2954 ± 941 | 1075 ± 626 |
| 9 | 3623 ± 527 | 1564 ± 429 |
| 10 | 3256 ± 711 | 1409 ± 352 |

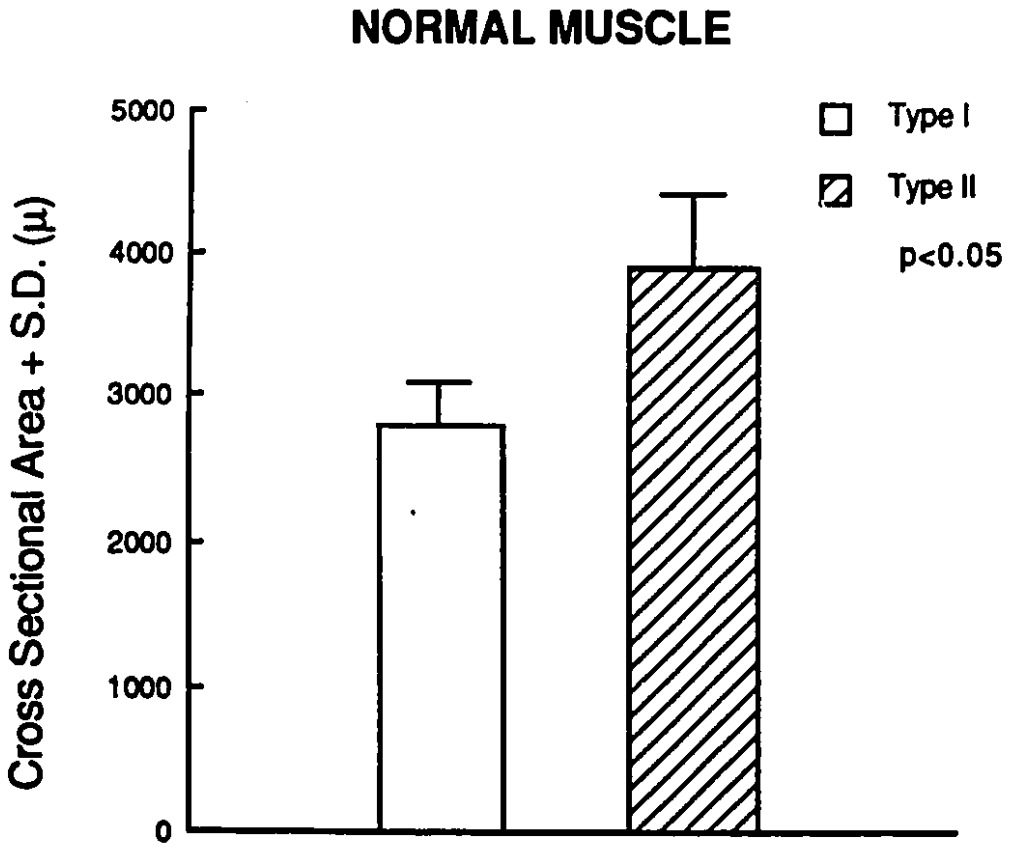



**Figure 16:** Mean diameters for type I and type II myofibers in normal muscle.

ATPASE × 100

pH 4.3

Normal

Stimulated

Non-stimulated

**Figure 17:** ATPase stained sections of normal, stimulated, and non-stimulated muscle. Magnification X100. The dark fibers are type I, and the light fibers are type II.

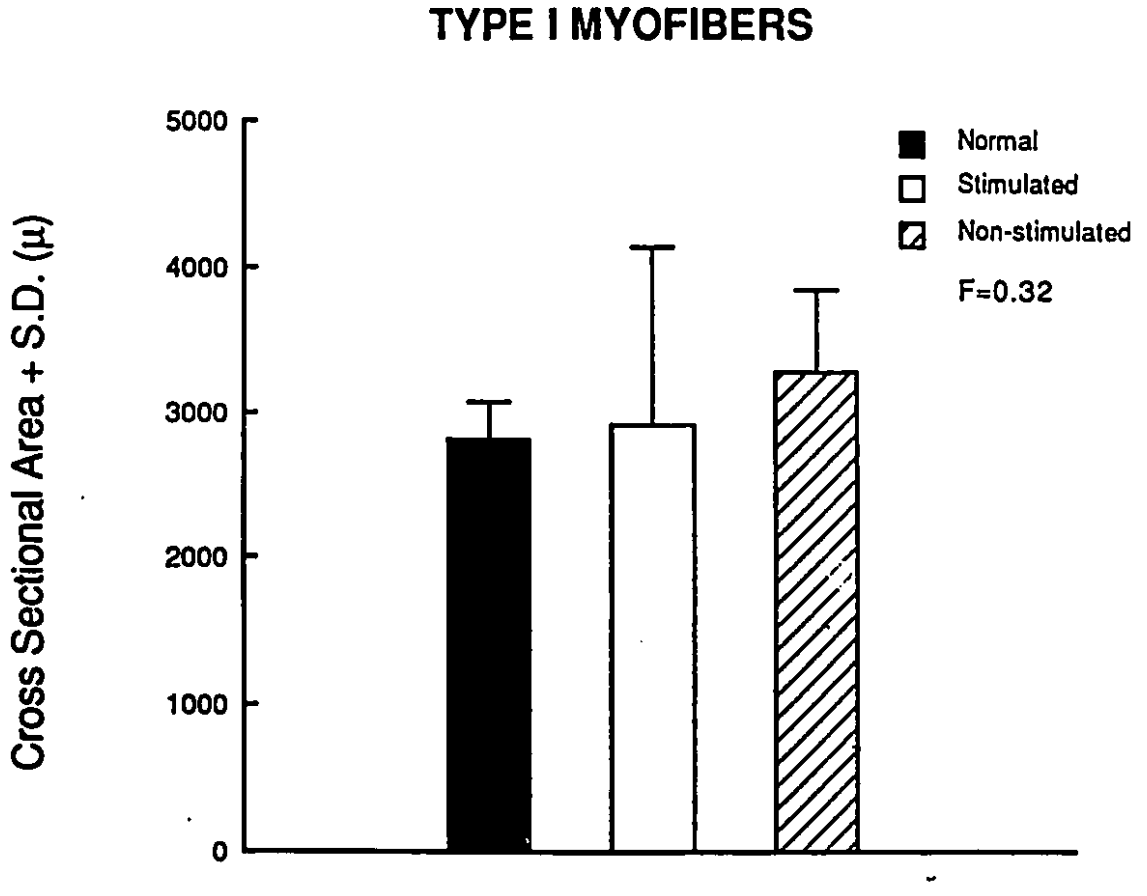



**Figure 18:** Mean diameter of type I myofibers: normal = 2807 ± 274(SD)u, stimulated = 2913 ± 1224(SD)u, non-stimulated = 3280 ± 554(SD)u. There is no significant difference between the three groups.

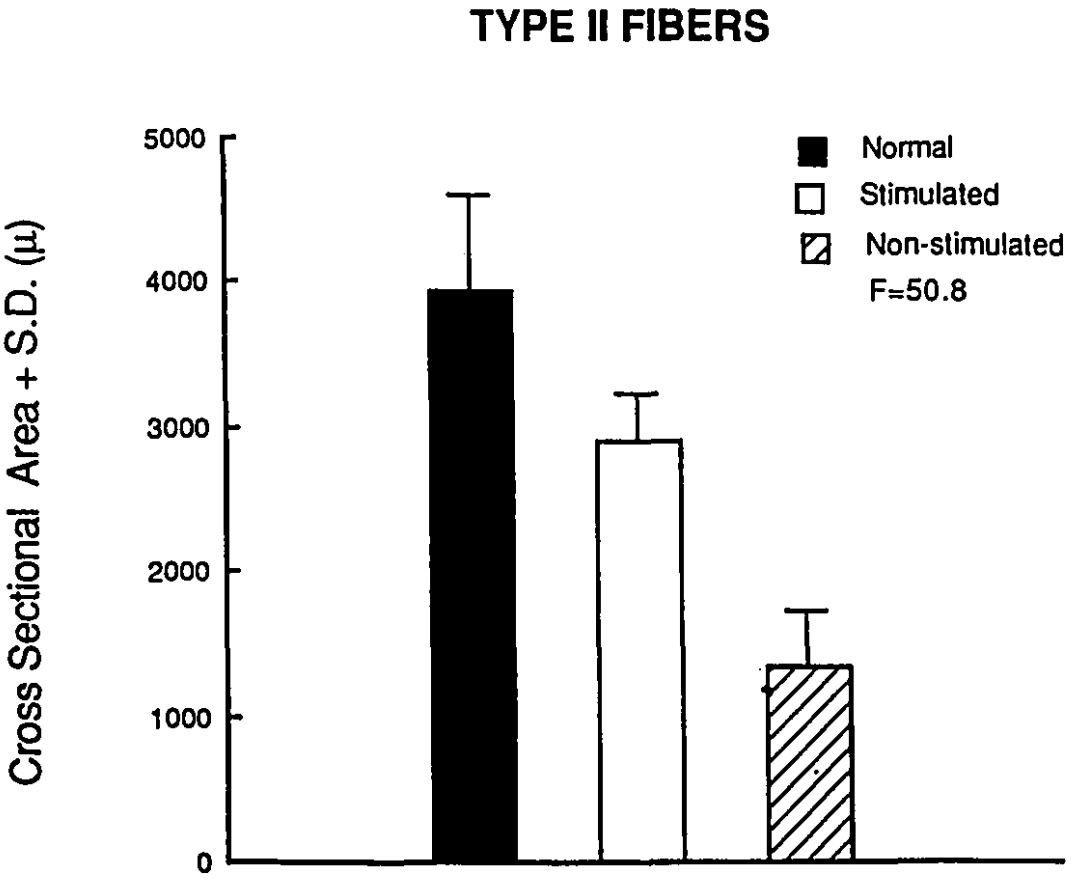



**Figure 19:** Mean diameter of type II myofibers. Normal = 3916 ± 661(SD)u, stimulated = 2887 ± 321(SD)u, non-stimulated = 1343 ± 386(SD)u. There is a significant difference between all three groups ($p<0.001$).

muscle, and non-stimulated muscle (figure 19). The diameter of type II fibers in normal muscle was found to be significantly larger than that of stimulated muscle, which was significantly larger than that of non-stimulated muscle. Comparison of the population means was carried out by analysis of variance. Hartly's F-Max test yielded a value of 50.8. The F value required for significance at the 0,001 level in this case was 13.0. This F value therefore is highly significant across all three groups (i.e. $p<0,001$). In sum, the application of electrical stimulation would seem to have prevented, mainly, the atrophy of type II muscle fibers.

## 6. Electron Microscopy:

By examining electron micrographs of normal, stimulated, and non-stimulated muscle, several alterations in both the morphology and the cytoarchitecture of muscle fibers were found. Interestingly, some of these changes were present in both the stimulated and non-stimulated groups; and were thus termed **non-specific** ultrastructural changes (of denervation). On the other hand, numerous other alterations in the myofiber's cytoarchitecture were present solely in specimens of non-stimulated muscle. These have been termed as **specific** changes.

## NON-SPECIFIC ULTRASTRUCTURAL ALTERATIONS

Compared to normal muscle, the following ultrastructural alterations were found in both the stimulated and the non-stimulated muscle groups:

### Myofiber Contraction

Compared to normal innervated muscle, both of the denervated muscle groups showed signs of myofiber contraction as evidenced by a decrease in the sarcomere length by approximately one half. The normal length from one Z-line to the next averages 2.25 microns in normal muscle[57] (figure 20); whereas the denervated specimens showed this measurement to have decreased to just over 1 micron in both the stimulated (figure 21) and non-stimulated (figure 22) muscles.

### Irregular Plasma Membrane

Muscle fibers in both groups exhibited a wavy outline to their plasma membrane; this, in addition to the finding that the sarcolemma frequently exhibited interruptions in it's continuity. Within these cytoplasmic projections were identified increased numbers of mitochondria, glycogen granules, lipid bodies, and autophagic vacuoles (figure 22 & 23). This is in contrast with the straight and continuous

plasma membrane found in normal muscle fibers.

**Nuclear Indentations**

Just as irregularities of the plasma membrane were found, the nucleus of the denervated muscle fibers demonstrate numerous indentations and appear contracted compared to normal muscle. Figures 20 and 24 show the contour of a normal myofiber nucleus, whereas figures 21 and 25 show the irregular nuclear indentations in stimulated and non-stimulated muscle respectively.

**Sarcotubular Dilatations**

Denervated muscle uniformly exhibited focal dilatations of the sarcoplasmic and T-tubular systems which are located at the junction of the A and I bands of each myofibril. Each T-tubule is surrounded by two terminal cisternae of the sarcoplasmic reticulum, such that triads of these structures are identified on longitudinal sections. These dilatations are as pronounced in the stimulated group as they are in the non-stimulated group (figure 26).

**Glycogen Granules and Ribosomes**

Denervated muscle contained an overabundance of glycogen granules which were deposited mainly between the myofibrils and just beneath the plasma membrane (figure 27). These are difficult to differentiate from the surplus of ribosomal

particles which are also present; and most likely result from the compensatory hypertrophy of the sarcoplasmic reticulum.

## Lipid Bodies

Lipid bodies occur naturally in normal muscle, but appear in increased numbers in denervated muscle (figure 4). Although they occurred in both stimulated and non-stimulated, their size and number were greater in the non-stimulated muscle.

## Mitochondria

The size and number of mitochondria decreased in both groups of denervated muscle. Specific alterations in the morphology of those contained in non-stimulated muscle are described in the following section .

## SPECIFIC ULTRASTRUCTURAL ALTERATIONS

The following are cytoarchitectural and morphological changes in myofibers which were observed to have occurred solely in non-stimulated, denervated muscle; and were observed not to have occurred in stimulated muscle.

### Central Nucleus

In non-stimulated muscle we note that the myofiber nuclei, which usually occupy a peripheral location just beneath the plasma membrane, have migrated towards the center of their myofiber. Figure 25 shows such a myofiber and it's nucleus in cross section; which contrasts sharply with the peripheral location of such nuclei in stimulated as well as normal muscle (figures 21 and 24 respectively).

### Myofibrillary Degeneration

In non-stimulated muscle there is evidence of myofibrillary degeneration, which can be observed in figures 22 and 26 as irregularities and an interruption of the continuity of the myofibrillar and myofilamentous elements throughout the long axis of each myofiber. Stimulated muscle did not manifest such interruptions in the contractile

elements (figure 21). The loss of myofilaments and the subsequent diameter of the myofibrils results in a relative increase in the amount of intervening sarcoplasm (figure 22). Interestingly, this widened intermyofibrillary space becomes occupied with: a) dilated sarcotubular systems, b) larger and increased numbers of lipid bodies, c) increased numbers of glycogen granules, d) degeneration products of myofibrils, e) myelin bodies, and f) mitochondria which have reoriented themselves in space.

**Myofilamentous Distraction**

Figures 28 and 29 show several myofibrils from stimulated and non-stimulated muscle respectively, both magnified 39 500 times. The non-stimulated fibrils show evidence of fragmentation, due to distraction of their myofilaments by the interposition of mainly ground substance and glycogen granules. Conversely, the stimulated muscle shows no such breach of the structure of it's myofilamentous or myofibrillar elements. In sum, the basic contractile elements of muscle have become disrupted in the non-stimulated specimen, whereas in the stimulated muscle these have been preserved.

**Mitochondria**

The mitochondria observed in samples of non-stimulated muscle display significant morphological differences from the same organelles observed in samples of normal or of stimulated muscle. Notably, the number of mitochondria decreases in the non-stimulated specimens; in addition to which the remaining mitochondria are smaller than those in either normal or stimulated muscle. The orientation of mitochondria in normal muscle is such that the long axis of the organelle aligned parallel to that of the myofibrils (figure 20), whereas in non-stimulated muscle the mitochondria appear to assume a more spherical shape (figure 23). Specific changes were also seen with respect to the content of the mitochondria: those found in non-stimulated muscle have much less prominent cristae; which have become less electron dense, undergo fragmentation, and eventually disappear (figure 26).

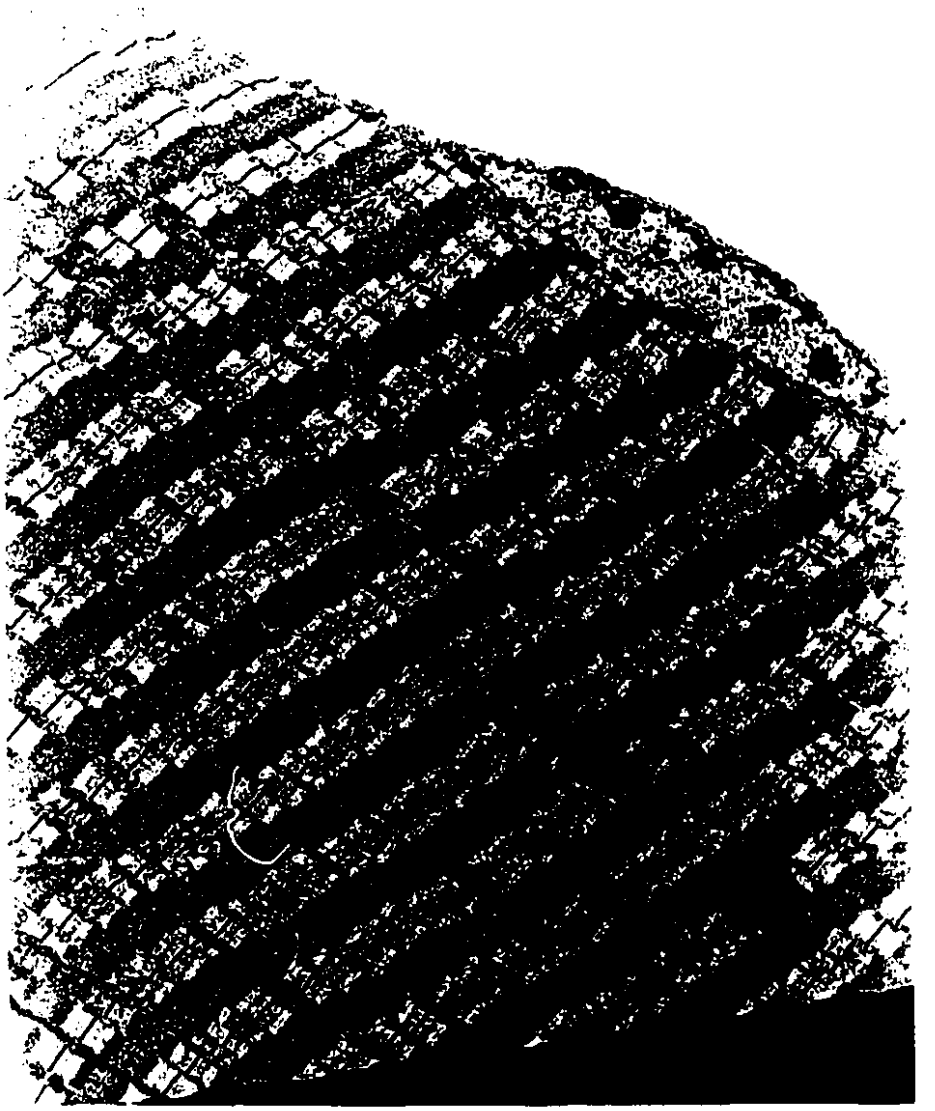


**Figure 20:** Electron photomicrograph of normal muscle, longitudinally oriented, magnified 14 000 X.

**Figure 21:** Electron photomicrograph of stimulated muscle, longitudinally oriented, magnified 14 000 X.

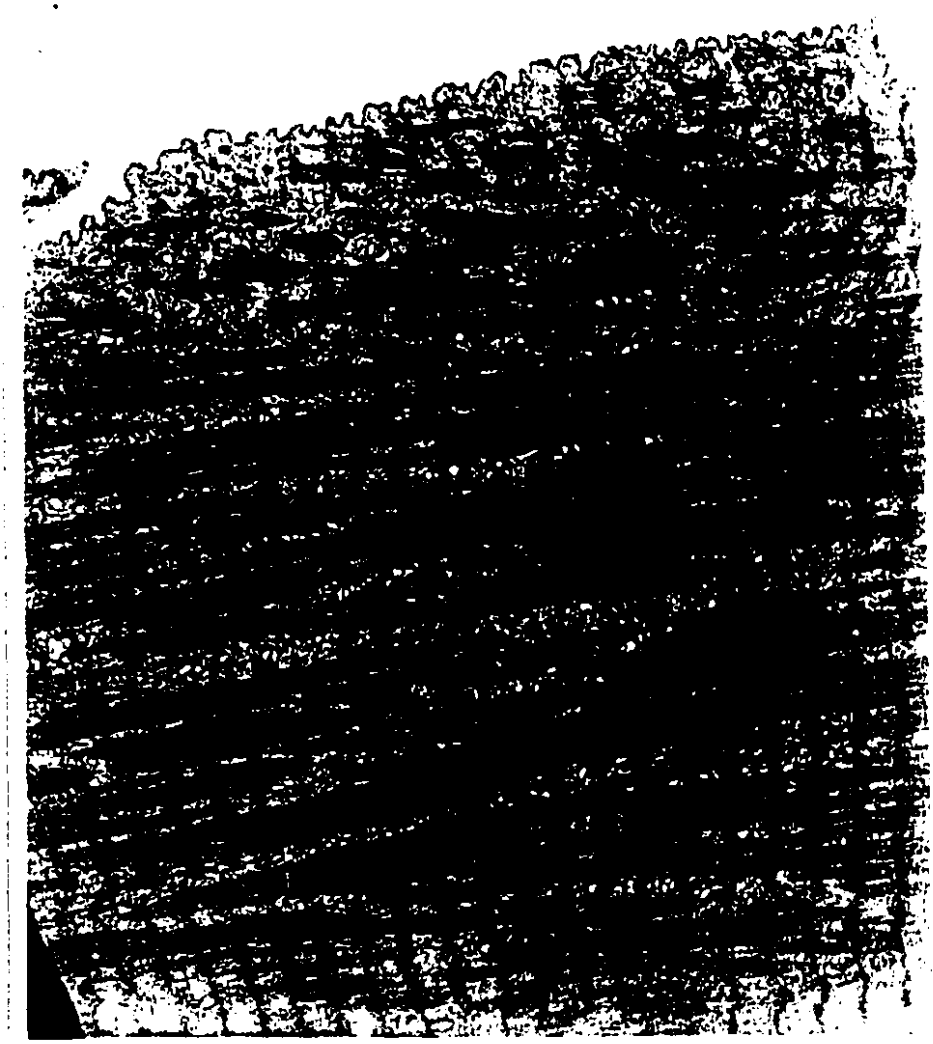


**Figure 22:** Electron photomicrograph of non-stimulated muscle, oriented longitudinally, magnified 14 000 X.

**Figure 23:** Electron photomicrograph of non-stimulated muscle, oriented longitudinally, magnified 15 000 X.

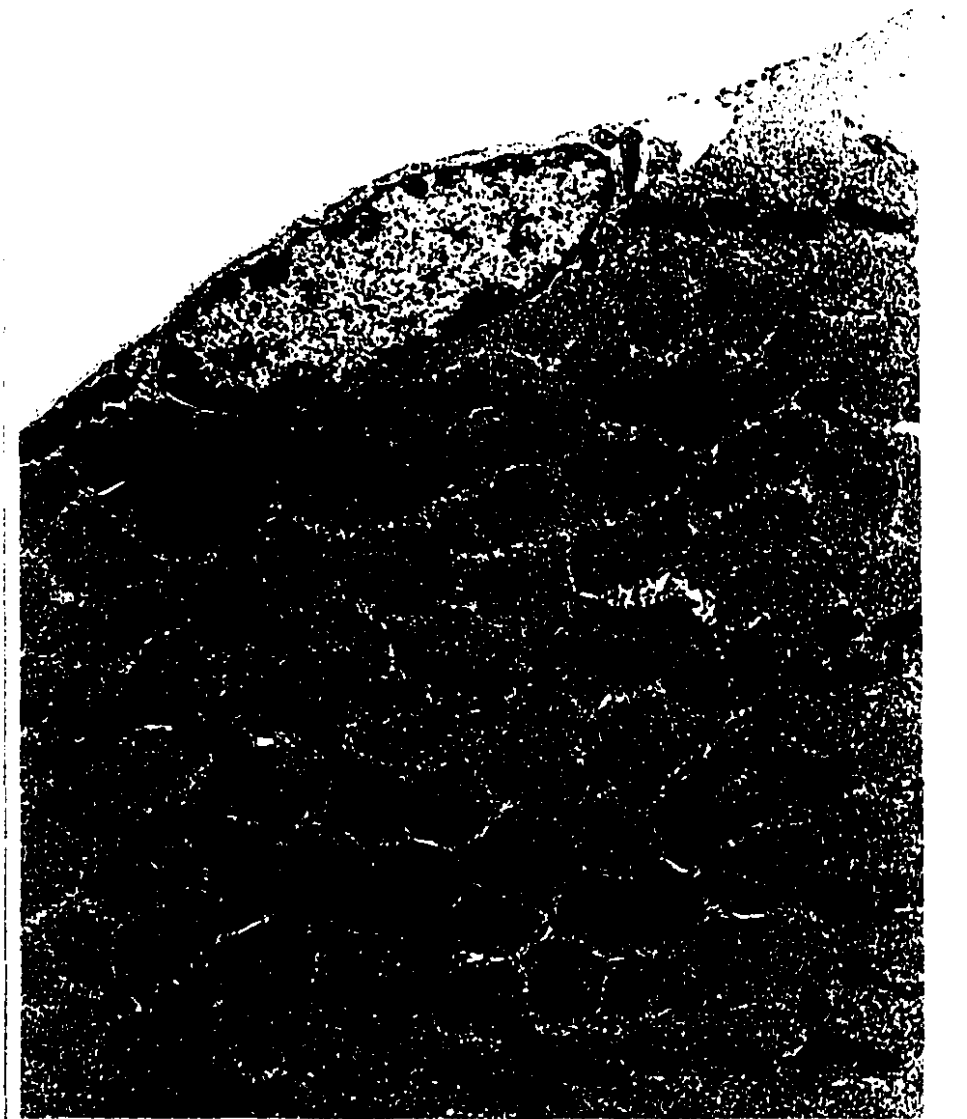


**Figure 24:** Electron photomicrograph of normal muscle, seen in cross section, magnified 23 000 X.

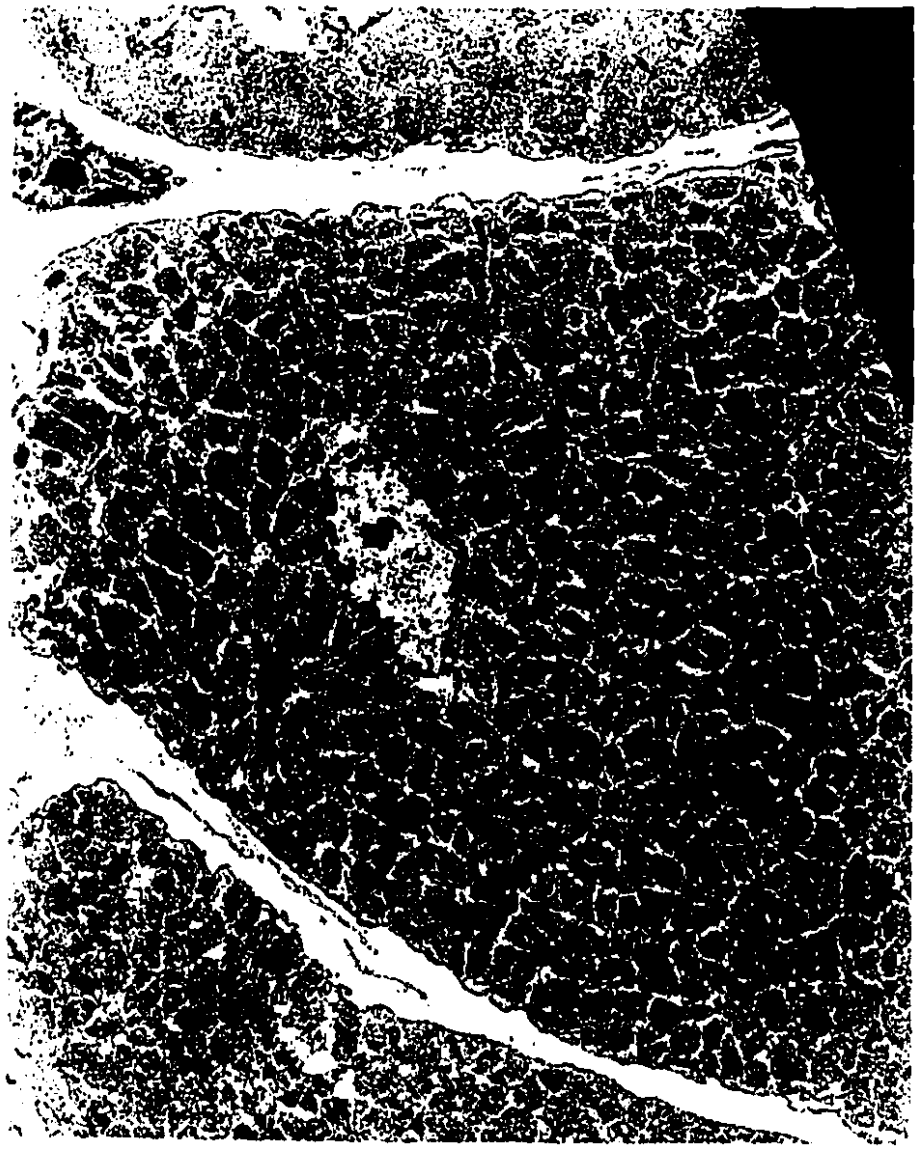


**Figure 25:** Electron photomicrograph of non-stimulated muscle, seen in cross section, magnified 11 000 X.

**Figure 26:** Electron photomicrograph of non-stimulated muscle, oriented longitudinally, magnified 15 000 X.

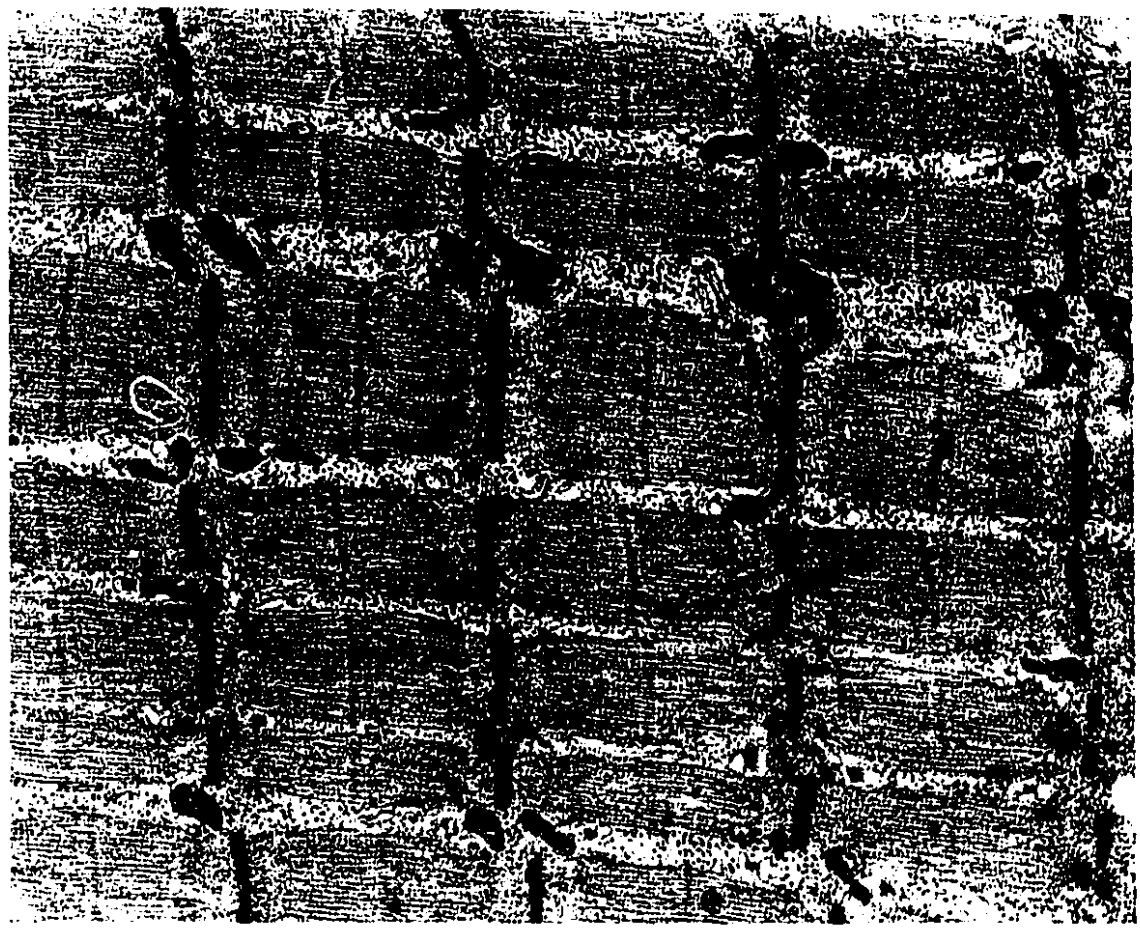


**Figure 27:** Electron photomicrograph of stimulated muscle, oriented longitudinally, magnified 35 000 X.

**Figure 28:** Electron photomicrograph of stimulated muscle, oriented longitudinally, magnified 39 500 X.

**Figure 29:** Electron photomicrograph of non-stimulated muscle, oriented longitudinally, magnified 39 500 X.

# DISCUSSION

In this section we will review the significance of the results obtained in this study. From there we will go on to explore the direction that this area of research is likely to assume in the near and not too distant future. We will begin by summarizing the results generated in this study and comment upon their significance.

## Analysis of Results

The electromyographic studies disclosed no significant difference between the compound action potential waveform or amplitude between the stimulated and non-stimulated groups eight weeks post-op. This is not surprising, and is even expected since this suggests that the application of electrical stimulation to a muscle following the repair of it's motor nerve did not interfere with or impede the regeneration of axons across the neurorrhaphy site. In short, reinnervation of the muscles was complete as well as comparable between the two study groups.

When we consider the maximum force of tetanic contraction that could be generated by stimulated and non-stimulated muscle, we note that although the stimulated group was able to generate more than twice the amount of force that could be generated by the no-stimulated muscle, this force was still well below (i.e. 41 ± 5 SEM%) that which could be generated by normal muscle. There are two possible explanations for

this: one is that although electrical stimulation resulted in providing a muscle that was capable of generating more force than a non-stimulated muscle, the electrical stimulation, alone, was not sufficient to prevent outright the functional deleterious physiological effects associated with the state of denervation. The other possibility is that the muscle had not fully recovered by the time it was tested with regard to the maximum force of contraction. Regardless of the explanation that one chooses to accept for this discrepancy, the fact still remains that, with respect to the functional capacity of the muscle, a clinically significant beneficial effect was noted to have resulted from the application of electrical stimulation to denervated muscle.

The same line of reasoning applies to the evaluation of the wet muscle weight of stimulated versus non-stimulated muscle. Even though the stimulated muscle maintained but 79 $\pm$ 5 (SEM)% of the mass of normal muscle (compared to the 42 $\pm$ 3 (SEM)% in the non-stimulated group) this study presents statistically significant results to suggest that the application of electrical stimulation served to minimize the atrophy of the muscle which would otherwise have been inherent to the course of denervation. The choice of explanation for even the loss of mass observed in the stimulated muscles is, as mentioned earlier, up to individual interpretation at this point in time; and quite possibly may become the focus of future research.

The histological analysis of each of the muscle samples showed that the application of electrical stimulation to denervated muscle helped to maintain the morphology and integrity of the myofiber as measured by light and electron microscopy. Whether this effect is a temporary delaying of an eventual complete atrophy or a permanent therapeutic state is a question which is beyond the scope of this particular study. However, there was an indisputable effect of electrical stimulation in helping to maintain the essential muscle cell integrity as shown by this experiment. Histological sections examined with histochemical stains, on the other hand, showed that the type II (fast twitch) muscle fibers were more sensitive to the state of denervation---and subsequently atrophied preferentially---compared to the type I (slow twitch) muscle fibers. These findings are in keeping with those of previous studies which, for the most part, suggest that, in the state of denervation, type II fibers atrophy earlier and to a greater extent than do slow twitch fibers[22,43,45,46]. In fact, some studies even report that type II fibers are more affected---and thus more liable to undergo atrophy---in the presence of denervation as well as disuse[7] when compared to type I fibers.

## Electrical Stimulation Vs Neurotrophic Factors in the Prevention of Post-Denervation Muscle Atrophy

In pursuing research in this field, the ultimate therapeutic goal would be to develop a working system which will prevent entirely the atrophy which occurs after a muscle is cut off from it's motor nerve supply. In the present study we find results which suggest that the application of electrical stimulation will minimize the atrophy that would otherwise occur in it's absence. In other words, providing electrical stimulation to denervated muscle appears helpful and beneficial from a potential therapeutic standpoint, but it may not be the complete answer to the problem of post-denervation atrophy. The question, then, is: what more is needed in order to achieve the prevention of atrophy. The answer definitely lies in the analysis (and subsequent recreation) of those same factors which are responsible for maintaining the integrity of the muscle cell in the normal state; as well as the recognition that the interaction of these factors is responsible for the differences in the degree of atrophy that a muscle undergoes when it is completely denervated, as opposed to when it is simply in a state of disuse.

Some researchers have theorized that the atrophy observed following denervation results not only from a lack of use of the muscle involved, but also from the depletion of trophic

substances secreted into the muscle fibers of their motor unit[5,30,41,67-69]. It is conceivable, then, that certain aspects of muscle integrity are controlled by chemical substances which may be produced in anterior horn cells and are delivered to the muscle via an axonal transport mechanism[70,71].

In the early part of this century it was commonly believed that the atrophy which resulted after the transection of a peripheral motor nerve was the result of inactivity only[75]. However, in a landmark study, Tower was the first to challenge this notion in 1935, when she noted that the atrophy of paralysed muscles in dogs was not as great as that of denervated muscles[60]. Tower suggested that since the paraplegic dogs' muscles do not contract voluntarily but do remain connected to normal neurons, the greater atrophy of denervated muscles was due to the loss of substances which are normally secreted by the intact motor nerve. Demonstrating marvellous foresight, she submitted that these neural influences would be in addition to the release of acetylcholine. Today it is generally agreed that, in addition to the release of neurotransmitter, motor nerves actively secrete substances, which act by way of an as yet unknown mechanism, to maintain the integrity of the muscle cell. These are generically referred to as <u>neurotrophic</u> substances.

An interesting group of studies supporting the existence of neurotrophic substances demonstrated that when a motor nerve is transected, the changes associated with denervation

could be delayed by leaving a longer distal nerve stump attached to the muscle. This resulted in a delayed onset of (a) supersensitivity to acetylcholine, (b) depolarization of the resting membrane potential, and (c) appearance of fibrillations[76-80]. Authors have explained this on the grounds that there is a progressive depletion of trophic substances contained in the nerve segment still attached to the muscle. This acts to retard the onset of denervation changes.

During the first few weeks following denervation, large numbers of de novo acetylcholine receptors are produced and incorporated into the plasma membrane of the muscle cell[48,63,64]. The acetylcholine receptors, which are normally all located on the post-synaptic side of the motor end plate, become distributed throughout the surface of the muscle fiber in denervation. When this occurs, the muscle cell is said to be hypersensitive---or supersensitive---to acetylcholine; and even very low concentrations of the neurotransmitter may elicit contractions[48,64]. Jones and Vrbova, in 1973, reported that the two main factors responsible for this hypersensitivity were: (a) the effect of inactivity per se, and (b) the presence of degenerating nerve tissue in proximity to the motor end plates[48]. In their study the application of electrical stimulation was found to have a beneficial effect in reducing the sensitivity of denervated muscles to acetylcholine, but could not totally prevent it.

It has been mentioned above that axoplasmic flow may

possibly play a role in the mechanism of neurotrophic support to the skeletal muscle. Results suggesting that this is indeed the case first became known in the mid-1970s[72-74]. A representative study[74] shows that the application of vinblastine or colchicine (agents known to interrupt axoplasmic flow) to motor nerves results in muscle atrophy, hypersensitivity to acetylcholine, a decrease in the end-plate acetylcholinesterase activity, and depolarization of the muscle cell membrane. These changes are usually associated with denervation.

In an effort to qualify which physiologic changes are due to denervation as opposed simply to disuse atrophy, researchers have designed experiments to try and dissociate, and thus contrast, disuse from denervation atrophy. Perhaps the simplest way of preventing voluntary or involuntary contractions of skeletal muscle without denervating them is by mechanically restraining these muscles. In two such studies, approximately ten years apart, different research groups were able to independently show that the disuse atrophy of skeletal muscle (as afforded by either pinning or casting) resulted in: (a) less of a decrease in the absolute mass and protein content compared to denervated muscle, (b) only a modest increase in acetylcholine supersensitivity, (c) no effect at all on the resting membrane potential, and (d) no effect on the acetylcholinesterase activity or that of the oxidative enzymes[81,82]. In a similar study, Stanley and Drachman

reported that after effecting disuse with the application of local anaesthetics or tetrodotoxin to the motor nerve, the density of acetylcholine receptors was found to be less in the paralysed muscle than in denervated muscle[83].

Faced then with the mounting evidence for the existence of neurotrophic support of skeletal muscle, it was only a matter of time, as well as natural progression, until researchers began to try and supply these substances to denervated muscles in an attempt to prevent denervation atrophy. The major problem inherent to this plan, however, is that the substance(s) responsible for the trophic effects have been quite difficult to isolate and/or identify. As a result, those investigators wishing to experiment with neurotrophic substances have had to use rather crude extracts of protein from nervous tissue. It has since been shown that such extracts have the capacity to promote the maturation and differentiation of embryonic muscle cells grown in tissue cultures[84-86]. In addition, Davis and Kiernan have shown that extracts of peripheral nerve tissue demonstrates neurotrophic effects in vivo as well as in vitro [87]. In a subsequent paper the same authors compared three groups of rats: (1) one in which the extensor digitorum longus muscle was denervated, (2) one in which the muscle was immobilized, and (3) a group in which the muscle was denervated and the animal was injected with sciatic nerve extract. By comparing and contrasting the degree of atrophy between the three groups, their results

suggest that forty percent of the denervated muscle atrophy (as measured by wet muscle weight, total protein content, and cross-sectional area of type II fibers) was attributable to the loss of trophic effects on the muscle. The remaining sixty percent of the atrophy was due to disuse. Furthermore, their results show that the portion of denervation atrophy which was due to the loss of trophic effects could be completely prevented by injections of nerve extract[86]. Davis and Kiernan have isolated a molecule (or group of molecules) which they believe may be responsible for the neurotrophic effects observed following the injection of peripheral nerve extract. This compound has been characterized as having a molecular weight of 84 000, and is still under investigation.

## The Role of Electrical Stimulation

This study reveals that electrical stimulation is beneficial in minimizing the amount of atrophy that occurs after a skeletal muscle is denervated. However, since minimizing (or retarding) is not the same as the prevention of atrophy, stimulation alone may be only a delaying action. If enough time passes, will treated muscle become as atrophic as non-treated muscle? If so, then there must be a logical explanation why electrical stimulation cannot substitute entirely for normal muscle activity and maintenance. There are at least two possible explanations:

1. The first is that the amount and the parameters of stimulation employed in the present study (or any previous study) did not simulate closely enough the level of exercise that a normal muscle undergoes.
2. The state of denervation results not only in a state of relative inactivity (which is partly what electrical stimulation tries to substitute for) but also deprives muscles of trophic substances which are secreted by the motor nerves. If this is true, electrical stimulation is providing for only a part of the problem; and it's inability to maintain

denervated muscles fully---or indefinitely---becomes understandable.

In a field that is, for the most part, an uncharted area of muscle and neurophysiology, we believe that by defining the advantages as well as the true limitations of electrical stimulation, this study contributes to the further understanding of the value of electrical stimulation in the treatment of denervated muscle. It also promotes continuing research involving the combined effects of electrical stimulation and neurotrophic factors in the treatment of denervated muscle.

## CONCLUSIONS

Several conclusions make themselves evident by this study. These are best expressed in point form:

1. The decrease in wet muscle weight that is noted in atrophic muscle is mostly due to a decrease in the size of individual muscle fibers.
2. Fatty infiltration, interfascicular fibrosis, and myofiber atrophy were minimized through the application of electrical stimulation to denervated muscle.
3. Type II (fast twitch) muscle fibers undergo preferential atrophy in the absence of electrical stimulation.
4. Electrical stimulation prevents the disruption of the myofibrillar elements at the ultrastructural level.
5. This model is an effective method of retarding denervation changes after peripheral motor nerve transection and repair.
6. Atrophy and the physiologic consequences of denervation could not be prevented using this method only.

## Clinical Applications of Electrical Stimulation

One of the key points brought out by this experiment was that type II muscle fibers undergo a preferential atrophy in the face of denervation. This preferential atrophy did not occur when electrical stimulation was applied to the muscles post-denervation. This is an extremely interesting issue; especially if one considers that the majority of muscle cells in the human body are composed of type II fibers. If, according to this model, we can preferentially prevent, or retard, the atrophy of these (type II) muscle fibers, then the results brought forth by this experiment clearly lend themselves to potential clinical applications in human beings. Furthermore, we believe that the results generated by this experiment go on to suggest that retarding or preventing muscle atrophy through the use of electrical stimulation, following peripheral motor nerve transection and repair, may lead to an improved functional result that may be of benefit not only in cases of peripheral nerve injuries, but also to patients who have sustained brachial plexus injuries, facial nerve paralysis, and those undergoing free muscle transfers. It also offers a ray of hope to those patients who have been affected by paraplegia, quadriplegia, and cerebrovascular accidents.

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