Electrotherapy for crushed radial nerve in the dog

by

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TABLE OF CONTENTS

I. INTRODUCTION

Even while I was a physical therapy student I wondered how effective many of the procedures used daily by physical therapists actually are. And, as I began working in the clinic and seeing the difference which good patient rapport makes, my skepticism increased.

Then, as now, I believed that physical therapy does help the patient. But in what ways? Does that gentle push at the end of the range of a joint really stimulate the stretch receptors which activate the agonists which eventually get stronger because of the therapist's graded manual resistance? Or is it the auditory and visual commands which stimulate the patient instead of the tactile input? Or is it the fact that the therapist also has the time and opportunity to discuss with the patient ways of coping with physical disability?

I guess these questions belong as much in the realm of psychology and philosophy as pure science at this time. But I still wanted to take a tiny bit of physical therapy practice and subject it to the rigors of the experimental method. This can't be done with human subjects because of a lack of control subjects and limited measurements, but in lower animals it is possible. The dog seemed to be

a good compromise. And electrical stimulation for nerve injuries is about as measurable as any aspect of the art of physical therapy at this time.

With that, I begin--

II. REVIEW OF LITERATURE

A. Degeneration and Repair of Traumatized Peripheral Nerve

Changes in the anatomy, physiology, and biochemistry of damaged peripheral nerve can only be understood in light of the structure and function of normal nerve. I shall follow the classification scheme of Ducker (1) and Ducker et al. (2) in reviewing some of these changes seen in a motor neuron in response to injury. The components used in this scheme include the soma, glial cells, proximal nerve trunk, proximal and distal nerve stumps, mesenchymal tissue, distal nerve trunk, and the muscle cell.

1. Soma

The soma, or cell body, of a motor neuron controls the metabolism of the neuron. Transcription and translation occur in the nucleus and proteins are made in the axoplasm. The newly-synthesized proteins travel from the soma along the axon by a process called axoplasmic flow.

When the nerve is injured, the cell body enlarges as it attempts to meet the increased metabolic needs. The nucleus moves from the center of the soma towards the edge (3). Somal enlargement begins by the third or fourth day and reaches a peak between the tenth and twentieth days after injury. This increased mass persists as long as

regeneration occurs, and then the soma gradually returns to its original size.

A large portion of the injury-induced enlargement, beginning soon after injury, is due to increased amounts of ribonucleic acid (RNA) in the cytoplasm of the neuron. Simultaneously, the RNA breaks into smaller units which migrate to the edge of the cell, where it is thought to be a more active form of RNA. The new RNA is then made into proteins which are used to form new plasma membrane. In a successful repair, there may be 50 to 100 times the normal amount of protein and organic material produced in the soma.

The lesion site is one very important factor in the successful repair of a peripheral nerve. Because a greater portion of the total cell mass is lost with a proximal injury, the metabolic needs are greater for survival and regeneration and the prognosis is poorer, than in a distal injury. If the injury is too extensive for successful repair, the neuron will atrophy or die.

2. Supportive glial cells

These cells surround the neuron and axons in the central nervous system and provide essential nutrients such as amino acids and sugars to the cell. In this manner they act as regulatory agents for the neuron.

When the neuron is injured, the astroglia have increased

enzyme activity, increased amino acid uptake, cell hypertrophy, and cellular proliferation. The increased metabolic demands cause the proliferation of oligodendroglia. Glial DNA synthesis increases starting 2 to 5 days post-injury and continues throughout the duration of axonal repair. Both the soma and the supportive glial cells must be active for successful regeneration to occur.

3. Proximal nerve trunk

This portion of a normal peripheral nerve has more than one rate of axoplasmic flow. A slow flow proceeds at a rate of 1 mm/day by peristaltic waves in the nerve trunk membrane, while a faster flow (100 mm/day) occurs in microtubules. Hubbard (4) reports that new proteins can be transported as fast as 400 mm/day. Some slowly-transported proteins are used to replace catabolized enzymes, but a significant amount of protein reaches the distal portion, or synaptic region, of the axon. The fast transport system provides sudden nutritional needs which often occur at the synaptic regions.

Miani et al. (5) report that the flow of the slow component speeds up during peripheral nerve regeneration, but the overall rate of transport appears to be unchanged. The metabolic requirements at the synaptic junction are decreased, but the metabolic demands of the regenerative

effort are increased, resulting in approximately the same amount of transport as occurs in normal nerve.

In addition to changes in axoplasmic flow, axon diameters proximal to the lesion site may shrink to 20 to 50% of their original size, and may grow to only 80% of their original diameter after regeneration is complete (4). Cragg and Thomas (6) report that axon diameters after a crush injury decrease to 86% of normal diameter at 50 to 150 days, then begin to increase and have regained their normal diameter by 225 days post-injury.

4. Proximal and distal nerve stumps

Within one hour of nerve injury, the proximal and distal nerve stumps (about 0.5 to 1.0 cm proximal and distal to the site of the lesion) swell, with the total cross-sectional area increasing three to four times. The edema is both intracellular and extracellular and consists primarily of a gel-like substance with mucopolysaccharides, and with blood plasma and serum also accumulating. The edema lasts about 1 week.

There is also increased alkaline phosphatase activity in the proximal and distal stumps within 24 hours after a nerve transection. This activity is the result of capillary budding which persists through the 30th day (7).

In man, if the cut is a clean one, axon sprouting may begin as early as the 4th day post-injury, and by 10 to 20

days post-injury there is vigorous axon sprouting. In a clean nerve section, this occurs a few millimeters retrograde to the last intact node of Ranvier. Axon buds cross the anastomosis of a primary neurorrhaphy at 2 to 3 weeks post-injury. By 6 weeks, a large number of axons are present distal to the lesion site; however, the delay is longer in a proximal lesion than in a distal lesion.

5. Supportive mesenchyme and neuroectoderm at the injury site

Within hours of injury, there is an increase in the metabolism of the Schwann cells, the perineural epithelial cells, and the epineurium. These cells arrive at the injury site from both the proximal and distal stumps (8), and their metabolic processes dominate the activity immediately post-injury.

Two to three days after the injury there is cellular proliferation of nearly all elements within the proximal and distal stumps. By the end of the first week the Schwann cell is the most active element. It is phagocytizing breakdown products, with the magnitude of its response proportional to the degree of the injury. After a nerve crush or section, the newer outer myelin lamellae are the first to break down. While Schwann cells begin breaking down their own myelin, later, myelin is found in macrophages. There is debate as to the origin of the large

numbers of phagocytes, whether from Schwann cells, histiocytes, or other cells. Asbury and Johnson (8) believe the macrophages seen in nerve degeneration come from blood-borne monocytes.

As debris is cleared, mesenchymal and neuroectodermal scar tissue remains. Early axon sprouting occurs by ameboid extension, depending upon contact guidance of adjacent structures rather than on intrinsic guidance factors. Since the scar tissue is not longitudinally aligned, this early axon growth may become tangled and result in neuroma formation. Several axons may grow through a single endoneurial tube, resulting in limitations in myelin sheath diameter and in conduction velocity. Sensory and sympathetic fibers can also become tangled with the motor fibers, sometimes blocking reinnervation. It is not until axon budding and regeneration take place that action potentials occur.

6. Distal nerve trunk

Upon injury, the axon separates from the soma and dies, so the distal nerve trunk reacts only with supportive structures. Proliferating supportive elements can inhibit penetration of regenerating axons.

Wallerian degeneration occurs distal to the lesion site. The axon degenerates before the myelin sheath does,

with macrophages beginning digestion of the axon within 4 to 7 days post-injury (3). Although isolated sections may live up to 2 weeks, most of the axon dies within 1 week. After the neuronal elements have degenerated, the myelin is broken up and digested by Schwann cells and by macrophages. This process is essentially completed at 3 weeks, and debridement is complete at 6 weeks post-injury.

Endoneurial sheaths shrink to 10% of their original diameter while portions of some fascicles, or groups of axons, persist. Fragments of axons and myelin remain in digestion chambers, where ingestion may continue several months. The nerve shrinks, and this shrinkage may become permanent.

The penetration of new axons across the lesion site results in increased Schwann cell production of new myelin. Soon afterward, somal enlargement peaks a second time as the myoneural junctions are reformed. Because of the need for metabolites for regeneration, new myoneural junctions, membrane maturation, etc., this second peak in cell body size may be greater than the initial one immediately post-trauma.

Regeneration along the distal nerve trunk progresses at about 1 mm/day or 1 inch/month. There is an initial delay until the axon crosses the anastomosis, then growth occurs at a rate of 3 (9) to 4 (10) mm/day. Regeneration slows again as motor junctions are formed.

Even if regeneration succeeds, the nerve is not exactly like normal, uninjured nerve. Asbury and Johnson (8) report that even if the fibers have crossed to the distal segment and the nerve appears relatively normal on cross section, the number of cell nuclei/unit area is increased, the amount of endoneurial collagen is increased, and the myelin sheaths are thinner.

7. Muscle cell

These cells depend upon the central nerve cell to maintain normal function. The motor end plates disappear 3 to 5 days after the injury. Held (11) reports that beginning 2 to 3 days after denervation, increased RNA activity is seen in muscle cell nuclei. This declines by the 5th day after denervation. Eventually the muscle cell shrinks, the muscle spindles atrophy, and the endomysium and perimysium thicken. This process begins at 3 to 4 months in animals and later in man, and it may continue as long as 2 years. Bowne (12) found that in dogs which had neurectomies at various portions of the brachial plexus, muscle atrophy began as early as 10 days following denervation and peaked at 3 months after denervation. These changes occur even with treatment by electrical stimulation. The thickened muscle sheath retards end plate formation. The growth of fibrous tissue in and around

muscle interferes with nerve growth and muscle contractions. The process of denervation atrophy ceases only with re-innervation.

8. Other factors affecting recovery

Younger animals have a better chance of recovery from nerve injury than older animals. Lower mammals such as the rat and dog can have regrowth of severed nerves without anastomosis, whereas primates have a much greater chance of success when the nerve is repaired (13). The delay in crossing the injury site is longer in primates than in dogs and cats (13, 14). The wounding agent and whether the wound is a clean one also affects the success of regeneration.

B. Types of Nerve Injuries

A crush injury produces minimal cell loss and transient chromatolysis, while a nerve transection results in a loss of two-thirds of the cells within 60 days (4). Guttman and Sanders (15) showed that a crushed nerve has better functional recovery and better fiber diameter restitution in the distal stump than a transected nerve. The latent period prior to the appearance of axons in the distal stump is also shorter in a crush injury. With a nerve transection there is interlacing of regenerating axons at the suture site, whereas in a crushed nerve the endoneurial sheaths persist

and guide the regenerating axons.

McQuarrie and Grafstein (16) crushed a rat peripheral nerve for 20 sec using a smooth-tipped jeweler's forceps. They found the onset of regrowth to be 4 days with a growth rate of 0.15 mm/day.

Haftek and Thomas (17) crushed the rat sural and peroneal nerves for 10 sec with smooth-tipped watchmaker's forceps. They observed greater changes in the larger diameter fibers, with smaller fibers often remaining normal. Nagatsu et al. (18) also saw little change in the smaller nerve fibers after ligature of the rat sciatic nerve. The larger myelinated fibers showed early changes both 0.5 mm and 1.0 mm distal as well as proximal to the injury site.

C. Clinical Changes in Nerve and Muscle

A complete nerve lesion results in a loss of motor function, muscle atrophy, and loss of sensation in the area supplied by that nerve. Responses to electrical current also change. The rheobase, the amount of current required to produce a minimal visible contraction at a duration of 100 msec, increases. The chronaxie, the time required for a current having twice the intensity of the rheobase to produce a minimal visible contraction, increases from less than 1 msec in innervated muscle to 50 msec or more

in denervated muscle (19). Stimulation of a denervated muscle causes a sluggish, wormlike contraction instead of the usual brisk contraction. A significantly larger amount of current is required to produce the same magnitude of contraction, and the motor point is often displaced distally when the nerve is damaged. With complete denervation, electrical current must stimulate the muscle directly to produce a visible response rather than acting on the muscle by way of the nerve.

Griffiths and Duncan (20) report that in denervated dog muscle the nerve conduction velocity remains normal for 5 to 7 days, then ceases. While normal muscle can respond to a stimulus with a frequency of 60 Hz or more, denervated muscle requires frequencies of 40 Hz or less. Completely denervated muscle may require a stimulus rate of 1/2 to 20 Hz (21). Bouman and Shaffer (22) report that normal axons require current flow in one direction for 0.1 to 1.0 msec to produce depolarization. Denervated muscle fibers require 2.5 to 3.0 msec for depolarization, while chronically denervated fibers may require up to 10 msec flow in one direction.

Jacobson and Guth (9) crushed the rat sciatic nerve with a hemostat and tested for signs of regeneration for 56 days. They recorded the action potentials millimeter by millimeter

along the nerve. Immediately post-crush, action potentials were recorded only as far as 2 mm proximal to the crush site, whereas by 4 days afterward, action potentials were recorded 4 mm distal to the crush site. The regeneration rate accelerated from 0.3 mm/day initially to 3.0 mm/day at 18 days. By the 28th day, nerve fibers had regenerated to the muscles of the hindlimb. The amplitude of the nerve action potential increased steadily but only returned to 20% of normal at 56 days. The conduction velocity increased to 75% of normal by 28 days with no further increase noted thereafter. Jacobson and Guth (9) hypothesized that the slower conduction velocity might be due to smaller axons.

Cragg and Thomas (6) in an earlier study, followed recovery from a crush injury for a longer period than Jacobson and Guth (9). They reported a conduction velocity of 90% normal 25 to 30 days post-injury, then a decrease to 80% normal 50 to 100 days post-crush, with recovery to 100% normal by 200 days post-injury. During this same 50 to 150-day period, the axon and nerve fiber diameters also were 85 to 90% normal, and these too increased to normal by 225 days post-injury.

Mira (23) froze rat peripheral nerves several times and found that 18 months post-injury, the myelinated nerve fibers were only 70% of the diameter of normal fibers.

D. Treatment of Peripheral Nerve Injuries by Electrical Stimulation

1. Rat

Electrical stimulation using implanted electrodes has been shown to be beneficial in rat denervation studies. Melichna and Guttman (24) stimulated denervated rat limbs using implanted electrodes at a duration of 300 msec, a frequency of 60 Hz, and a rate of 4/min, followed by a 2-minute rest period. Treatment for 3 days resulted in decreased muscle weight loss and shorter contraction times. However, if the stimulation was not started until 7 days after denervation, a shortened contraction time was not observed. They hypothesized that denervation changes may be caused by: a lack of release of neurotrophic agents; activity loss; or other factors such as the change in resting length or in passive tension. They believe the amount of muscle activity (or the frequency of stimulation) is the most important factor in the treatment of muscle atrophy. While they did not specify the frequency which would be best, their experimental protocol implies a fairly continuous amount of electrical stimulation for several days.

Axelsson and Thesleff (25) showed that while the normal muscle membrane is sensitive to acetylcholine (Ach) only at the synaptic junction, this sensitivity spreads to the entire

membrane with denervation. Jones and Vrbova (26) were able to prevent denervation hypersensitivity by stimulation with implanted electrodes at a rate of 40 Hz for 500 msec every 3 sec for 7 hrs daily.

Drachman (27) gave continuous electrical stimulation to denervated rat diaphragm and was able to prevent the spread of Ach sensitivity. He reported that the presence of a small amount of Ach near muscle fibers prevents the establishment of accessory end plates. Thomson (28) not only found decreased sensitivity to Ach, but also prevented prolonged twitch times in rats receiving electrical stimulation for 14 days following a nerve crush.

Lomo and Rosenthal (29) stimulated denervated rat soleus and extensor digitorum longus muscles using implanted electrodes. The stimulated muscles had greater weight, larger fiber diameters and lower input resistance than unstimulated muscles. They also reported that not all the denervated muscle fibers became sensitive to Ach in 1 to 2 days; some stimulated fibers did not show increased Ach sensitivity until 4 to 11 days post-injury. Treatment by electrical stimulation for 5 to 8 days produced a lowered sensitivity to Ach. Lomo and Rosenthal (29) and Lomo and Westgaard (30) showed that increased sensitivity to Ach was not related to the underlying atrophy, and like Melichna and Gutmann (24), suggested that Ach sensitivity is regulated

by muscle fiber activity.

However, there is evidence that loss of activity does not completely account for denervation hypersensitivity. Buchthal and Schmalbruch (31) discussed the disagreement over this point in a recent review article. Whereas Lomo and Westgaard (30) believe that muscle fiber activity is solely responsible for the maintenance of the muscle membrane, Guth and Albuquerque (32) postulate that a neurotrophic transmitter is the factor which maintains the properties of the muscle membrane. A third factor has been studied by Brown et al. (33), amongst others. They suggest that the muscle membrane becomes sensitive to Ach after denervation secondary to the presence of nerve degeneration products along the membrane. Brown et al. showed that the presence of nerve degeneration products can cause innervated muscle fibers to become sensitive to Ach (33).

Vrbova et al. (34) also review this controversy. Although Lomo and Westgaard (30) were able to delay denervation hypersensitivity with electrical stimulation, nonetheless, it still developed. They also report that increased Ach sensitivity can be experimentally induced in active muscle. Moreover, muscle with an intact nerve supply and with activity prevented by experimental procedures, hypersensitivity was not as great as with denervation. According to Vrbova et al. (34), the length of the peripheral stump influences

the rate of development of denervation hypersensitivity. Typically, in normal muscle hypersensitivity to Ach decreases by the 12th day post-injury (26). This decrease does not occur in the absence of muscle activity, as in denervation. This study suggests that degenerative changes in the muscle, along with muscle inactivity, are responsible for denervation hypersensitivity.

Lomo and Slater (35) more recently presented evidence that 2 days of inactivity are needed to allow the formation of functional neuromuscular junctions. Denervated rat muscles which were stimulated immediately after the lesion occurred resulted in fewer innervated muscle fibers than the unstimulated controls. This apparently conflicts with the results by Melichna and Gutmann previously stated (24).

2. Rabbit

Pette et al. (36) reported that long-term stimulation using implanted electrodes in the rabbit results in a shortened contraction time and a change of the mosaic pattern of fast muscle fibers to a more uniform pattern as seen in slow fibers.

Thomson (37) suggested that stimulation of a fatiguing muscle may delay or arrest nerve regeneration, and Smith and Steinberger (38) reported that this problem is minimized in the rabbit if the muscle is stimulated five to ten times

during four to five periods each day rather than stimulating 20 to 100 times in one period.

3. Man

Electrical stimulation is a widely-used treatment for denervated muscle in man. The most common type of stimulation used clinically is motor point stimulation using surface electrodes. A motor point for a nerve or muscle is the point at which the nerve or muscle is most easily stimulated by electrical current, or the point at which the least amount of current is required to produce a visible muscle contraction. The motor point for any nerve is usually where the nerve lies closest to the skin. A muscle motor point is usually near the origin of the muscle where the nerve enters the muscle belly.

Amongst the commonly seen denervated conditions in man are Bell's palsy, a unilateral facial paralysis of unknown etiology, and traumatic nerve injuries. The most common nerve injuries involve the upper extremities, either at the level of the brachial plexus, at the portion of the radial nerve just proximal to the elbow joint, or at the portions of the radial and median nerves lying just proximal to the wrist joint. The rationale for use of electrical stimulation for denervated muscle is that it helps maintain muscle elasticity, retards the infiltration of muscle fibers

by fibrous connective tissue, and improves circulation. Electrical stimulation does not decrease the regeneration time due to axonal growth, but it can shorten the interval between the first electromyographic (EMG) evidence of re-innervation and the appearance of voluntary contractions (4). Maintenance of muscle integrity is considered very important so that if the nerve regenerates the nerve-muscle complex will be functional.

Dolenc and Janko (39) and Donoso et al. (40) used EMG findings along with clinical evidence to evaluate the rate of regeneration of injured nerves. However, studies of electrical stimulation for injured peripheral nerves to date on man have included only clinical evidence. For example, Larsen and Posh (41) used electrical stimulation two to three times/week in persons with peripheral nerve injuries, and they saw no benefits for their patients, but they did not report any objective testing as a basis for their conclusions. Griffin and Karselis (21) and Bateman (42) suggest that electrical stimulation must be given at least daily to produce any benefit.

More recently, implanted electrodes have been used to improve motor function (3). This technique is similar to that used extensively in denervation studies performed on lower mammals. Preliminary findings have shown this can be a valuable adjunct to other forms of therapy (43, 44).

Motor point stimulation also is used in the diagnosis of the integrity of a nerve by tests such as rheobase and chronaxie determination, reaction of degeneration, and strength-duration curves.

4. Dog

The first published use of electrical stimulation in the dog was by Allam et al. (45) in 1949. They described motor point locations for several major peripheral nerves and a procedure for motor point stimulation using implanted electrodes. More active research in this area did not occur until the 1970's (46-48). Thomson and Bowen (46) made complete motor point maps for the dog similar in form to those existing for the human. Other than the work of these two authors, literature on the use of motor point stimulation in animals is almost nonexistent. Downer (49) has written a procedure guide for the use of motor point stimulation in animals, but she does not discuss the physiological basis for the use of this technique, nor does she give any evidence for its effectiveness in the indicated conditions.

Thomson and Bowen (46) report that surface electrodes require about ten times more current than needle electrodes do, to obtain equal-sized muscle contractions, or a minimum of 5 to 50 v in the dog.

III. STATEMENT OF THE PROBLEM

The goal of this project is to determine whether motor point stimulation as it is used in human medicine actually helps to restore motor function. To achieve this goal, dogs will be used as subjects. The use of dogs permits the application of routine sensory and motor function tests, and provides the following advantages over human studies: 1) allows for the creation of a control group and 2) provides for the use of histopathological techniques to analyze the involved nerve and muscle tissue.

It is hypothesized that motor point stimulation retards the degeneration process following a radial nerve injury. It is anticipated that the animals treated by motor point stimulation will exhibit more normal strength-duration curves, more normal rheobase and chronaxie values, larger and more numerous muscle fibers and greater capillary density than will untreated control animals.

IV. METHOD

A. Animals

Three Weimaraners and six Beagles were used as subjects. The dogs ranged in weight from 23 to 40 lbs. In each dog the left radial nerve was crushed, with the intact right forelimb serving as a control.

The nine dogs were assigned so that one Weimaraner and two Beagles were in each of three groups: 1) control-crush the radial nerve; no electrical stimulation given; 2) crush the radial nerve; stimulate the radial nerve motor point and the motor points of the extensor muscles supplied by the nerve starting the first day post-injury; and 3) crush the radial nerve; wait 7 days, then stimulate the radial nerve motor point and the motor points of the extensor muscles supplied by the nerve beginning on the 8th day.

B. Surgical Procedure

All dogs were premedicated with 1 cc (1/120 g/ml) of atropine and anesthetized with sodium pentobarbital (1 cc/5 lbs body wt of 5% solution). A 2-inch incision was aseptically made on the lateral surface of the arm from the mid-humerus level to just proximal to the elbow joint. The radial nerve was exposed so that the branch of the

radial nerve to the lateral head of the triceps and the site where the radial nerve splits into superficial and deep branches were visible.

The radial nerve was clamped with a needle holder at a level between the origin of the branch to the lateral head of the triceps and the origin of the superficial and deep branches. The clamp was held closed for approximately 30 sec.

The surgical site was then sutured. Vetafil was used for skin closure in the Weimaraners while surgical steel was used in the Beagles. Beagles 3-6 had the crush site marked by the placement of one Vetafil stitch.

C. Stimulation Procedure

A Grass SD9 stimulator was used for the motor point stimulation treatments. The machine was calibrated twice. The actual output was within 9.5% of the stated voltage setting at all voltages, and within 4% of the stated voltage at all settings less than 50v. All deviations were due to the actual output being less than the stated value on the machine.

The stimulator was set at a frequency of 1.05 Hz and a duration of 0.2 msec to elicit a response. In several instances the duration had to be increased to 2.0 msec.

Alternating current (AC) was used, with a negatively charged active electrode and a positively charged dispersive electrode. The active electrode is 1.5 cm in diameter, and the dispersive electrode is a square of 9.5 cm/side. The amount of current varies with the type and quality of contractions produced, the subject's skin resistance, and the subject's comfort.

Because preliminary tests showed that the usual technique of covering the electrodes with saline-soaked padding required at least 40v of current to produce a visible contraction in a normal dog, I substituted a method which has been used satisfactorily both in man (50) and in the dog (48). The padding was removed from the electrodes and a thin layer of Aquasonic electrode gel was spread over the metal surface of both electrodes.

The locations for both electrode placements were rubbed with alcohol prior to electrode placement to decrease skin resistance. The location of the dispersive electrode, the left scapula, had not been shaved.

The motor points stimulated included those of the radial nerve, accessory head of the triceps (intact innervation), extensor carpi radialis, common digital extensor, lateral digital extensor, and ulnaris lateralis muscles. These motor points were obtained from Thomson and Bowen (46) and from previous pilot studies. When a motor point

was located, the output was adjusted to a comfortable level which produced a strong contraction and the current was applied briefly to produce 10 to 15 contractions.

Treatments were given 6 days per week for approximately 8 weeks, at which time the dogs were euthanized. To eliminate possible benefits derived from handling the dogs, control as well as experimental animals were taken to the treatment area and given about the same amount of physical handling each day.

The Weimaraners were treated in a room separate from their cages. The dogs were laid on the right side, with an assistant holding the dogs during each treatment.

The Beagles were given a 2-month acclimation before the experimental period in which they were placed daily in a suspended canvas sling for several minutes. This sling was used during the experimental period and no assistance was needed in holding these dogs.

D. Measurements

1. Clinical

All dogs had normal sensory and motor systems prior to the experiment. No formal sensory and motor examination was made prior to euthanitizing the animals. Each dog was weighed prior to surgery, and range of motion measurements were taken at the wrist joints before surgery

and again before the animals were euthanitized.

The voltage required to produce a visible contraction at each motor point was recorded daily for each dog. In addition, the rheobase and chronaxie values were recorded periodically from the left common digital extensor motor point of each dog. Strength-duration curves (S-D curves) were also made from the left common digital extensor motor point during the 5th and 7th weeks for the Weimaraners, and prior to surgery and during the 5th and 8th weeks for the Beagles. This curve provides more information about the integrity of the nerve than do the rheobase and chronaxie, because the voltage required to produce a minimal visible contraction is recorded at 16 durations, ranging from 100 msec to 0.05 msec.

Any unusual behaviors or responses to treatment were also recorded.

2. Histological

a. Muscle At the time each dog was euthanitized, a portion of the common digital extensor muscle was removed from each forelimb. The muscle was cut approximately 1 cm distal to the elbow joint. The muscle was immediately cooled by placing it in isopentane which was precooled in liquid nitrogen to -160°C. This allows for more rapid freezing than if the specimen were placed directly into

liquid nitrogen (51). The specimen was then placed into an airtight polythene container and stored in an ultra-cold chest (-95°F).

The muscle cross sections were cut at 9 microns with a cryostat microtome at -20°C. They were mounted on slides and allowed to dry overnight. The sections were stained by a modified myosin ATPase technique (pH 4.3) to demonstrate muscle fiber types (52) and by a PAS alpha-amylase technique to demonstrate capillary density (53). For each muscle, three ATPase and two PAS slides were made.

b. Nerve A portion of the radial nerve was removed from each forelimb at the same time as the muscle samples were taken. Nerve samples were taken just proximal to the crush, at the beginning of the superficial branch of the radial nerve, and at the beginning of the deep branch of the radial nerve.

Each nerve section was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% OsO_4, stained en bloc with 2% uranyl acetate, dehydrated in ethanol and acetone, and embedded in epon-araldite mixture according to the protocol of Asbury and Johnson (8). Semithin sections (2 microns) were stained with Azure blue and examined with the light microscope.

E. Data Analysis

1. Clinical

The right and left carpal joints were compared for range of motion for each dog and among groups. Daily voltages were recorded for the radial nerve, the accessory head of the triceps, and the extensor muscles. Because all four extensor muscles usually required the same voltage to produce a fairly strong contraction, one voltage was recorded for all extensors. The voltages for each motor point were then averaged for the week (Sunday to Saturday) to give a mean value for the week. These values were analyzed using a one-way analysis of variance (ANOVA) (54), with each week's mean voltage subtracted from the mean voltage of Week 2 (the first week in which both Groups 2 and 3 received stimulation), to give a mean change in voltage or a mean voltage difference.

Rheobase and chronaxie values were compared using ANOVA among groups during the same week. Strength-duration curve values were compared among groups at each duration setting during the same testing period (pre-injury, one month post-injury, and two months post-injury) using ANOVA.

2. Histological

Muscle fiber types were classified according to the protocol of Dubowitz and Brooke (51) as I, IIA, or IIC. Type I fibers stain dark with ATPase preincubation at pH 4.3; IIA fibers appear white, and IIC fibers appear intermediate in shade (52).

A photomicrograph was taken from an ATPase-stained slide of each muscle section at a magnification of 64 times. A 5x7 enlargement was made from each negative and was used for counting muscle fibers. The fibers were counted by type and the frequency of each type was determined. The diameter and circumference of each muscle fiber were also recorded. A Modular Optical Analysis System (MOP-3) was used in making all measurements.

A photomicrograph from a PAS slide of each muscle section was made and enlarged in the same manner as the ATP slides. Capillary density was calculated for each section using the MOP-3.

Statistical analysis was performed on the muscle fiber numbers, diameters, areas, and capillary numbers using ANOVA. Calculations were made using the Statistical Analysis System (SAS) on a computer.

Photomicrographs were made from each nerve sample at a magnification of 160 times on the negative. The nerve

sections were examined for the presence or absence of degeneration.

V. RESULTS

A. Clinical

The range of motion measurements of the carpal joints for the dogs are recorded in Table 1. The Weimaraners did not have pre-injury range of motion measurements. As can be seen from the table, there was a 5° difference in only two dogs, the post-injury measurements on the control Beagles. A 10° difference was obtained on the post-injury measurements for Beagle 5 (Group 2), but the other Beagle in Group 2 had no right-left difference for that measurement. Group 3, which started receiving electrical stimulation 1 week post-injury, showed no right-left differences.

The between-group comparisons of the daily voltages necessary to elicit a muscle contraction are listed in Table 2. The only significant difference between the two treatment groups was at Week 4 for the triceps brachii motor point. Group 3 had a larger decrease in the mean voltage difference (11.2 v) during Week 4 than Group 2 (5.9 v), with an F-value of 8.63 ($p<0.05$). Group 3 had no daily voltage values for Week 1 because treatment was not yet begun on these dogs. The lowest stimulus voltages for both treatment groups at all the motor points occurred during Week 4. The mean voltages increased for all

Table 1. Range of motion of the carpal joint

			Left (degrees)	Right (degrees)
Group 1 (Control)	Pre-injury	Beagle 3	35	35
		Beagle 4	35	35
	Post-injury	Beagle 3	30	35
		Beagle 4	25	30
Group 2 (1st Day treatment)	Pre-injury	Beagle 5	40	40
		Beagle 6	40	40
	Post-injury	Beagle 5	30	40
		Beagle 6	45	45
Group 3 (8th Day treatment)	Pre-injury	Beagle 1	35	35
		Beagle 2	30	30
	Post-injury	Beagle 1	35	35
		Beagle 2	40	40

Table 2. Between-group comparisons of daily voltages

	Week[a]						
	1	2	3	4	5	6	7
Triceps brachii							
Group 2 (1st day)	27.3	25.0	24.3	19.1[b]	20.3	18.3	16.9
Group 3 (8th day)	-	34.4	27.6	23.2[b]	31.3	28.7	23.4
Radial nerve							
Group 2	32.8	25.8	23.6	22.3	28.8	29.1	30.4
Group 3	-	31.3	24.7	22.2	34.8	32.0	30.8
Extensor muscles							
Group 2	31.0	37.4	27.1	25.2	32.8	33.5	32.9
Group 3	-	42.2	31.9	29.4	40.1	37.2	33.6

[a]All values are mean voltages for 3 dogs/group for each week (Sun.-Sat.). Group 1 (control) received no daily stimulation.

[b]$F(1,4) = 8.63$ ($p < 0.05$).

dogs during Week 5, then decreased again in either the 6th or 7th weeks.

Values for the weekly recordings of rheobase and chronaxie on the Beagles are given in Table 3. The rheobase and chronaxie were not recorded on the Weimaraners at the same time intervals as on the Beagles, so these values were not included in the statistical analysis. All recordings were made from the left common digital extensor muscle motor point. To determine the sequential time differences, the rheobase values for all six Beagles were pooled. There were no differences among the three groups, but there were highly significant sequential time differences within groups. The mean rheobase values ranged from a low of 6.8 v three weeks post-injury to a high of 22.7 v 7 weeks post-injury ($F=18.29$, $p<0.0001$). The mean rheobase values before the nerve crush (8.4 v) were significantly lower than 8 weeks post-injury (20.8 v, $p<0.001$). However, there was no significant difference between the pre-injury mean rheobase voltages and the mean voltages at 4 weeks post-crush (7.0 V). Mean chronaxie values ranged from 0.03 msec during the pre-injury measurement to 19.75 msec 2 weeks post-injury ($F=6.94$, $p<0.0002$). There is a large difference between the two Beagles in Group 2 during the entire post-treatment period. Groups 1 and 3 do not show this consistent difference between dogs.

Table 3. Weekly rheobase and chronaxie values[a]

		Week	Pre-injury	2	3	4	5	6	7	8
Group 1 (Control)	Beagle 3	Rheobase (v)	7.0	14.0	14.0	12.0	19.0	25.0	28.0	26.0
		Chronaxie (msec)	<0.05	8.0	0.08	0.1	0.05	0.04	0.03	0.04
	Beagle 4	Rheobase (v)	7.3	14.0	5.0	5.0	14.0	20.0	22.0	14.0
		Chronaxie (msec)	<0.05	6.5	0.14	20.0	0.1	0.04	0.03	0.04
Group 2 (1st day treatment)	Beagle 5	Rheobase (v)	8.5	8.5	5.0	5.0	24.0	22.0	26.0	25.0
		Chronaxie (msec)	<0.05	8.0	0.03	14.0	0.05	0.05	0.04	0.03
	Beagle 6	Rheobase (v)	11.0	4.0	6.0	6.0	9.0	10.0	10.0	12.0
		Chronaxie (msec)	<0.05	40.0	10.0	18.0	30.0	12.0	9.0	10.0
Group 3 (8th day treatment)	Beagle 1	Rheobase (v)	9.0	7.0	5.0	7.0	16.0	26.0	28.0	26.0
		Chronaxie (msec)	<0.05	40.0	14.0	18.0	0.6	0.03	0.03	0.04
	Beagle 2	Rheobase (v)	7.5	9.0	6.0	7.0	19.0	20.0	22.0	22.0
		Chronaxie (msec)	<0.05	16.0	14.0	18.0	0.08	0.06	0.05	0.04

[a]Taken from the left common digital extensor motor point.

(Beagle 5 was quite apprehensive, while Beagle 6 was the calmest of the nine dogs during the actual treatments.)

Strength-duration curve values are listed in Table 4. No pre-injury curve was recorded for the Weimaraners, so these dogs were not included in the group comparisons. All recordings were made from the left common digital extensor muscle motor point. There were no differences among groups for the pre-injury recordings and for the 2 months post-injury recordings. At 1 month post-injury, greater differences occurred among the groups at the shorter durations. At 0.05 msec, the voltage required to produce a minimal visible contraction ranged from mean value of 33.5 v for Group 2 to a mean of 45.5 v for Group 3. The F-value of 9.18, while not significant at the 0.05 level, approaches the critical value (9.55). At 0.1 msec and at 0.05 msec, the dogs in Group 3 required the greatest voltage to produce a minimal visible contraction, while the dogs in Group 2 required the least voltage of the three groups. The voltages for all groups are much greater in the two post-injury tests when compared to the pre-injury test. For example, the mean voltage at 0.05 msec for all groups during the pre-injury test is 6.4 v, at 1 month post-injury is 38.5 v, and at 2 months post-injury is 34.2 v. These differences are significant at the 0.05 level. Even though there is a slight decrease between the first and second months post-injury,

Table 4. Between-group comparisons of strength-duration curves

Duration (msec)	Group	Pre-injury[a]			One month post-injury[a]			Two months post-injury[a]		
		1	2	3	1	2	3	1	2	3
100		4.35	5.3	5.0	16.5	16.5	17.5	20.0	17.5	25.0
50		4.35	5.3	5.0	16.5	19.0	17.5	20.0	17.5	25.0
10		4.35	5.3	5.0	21.5	26.5	26.5	20.0	19.0	25.0
7		4.35	5.5	5.0	21.5	25.0	26.5	20.0	19.5	25.0
5		4.35	5.55	5.0	21.5	26.0	28.0	20.0	21.0	25.0
3		4.35	5.55	5.0	21.5	26.0	28.0	20.0	22.0	25.0
2		4.35	5.55	5.0	21.5	26.0	29.0	20.0	22.0	25.0
1		4.75	5.55	5.0	21.5	26.0	29.0	20.0	22.0	25.0
0.8		4.85	5.55	5.1	22.0	26.5	31.0	20.0	22.0	25.0
0.6		4.85	5.65	5.1	22.0	26.5	32.5	20.0	22.0	25.0
0.4		4.9	5.65	5.1	22.0	26.5	32.5	20.0	22.0	25.0
0.3		5.5	5.65	5.1	22.0	26.5	32.5	20.0	22.0	25.0
0.1		5.65	5.75	5.4	28.0	27.5	35.5	24.0	25.5	28.0
0.05		6.95	6.5	5.75	36.5[b]	33.5[b]	45.5[b]	32.5	31.0	39.0

[a]All values listed as mean voltages for 2 beagles/group. Recorded from the left common digital extensor motor point.

[b]F value = 9.18 ($p<0.1$; $F(0.05) = 9.55$).

the voltage at 0.05 msec at 2 months is still over five times greater than the initial value.

The S-D curves at 1 month post-injury and at 2 months post-injury are illustrated in Figures 1 and 2, respectively. Note not only the larger voltages in the two post-injury trials, but also the differences in the shapes of the curves. The lack of smoothness in the curves of all groups at 4 weeks post-injury indicates that reinnervation has begun (19). Note that the curves for all groups at 8 weeks post-injury are smoother than the curves at 4 weeks.

B. Histological

The number of capillaries/57.5 mm^2 were counted from the PAS slides made from the common digital extensor muscles. Examples of the PAS-stained capillaries from each of the three groups can be seen in Figures 5, 6, 9, 10, 13 and 14. The numbers of capillaries/57.5 mm^2 for each group are shown in Table 5, and values for individual dogs are listed in Table A10 in the Appendix. Although no differences were seen among the treatment groups, the number of capillaries on the left side (297.1) compared to the right side (176.7) is highly significant ($F=19.93$, $p<0.004$).

Representative samples of the common digital extensor

Figure 1. Strength-duration curves, 1 month post-injury. The three curves represent the mean values of 2 Beagles/group. Group 1 has circles at individual points (●—●), Group 2 has squares (■—■), and Group 3 has triangles (▲—▲)

STRENGTH DURATION CURVE

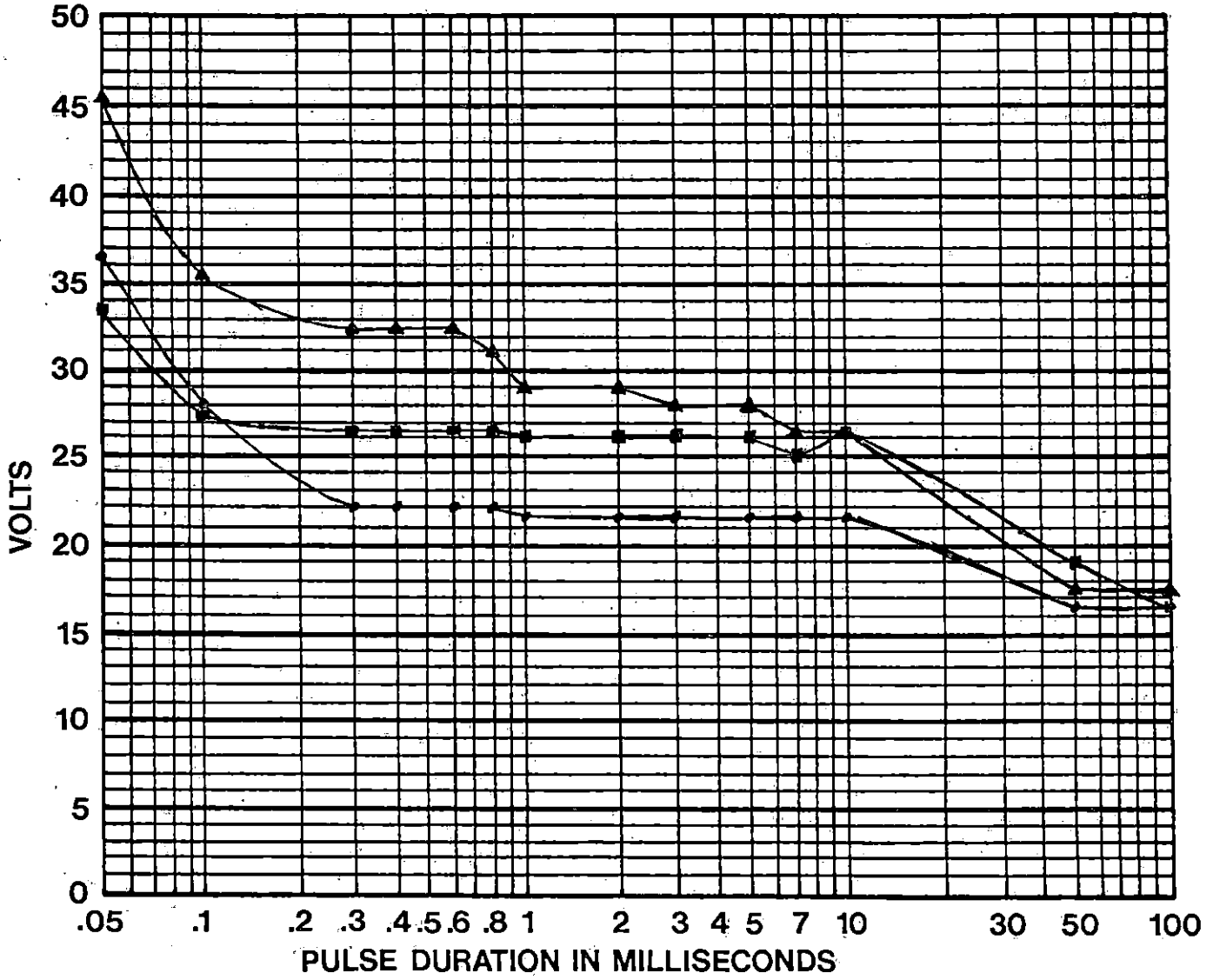

Figure 2. Strength-duration curves, 2 months post-injury. The three curves represent the mean values of 2 Beagles/group. Group 1 has circles at individual points (•—•), Group 2 has squares (■—■), and Group 3 has triangles (▲—▲)

STRENGTH DURATION CURVE

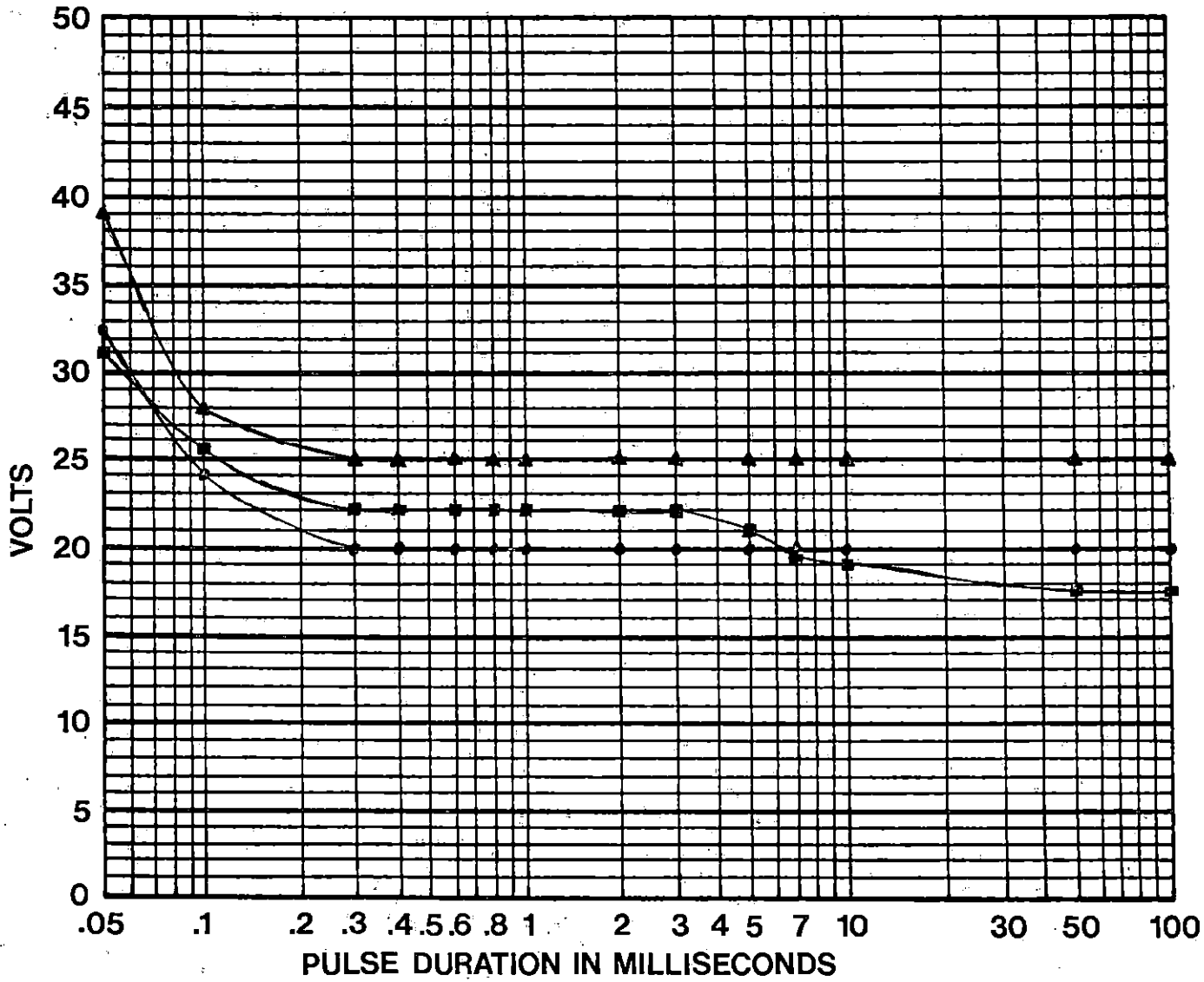

Figure 3. Photomicrograph of a portion of the right common digital extensor muscle from Beagle 4 in Group 1 (control) stained by a modified ATPase method at pH 4.3. The Type I muscle fibers are dark, taking up the stain, while the Type IIA fibers are light (448X)

Figure 4. Photomicrograph of the left common digital extensor muscle from Beagle 4 in Group 1 stained by a modified ATPase method. Note that in addition to the Type I and Type IIA fibers seen in Figure 3, Type IIC fibers, which stain intermediate in darkness, are also present. The muscle fibers in this figure are smaller than in Figure 3 (448X)

Figure 5. Photomicrograph of a portion of the right common digital extensor muscle from Beagle 4 in Group 1 with a PAS stain. The arrow points to a capillary (448X)

Figure 6. Photomicrograph of the left common digital extensor muscle from Beagle 4 in Group 1 with a PAS stain (448X)

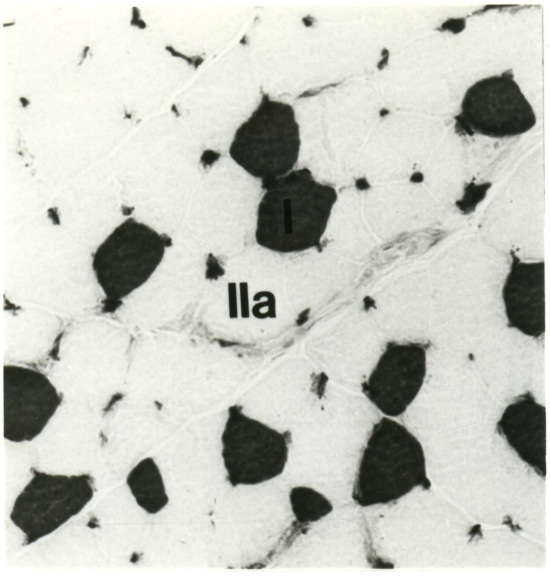

Figure 3

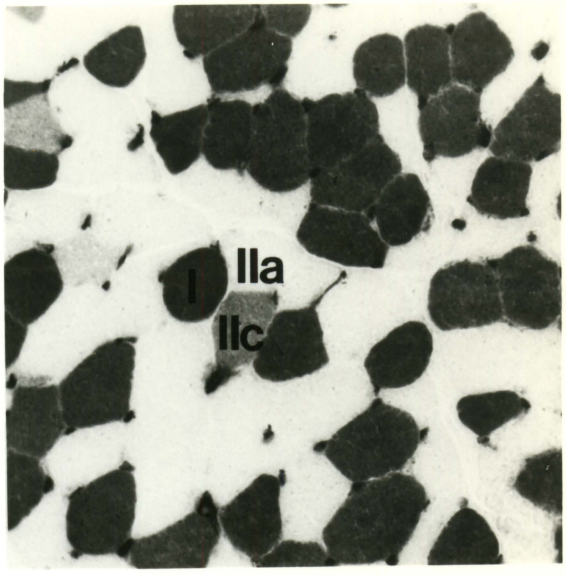

Figure 4

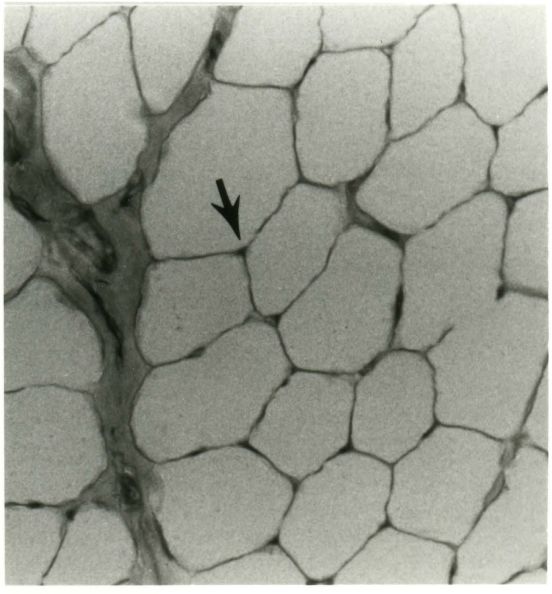

Figure 5

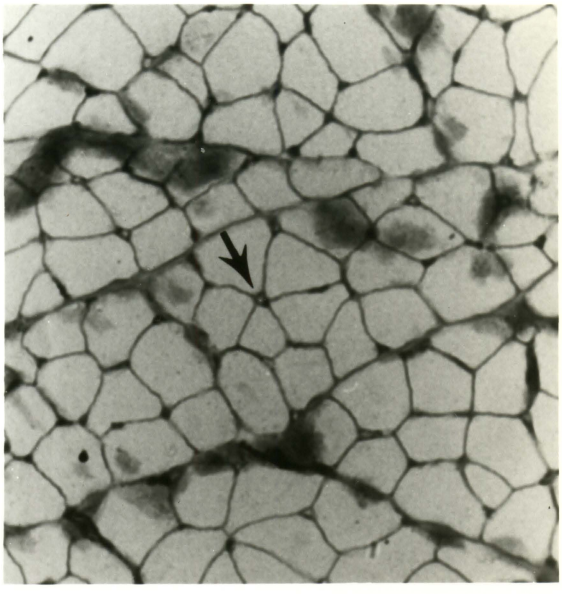

Figure 6

Figure 7. Photomicrograph of the right common digital extensor muscle, from Beagle 6 in Group 2 (1st day treatment). ATPase stain at pH 4.3. A Type IIC fiber is present in this section (448X)

Figure 8. Photomicrograph of the left common digital extensor muscle, from Beagle 6 in Group 2, with an ATPase stain (448X)

Figure 9. Photomicrograph of the right common digital extensor muscle, from Beagle 5 in Group 2, with a PAS stain (448X)

Figure 10. Photomicrograph of the left common digital extensor muscle, from Beagle 5 in Group 2, with a PAS stain (448X)

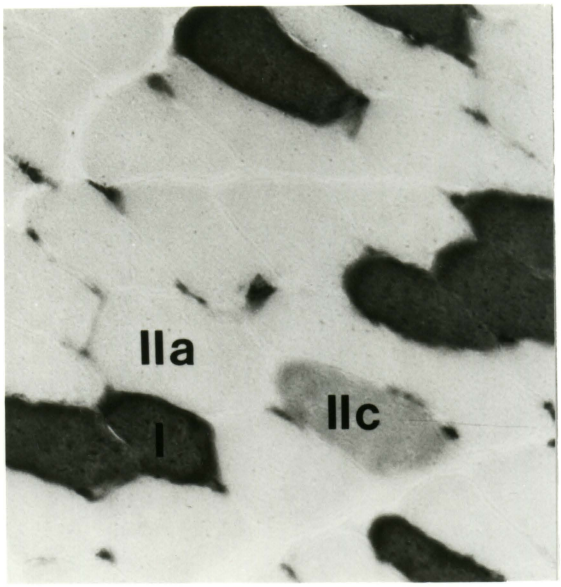

Figure 7

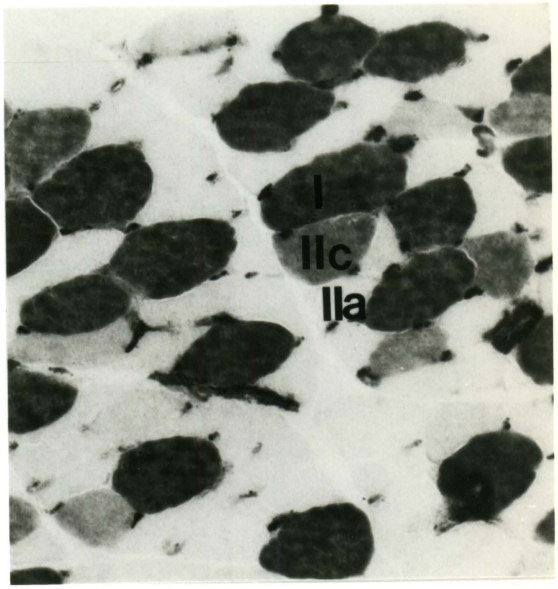

Figure 8

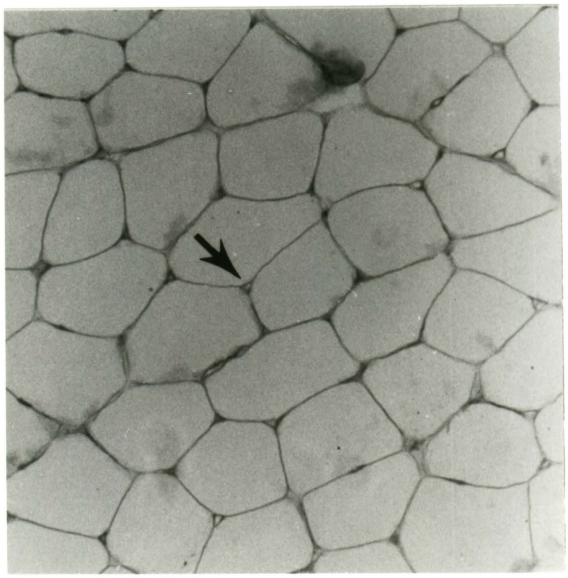

Figure 9

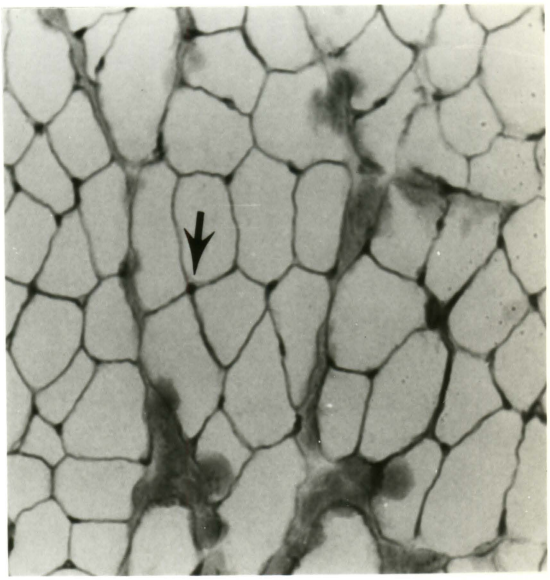

Figure 10

Figure 11. Photomicrograph of the right common digital extensor muscle from Beagle 2 in Group 3 (8th day treatment). ATPase stain at pH 4.3 (448X)

Figure 12. Photomicrograph of the left common digital extensor muscle from Beagle 2 in Group 3. ATPase stain (448X)

Figure 13. Photomicrograph of the right common digital extensor muscle from Beagle 2 in Group 3, with a PAS stain (448X)

Figure 14. Photomicrograph of the left common digital extensor muscle from Beagle 2 in Group 3, with a PAS stain (448X)

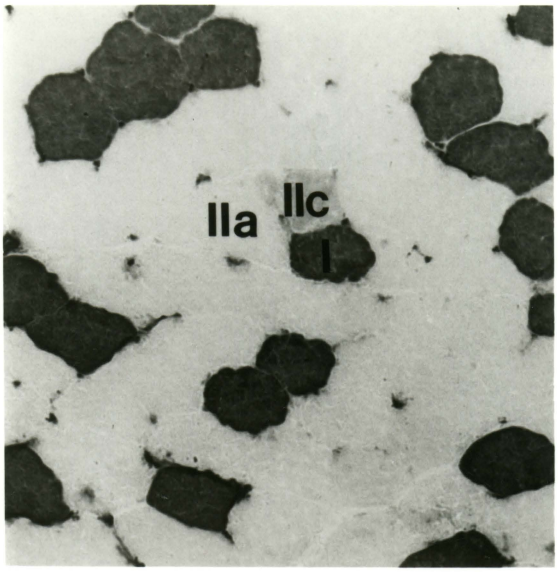

Figure 11

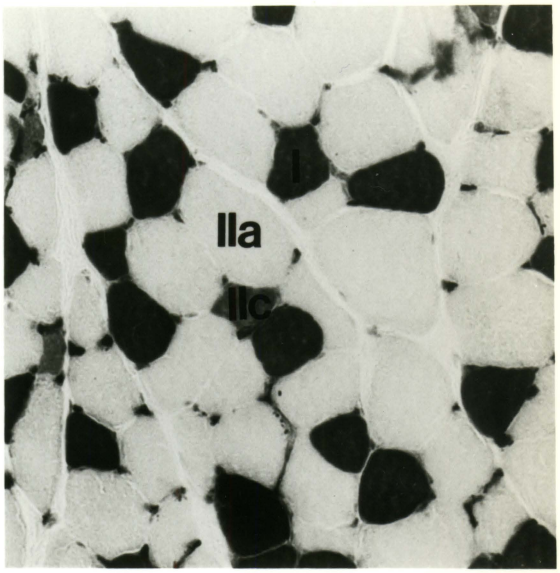

Figure 12

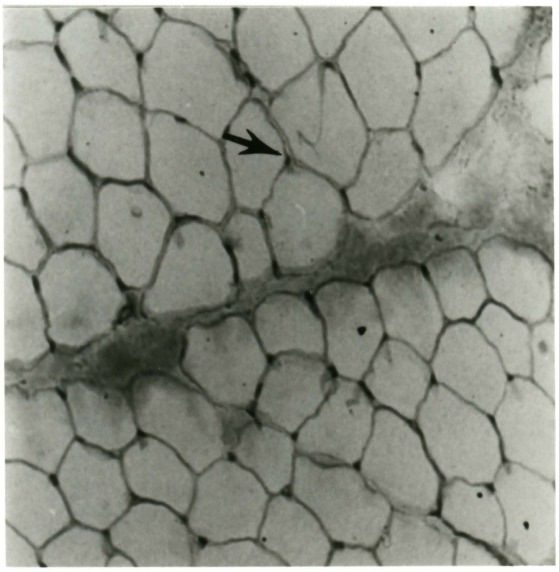

Figure 13

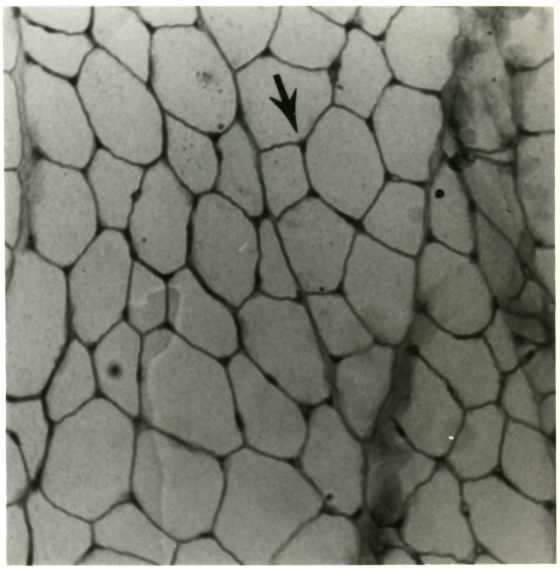

Figure 14

Table 5. Number of capillaries/57.5 mm^2

	Left[a]	Right[a]
Group 1 (Control)	296	160.7
Group 2 (1st day treatment)	317	150
Group 3 (8th day treatment)	278.3	219.3
Groups combined[b]	297.1	176.7

[a]All values are means for the three dogs in each group. Capillaries counted from a portion of the common digital extensor muscle with a PAS stain.

[b]F value = 19.93 for the left side vs. the right ($p < 0.004$). Mean square error for the groups is 682.1 (2 df) and between sides is 65280.9 (1 df).

muscle stained by the modified ATPase method at pH 4.3 can be seen in Figures 3, 4, 7, 8, 11 and 12. The mean number of muscle fibers/57.5 mm^2 for each group and muscle type are shown in Table 6. No significant differences occurred among the treatment groups, as had been anticipated. Recognizing the large mean square errors, Groups 2 and 3 had fewer Type I and Type II fibers on the left side than the controls. In contrast, Groups 2 and 3 each had almost twice as many Type IIC fibers on the left side as did the control group. The same relationships do not exist for the right side except in the case of the Type IIC fibers. In the latter case, it was observed that the mean number of IIC fibers increased on the right side in

Table 6. Mean number of muscle fibers/57.5 mm^2

	Type I[a]		Type IIA		Type IIC[b]	
	Left	Right	Left	Right	Left	Right
Group 1 (control)	76.3	35.3	116.0	96.0	9.7	0
Group 2 (1st day treatment)	54.3	26.3	93.7	79.0	20.7	0.3
Group 3 (8th day treatment)	62.0	43.7	94.7	104.0	16.7	6.0
Groups combined $\bar{x}$ =	64.2	35.1	101.5	93.0	15.7	2.1

[a] F value = 26.32 for the number of Type I fibers on the left vs. right side ($p<0.002$). The mean square error among the groups is 405.5 (2 df) and between sides is 3813.6 (1 df).

[b] F value = 11.07 for the number of Type IIC fibers on the left vs. right side ($p<0.02$). The mean square error among groups is 75.1 (2 df) and between sides is 826.9 (1 df).

treated groups, whereas no IIC fibers were observed in control animals. There were significantly more ($F = 26.32$; $p<0.02$) Type I fibers on the left side (64.2) than on the right (35.1). Similarly, there were significantly more Type IIC fibers ($F=11.07$; $p<0.02$) on the left side (15.7) than on the right (2.1). Values for the individual dogs are included in the Appendix.

The mean muscle fiber diameter for each fiber type for each group is shown in Table 7. Although no differences

Table 7. Mean diameter of muscle fibers

	Type I[a]		Type IIA[b]		Type IIC	
	Left (mm)	Right (mm)	Left (mm)	Right (mm)	Left (mm)	Right (mm)
Group 1 (control)	31.5	38.4	33.9	44.6	28.7	-
Group 2 (1st day treatment)	38.7	47.2	35.8	48.5	35.2	58.6
Group 3 (8th day treatment)	33.1	38.3	38.1	41.7	29.5	33.0

[a]F value = 5.14 for the diameter of Type I fibers on the left vs. right side ($p<0.06$). Mean square error among the treatment groups is 116.8 (2 df) and between sides is 210.1 (1 df).

[b]F value = 6.61 for the diameter of Type IIA fibers on the left vs. right side ($p<0.04$). Mean square error among treatment groups is 13.9 (2 df) and between sides is 365.4 (1 df).

were seen among the treatment groups, the left-sided mean diameters of all muscle types from the animals in the treated groups were larger than the mean diameters of all muscle types for control dogs. There were larger Type I ($F=5.14$, $p<0.06$), and Type IIA fibers on the right side than on the left side ($F=6.61$, $p<0.04$).

The mean areas of the muscle fibers are listed in Table 8. No treatment differences occurred; however, the

Table 8. Mean area of muscle fibers

	Type I[a]		Type IIA[b]		Type IIC	
	Left (mm^2)	Right (mm^2)	Left (mm^2)	Right (mm^2)	Left (mm^2)	Right (mm^2)
Group 1 (control)	71.1	102.3	75.3	138.3	57.9	-
Group 2 (1st day treatment)	100.5	146.0	85.7	151.1	81.3	199.6
Group 3 (8th day treatment)	75.9	106.4	100.2	118.3	60.0	72.8

[a] F value = 6.42 for the area of Type I fibers on the left vs. right side ($p<0.04$). Mean square error among the treatment groups is 2388.9 (2 df) and between sides is 5749.5 (1 df).

[b] F value = 7.6 for the area of Type IIA fibers on the left vs. right side ($p<0.03$). Mean square error among the treatment groups is 222.7 (2 df) and between sides is 10750.7 (1 df).

common digital extensor muscle Type I ($F=6.42$; $p<0.04$) and Type IIA ($F=7.6$; $p<0.03$) fibers were significantly larger on the right than on the left. Diameters and areas for the individual dogs are listed in the Appendix (Tables A1-9).

Photomicrographs of representative radial nerve sections are shown in Figures 15-17. A sample of nerve from the right (intact) limb of a dog is presented in Figure 15 while in Figures 16 and 17, sample sections from the left

Figure 15. Photomicrograph of a portion of normal nerve from the right superficial radial nerve from Beagle 6 in Group 2 (1120X)

Figure 16. Photomicrograph of injured nerve from the left superficial radial nerve of the Silver Weimaraner in Group 1 (control) (1120X)

Figure 17. Injured nerve taken from the left proximal radial nerve of Beagle 3 in Group 1. Note the large number of unmyelinated nerve fibers and the irregular-shaped myelinated fibers. There is also a large amount of undifferentiated material located among the nerve fibers (1120X)

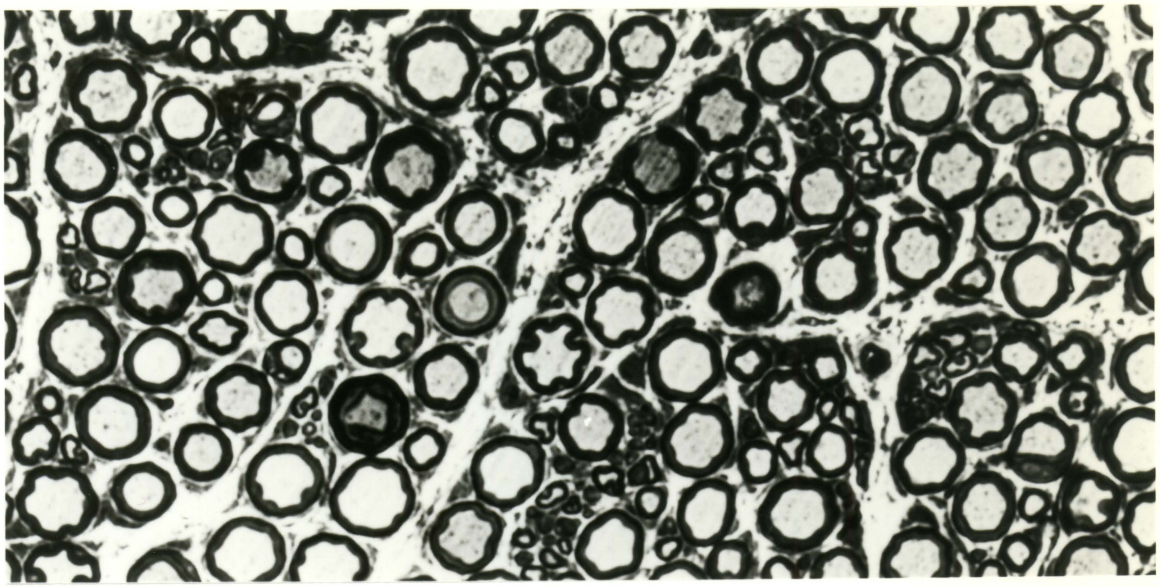

Figure 15

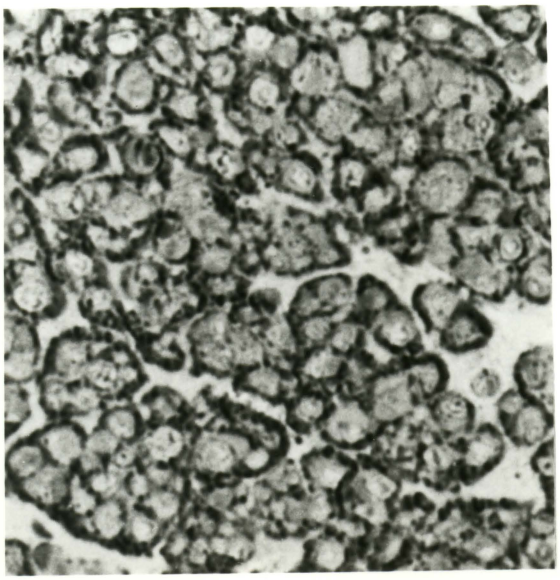

Figure 16

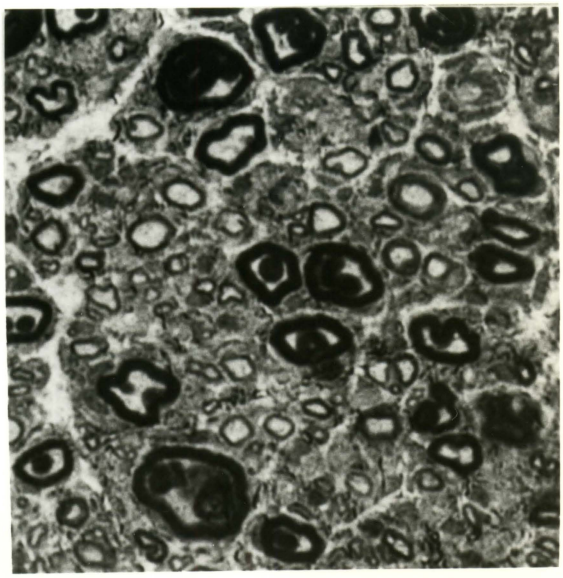

Figure 17

radial nerve of two dogs are shown. No quantitative measurements were possible because not all the nerve fibers were oriented in true cross sections. Because of the pattern of regrowth of the crushed nerves, cross sections on the crushed side were not similar to those obtained from the intact side. In general, in contrast to normal nerve, the nerve sections on the crushed side exhibited poor myelinization with vacuolization of the myelin, considerable gliosis, many neutrophils, and the occasional presence of macrophages.

VI. DISCUSSION

Based on these findings it would appear that electrical stimulation has no effect on nerve regeneration or on the prevention of muscle atrophy, and further, there is no difference whether stimulation is begun immediately followed a nerve crush or 1 week later. In general, the only significant differences found were between the injured and uninjured sides for the various parameters measured. However, because of the small sample size and the large mean standard error in many cases, it is worthwhile to look again at the data to examine any trends.

The differences amongst the groups in some of the clinical measurements at approximately 4 weeks post-injury are of interest. At this point, all dogs in the two treatment groups required less voltage during the daily motor point stimulation treatments and lower rheobase voltages than at any time earlier or later. And although the voltages at the shorter duration (0.8 msec or less) on the S-D curves are lower at 2 months post-injury than at 1 month post-injury, there are large differences in the mean voltages of the two treatment groups at 0.05 msec at 1 month post-injury (Group 2 = 33.5 v, Group 3 = 45.5 v).

In reviewing the literature, it seems that about 3 to 6 weeks post-injury the regeneration process is progressing

rapidly. The edema around the crush site has subsided, vigorous axon sprouting is occurring, axons are crossing the anastomosis, and somal enlargement has peaked in order to form new end plates (1). The axons which have crossed the injury site are now growing 3 (9) to 4 (10) mm/day, and although the old myelin has been almost completely digested and debridement continues, the Schwann cells are now laying down new myelin. Bowne (12) reports that in dogs with neurectomies, muscle atrophy begins as early as 10 days post-injury, but the thickening of the endomysium and perimysium, which retard end-plate formation, begin later. By 1 month post-injury in the rat, the conduction velocity peaks at 75% of normal (9).

Cragg and Thomas (6) reported that 25 to 30 days after a crush injury the conduction velocity was 90% of normal, at 50 to 100 days following the nerve crush it was 80% of normal, and by 200 days it was 100% of normal. The mean daily voltages and rheobase values obtained in the present study correlate with their findings. These voltages were lower at 4 weeks (or 25 to 30 days) post-injury than anytime later, including 8 weeks (about 56 days) post-injury. If the experiment had been continued for about 8 months, these voltages might have significantly decreased from the voltages at 8 weeks.

The lower voltages in the daily treatments and in the

rheobase and chronaxie tests at 4 weeks post-injury suggest that regeneration is occurring in all the dogs. The significantly lower voltage at 0.05 msec in Group 2 seen in the S-D curve at 1 month post-injury supports Melichna and Gutmann's (24) contention that stimulation should begin immediately to be effective. Although the subsequent increase in the daily voltages suggests that some fibrosis may be occurring, the S-D voltages continued to decrease at 2 months post-injury. The clinical evidence suggests that by 4 weeks post-injury the regeneration process was well-established in all dogs.

It should be noted that the hypothesis tests on the rheobase and chronaxie values were run using 7, 21 degrees of freedom (df) for the sequential time differences. If conservative df had been used (1,3), the mean rheobase values still would have been significant at the 0.05 level, but the chronaxie values would not have been significant.

Another interesting observation is the presence of relatively large numbers of IIC fibers on the injured side. It has been hypothesized that Type IIC fibers are primitive fibers which differentiate into Type IIA or Type IIB fibers in neonatal human muscles (55). If so, then their relative abundance on the injured side compared to the intact side in these dogs is of interest. Braund et al. (52) report that the presence of Type I and IIA fibers in normal dogs

is constant, but Type IIC fibers are sometimes absent and generally comprise less than 10% of the total fiber population. However, on the left side in Group 2, Type IIC fibers represent over 12% of the total population. In addition, Braund et al. (52) report preliminary findings of variance in the distribution of all fiber types among individual muscles of the same dog and between homologous muscles of different breeds. In the present study, when the Type IIC data is broken into data for individual dogs, the Weimaraners have more IIC fibers than the Beagles with a mean population of 30.7 in the Weimaraners compared with a mean of 8.2 in the Beagles. This difference is significant at the 0.01 level ($F=13.2$). While the Weimaraner in the Control group had 8.5% of the total muscle fiber population composed of Type IIC fibers, the Weimaraners in Groups 2 and 3 had a total of 34 fibers/57.5 mm^2 or 18.5% and 40 fibers/57.5 mm^2 or 21% Type IIC fibers, respectively. These percentages are well-above the 10% figure reported by Braund et al. (52) in normal dogs.

The mean diameters and mean areas for the Type IIC fibers were not significantly different between sides or amongst groups, but the presence of zero IIC fibers in some of the dogs made statistical analysis of this data more difficult. The F value of 2.26 ($p<0.13$) for the difference in mean diameter and the F value of 1.87 ($p<0.18$)

for the difference in mean area amongst the three treatment groups are the closest to a significant difference amongst the three treatment groups seen in the muscle data. It would be interesting to follow these IIC fibers in a longitudinal study, particularly 4 weeks post-injury, to learn when their number peaks and what type of fibers they become. Possibly the presence of Type IIC fibers correlates with the regeneration process.

Although there are significantly more Type I and Type IIC muscle fibers on the left side than the right when the three groups are combined, there are no significant differences in the numbers of Type IIA fibers between sides. According to Pette et al. (36) long-term electrical stimulation in the rabbit produces more slow, or Type I muscle fibers than fast, or Type II fibers. Dubowitz and Brooke (51) also report selective atrophy of Type II muscle fibers with nerve injuries. This apparently is the type of change seen in the present experiment. The Type IIA fibers, by far the greatest proportion of Type II fibers in these dogs, are not found in significantly greater numbers in the crushed limbs, so their proportion relative to the number of Type I, or slow twitch fibers, has actually decreased in all groups.

This increase in the proportion of Type I fibers may have an effect on the Ach sensitivity of the muscle. Vrbova

et al. (34) report evidence that Type I, or slow twitch fibers may be more sensitive to Ach over their entire surface than Type II fibers. They further suggest that this sensitivity may be determined by the activity of the motor nerve supplying the muscle, because the extrajunctional sensitivity to Ach disappears when the muscle is supplied by a nerve which normally innervates a fast (Type II) muscle. So the relative decrease in Type II fibers reported by Pette et al. (36) and Dubowitz and Brooke (51) may be a secondary effect of the change in the crushed nerve. The question then is whether the proportion of Type II fibers would again increase as the nerve regenerated more completely.

This study did not show any significant differences between Groups 2 and 3 with the exception of the lower S-D curve voltages in Group 2 and the lower mean daily voltage in Group 3 at the triceps motor point 1 month post-injury. Thus, one clinical parameter suggests that electrical stimulation is more beneficial during the 4 week post-injury period if it is started immediately while another parameter suggests that a week should elapse before starting treatment. So there is no support for either Melichna and Gutmann's (24) contention that stimulation must be started immediately after a nerve injury, or for Lomo and Slater's (35) contention that several days should lapse to allow the formation of

functional neuromuscular junctions. However, both these studies were done on the rat and both involved the use of implanted electrodes almost continuously for no more than a week, so the comparisons of those studies with mine must be limited. I chose to stimulate some dogs beginning 8 days after the injury partly because there is often a delay before starting stimulation in human patients with nerve injuries.

The number of capillaries did not differ among groups, although there were more present on the left (crushed) side. The cause of this increased capillary number on the left side is unclear, however. Although more capillaries would be expected during nerve regeneration, Andersen and Henriksson (56) also report that in normal skeletal muscle more capillaries are present in Type I muscle fibers than in Type II fibers. Because there were also significantly more Type I muscle fibers on the left side than on the right, and because I was not able to determine which muscle fiber type a given capillary supplied, it was not possible to know whether the increased number of capillaries were associated with the increased number of Type I fibers on the injured side.

The modified ATPase staining method at pH 4.3 was chosen in this study because of the success Braund et al. (52) had with this technique in the dog as compared with

other histochemical techniques. After experimenting with different preincubation pH's, they concluded that the pH 4.3 preincubation was the best choice because of its excellent differentiation of muscle fiber types I, IIA and IIC.

In agreement with Ducker's (1) findings, muscle cells were smaller on the injured side in the present study. However, I did not observe any thickening of the endomysium and perimysium, as he reported. Perhaps this could be due to the apparent regeneration of the injured nerve in the present study, and therefore, few muscle changes occurred.

In choosing to perform a nerve injury on the dog, several types of injuries and several sites were considered. A crush injury was chosen for a couple of reasons: first, it is relatively simple to perform and is a reliable procedure, and second, it is more typical of nerve injuries seen clinically (57). The radial nerve was selected because of its size, its accessibility for crush, its accessibility for motor point stimulation, and its motor and sensory fiber components. Also, Sunderland (57) reports that the radial nerve is injured more frequently than any other nerve in man. He also reports that radial nerve injuries which are not associated with a fracture predominate in the lower-

third of the upper arm. This is the portion of the radial nerve which was used as the crush site in the present study.

The duration of the experiment also was carefully considered. Radial nerve injuries in man may be treated by electrical stimulation from 2 weeks to 1 year. If electrical stimulation is to be given for an extended period of time, it is usually given two to three times/ week. Sunderland (57) reviews many studies of the onset of recovery following a radial nerve lesion in man. Clinical signs of recovery vary from 2 to 10 weeks for a mild compression injury to 8 to 40 weeks following a second degree injury (where recovery depends on regenerating axons). Recovery is generally faster and more complete in the dog than in man (13,14), so these periods would be expected to be shorter in the dog. Jacobson and Guth (9) reported that when the rat sciatic nerve was crushed, the nerve had regenerated to the hind limb by the 28th day post-injury. In the present study it was expected and found that clinical signs of regeneration were present by 8 weeks post-crush.

Clinical signs of improvement were observed in these dogs within several days after the nerve crush. Within a week post-injury, all the dogs had an apparently normal gait pattern during slow walking, and none of the dogs held the injured limb in the flexed posture classically described

following a nerve lesion (12) after several days. But the clinical and histological data show that the nerve is far from normal. Even 8 weeks post-injury the S-D values are over five times the pre-injury values, the rheobase and chronaxie values are higher, and all the muscle and capillary data show significant right-left differences. Thus, the dogs are able to function almost normally in nonstress situations despite major nerve damage in one limb.

One possible concern was the use of two breeds in the study. The three Weimaraners were assigned randomly, one to each treatment group, and the Beagles were likewise assigned two to each treatment group. The total number of muscle fibers and capillaries, and mean fiber areas and diameters were tested for interbreed differences and none were found. However, there were significantly more Type IIC muscle fibers in the Weimaraners. In all clinical measurements except for the mean daily voltages the Weimaraners were not included, so interbreed differences were not a potential source of error in those comparisons.

One unexpected problem arose because of the use of steel sutures for skin closure for the Beagles. I removed all the sutures from all the dogs on the same day (in the second post-injury week), and beginning the next day all the Beagles receiving electrical stimulation required

more current than before. The Weimaraners had skin closure with Vetafil so this did not happen to them.

The nine dogs exhibited a wide variety of responses to the experimental procedure. Several of the dogs were very calm at all times, while two others were quite active each day. Beagle 5 in Group 2 was hypersensitive to stimulation of the radial nerve and the extensor motor points during Weeks 2 and 3. Less current was required during this period, but the dog still acted quite uncomfortable. Because alternating current (AC) was used, there was no ion buildup, so this was not the cause of the dog's discomfort. The dog gradually lost this hypersensitivity and in the 2nd month responded to the treatments no differently from the other dogs. Beagle 5 was the only dog who had 10° less range of the left carpal joint than the right two months post-injury. The rheobase and chronaxie values for Beagles 5 and 6 showed larger differences than the Beagles within the other two groups. Beagle 5 also had fewer muscle fibers of all three types on the left side than the other dogs in Group 2. This is particularly obvious in the Type IIC fibers where Beagle 5 had 5 fibers on the left side compared with 34 and 23 fibers for the other two dogs. The results seen in Beagle 5 may have led to misleading conclusions for Group 2 as a whole.

Whether the conclusions drawn from this study can be

applied to man is unknown. Kline et al. (13, 14) have documented the differences in recovery from nerve lesions in the dog and in primates. Whereas spontaneous recovery from nerve transection is commonly seen in the dog, this seldom occurs in monkeys and chimpanzees. This suggests that the applicability of any results found in the dog to man may be limited.

In summary, the main differences seen in this experiment are between the injured and uninjured sides. The electrical stimulation treatments did not result in any consistent improvement over the untreated controls. And there were no consistent differences between the two treatment groups. From these data it appears that electrical stimulation, as it is clinically used on humans, does not result in more normal strength-duration curves, more normal rheobase and chronaxie values, larger muscle fibers and greater capillary density than untreated controls. The possible facilitation of nerve regeneration by electrical stimulation must be weighed against the costs involved. This study suggests the time and effort may be better spent in other ways.

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IX. APPENDIX

Table A1. Muscle fibers - Type I

Group 1 (Control)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area $(mm)^2$
Left	Weimaraner -Silver female	100	32.6	76.4
	Beagle 3	38	33.5	79.4
	Beagle 4	91	28.5	57.6
	$\bar{X}$	76.3	31.5	71.1
Right	Weimaraner -Silver female	38	34.2	79.0
	Beagle 3	23	52.0	166.9
	Beagle 4	45	29.1	61.1
	$\bar{X}$	35.3	38.4	102.3

Table A2. Muscle fibers - Type I

Group 2 (1st day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area $(mm)^2$
Left	Weimaraner -Blue male	61	32.9	73.3
	Beagle 5	43	45.3	131.4
	Beagle 6	59	38.0	96.9
	$\bar{X}$	54.3	38.7	100.5
Right	Weimaraner -Blue Male	33	48.1	158.2
	Beagle 5	27	40.9	120.2
	Beagle 6	19	52.5	159.7
	$\bar{X}$	26.3	47.2	146.0

Table A3. Muscle fibers - Type I

Group 3 (8th day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area (mm)^2
Left	Weimaraner -Silver Male	79	31.1	62.7
	Beagle 1	40	39.0	105.8
	Beagle 2	67	29.2	59.1
	$\bar{X}$	62.0	33.1	75.9
Right	Weimaraner -Silver male	75	31.2	68.5
	Beagle 1	15	45.6	150.9
	Beagle 2	41	38.0	99.8
	$\bar{X}$	43.7	38.3	106.4

Table A4. Muscle fibers - Type IIA

Group 1 (Control)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area (mm)^2
Left	Weimaraner -Silver female	95	35.1	82.5
	Beagle 3	123	35.0	87.9
	Beagle 4	130	31.7	55.4
	$\bar{X}$	116.0	33.9	75.3
Right	Weimaraner -Silver female	111	39.2	104.4
	Beagle 3	46	58.4	219.8
	Beagle 4	131	36.3	90.9
	$\bar{X}$	96.0	44.6	138.3

Table A5. Muscle fibers - Type IIA

Group 2 (1st day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area $(mm)^2$
Left	Weimaraner -Blue male	89	33.2	75.4
	Beagle 5	79	44.1	126.0
	Beagle 6	113	30.1	55.6
	$\bar{X}$	93.7	35.8	85.7
Right	Weimaraner -Blue male	61	46.9	150.5
	Beagle 5	108	42.9	125.2
	Beagle 6	68	55.8	177.5
	$\bar{X}$	79.0	48.5	151.1

Table A6. Muscle fibers - Type IIA

Group 3 (8th day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area $(mm)^2$
Left	Weimaraner -Silver male	74	39.4	104.6
	Beagle 1	95	39.1	106.0
	Beagle 2	115	35.8	89.9
	$\bar{X}$	94.7	38.1	100.2
Right	Weimaraner -Silver male	102	38.6	95.1
	Beagle 1	94	48.7	159.9
	Beagle 2	116	37.8	99.9
	$\bar{X}$	104	41.7	118.3

Table A7. Muscle fibers - Type IIC

Group 1 (Control)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area$_2$ (mm)
Left	Weimaraner -Silver female	18	34.3	77.6
	Beagle 3	3	27.0	52.2
	Beagle 4	8	24.7	43.8
	$\bar{X}$	9.7	28.7	57.9
Right	Weimaraner -Silver female	0	-	-
	Beagle 3	0	-	-
	Beagle 4	0	-	-
	$\bar{X}$	0	-	-

Table A8. Muscle fibers - Type IIC

Group 2 (1st day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area$_2$ (mm)
Left	Weimaraner -Blue male	34	33.0	76.0
	Beagle 5	5	38.6	93.9
	Beagle 6	23	33.9	74.0
	$\bar{X}$	20.7	35.2	81.3
Right	Weimaraner -Blue male	0	-	-
	Beagle 5	0	-	-
	Beagle 6	1	58.6	199.6
	$\bar{X}$	0.3	58.6	199.6

Table 9. Muscle fibers - Type IIC

Group 3 (8th day treatment)		Number of fibers	Mean fiber diameter (mm)	Mean fiber area (mm)^2
Left	Weimaraner -Silver male	40	34.3	75.1
	Beagle 1	4	34.1	78.2
	Beagle 2	6	20.0	26.7
	$\bar{X}$	16.7	29.5	60.0
Right	Weimaraner -Silver male	14	31.0	66.2
	Beagle 1	0	-	-
	Beagle 2	4	35.0	79.3
	$\bar{X}$	6	33.0	72.8

Table A10. Number of capillaries/57.5 mm^2[a]

Group	Dog	Left	Right
1 (Control)	Weimaraner -Silver female	304	250
	Beagle 3	274	98
	Beagle 4	310	134
	$\bar{X}$	296	160.7
2 (1st day treatment)	Weimaraner -Blue male	405	148
	Beagle 5	230	187
	Beagle 6	316	115
	$\bar{X}$	317	150
3 (8th day treatment)	Weimaraner -Silver male	312	296
	Beagle 1	217	105
	Beagle 2	306	257
	$\bar{X}$	278.3	219.3
Groups combined	$\bar{X}$	297.1	176.7

[a] F value = 19.93 for number of capillaries left side vs. right $p > F = 0.004$.