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Dedication

To my Mother, Mrs. Mary Jane Linn, who inspired my interests in science and medicine.

Acknowledgements

I would like to thank Dr. Bill Garoutte and Dr. Henry Ralston for their helpful advice and recommendations. I want to extend my deepest gratitude to Dr. Parry, who has been an exceptional mentor. His patient guidance and encouragement have been invaluable. I am also obliged to Ms. Ellyn Maulhardt for expert technical assistance and instruction, and to Ms. Debbie Crumrine for her many helpful suggestions. I am especially indebted to Mr. Steven Kepler, my cousin, for his care and patience in preparing this manuscript; and Mr. Gary Lake, my fiance, for his continual support.

I am grateful to each of these individuals and sincerely appreciate their generous contributions of time and their many excellent suggestions.

Summary

The effects of ischemia on peripheral nerve structure and function were studied in rats following injection of 0.1 umole arachidonic acid into the femoral artery. The compound muscle action potential (CMAP) was recorded from the plantar intrinsic foot muscles following stimulation proximal and distal to the ischemic region. The amplitude and latency of the motor responses and the amplitude of the compound nerve action potential (CNAP) were measured. The maximal motor conduction velocity (MCV) and the ratio of proximal to distal amplitudes (R) were calculated. Epoxy-embedded sections and single teased fibers were examined by light microscopy.

CMAP amplitudes began to fall 5 to 10 minutes after the injection of arachidonic acid. The proximally elicited CMAP fell earlier than the distal response and was more profoundly reduced, indicating conduction block. The MCV was significantly slowed only through the ischemic segment of nerve. These early electrophysiological abnormalities were unaccompanied by gross anatomical changes suggesting that a greater supply of energy must be needed for impulse conduction than is required to maintain morphological integrity. A small portion of rats had normal electro-physiology and/or morophology 1 - 7 days later.

The majority of animals developed partial nerve infarction characterized by low amplitude CMAP and mild conduction slowing at the site of the infarct in the proximal tibial nerve. Degenerating axons were concentrated in the center of the fascicle and subperineurial axons were spared. Approximately half of these nerves showed conduction block at 24 hours. The mean R value for this group increased during the first week as the proximal CMAP improved. Paranodal retraction of myelin and segmental demyelination were too rare to account for the conduction block.

Only slight improvements in CMAP amplitudes, MCV, distal motor latency and CNAP were observed between 1 and 3 weeks. Regeneration of axons is preceeded by revascularization and phagocytosis, and the recovery of nerve function parallels regeneration.

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List of Abbreviations

ATP	- adenosine triphosphate
BF	- basic fuchsin
°C	- degrees centigrade
cm	- centimeter
CMAP	- compound muscle action potential
CNAP	- compound nerve action potential
D	- distal stimulation site and response
dd H_2O	- double distilled water
DML	- distal motor latency
EBA	- Evans blue - albumin
g	- gram
IGA	- inferior gluteal artery
kg	- kilogram
LT	- leukotriene
m	- meter
M	- molar
M_1	- stimulation site in leg and response
M_2	- stimulation site in thigh and response
MB-azure II	- methylene blue - azure II
MCV	- maximal motor conduction velocity
mg	- milligram
ml	- milliliter
mm	- millimeter
mM	- millimolar
msec	- millisecond
mV	- millivolt
NCV	- maximal nerve conduction velocity
ND	- not determined
OsO_4	- osmium tetroxide
P	- proximal stimulation site and response
PG	- prostaglandin
PO	- propylene oxide
PO_4	- Sorenson's phosphate buffer
R	- ratio of proximal to distal amplitudes
SD	- standard deviation
sec	- second
TXA_2	- thromboxane A_2
ul	- microliter
um	- micrometer
umoles	- micromoles
uV	- microvolt
v	- volume

I. Introduction

A. Literature Review

The effects of ischemia on peripheral nerve structure and function have been examined since the turn of the century (Okada 1905). The effects of anoxia on nerve conduction were initially investigated in vitro during the 1920's and 30's. In vivo studies were impeded by the apparent resistance of peripheral nerves to ischemia. Either the vasa nervorum provided such a rich anastomosis that is was difficult to induce ischemia, or the axons were intrinsically resistant to ischemia damage. Nerve conduction studies and morphological examinations have been utilized to assess models of peripheral nerve ischemia induced by tourniquets, arterial ligation and microvascular occlusion.

1. In Vitro Studies

The effects of anoxia on frog nerve were first studied in vitro by Heinbecker (1929). Replacing oxygen in the bathing medium with hydrogen or nitrogen initially decreased the stimulus threshold and increased the action potential amplitude and conduction velocity. Within minutes the conduction velocity decreased and after 2 to 7 hours of anoxia the action potential was extinguished. Heinbecker also found that nerve fibers have different susceptibilities to anoxia. The smallest, slowest conducting fibers failed within the first 30 minutes, and by 2 hours only the largest

fibers continued to respond. Following reoxygenation, some nerves completely recovered even after 2 to 3 hours of anoxia. Administering carbon dioxide or lactic acid produced identical alterations in the recorded electrical activity, suggesting that the effects of anoxia are at least partially due to the accumulation of acidic metabolites generated by anaerobic glycolysis.

Gerard (1930), Lehmann (1937) and Wright (1946) extended these observations in amphibian, crustacean and mammalian nerve preparations. The survival time of frog nerve varied inversely with temperature: raising the temperature of the bathing medium from 25°C to 35°C diminished the survival time by about one hour. Mammalian nerves were more susceptible to anoxia than amphibian or crustacean nerves and conduction failed within 15 to 45 minutes. The differential susceptibility of mammalian nerve fibers parallels the response seen in frog nerves. Large, myelinated Group A axons (less than 20 u diameter) which conduct at a rate of up to 100 m/sec were found to be more resistant to the effects of anoxia and to recover more quickly than smaller myelinated fibers. Small, myelinated Group B axons (less than 3 u diameter) with conduction velocities of 3 to 14 m/sec were the most susceptible to anoxia and the slowest to recover (Grundfest, 1939; 1940). On the other hand, the small unmyelinated Group C axons with conduction velocities less than 2 m/sec were the most resistant to anoxia and the first to recover.

If oxygen was reintroduced subsequent to conduction failure, recovery began within 30 to 120 seconds and was complete within 5 to 10 minutes. When anoxic conditions were maintained for 1 to 2 hours, the recovery was delayed and, in some nerves, the amplitude was permanently reduced. It was found that the effects of repeat asphyxiation progressed more rapidly, even if nerve potentials had been restored to pre-ischemic levels. This suggests that the nerve had been permanently damaged in some way or that an energy store within the nerve had been depleted.

Conclusions: In Vitro Studies

i. Nerves are intrinsically resistant to anoxia. Mammalian nerves continued to conduct impulses for 15 to 45 minutes in the absence of oxygen.

ii. Anoxia produced a drop in the conduction velocity of peripheral nerves in vitro. With prolonged anoxia the action potential was extinguished.

iii. In mammalian nerves unmyelinated axons were the most resistant to anoxia, large myelinated axons were more susceptible, and the smaller, slower conducting myelinated axons were most susceptible.

iv. Following short periods of anoxia, nerves could recover completely. Following longer periods, conduction was permanently impaired.

2. In Vivo Studies

One of the earliest studies of peripheral nerve ischemia was published in 1905 by Okada and purported to show that ligation of the inferior gluteal artery (IGA) invariably produced degeneration of the sciatic nerve. Okada's work was refuted by Adams (1943), who found that ligation of the IGA of rabbits produced little or no clinical or histological change. If the IGA, profunda femoris and small saphenous arteries were ligated, only mild degeneration was observed. Multiple ligations performed in conjunction with mobilization of the nerve to disrupt the regional blood supply produced paralysis and extensive degeneration. Because of this demonstrated difficulty in reliably producing ischemic nerve degeneration, other methods were employed.

a. Tourniquet Studies

Tourniquets were utilized by Bentley and Schlapp (1943) to produce ischemia in branches of cat sciatic nerve. A pneumatic cuff encircling the hindlimb decreased the Group A fiber action potential after 18 minutes and extinguished the response by 30 minutes. Increasing the temperature of the limb caused the response to be extinguished earlier. The sciatic nerve could be protected from direct compression by applying tourniquets around the tissue on either side of the nerve. Ischemia was not complete and the action potential was maintained for 45 minutes. Nerves rendered ischemic for

less that 1.5 hours began to recover within 30 seconds after tourniquet removal, and recovery was complete after 6 minutes. Although delayed, partial recovery was still possible after 4 hours of ischemia.

The small, slowly conducting C fibers appeared to be most resistant to ischemia (Clark et al, 1935). Within 45 minutes after the application of a sphygmomanometer cuff, the electroneurogram tracings from A and B fibers of cat saphenous nerve were extinguished. However, the C elevation was still present and stimulation continued to elicit reflex changes in respiration. After more than an hour of ischemia the C fibers were also blocked but this effect was reversible with removal of the cuff.

Fox and Kenmore (1967) produced cardiac arrest in dogs by injecting air intravascularly or used pneumatic tourniquets to induce ischemia, then studied nerve conduction in the hind limb. Compound muscle action potentials recorded in the foot disappeared 18 to 21 minutes after tourniquet application. More proximal segments of nerve were more susceptible. Nerve action potentials persisted for an average of 43 minutes suggesting that neuromuscular transmission was especially susceptible to ischemia. This finding was confirmed in an in vitro rat phrenic nerve-diaphragm preparation (Hubbard and Løyning, 1966).

Morphological examinations of rat muscle following 2 to 8 hours of tourniquet-induced ischemia (Thomason and

Matzke, 1975) revealed a profound reduction in the number of motor end plates. Nerve fiber density was reduced but the largest myelinated axons were spared in milder lesions. Slight chromatolysis was found and the axons appeared to be more susceptible than the myelin.

Mäkitie and Teräväinen (1977) used tourniquets to produce ischemia for 1 to 6 hours in the rat hindlimb. Nerve conduction studies were performed 1 to 90 days later. They found mildly reduced motor and mixed nerve conduction velocities following 1 to 2 hours of ischemia and profound slowing or complete conduction failure if the tourniquet had been maintained for 3 to 6 hours. One hour of ischemia produced no permanent morphological abnormalities. Wallerian degeneration was evident after more than two hours of ischemia and myelinated fibers of all sizes were proportional damaged. No paranodal or segmental demyelination was observed.

Denny-Brown and Brenner (1944) used a mercury pressure bag to achieve direct compression of the sciatic nerve. A specially designed pneumatic cuff was employed by Ochoa, Fowler and Gilliatt (1972) to compress the medial popliteal nerve of baboons, and Baba, Fowler, Jacobs and Gilliatt (1982) compressed the tibial nerve of rabbits with silk ligatures. These three models produced conduction failure as well as paranodal demyelination at the site of constriction; intra-myelinic and periaxonal edema, and shrunken, swollen or degenerating axons. Harriman (1977) reviewed the literature

on peripheral nerve ischemia and also concluded that the demyelination and local conduction block were the result of direct nerve compression rather than ischemia.

Tourniquet studies have also been performed on human volunteers. Lewis, Pickering and Rothschild (1931) applied sphygmomanometer cuffs at various levels of the arm. Numbness appeared within 3 minutes and paralysis began after 25 to 30 minutes. Sensory and motor loss began concurrently in the fingers and spread centripetally. The longer the length of nerve rendered ischemic, the more quickly paralysis occurred, suggesting that the proximal portion of nerve was more susceptible to ischemia.

Hershey and Wagman (1966) placed blood pressure cuffs around one thigh of normal volunteers. They found the small motor fibers to be more susceptible as the minimal conduction velocity was reduced by 32 to 50%, compared to a 16 to 20% reduction in the maximal conduction velocity. In one subject after 18 to 23 minutes of ischemia, the compound muscle action potential was reduced following stimulation at the knee but not the ankle, supporting the theory that the proximal region of nerve was more susceptible.

Kamp-Nielson and Kardel (1974) and Lütschg and Ludin (1982) found decremental impulse propagation in the human median nerve during tourniquet-induced ischemia. Electrodes were placed at 3 or 4 sites along the nerve and it was found that the sensory nerve conduction velocity decreased

as a function of distance from the site of stimulation in the hand. The conduction velocity diminished to 80% of pre-ischemic values at the elbow after 9 to 12 minutes and at the wrist following 19 to 21 minutes. No differences from the rates of conduction were observed if a second cuff was placed around the mid-forearm. The proximal motor latency increased earlier that the more distal latencies and recovery at the elbow was delayed compared to the wrist. Lütschg and Ludin proposed that the greater susceptibility of proximal segments may be due to the presence of longer internodes.

Conclusions: Tourniquet Studies

i. Tourniquet studies have confirmed in vitro findings on the effects of anoxia. The smaller myelinated fibers appeared more susceptible to ischemia and the largest myelinated axons were spared in subtotal lesions. Unmyelinated axons were especially resistant. Conduction velocity was decreased and conduction failed in 18 to 30 minutes. Following 90 minutes of ischemia, recovery began within 30 seconds after restoration of the blood supply and was complete by 6 minutes.

ii. Neuromuscular transmission appeared to be the component most susceptible to ischemia and the proximal portion of nerve was affected more than the distal segment.

iii. Tourniquet models of ischemia present several problems. It is difficult to produce focal infarcts which resemble clinical ischemic neuropathies. Furthermore, tourniquets probably also cause damage through direct nerve compression.

Arterial ligation models of ischemia were developed in an attempt to produce focal infarction without nerve compression.

b. Arterial Ligation Studies

The diffusion of nutrients from well-vascularized muscle tissue may be an important component in the prevention of nerve infarction. After cutting the nutrient vessels, Blunt (1960) encased nerve in a polyethylene sheath. The ensheathed nerves showed swollen axons, intramyelinic edema and paranodal retraction to a much greater extent than those nerves subjected to ligation alone. These findings support the view that surrounding tissues may help to maintain a nerve which has lost its vascular supply; however, direct physical trauma may have contributed to the morphological abnormalities.

Korthals and Wiśniewski (1975) developed the first reproducible model of peripheral nerve ischemia using ligation of the abdominal aorta and one femoral artery. Following ligation, the cats developed paralysis below the knee and lost the claw-spread reflex. Morphological studies

revealed total necrosis of all cellular elements in the tibial and peroneal nerves. At the proximal and distal margins of the zone of necrosis, axonal organelles had accumulated, degenerating fibers were concentrated in the center of the fascicle, and paranodal demyelination was found between the segments of normal and degenerating nerve (Korthals et al, 1978). Neither the proximal sciatic nor the nerves in the foot suffered primary ischemic damage.

Peripheral nerve ischemia can also be induced by ligating the internal iliac and the external or common iliac arteries. Hess et al (1979) used this model in rabbits and produced central-fascicular Wallerian degeneration, proximal paranodal demyelination and rare internodal demyelination. All sizes of myelinated fibers were affected but the unmyelinated axons were spared.

Fowler and Gilliatt (1981) examined the effects of large artery ligation on impulse conduction in the rabbit sciatic nerve. The severity of ischemic damage ranged from mild reduction of the muscle and nerve action potential amplitudes to complete conduction block. Conduction block, which occurred in 50% of rabbits with partial infarcts, persisted for as long as 20 days. The maximal motor conduction velocity was reduced in many animals through the twentieth day. Paranodal and segmental demyelination were seen proximal to degeneration and in fibers which were not degenerating

distally. Demyelination was more common in the nerves with conduction block.

Following temporary compression of the terminal aorta and femoral artery of cats, Korthals et al (1984) found demyelination and remyelination. They concluded that reperfusion of vessels may cause demyelination via edema or the toxic effects of acidic metabolites. This demyelination in the absence of distal degeneration was not seen with permanent arterial ligation (Korthals and Wiśniewski, 1975).

Parry et al (1984 a) produced acute transient conduction block by ligating the femoral artery. Conduction abnormalities appeared in the majority of rats after 10 to 20 minutes. The evoked motor and mixed nerve compound action potentials were reduced and occasionally extinguished, and the ratio of proximal to distal amplitudes decreased. The impulse conduction velocity was only mildly reduced (12%). All conduction abnormalities were reversed by 24 hours and the nerves appeared normal morphologically. They found that small myelinated fibers were more susceptible to ischemia than the larger, faster conducting axons, and suggested that transient conduction failure had a metabolic rather than a morphologic basis.

Conclusions: Arterial Ligation Studies

i. Resistance of nerves to ischemia in vivo may be

aided by the diffusion of nutrients from surrounding, well-vascularized muscle tissue.

ii. Ligation of large arteries produced clinical, morphological and electrophysiological abnormalities. Milder lesions resulted in central-fascicular degeneration but total necrosis of all cellular elements accompanied more severe infarcts. Paranodal demyelination commonly occurred in fibers proximal to Wallerian degeneration but segmental demyelination was uncommon and may have been associated with reperfusion of the ischemic nerve segment.

iii. Transient ischemia decreased action potential amplitudes, produced conduction block and mildly reduced conduction velocity. The small myelinated axons may have been more susceptible to ischemia than larger axons.

iv. Nerve infarction caused low amplitude CMAP, universal conduction slowing, and conduction block in the surviving axons of some animals.

v. Although technically difficult, this method has the advantage that the ischemia may be reversed by removing the arterial ligatures. However, large artery ligation allows little control over the severity of the infarct. Effects of the resultant ischemia may range from transient disturbances to complete necrosis. In addition, the lesion may differ morphologically and electrophysiologically from microangiopathic infarction because of the possibility for early reperfusion via collaterals.

To overcome these problems methods were developed by which the vasa nervorum could be occluded.

c. Microvascular Occlusion Studies

J. T. Roberts (1948) compared a variety of methods for producing ischemia. He was the first to create emboli in the vasa nervorum using air, sterile powered graphite or lycopodium spores. These emboli filled the small blood vessels in the center of the nerve as well as epineurial and nutrient vessels.

Inspired by Roberts' success with lycopodium spores, Parry and Brown (1981, 1982) developed a simple and reproducible method of producing experimental nerve infarction. Arachidonic acid had been proven to cause intravascular platelet aggregation and vasoconstriction of the arterioles and large capillaries (Furlow and Bass, 1975 a, b). Following injection of 0.2 to 0.5 umoles arachidonic acid into the femoral artery of rats, the ipsilateral foot subsequently developed some degree of paralysis and diminished responsiveness to painful stimuli.

The proximal tibial, fibular and sural nerves exhibited a zone of focal necrosis. A transition zone of central-fascicular degeneration was seen more proximally. Distally, the degenerating axons were scattered throughout the fascicle and morphometric analysis revealed a disproportionate decrease in small myelinated and unmyelinated axons. Many

surviving unmyelinated axons were swollen. However, segmental demyelination was found in the same proportion of single nerve fibers teased from experimental and control animals. Related 20-carbon fatty acids failed to produce paralysis or morphological abnormalities. In contrast to central nervous system injections (Chan et al, 1983 a; Chan and Fishman, 1984), little or no axonal degeneration was seen one week after injecting arachidonic acid into the sciatic nerve.

By injecting radio-labelled amino acids into the dorsal root ganglia before or after intra-arterial arachidonic acid injection, Parry et al (1982 a, b) showed that fast axonal transport was blocked at a time when the ischemic nerve still appeared intact. Orthograde and retrograde transport in the segment of nerve distal to the infarct was normal. After 60 minutes of ischemia the earliest ultrastructural changes were observed, but the blockage of transport could still be reversed if the nerve was excised and bathed in oxygenated medium.

Nukada and Dyck (1982, 1983) produced an ischemic neuropathy with similar morphological characteristics by injecting 15 um microspheres into the internal or external iliac or superior gluteal arteries. The highest concentration of microspheres was found in endoneurial capillaries in the distal sciatic, and the proximal tibial, peroneal and sural nerves. Again, a central-fascicular pattern of

degeneration was observed which was more diffuse and severe distally. Even though paranodal demyelination was observed, the axons appeared to be more susceptible to ischemic damage than the Schwann cells.

Conclusions: Microvascular Occlusion Studies

i. Focal nerve ischemia can be produced by occluding the vasa nervorum with intra-arterial injections of lycopodium spores, arachidonic acid or microspheres.

ii. Microvascular occlusion arrested fast orthograde axoplasmic transport.

iii. Microangiopathic ischemic lesions were characterized by proximal central-fascicular degeneration, focal necrosis, and scattered degenerating axons distally. Small myelinated and unmyelinated axons appeared more susceptible. Focal ischemia may also produce paranodal demyelination.

B. Aims of the Study

Controversy remains concerning the effects of ischemia on the structure and function of peripheral nerve. This debate partially results from the differences in methodology (in vitro anoxia, tourniquet, arterial ligation and microvascular occlusion). The present study was intended to define the morphological and physiological parameters of ischemic neuropathy produced by microvascular occlusion.

The results were compared with the effects of ischemia reported above.

1. Project I - Transient Conduction Abnormalities

Hypothesis: Ischemia of peripheral nerve can produce transient functional effects which lack a morphological basis.

Nerve conduction studies were performed on rats during the first 24 hours after the onset of ischemia to monitor any changes in function. A representative sample of nerves were examined by light microscopy and the animals were divided into 2 groups.

a. Group A: Animals in which the ischemia was of insufficient severity or duration to cause detectable axonal loss. The duration of conduction abnormalities was investigated in nerves which retained normal morphology.

b. Group B: Animals in which ischemia caused nerve degeneration. The function of the surviving axons was studied.

2. Project II - Persistent Focal Conduction Block

Hypothesis: Incomplete peripheral nerve infarction (ischemic necrosis which spares some axons) can produce persistent focal conduction block in some surviving axons. This temporary abnormality may have a metabolic or morphological basis, but is not due to demyelination.

Serial nerve conduction studies were performed during the first week after infarction and observed electrophysiological abnormalities were correlated with nerve morphology.

3. Project III - Recovery After Infarction

Hypothesis: Functional recovery in partially infarcted nerves parallels regeneration of axons.

Nerve conduction studies and morphological examinations were performed 1, 2 and 3 weeks after infarction to correlate the recovery of function with repair and regeneration of axons.

II. Materials & Methods

A. Experimental Methods

Male Sprague-Dawley rats weighing 200-325 grams were anesthetized with intraperitoneal sodium pentobarbital (33-75 mg/Kg) or chloral hydrate (466 mg/Kg). For prolonged studies anesthesia was maintained with repeated Nembutal injections of 25-50% of the initial dose. Some rats were given 0.1-0.2 ml subcutaneous or intramuscular injections of atropine sulfate (0.4 mg/ml) to reduce bronchial secretions.

The limb temperature was constantly maintained at 37-38°C using a heat lamp coupled to a YSI Thermistemp Temperature Controller. The thermistor probe was placed subcutaneously in the dorsum of the foot or superficial to the gastrocnemius muscle in the leg.

Compound muscle action potentials (CMAP) were recorded with steel needle recording electrodes inserted percutaneously over the plantar intrinsic foot muscles (Appendix 1). The recording electrode was positioned between the first and second toes or lateral to the medial walking pad and adjusted to give a CMAP of maximal amplitude with an initial negative deflection. On the rare occasions when an acceptable baseline (pre-ischemic control) response could not be obtained from the plantar muscles, the recording electrode was placed over the dorsal muscles of the foot. The reference electrode was inserted into the apical walking pad of one toe. The ground was placed subcutaneously in the medial or

lateral aspect of the foot, between the distal stimulating cathode and the recording electrode.

Stimulating electrode pairs with cathode distal and 2-3 mm inter-electrode distance were placed alongside the sciatic nerve and its branches at two or four locations (Greene, 1955; Hebel and Stromberg, 1976). With a rat in the supine position, the most distal stimulating electrodes (D) were inserted into the ankle over the tibial nerve, between the medial malleolus and the calcaneus. The most proximal electrodes (P) were placed in the upper thigh at the sciatic notch, between the acetabulum and the ischial tuberosity. The middle pairs were placed 10-15 mm proximal to the medial malleolus, between the tibia and the gastrocnemius muscle (M_1); and 10-15 mm distal to the sciatic notch in the mid-thigh, just above the sciatic trifurcation (M_2) (Figure 1). When the rats were studied in the prone position, the electrodes were placed percutaneously in the corresponding sites on the dorsal aspect of the leg (Figure 2).

The femoral artery was exposed using blunt dissection through a groin incision. The subcutaneous fascia was divided with blunt scissors and the neurovascular bundle was carefully dissected. Two 5-0 silk ligatures were placed around the artery. A double knot was tied loosely around the distal end of the exposed segment. The proximal ligature was tied tightly with a double knot and the silk was held taut with a pair of hemostats. Under the Zeiss

binocular dissecting microscope, the artery was opened with straight micro-scissors and a plastic cannula (Clay Adams Intramedic PE 10) connected to a flat 30 gauge needle was inserted. The cannula was secured in place with the distal ligature and flushed with 0.05-0.10 ml distilled water to prevent blood clotting within the tubing.

Arachidonic acid (Sigma A6382) was obtained in 10 mg aliquots as 99% pure free acid from porcine liver. It was stored frozen until used. The arachidonic acid was dissolved in a diluent of 154 mM sodium chloride and 65.8 mM sodium carbonate mixed with 95% ethanol (10% v/v) to make a 0.5 or 1.0 mM solution of sodium arachidonate (Appendix 2). The arachidonic acid solution was made up fresh daily and kept cold. It was usually used within 6 hours but occasionally was used as much as 24 hours later.

An aliquot containing 0.015-0.11 umoles arachidonic acid was rapidly injected into the artery, and the cannula flushed with 0.05-0.10 ml distilled water. The cannula could be left in place to permit additional injections to be administered if necessary. The amount of arachidonic acid needed to produce partial ischemia varied with the Sigma lot number and the size of the rat, as well as with individual differences in susceptibility. Following injections, the cannula was removed and the distal arterial ligature was tied. The incision was closed with 9 mm stainless steel Autoclip woundclips (Clay Adams).

Nerve conduction studies were performed using a TECA TE4 Electromyograph with AA6 MKII amplifier, Dav 6 signal averager and NT6 stimulator (Jabre and Hackett, 1983; Hammer 1982; TECA). Compound muscle action potentials were stored on a single beam storage oscilloscope. Two to six responses were superimposed and recorded on type 084 black and white Land film using a Polaroid C-5 oscilloscope camera. The calibration signal was included in each photograph. The gain was set to obtain maximal deflection of responses, usually 2 mV/division for normal rats. The sweep was maintained at 1 msec/division. Nerves were stimulated at each site with a 0.05 msec square wave pulse of supramaximal intensity. Maximal intensity was determined by increasing the voltage in 25 volt increments until no further increase in the amplitude of the CMAP occurred. The voltage was then increased by approximately 25% to assure supramaximal current. If baseline studies required a stimulation voltage over 100 volts, the stimulating electrodes were repositioned. For a normal rat, the stimulus voltages for D, M_1, M_2 and P were 25, 50, 75 and 100 volts respectively. During the period of ischemia the stimulus threshold of the nerve increased and the voltage and stimulus duration were increased as necessary to maintain a maximal response.

For each CMAP studied the latency to the onset of the negative deflection and the amplitude of the response (from

baseline to negative peak) were measured and the ratio (R) of proximal to distal amplitudes was determined.

Equation 1. $R = \frac{\text{proximal amplitude}}{\text{distal amplitude}}$

Reduction in this ratio suggests focal conduction block. The affected axons retain the ability to transmit impulses distal to the site of the block, but not through the block.

The maximal motor conduction velocity (MCV) between stimulus sites was determined.

Equation 2. $MCV = \frac{\text{inter-electrode distance}}{\text{proximal - distal latency}}$

The distance between stimulating electrodes was measured with the rat's leg in maximal extension using a caliper.

Potential duration was determined by measuring the widths of CMAP in the photographs. Width in millimeters was converted to duration in milliseconds using the calibration signal. The measurements were compared over time. Any widening of responses indicated temporal dispersion.

Reference CMAP were obtained for each rat preceding injection of arachidonic acid (Figure 3). Following injection, rats were studied at 5 minute intervals for 30 minutes, at 10 minute intervals for the next 30 minutes, and at 30 minute intervals until 2-4 hours post-injection. Rats were again studied 1, 2 and 3 days and 1, 2 and 3 weeks later (Appendix 3). Not all rats were studied at all times.

Before each follow-up nerve conduction study rats were examined clinically for signs of cyanosis, edema or infection.

The strength of intrinsic foot muscles was assessed by lifting the rat by its tail and provoking the toespread reflex.

During follow-up studies, compound nerve action potentials (CNAP) were recorded in some rats to determine if CMAP abnormalities were due to nerve conduction or neuromuscular transmission failure. The tibial nerve was stimulated at the ankle (site D) and the nerve response was recorded from the proximal sciatic nerve (site P). Depending on the amplitude of the CNAP, 4-16 responses were averaged with an electronic signal averager. The image was recorded on Kodak light-sensitive photographic paper. For each CNAP the latencies to the onset and peak of the negative deflection were measured, and the amplitude from the peak of the positive to the peak of the negative deflection was measured.

Nerves were harvested for morphological studies at the onset of conduction block and 15 minutes, 30 minutes, 1 hour, 3-4 hours, 1 day, 6-7 days, 12-16 days or 21-22 days later. Following nerve conduction studies, the sciatic and tibial nerves were exposed and fixed in situ with 3.6% glutaraldehyde (Appendix 4) in 0.1M Sorenson's phosphate buffer (pH 7.6) (Appendix 5). The glutaraldehyde was replaced at least twice during the first 5 minutes and twice more during the total 10-15 minute fixation. The glutaraldehyde was changed more frequently if the limb was bleeding heavily. The nerve was then carefully removed and

stored in glutaraldehyde and the rats were killed by cardiotomy.

The sciatic and tibial nerves were cut into 6 mm segments with a surgical blade. Each segment was further divided into a 3 mm proximal piece for teasing, a 2 mm piece for cross sectioning, and a distal 1 mm piece for longitudinal sectioning. These segments were stored in glutaraldehyde in the cold for a minimum of 2 hours. The segments of nerve to be sectioned were post-fixed in reduced osmium tetroxide, dehydrated in a graded series of ethanol and propylene oxide, and embedded in Poly/Bed-Araldite (Appendix 6). The segments for teasing were osmicated and stored in glycerol (Appendix 7). One and 2 um cross and longitudinal sections were cut using an LKB Nova ultramicrotome. Two micron sections were mounted on glass slides and examined to determine preliminary results. One micron sections were stained with methylene blue - azure II - basic fuchsin (Appendix 8). Coverslips were added using Permount or Pro-Texx and the sections were carefully examined on a Zeiss Universal microscope. Photographs were taken with a green filter using Kodak Plus X-Pan or Panatomic-X film.

Single myelinated fibers from the segments of nerve determined to be at or near the site of the infarct were teased with fine jeweler's forceps. The nerve fascicles were separated and the perineurial sheath was removed. Individual fibers were teased in glycerol, transferred to

creosote or Histo-Clear (National Diagnostics) and arranged on a glass slide. The slides were dried at 60°C, coverslipped with Permount or Pro-Texx, and examined for evidence of segmental and paranodal demyelination, remyelination and Wallerian degeneration.

Student's t test was used to compare group data. Repeated measures analysis of variance was performed on smaller groups of animals. Rats were selected for this test only if nerve conduction studies had been performed at each recommended time, and data were available for proximal and distal CMAP amplitudes, R and MCV (Glantz, 1981).

B. Controls

Controls were needed to (1) establish normal maximal motor conduction velocity and CMAP and CNAP amplitudes, (2) determine the time to conduction failure following nerve transection and (3) assess the direct toxicity of arachidonic acid on peripheral nerve.

1. Nerve Conduction Controls

Nerve conduction studies were performed on normal, anesthetized rats. The CMAP amplitude and latency, MCV, R, and CNAP amplitude were determined.

2. Transection Controls

Transections were performed on deeply anesthetized rats to determine the length of time required for conduction failure in transected axons.

a. In the first group, the transection was performed at a site corresponding to the proximal margin of the arachidonate-induced infarct. The sciatic nerve was exposed at the sciatic notch by the separation of muscle planes. A silk thread was slipped beneath the nerve to ensure that all nerve fibers were severed, and the nerve was quickly transected with a pair of scissors.

b. In the second group the transection was performed at a site corresponding to the distal margin of the infarct. The posterior tibial nerve was exposed where it exits from beneath the gastrocnemius muscle, and cut with scissors.

c. The rats in the third group had the nerve transected at both sites.

Nerve conduction studies were performed before transection and immediately afterward to ensure that all nerve fibers had been cut. The incisions were closed with metal clips and the animals were studied electrophysiologically at various times during the next 72 hours.

3. Nerve Injection Controls

To determine whether there was a direct toxic effect on nerves, arachidonic acid was injected onto or directly

into nerve. Fifty microliters of 0.05 mM or 25 ul of 0.10 mM arachidonic acid or diluent alone were injected either beneath the perineurium or onto the epineurium of the sciatic nerve near the sciatic notch, the origin of the tibial nerve in the thigh or the tibial nerve beneath the gastrocnemius muscle. Nerve conduction studies were performed before the injection, every 5 minutes for the first 20 minutes following the injection, and at 1, 2, 3, 4 and 7 days. Rats were sacrificed for morphological examination at 4 or 7 days.

C. Study Design

1. Project I - Transient Conduction Abnormalities

Nerve conduction studies were performed at frequent intervals for up to 3 hours following the intra-arterial injection of arachidonic acid. A sample of rats was sacrificed for morphological examination within hours after the onset of ischemia. Follow-up nerve conduction studies were again performed 1 to 7 days later. The animals were divided into two groups on the basis of the morphological and physiological findings.

a. Group A: Rats without evidence of axonal loss (N=18). This group included all animals which experienced transient functional deficits, but were either morphologically or electrophysiologically normal.

b. Group B: Rats with evidence of axonal loss (N=37). All these animals had abnormal nerve conduction studies and nerve morphology.

2. Project II - Persistent Focal Conduction Block

A proportion of the rats with partial nerve infarction and evidence of axonal loss (Group B of Project I) were studied at 1, 2, 3 and 7 days. The structure and function of the surviving axons were followed and these animals were further subdivided into two groups.

a. Group B1: Rats with low amplitude potentials (N=17). All animals in this study had reduced compound muscle action potentials, without evidence of conduction block. Animals were assigned to this group if R was within 2 standard deviations of the control mean ratio.

b. Group B2: Rats with persistent conduction block (N=20). Following ischemia, some nerves continued to transmit compound muscle action potentials when stimulated at the ankle, but gave disproportionately diminished potentials following proximal stimulation. Animals were assigned to this group if R was less than 0.66 (control mean ratio - 2 SD). The nerves from these animals were carefully examined for any morphological basis for the conduction block in surviving axons.

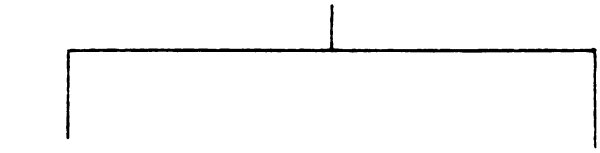
3. Project III - Recovery After Infarction

A proportion of the rats from Groups B1 and B2 were studied 1, 2 and 3 weeks after arachidonic acid injection to monitor the long-term electrical and morphological changes associated with ischemia and the early stages of recovery.

4. Project Outline

Project I - Transient Conduction Abnormalities

Nerve conduction studies were performed before and after injection of arachidonic acid.



Group A

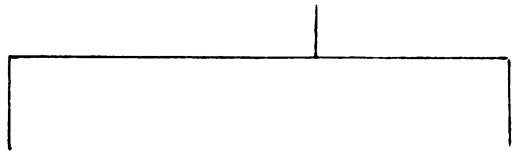
Normal electrophysiology and/or morphology 1-7 day after injection.

Group B

Partial nerve infarction: Diminished CMAP amplitudes and/or axonal degeneration 1-7 days after injection.

Project II - Persistent Focal Conduction Block

Group B animals from Project I were examined for evidence of conduction block.

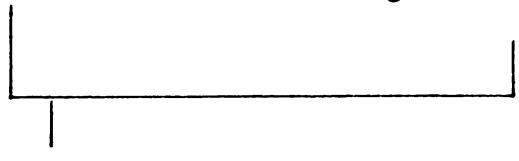


Group B1

Partial nerve infarction without conduction block.

Group B2

Partial nerve infarction with evidence of conduction block during first follow-up study.



Project III - Recovery After Infarction

Remaining Group B1 and B2 animals were studied 1-3 weeks after injection of arachidonic acid.

III. Results

A. Control Animals

1. Nerve Conduction Controls

Nerve conduction studies were performed on 11 legs of 7 rats and the results were averaged. The distal and proximal compound muscle action potential amplitudes were 6.1±0.8 mV and 5.3±1.0 mV respectively. The R value averaged 0.86±0.06. The maximal motor conduction velocity between the ankle and hip was 46±5 m/s. The compound nerve action potential amplitude was 17.8±5.2 uV.

2. Transection Controls

a. Proximal Transections

The sciatic nerve was transected above the trifurcation in the lower thigh of three rats and near the sciatic notch in four rats. This site corresponds to the upper margin of the experimental infarct. Only slight reductions were observed in the distal amplitude of any rat during the first 6 hours of study (67 to 130% of pre-transection values). Between 19 and 24 hours the CMAP amplitude was 0 to 0.2 mV and the mean amplitude was reduced to 7% of baseline. No CMAP was elicited in the one rat re-examined at 48 hours.

b. Distal Transections

The tibial nerve was transected below the gastrocnemius muscle in four animals. This site roughly corre-

sponds to the distal margin of an arachidonic acid-induced infarct. The post-transection amplitude was 81 to 91% of baseline and no additional reduction was detected for up to 7 hours. The distal amplitude was reduced 7 to 27% by 16 hours and by 24 hours the mean amplitude was only 8% of baseline. No further reduction was observed by 48 hours.

c. Double Transections

Double transections were performed on four rats. First the sciatic nerve was cut near the sciatic notch, then the tibial nerve was transected below the gastrocnemius muscle. The 48 hour electrophysiological results following distal transection alone suggested that collaterals of the fibular nerve or other branches of the sciatic nerve might also be supplying the plantar intrinsic foot muscles. The double transection procedure allowed the degeneration of the tibial nerve to be monitored without interference from collateral innervation. As before, very slight reduction of the distal amplitude was observed during the four hours following transection (86 to 161% of baseline). Between 19 and 24 hours the four rats had CMAP amplitudes which were 0 to 0.4% of baseline and no responses were detected in the three rats examined up to 48 hours.

3. Nerve Injection Controls

To investigate the direct toxic effects of arachidonic acid, 16 nerves were exposed to arachidonic acid in solution or to the diluent alone.

a. Diluent Injections

Diluent was injected beneath the perineurium or onto the epineurium of the tibial nerve or the sciatic nerve near the sciatic notch. In three of five animals there was a small immediate reduction in the CMAP amplitude of both the proximally (10 to 14%) and distally elicited responses (13 to 20%). No decrease was observed in the other two rats. Nerve conduction studies were performed on four rats 4 or 7 days later at which time all animals had normal electrophysiological responses. Following these studies, the nerves were removed and processed for morphological examination. Two nerves had normal morphology; one had occasional, scattered degenerating myelinated axons; and the fourth showed mild endoneurial edema and intramyelinic edema of some subperineurial axons without axonal degeneration.

b. Intraneural Arachidonic Acid Injections

Arachidonic acid was injected into the sciatic nerve at the sciatic notch or above the trifurcation in the thigh of three rats. An immediate reduction (8 to 30%) in the

proximal and distal CMAP amplitudes occurred. This decrease was accompanied by an increase in the stimulus threshold. All three rats had normal nerve conduction studies 3, 4 or 7 days later. Morphological examination of one nerve revealed a small subperineurial region of focal degenerating and regenerating axons. In the other two animals the nerve was cleaned, separated from underlying tissue, and a small piece of silk was placed beneath the injection site. These two nerves had more severe lesions. One nerve had a wedge one fourth the size of the fascicle which exhibited myelinated axon degeneration and endoneurial edema. The other had panfascicular degeneration. The largest myelinated axons and those in the center of the fascicle were spared.

Arachidonic acid injection into the tibial nerve of three rats gave varied results. In the first rat there was a slight reduction in the proximal CMAP amplitude (5%) and an increase in threshold. The responses were normal at three and five days and the nerve appeared normal by light microscopy. The second rat experienced a more profound drop in the proximal amplitude (16%), an increase in latency as well as threshold, and reduced CMAP amplitudes at 3 and 7 days. This nerve showed moderately severe panfascicular degeneration. The third rat showed severe reduction of both the proximal (84%) and distal (32%) amplitudes, increases in threshold and latency, and low amplitude responses at 1, 2, 3 and 7 days. The tibial nerve was grossly swollen and

edematous. There was severe panfascicular degeneration with scattered surviving myelinated axons.

c. Epineurial Arachidonic Acid Injections

To avoid the effects of trauma induced by injecting into the endoneurium, arachidonic acid was injected onto the epineurium of 5 nerves. Three tibial nerves were exposed below the gastrocnemius muscle and two sciatic nerves were exposed, one above the trifurcation and one immediately distal to the sciatic notch.

One rat showed no electrophysiological changes immediately after injection or one week later. This nerve had normal morphology. Another rat maintained normal amplitudes but had increased thresholds and latencies. Nerve conduction studies were normal at 4 days and morphological examination revealed intramyelinic edema of subperineurial axons. Three animals showed reduced proximal and distal CMAP amplitudes which averaged 80 and 85% of respective baselines. All nerve conduction studies were normal at 1, 4 or 7 days. Of these three nerves, one was normal, one showed mild endoneurial edema and one had endoneurial edema accompanied by intramyelinic edema of subperineurial axons.

All nerves which had been exposed to arachidonic acid by injection or topical application developed a thickened and very cellular epineurium with an increase in the size

and number of epineurial blood vessels. These changes were occasionally observed in the diluent controls.

B. Experimental Animals

1. Clinical Findings

As the arachidonic acid was being injected into the femoral artery the rats hyperventilated. Immediately afterward, the ipsilateral foot became pale and cold. In some animals the appearance of the foot quickly returned to normal and in others, the pallor was maintained throughout the initial conduction studies.

At 24 hours about a third of the animals had obvious cyanosis of the foot while the rest had regained a healthy pink appearance. By 3 days the vast majority of rats had apparently normal circulation but rarely pallor and cyanosis persisted for 1 - 2 weeks. Occasionally minimal gangrene appeared at the tips of the toes. In some animals with severe ischemia the foot became infected necessitating removal from the study.

At one day the majority of rats had lost the toespread reflex and in all rats the responses were at least mildly reduced. The toespread reflex improved in most animals and returned to normal in a few days. One animal examined as long as three weeks after arachidonic acid injection still showed no response.

2. Project I - Transient Conduction Abnormalities

a. Initial Studies (See p. 30)

Despite the immediate clinical response to arachidonic acid, no immediate changes in nerve conduction were detected (Table 1).

Five rats were studied 2 and 4 minutes after injection. The mean distal amplitudes at these two times were not significantly different from pre-ischemic values (baseline). At 5 minutes, the majority of responses were still unchanged, but in 8 out of 41 animals, the distal CMAP had fallen. The mean amplitude for the entire group was 5.5± 1.6 mV, barely reduced from a baseline value of 5.8±1.5 mV ($P < 0.05$). By 10 minutes, nearly 50% of the animals had a reduced distal CMAP and the mean amplitude had fallen to 4.1±2.2 mV ($P < 0.001$). After 15 minutes, the majority of the rats had markedly reduced distal responses and the group mean had reached a nadir of 3.3±2.3 mV. In a few rats the amplitude continued to fall after 15 minutes, but in general the responses steadily increased during the remainder of the initial period of observation. By 90 minutes the distal amplitude had increased to 70% of that group's baseline (N=11) and at 3 hours the CMAP amplitude was 91% of that mean baseline (N=3) (Figure 4).

The proximal CMAP amplitude was more severely affected by ischemia (Table 1). No significant changes were seen at 2 or 4 minutes, but by 5 minutes the mean proximal amplitude

was reduced from a baseline value of 4.9±1.4 mV to 4.3± 1.7 mV ($P < 0.001$). Ten minutes after the onset of ischemia, more than half of the animals had proximal CMAP amplitudes below normal with a mean of 2.5±1.9 mV. The amplitude continued to fall and reached a low of 0.9±1.2 mV at 20 minutes. Only one of the 28 rats studied at 20 minutes had a proximal amplitude within the normal range (baseline ± 1 SD). The mean amplitude ranged between 0.6 and 1.1 mV during the remainder of the first 90 minutes, then increased slightly over the next 90 minutes to 1.3± 1.2 mV (N=3) (Figure 4).

If the proximal CMAP amplitude did not fall to 50% or less of that animals' pre-injection value by 20 minutes, either the study on that leg was discontinued or an additional injection of arachidonic acid was administered.

The greater drop in proximal amplitude was reflected in a fall of the ratio of proximal to distal amplitudes. R was significantly reduced at 10 minutes and had fallen to 0.27±0.28 at 20 minutes ($P < 0.001$). Little further change was recorded over the next 30 minutes, but between 1 and 3 hours R progressively fell because the amplitude of the distal response increased while the proximal amplitude remained relatively constant.

The CMAP amplitudes elicited by stimulation at M_1 closely paralleled the more distal responses. At 15 minutes the amplitude reached a low of 2.7±2.2 mV. This response

was reduced from baseline by almost the same percentage as the distal response. Both responses increased steadily thereafter. Similarly, the responses elicited by stimulation at M_2 paralleled the more proximal responses.

The mean baseline distal motor latency (DML) was 1.2±0.2 msec. During the first three hours after the onset of ischemia the mean DML never exceeded 1.4±0.2 msec. However, the latencies recorded at more proximal sites were prolonged due to a slowing of the maximal motor conduction velocity.

Conduction velocity between D and M_1 (MCV_1) was mildly slowed (Table 1). By 5 minutes MCV_1 was reduced from a baseline of 42±11 m/sec to 40±10 m/sec ($P < 0.20$). Conduction velocity reached a nadir of 35±10 m/sec at 90 minutes ($P < 0.05$), but had exceeded baseline by 2 hours (Figure 5).

The conduction velocity between M_1 and M_2 (MCV_2) was more profoundly reduced (Table 1). In the small group of animals studied at 2 and 4 minutes, slowing had already begun. In the majority of rats MCV_2 decreased from a baseline of 42±11 m/sec to 38±11 m/sec at 10 minutes ($P < 0.01$). A nadir of 32±14 m/sec was reached at 40 minutes ($P < 0.001$), and the conduction velocity had not returned to baseline by 3 hours (Figure 5).

The conduction velocity in the most proximal segment, between M_2 and P (MCV_3), was initially unchanged (Table 1). The baseline MCV_3 was 45±12 m/sec, and it was increased

between 15 and 40 minutes after the onset of ischemia. The lowest mean conduction velocity was 40±13 m/sec ($P < 0.20$), recorded at 60 minutes (Figure 5).

The nerves of 16 rats were fixed at the onset of conduction block or at various times during the next 4 hours. Eleven of these nerves were considered normal by light microscopy (Figures 6 and 7). A normal classification was assigned if (1) the myelinated fibers had the same density, distribution and fiber size spectrum as control nerves, (2) abnormal fibers and myelin debris were rare or absent, (3) no proliferation of Schwann cells and macrophages existed, and (4) capillaries were free of thrombi and were neither necrosed nor enlarged. Many nerves which were considered otherwise normal showed increased numbers of mast cells in the endoneurium. The other 5 nerves showed unusually prominent Schmidt-Lantermann incisures, mild intramyelinic edema and/or mild endoneurial edema in the region of ischemia. Proximal and distal segments of these nerves appeared normal.

b. Follow-up Studies (See p. 30)

Group A (12 rats) had normal nerve conduction studies during the first week (Figures 8 and 9). Six nerves from these animals were examined and appeared normal. Two additional rats had mildly reduced CMAP amplitudes but normal morphology.

Group B included the rest of rats with partial lesions, all of which had reduced CMAP amplitudes. The detailed electrophysiological and morphological findings on this group of rats will be discussed in the next section.

3. Project II - Persistent Focal Conduction Block (See p. 30)

Group B1 animals (partial infarction) had low amplitude CMAP without evidence of conduction block (Table 2). The mean distal CMAP amplitude on day 1 was 2.4±2.1 mV and it did not change significantly during the first week. The 24 hour proximal amplitude was reduced to 1.9±1.6 mV, with no significant change for 7 days (Figures 10, 11, 12, 13 and 14).

The R value at one day was 0.82±0.10, which was not significantly reduced from pre-ischemic controls. On the second day, 3 of these rats exhibited ratios below the normal range; the mean ratio was 0.74±0.27. No animal studied on day 3 or 7 had evidence of conduction block and R values were normal (Figures 10, 11, 12 and 13).

The distal motor latency was prolonged from a baseline of 1.2±0.2 msec to 1.4 msec at 1, 2 and 3 days ($P < 0.005$) and was 1.3±0.2 msec at 1 week ($P < 0.10$). By one day the mean MCV was slowed to 40±7 m/sec compared with 46±5 m/sec in controls, with no subsequent significant change during the first week (Figures 11, 12, 13 and 14).

The CNAP was recorded in 5 animals on day one. The mean amplitude was substantially reduced from a baseline of

17.8±5.2 uV to 2.6±2.7 uV ($P < 0.001$). No significant improvement was noted by day 7.

All Group B2 animals (partial infarction, conduction block) had evidence of conduction block on their first nerve conduction study (Table 2). The R value returned to normal in the majority of rats and only 2 nerves still showed conduction block at 1 week (Figure 15).

At one day the distal CMAP amplitude was reduced to 1.3±1.7 mV, which was markedly reduced from pre-ischemic controls ($P < 0.001$) but not significantly different from the Group B1 mean. No further change in the distal amplitude was observed during the first week. The CMAP following proximal stimulation at 1 day was 0.5±0.7 mV, a reduction from baseline ($P < 0.001$) and the Group B1 mean ($P < 0.02$). Slight improvement in the proximal amplitude was noted on days 3 and 7 (Figures 11, 12, 13, 14, 16 and 17).

The R value at one day was considerably reduced to 0.35±0.19 ($P < 0.001$). (Figure 11). Two rats with low ratios (0.28 and 0.20) were first examined on day 2. The ratio was normal in 8 of 16 rats studied on the second day and 10 of 13 rats by day 3. At 1 week the R value had increased to 0.81±0.15. The return toward a normal R can be accounted for by the gradual increase in the proximally elicited CMAP response. In a few animals there was also a transient or permanent decrease in the distal CMAP.

The DML at one day was 1.3±0.2 msec. The latency did not change significantly during the next 2 days, but it was mildly prolonged at one week (1.5±0.2 msec). The MCV was reduced to 40±6 msec on day 1 which was not different from the velocity recorded in Group B1 nerves, without marked change between day 1 and day 7 (Figures 11, 12, 13 and 14).

The mean CNAP ranged between 1.1±0.5 uV and 3.2±1.9 uV during the first week. The means at all times were profoundly reduced from control values. No two consecutive means were significantly different; however, the data suggest a gradual improvement in nerve conduction.

There was no evidence of temporal dispersion of the CMAP to account for the drop in the R value. The average distal and proximal pre-ischemic control widths were both 10±1 msec (N=13). The widths measured at the nadir of conduction (or the last recording before complete conduction failure) averaged 9±1 msec distally and 8±2 msec proximally. Between 1 and 7 days, no individual measurement exceeded 11 msec and the average never exceeded baseline.

Repeated measures analysis of variance was performed on 4 Group B1 rats without conduction block and 12 Group B2 rats with block. Distal and proximal amplitudes, R and MCV were analyzed for both groups at 1, 2, 3 and 7 days after injection whether there was a significant change in any parameter with time. No significant changes were seen in the Group B1 animals. In Group B2 animals, no change in

MCV occurred. However, there were significant increases in the proximal CMAP and R value, while the distal CMAP remained unchanged.

A fraction of the animals were sacrificed at 1, 2, 3 and 7 days. No outstanding differences were observed between the nerves taken from animals with and without conduction block.

One day after arachidonic acid injection, microscopic examination of the distal sciatic and proximal tibial nerves revealed early central-fascicular lesions (N=3). Myelinated axons were often shrunken or swollen, and intramyelinic edema was observed in one nerve. The number of mast cells associated with nerve infarction was increased over normal nerve (Figure 18).

Two days after injection, more advanced degeneration was evident (N=3). In addition to the abnormalities noted at 1 day, there was an increase in the number of Schwann cells and macrophages at the borders of the infarct. Occasional large myelin rings with watery axoplasm were seen. Degenerating myelinated axons were apparent and in some otherwise normal axons the axoplasm had a floccular appearance. Mild endoneurial edema was present in 2 nerves and rare myelin ovoids in the third.

The only additional changes at 3 days were an increased amount of myelin phagocytosis, more advanced axonal degeneration and occasional proliferation of endothelial cells.

The one week morphological results will be discussed with Project III.

Over 300 single teased myelinated fibers were examined from animals in each group 1 day to 3 weeks after injection (Table 3). Few fibers were teased from nerves with very mild or subtotal lesions (as determined by examination of cross and longitudinal sections).

Approximately one third of the fibers teased from each group showed Wallerian degeneration with or without a segment of normal nerve. A portion of these fibers had paranodal demyelination between the segments of normal and degenerating nerve [Group B1 (partial infarction): 5%, Group B2 (partial infarction, conduction block): 9%). Less than 1% of the fibers examined from either group showed paranodal retraction alone. Any fiber with an appearance suggesting demyelination was included in this category which totalled 2% of Group B2 fibers and less than 1% of Group B1 (Figure 19).

A few regenerating nerves were observed in each group.

4. Project III - Recovery After Infarction (See p. 30)

A portion of the animals with partial nerve infarction (Group B) were studied 1, 2 and 3 weeks after arachidonic acid injection (Table 4).

The mean 1 week distal CMAP amplitude was 1.9±2.2 mV. No change was seen at 2 weeks but slight improvement was

noted by 3 weeks (2.5±1.7 mV, $P < 0.20$). The average proximally elicited CMAP amplitude was 1.5±1.6 mV at one week, showed no improvement at 2 weeks, and was marginally increased by 3 weeks (2.1±1.3 mV, $P < 0.20$) (Figures 20 and 21).

By one week the mean ratio had returned to normal and there was no indication of conduction block in the majority of animals at 2 or 3 weeks (Figure 21).

The distal motor latency was perceptibly prolonged from baseline of 1.2 msec to 1.4 msec at 1 and 2 weeks, and had decreased to a mean of 1.3 msec at 3 weeks. The MCV was reduced from a control mean of 46±5 m/sec to 40±7 m/sec and did not change significantly over the next 2 weeks (Figure 22).

The CNAP was markedly reduced at 1 week (3.6±2.8 mV), unchanged at 2 weeks, and scarcely improved at 3 weeks (6.2±3.1, $P < 0.05$) (Figure 23).

Repeated measures analysis of variance was performed on 5 rats to determine whether there was a significant change in nerve conduction between 1 and 3 weeks. No changes were seen in the proximal or distal CMAP amplitudes or the ratio. The increase in MCV was marginally significant ($P < 0.05$).

At one week the proximal sciatic nerve was still normal by light microscopy (Figure 24).

The ischemic infarct was situated over 1 to 2 cm of the distal sciatic and proximal tibial nerves and was usually concentrated in the center of the fascicle (Figures 25 and 26). Mild endoneurial edema was commonly seen in the transition zone just proximal to ischemic necrosis and in the region of the infarct. Thinly myelinated regenerating axons were sometimes seen at the proximal border of the infarct. In the distal portion of the zone of infarction, necrosis was more severe and encompassed a greater area. This region was characterized by degenerating myelinated axons, intramyelinic edema, myelin ghosts and myelin ovoids. Schwann cells and macrophages had infiltrated the area and were proliferating. The severity of the lesions ranged from mild, central-fascicular degeneration to panfascicular necrosis with only occasional surviving myelinated axons. With the more severe infarcts the remaining fibers were predominantly large, subperineurial myelinated axons.

More distally, the tibial nerve is comprised of 2 or more fascicles. The degenerating axons were diffusely distributed and it appeared that the small myelinated axons were more severely depleted (Figure 27). Some surviving large, myelinated axons were swollen with watery axoplasm and occasionally swollen unmyelinated axons were visible with the light microscope.

More extensive regeneration had occurred by 2 and 3 weeks and thinly myelinated sprouts could be found in the

tibial nerve at the level of the medial malleolus (Figure 28). Numerous macrophages and Schwann cells were seen in the center of the infarct and there was more advanced myelin phagocytosis; however, myelin debris was still present near the infarct and more distally. Persistent, mild endoneurial and occasional intramyelinic edema were observed in several nerves.

IV. Discussion

Ischemia is implicated in the pathogenesis of many human neuropathies (Asbury and Johnson, 1978; Daube and Dyck, 1975; Wilbourn et al 1983). Impaired circulation may involve large vessel occlusion, usually resulting from thrombo-embolism, or occlusion of the capillaries which compose the vasa nervorum. Microangiopathy (disease of the small blood vessels) and concomitant peripheral neuropathy are most commonly associated with diabetes mellitus and vasculitis.

Peripheral nerve ischemia and infarction result from intra-arterial injection of arachidonic acid. Ischemia is a deficiency of blood which may be rapidly reversible or chronic. Severe or prolonged ischemia produces ischemic necrosis or infarction. The above projects describe the effects of transient ischemia and infarction on peripheral nerve.

Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is a precursor for thromboxane A_2 (TXA_2), prostacyclin, the prostaglandins (PG's) and the leukotrienes (LT's) (Moncada and Vane, 1979, Samuelsson, 1983). Cyclo-oxygenase is the first enzyme involved in the conversion of arachidonic acid to TXA_2, prostacyclin and the PG's. TXA_2 produces intravascular platelet aggregation and constriction of arterial smooth muscle resulting in occlusion of precapillary arterioles and large capillaries (Furlow and Bass, 1975 a, b).

Prostacyclin has opposite effects. Of particular interest are the prostaglandins F_2 and E_2 which cause venoconstriction and increased vascular permeability, respectively. Lipoxygenase converts arachidonic acid to the leukotrienes. In particular, LTC_4, LTD_4, and LTE constrict arterioles and produce leakage of macromolecules from post-capillary venules (Dahlèn et al, 1981; Gulati et al, 1983; Samuelsson, 1983).

Several lines of evidence support the conclusion that the phenomena investigated are the direct result of arachidonate-induced ischemia. First of all, cerebrovascular occlusion is seen following intra-carotid injection of arachidonic acid (Furlow and Bass, 1975 b). The components of the microvasculature occluded (small arterioles and capillaries) also comprise the vasa nervorum. Immediately following injection of arachidonic acid into the femoral artery the limb appears clinically ischemic. The rat's foot becomes pale and cold; the pallor lasts from a few minutes to several hours and is often replaced by cyanosis. The drop in temperature is indicated by the almost continual triggering of the heat lamp by the thermistor probe in the leg. Reduction in endoneurial blood flow has been demonstrated in this model by the injection of Evans blue - albumin (EBA) immediately after the arachidonic acid (Parry and Brown, 1984). EBA is detected by examination of sections under fluorescent light and the presence of intraluminal fluorescence is seen in all endoneurial capillaries in the

proximal sciatic and distal tibial nerves. Only occasional, faint fluorescence is detected in the endoneurial capillaries of the distal sciatic and proximal tibial nerves. No changes in the clinical appearance of the limb, blood flow, or nerve morphology are observed following injection of several closely related 20-carbon polyunsaturated fatty acids (Parry and Brown, 1981).

Intraneural injections do not produce the same abnormalities as intra-arterial injections. Parry and Brown (1981) report little if any axonal degeneration in nerves examined one week after injecting arachidonic acid into the sciatic nerve. In the present study, injections into the endoneurium caused an immediate reduction in the CMAP amplitudes. When present (4 or 7 days later), the degenerating axons were scattered throughout the fascicle, located in a wedge (probably at the site of injection), or concentrated at the periphery. Central-fascicular axonal degeneration characteristic of nerve infarction was never seen. The increased severity of the lesion may be partially due to attempts to clean, expose and mark the site and to the trauma of injection. Dyck et al (1981) report that intraneural injections of saline or nontoxic sera produce segmental demyelination and axonal degeneration even if 33 gauge needles and a micromanipulator are used.

Epineurial applications of arachidonic acid provide more suitable controls for direct neural toxicity because

there is less trauma. Transient mild decreases in CMAP amplitudes were recorded, but all nerve conduction studies were normal 1 to 7 days later. The only morphological abnormalities observed were mild endoneurial and intramyelinic edema. These same changes in structure and function were seen in some diluent controls.

Free fatty acids such as arachidonic acid are rapidly bound by albumin, which is the major vehicle for transport in the blood (Fitzpatrick et al, 1984). Serum albumin has six high energy binding sites and many weaker sites and studies show that 77 to 99% of the fatty acids tested are protein bound. (Saifer and Goldman, 1961; Shafrir et al, 1964; Spector et al, 1969). Chan et al (1980) report that binding radio-labelled arachidonic acid to bovine serum albumin diminishes its transport into rat cortical slices in vitro and reduces subsequent swelling and lactate production (Chan et al, 1983). Considering the high affinity binding of arachidonic acid to a readily available molecule like albumin, it appears unlikely that free fatty acid would be able to diffuse through capillary walls, as endoneurial capillaries have tight junctions and normally exclude large macro-molecules from the endoneurial compartment.

Finally, similar morphological findings of central-fascicular necrosis and distal Wallerian degeneration have been reported in other animal models of ischemia employing

tourniquets, arterial ligation or intra-arterial injection of microspheres (Korthals and Wiśniewski, 1975; Nukada and Dyck, 1982, 1983; Thomason and Matzke, 1957).

This evidence supports the conclusion that arachidonic acid produces ischemia. Due to its high-affinity binding to albumin, arachidonic acid is unlikely to gain access to the endoneurium and minimal nerve damage would be expected from any direct exposure if it did.

A. Project I - Transient Conduction Abnormalities

Within 5 to 20 minutes of the onset of ischemia CMAP amplitudes were substantially reduced and sometimes extinguished. The onset of conduction abnormalities in the majority of animals was earlier than the time to conduction failure reported in the literature (15 to 45 minutes) (Bentley and Schlapp, 1943; Fox and Kenmore, 1967). However in those studies ischemia was induced by tourniquet application which stops blood circulation but does not displace oxygen-carrying erythrocytes from the endoneurial capillaries. In addition, a heating lamp was used in our model to maintain the nerve temperature near 37°C, and other studies have shown that nerve survival time varies inversely with temperature (Bentley and Schlapp, 1943; Gerard, 1929).

The distal CMAP amplitude began to return toward normal within 25 minutes after the injection, and eventually reached baseline values in some animals. This relatively

rapid recovery suggests that there is some primary ischemia in the distal portion of the motor units which is rapidly reversed by blood flow reaching the distal tibial nerve or plantar muscles via non-femoral collaterals.

The proximal CMAP amplitude was more profoundly reduced and in some animals little recovery was observed even as long as 4 hours after the injection. The discrepancy in the amplitudes is due to conduction block of a portion of impulses traversing the nerve between the sciatic notch and the ankle. This block occurs in a focal nerve segment between M_1 and M_2, a site which corresponds to the area of infarction following injection of a higher dose of arachidonic acid (Parry and Brown, 1981). By stimulating the nerve at 4 sites we were able to localize conduction block to a focal segment of nerve. This finding conflicts with published reports of decremental conduction associated with ischemia (Hershey and Wagman, 1966; Kamp-Neilson and Kardel, 1974; Lewis et al, 1931; Lüthschg and Ludin, 1982). Tourniquet studies in humans showed earlier sensory and motor loss in ischemic nerve of increasing length and the proximally elicited CMAP amplitudes were diminished before distal responses. Decreased conduction velocity and increased latency were observed in the more proximal segments of ischemic nerve following stimulation in the hand.

In addition, the MCV was slowed soon after the onset of ischemia. The most profound slowing occurred through the

region of conduction block, between M_1 and M_2. Mean conduction velocity through the more distal segment was only mildly slowed which reflects the slight drop in limb temperature, animals in which a portion of the lesion occurred more distally, and some continued ischemia. Few animals exhibited slowed conduction through the proximal segment and, in fact, most had velocities which exceeded baseline. Continual heating of the distal ischemic portion of the leg probably results in an increase in the temperature of the upper thigh above 37°C, which would account for an increase in velocity.

Measurement errors account for some of the variability (large standard deviations) in MCV. Accurate determination of nerve length by surface measurements is impossible and even small errors (1-2 mm) can be reflected as major differences in MCV since very short segments of nerve are being measured (10-15 mm). An additional complication was encountered in follow-up studies when the limb was swollen and stiff, and full extension of the leg was impossible.

Ischemia produced electrophysiological abnormalities without morphological correlates, as demonstrated in 11 animals sacrificed within 4 hours after the onset of ischemia. Five additional nerves showed only mild intramyelinic or endoneurial edema. A portion of the rats re-examined 1 to 7 days after arachidonic acid injection had demonstrated only transient conduction abnormalities. These rats may

have had CMAP amplitudes which were still slightly reduced from pre-ischemic levels, but most were well within the normal range. These nerves were morphologically normal in that myelinated axons had the same fiber size, density and distribution as uninjected controls, degenerating axons and myelin debris were rare, Schwann cells were not proliferating and capillary lumina were patent. Even some nerves which showed severely reduced proximal CMAP amplitudes for as long as 3 hours exhibited no persistent changes in nerve conduction or morphological damage.

It appears that a greater supply of energy, nutrients or other substrates is needed to maintain normal impulse conduction than is required to uphold morphological integrity. Stimulating nerve fibers produces a measurable increase in oxygen consumption (Brink et al, 1952; Ritchie, 1967). Furthermore, anoxia produces almost immediate decreases in levels of ATP, glucose, glycogen and other peripheral nerve substrates (Stewart et al, 1965). These findings demonstrate the unusual resistance of peripheral nerve to infarction. Following arachidonic acid injection, oxygen and energy substrates drop below the level capable of supporting conduction, yet the nerve retains normal morphology and may even regain normal functioning.

B. Project II - Persistent Focal Conduction Block

Arachidonate-induced ischemia can produce persistent structural and function abnormalities; the functional deficit always exceeding the morphological abnormalities. With a sufficient dose of arachidonic acid, all animals develop nerve infarction in the region of the distal sciatic and proximal tibial nerves. In fact, the dose used in this study produced severe infarction with persistent conduction failure in a small number of animals which had to be eliminated from the study. As discussed in Project I (pp. 53-56) rats may recover completely from severe, prolonged conduction block due to ischemia. However, the majority of animals incompletely recover and are left with a long-term deficit of varying severity.

The principal abnormality was low amplitude CMAP from proximal and distal stimulation sites, as well as low amplitude CNAP. The decrease in amplitude reflects axonal loss and there is good correlation between the amplitude of the distal CMAP and the number of surviving myelinated axons seen morphologically. This early lesion was characterized by central-fascicular necrosis and endoneurial and intramyelinic edema in the distal sciatic and proximal tibial nerves. Subperineurial axons and large myelinated axons were most likely to survive the infarct. After 2 or 3 days, degeneration was more advanced. Macrophage and Schwann cells were proliferating and began to infiltrate

the region of infarct and degenerating myelin was apparent. Distally, degenerating axons were more evenly distributed throughout the fascicle.

Approximately half of the rats with infarction showed persistent conduction block. The diminished R value cannot be accounted for by dispersion of the proximal CMAP. The basis for this conduction abnormality was probably continued ischemia and the associated metabolic deficiencies. Although perfusion may be sufficient to maintain the integrity of the nerve and support electrical activity under normal conditions, a drop in blood pressure due to the anesthetic could produce temporary ischemia during conduction studies. The presence of endoneurial and intramyelinic edema would increase endoneurial pressure and might compress capillaries, thereby aggravating borderline perfusion. Improved perfusion via newly formed collaterals could coincide with the resolution of conduction block seen by one week (Garcia and Kamijyo, 1974).

The nerves with conduction block tended to have more severe lesions as shown by most electrophysiological parameters; however, these differences were not statistically significant. More importantly, the morphology of this group of nerves did not differ from nerves without block. Examination of single teased fibers revealed rare paranodal retraction in the absence of distal Wallerian degeneration. In the majority of these fibers the retraction was seen near

the end of the teased segment and distal degeneration could not be ruled out. Only 3 of the 14 nerves with conduction block showed evidence of segmental demyelination. Demyelinated axons totaled only 2% of the teased fibers examined from this group. It is difficult to conclusively diagnose segmental demyelination using the light microscope as strands of collagen from a fiber torn in the process of teasing may resemble a demyelinated axon. In this study any fiber with an appearance suggesting demyelination was classified as such, thereby giving an inflated estimate. No remyelinated axons with internodes of varying length were found. It is highly unlikely that the transient conduction block was due to paranodal or segmental demyelination, since the incidence of demyelination was too rare to account for the severity of the observed conduction abnormality.

These data contradict earlier reports from arterial ligation studies which show paranodal and segmental demyelination to be characteristic of ischemic lesions (Fowler and Gilliatt, 1981; Hess et al, 1979). Korthals et al (1984) found demyelination and remyelination following temporary compression of large arteries; these changes were not seen with permanent arterial ligation. Reperfusion of large vessels may promote demyelination via edema or infusion of toxic metabolites. Reperfusion of capillaries occluded

with platelet aggregates and accumulation of toxic metabolites within the nutrient arteries are unlikely.

The resolution of conduction block cannot be accounted for solely by the degeneration of infarcted axons. Transection of the tibial nerve of control rats produced little immediate change in the amplitude of the distally elicited CMAP, but by 24 hours the amplitude measured less than 8% of pre-surgical values. No response was detected after 48 hours. The distal segment of a transected axon can continue to transmit impulses for only as long as 24 hours; however, the conduction block observed following ischemia persisted for 2 to 7 days in some rats. In contrast to the gradual decline of the distally elicited CMAP amplitude following transection, the block resolves in the majority of ischemic animals as the amplitude of the proximal CMAP increases.

C. Project III - Recovery After Infarction

Animals with partial nerve infarction were also followed for longer periods of time. Minimal improvement was noted in nerves examined 1, 2 and 3 weeks after ischemia. Although small, most electrophysiological parameters do show improvement by 3 weeks. These improvements probably reflect the observed regeneration of axons.

By 3 weeks, thinly myelinated regenerating axons had advanced from the site of infarction in the distal sciatic or proximal tibial nerve to the level of the medial malleolus,

approximately 30 mm. The partially infarcted nerves in this study regenerated at a rate approximating regeneration in crushed nerves. Regeneration of myelinated axons following crush proceeds more rapidly than it does following complete ischemic necrosis due to injection of high doses of arachidonic acid (Parry, 1984). After 3 weeks, regenerating axons have extended 25 to 30 mm in crushed nerve and only 10 to 12 mm in severely infarcted nerve. This delayed regeneration was preceded by neovascularization of the infarcted region and proliferation and phagocytosis of debris by Schwann cells and macrophages. Because many blood vessels and Schwann cells are spared in partial ischemic lesions, revascularization and phagocytosis proceed more rapidly and present less of a delay to axonal regeneration. In addition, collateral sprouting of surviving axons could account for some of the thinly myelinated axons observed in the distal tibial nerve and contribute to improved nerve function.

In conclusion, arachidonate induced ischemia is a good model of microangiopathic neuropathy. Ischemia produced by microvascular occlusion produces both transient functional abnormalities and persistent structural and functional changes. Axons are more susceptible than Schwann cells; ischemic lesions are predominantly characterized by degenerating axons, and demyelination of surviving axons is extremely rare.

Table 1.

Amplitudes and MCV Before and After Arachidonic Acid Injection

Time After Injection (minutes)	Amplitude (mV) Mean ± SD Distal	Proximal	N	MCV (m/sec) Mean ± SD 1	2	3	N
Baseline	5.8±1.5	4.9±1.4	54	42±11	42±11	45±12	44
2	5.5±1.6	4.6±1.2	5	49±12	39±10	42±10	5
4	5.5±1.6	4.6±1.2	5	47±13	37±8	42±10	5
5	5.5±1.6	4.3±1.7	51	40±10	42±10	45±13	43
10	4.1±2.2	2.5±1.9	49	38±11	38±11	44±15	40
15	3.3±2.2	1.1±1.2	43	38±11	34±9	49±17	32
20	3.7±2.4	0.9±1.2	37	38±12	35±10	48±13	30
25	4.1±2.1	1.1±1.1	21	38±8	34±11	47±11	18
30	4.2±2.3	0.7±1.0	26	39±6	33±9	47±10	22
40	4.6±2.0	1.0±1.2	16	41±11	32±14	47±10	15
50	4.9±1.8	1.1±1.3	17	43±9	38±12	45±11	16
60	4.1±1.8	0.6±0.6	18	38±12	36±13	40±13	16
90	4.7±2.4	1.0±0.9	11	35±10	37±10	42±12	10
120	5.0±2.5	0.9±0.6	9	43±4	38±15	44±8	8
150	7.4±1.9	1.1±0.9	4	41±7	35±15	53±4	3
180	8.3±1.1	1.3±1.2	3	45±3	37±21	53±4	2
240	0.5	0	1	ND**	ND	ND	
1 day	1.9±1.9	1.2±1.4	36	ND	ND	ND	

**ND - not determined.

Table 2.

Nerve Conduction Studies 1, 2, 3 and 7 Days
After Arachidonic Acid Injection

Group (days)	Amplitude (mV) Distal	Proximal	R (P/D)	CNAP (uV)	MCV (m/sec)
Baseline	5.8±1.5	4.9±1.4	0.84±.09	17.8±5.2*	46±5*
Group B1 (Partial Infarction)					
(1)	2.4±2.1	1.9±1.6	0.82±.10	2.6±2.7	40±7
(2)	1.7±1.6	1.2±1.2	0.74±.27	2.0±0.5	41±6
(3)	1.8±1.9	1.4±1.5	0.84±.06	6.1±6.9	39±8
(7)	2.0±2.1	1.7±1.9	0.87±.12	4.4±4.4	41±6
Group B2 (Partial Infarction, Conduction Block)					
(1)	1.3±1.7	0.5±0.7	0.35±.19	1.9±1.6	40±6
(2)	0.7±1.0	0.3±0.4	0.58±.23	1.1±0.5	41±7
(3)	0.9±1.1	0.6±0.7	0.75±.23	1.9±1.4	38±8
(7)	1.8±2.3	1.3±1.3	0.81±.15	3.2±1.9	37±12

*Data from 11 normal nerve conduction studies.

All values expressed as $\bar{X}$ ± 1 SD.

Table 3.

Single Teased Fibers

	Group B1* (N=11)	Group B2** (N=14)
Normal	207	178
Wallerian degeneration	96	116
Paranodal retraction	3	2
Demyelination	1	7
Regeneration	2	9
Total	309	312

*Group B1: Partial Infarction

**Group B2: Partial Infarction, Conduction Block

Table 4.

Nerve Conduction Studies 1, 2 and 3 Weeks
After Arachidonic Acid Injection

Weeks	Amplitude (mV) Distal	Proximal	R (P/D)	DML (msec)	CNAP* (uV)	MCV (m/sec)
Baseline	5.8±1.5	4.9±1.4	0.84	1.2	17.8**	46±5**
1 (N=29)	1.9±2.2	1.5±1.6	0.84	1.4	3.6	40±7
2 (N=18)	1.4±1.7	1.2±1.3	0.92	1.4	3.1	41±9
3 (N=7)	2.5±1.7	2.1±1.3	0.86	1.3	6.2	45±4

*CNAP value was determined from a sample of 4-12 rats.

**Data from 11 normal nerve conduction studies.

All values expressed as $\bar{X} \pm 1$ SD or $\bar{X}$.

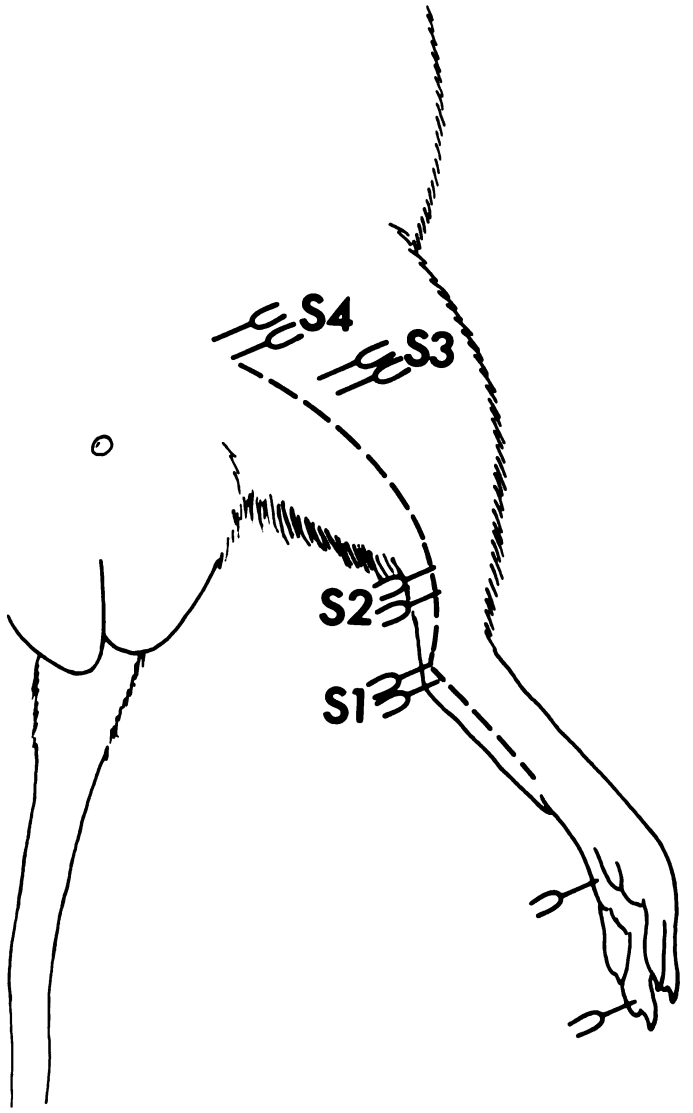


Figure 1.

Electrode Placement With the Rat in Supine Position

S1 = D, Distal stimulation site
S2 = M_1, Stimulation site in leg
S3 = M_2, Stimulation site in thigh
S4 = P, Proximal stimulation site

The recording electrode is positioned over the plantar intrinsic foot muscles and the reference electrode is placed in a toe.

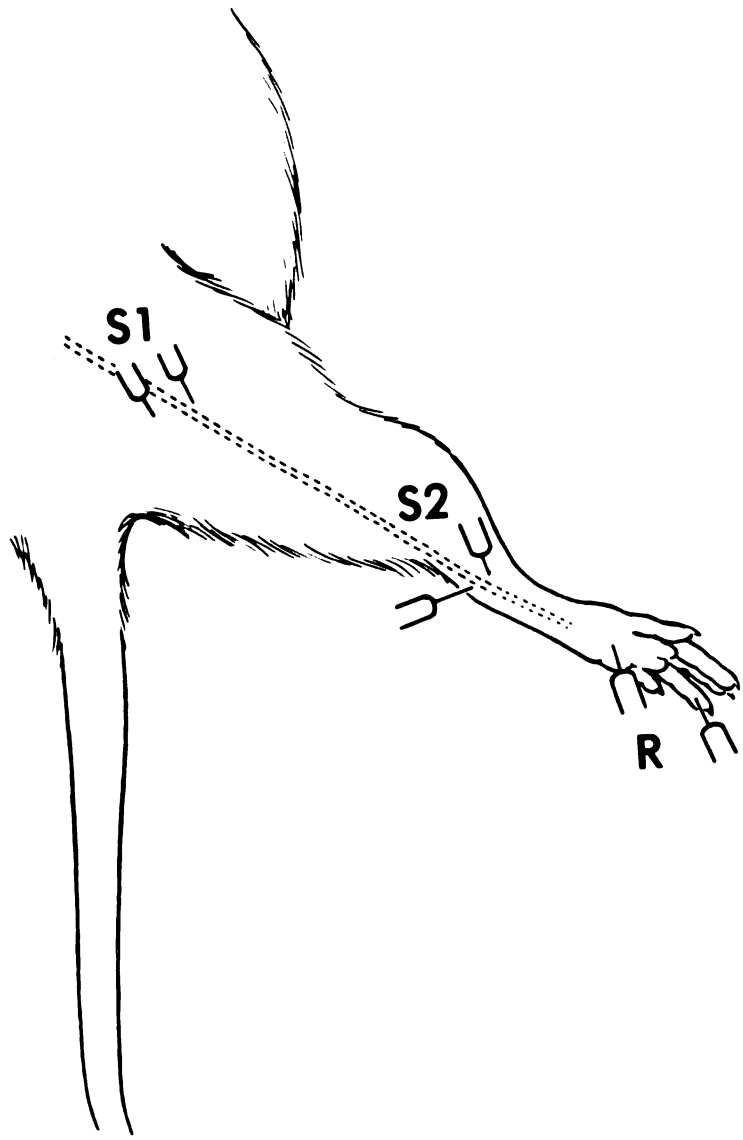


Figure 2.

Electrode Placement With the Rat in Prone Position

S1 = P, Proximal stimulation site
S2 = D, Distal stimulation site
R = Recording electrode pair

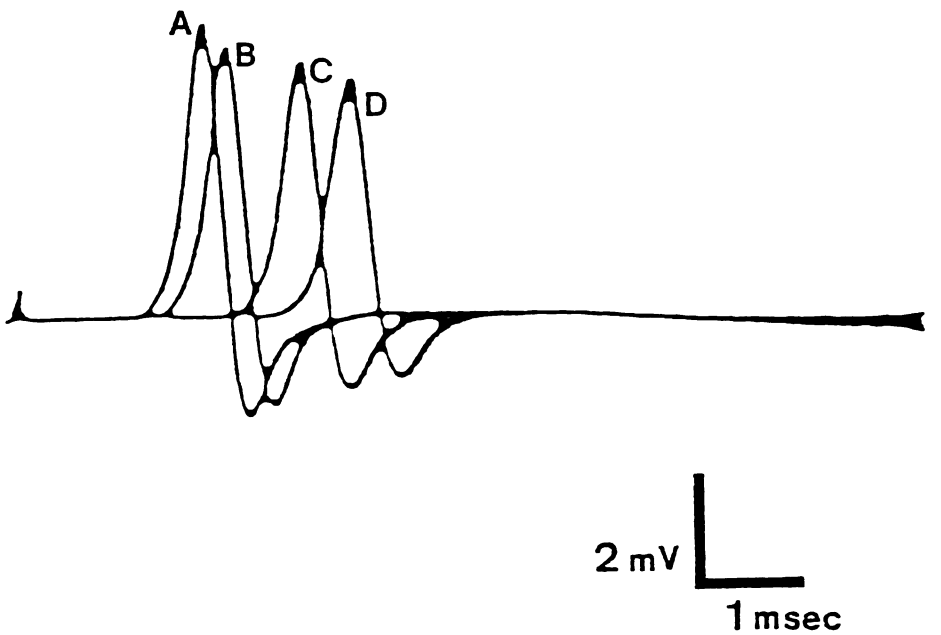


Figure 3.

Sample Baseline CMAP Recording

These peaks represent CMAP elicited from stimulation:

A. at the distal tibial nerve (D).
B. in the leg (M_1).
C. in the distal thigh (M_2).
D. at the proximal sciatic nerve (P).

Gain: 2 mV/division. Sweep: 1 msec/division.

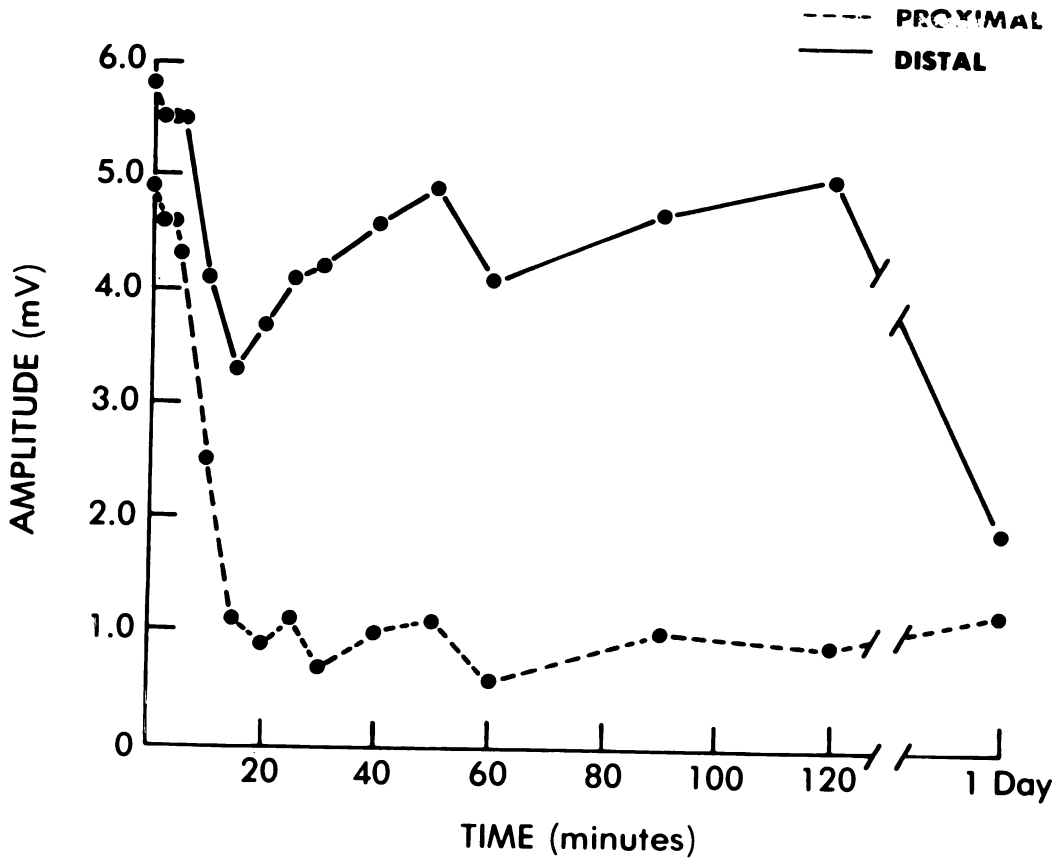


Figure 4.

Mean CMAP Amplitudes Up to 24 Hours After Arachidonic Acid Injection

The proximal CMAP amplitude is more severely reduced than the distal amplitudes indicating conduction block.

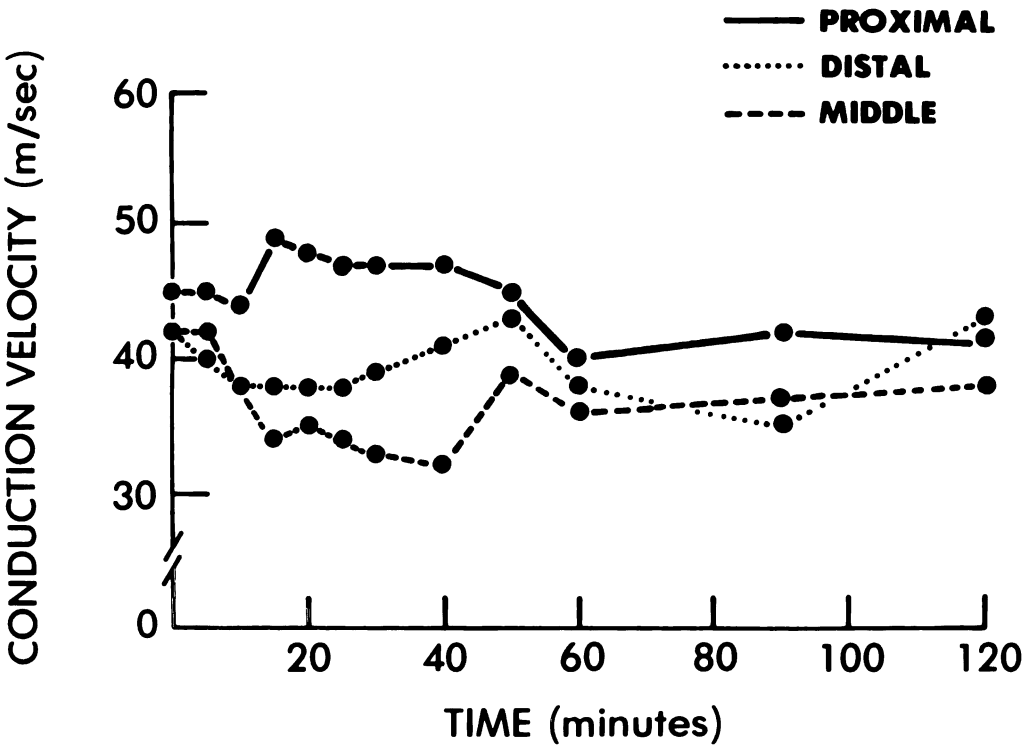


Figure 5.

Mean MCV Up to 24 Hours After
Arachidonic Acid Injection

There is greater slowing of MCV through the middle segment (between M_1 and M_2) which is the site of focal ischemia.

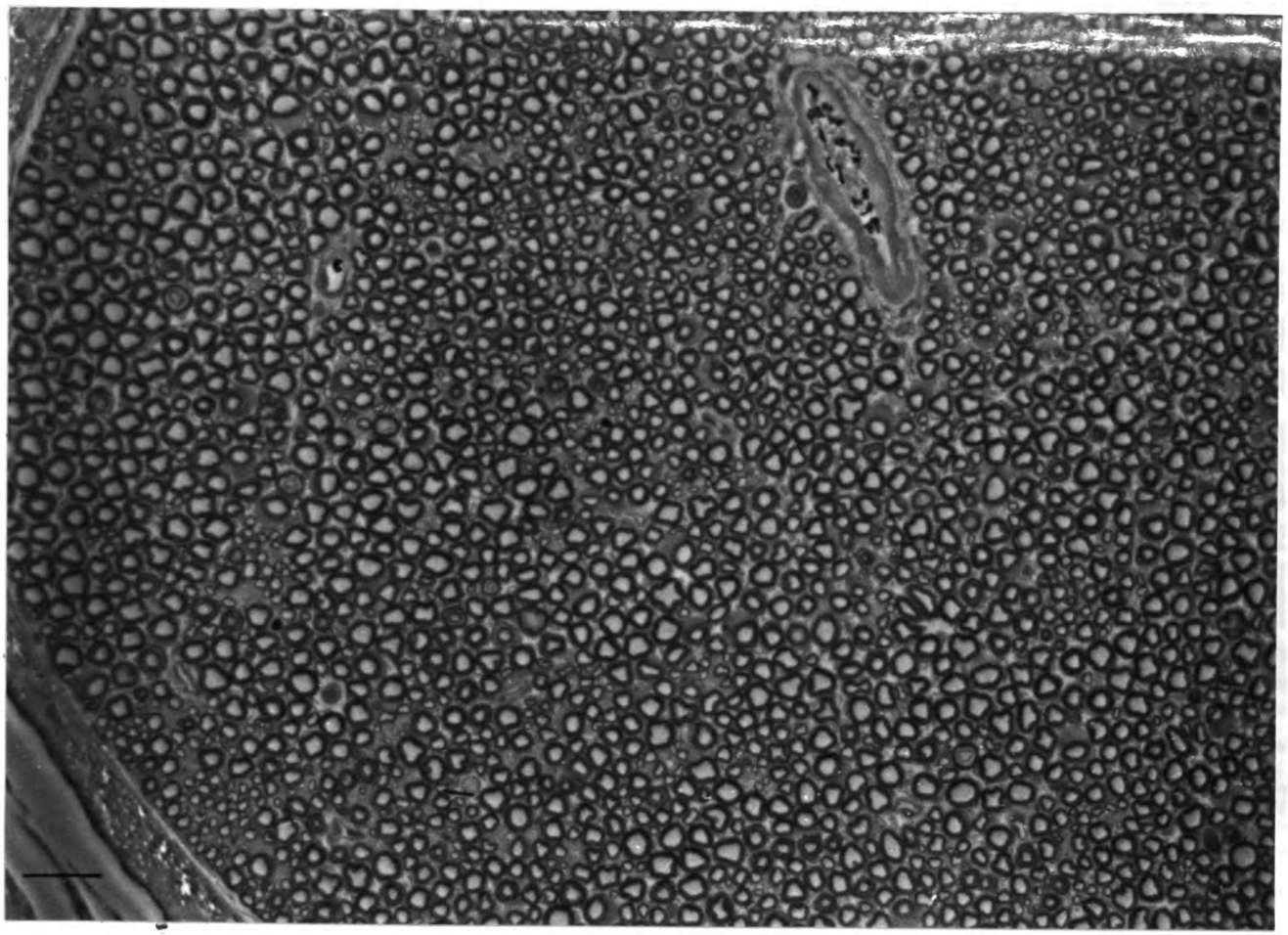


Figure 6.

Transverse Section of the Proximal Tibial Nerve, Within 30 Minutes After Arachidonic Acid Injection

Fiber morphology is normal. Bar = 100 um.

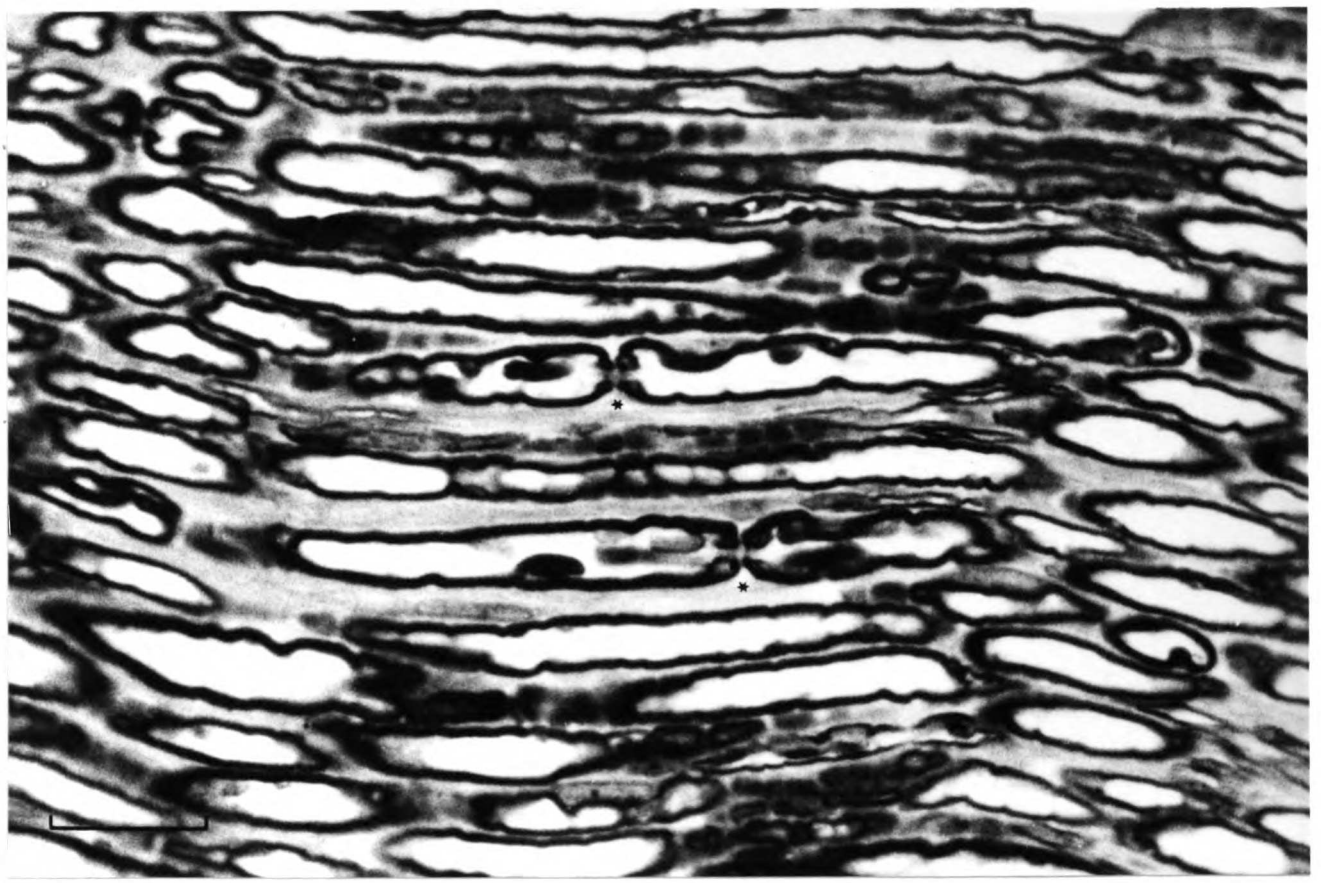


Figure 7.

Longitudinal Section of the Proximal Tibial Nerve, Within 30 Minutes After Arachidonic Acid Injection

Note normal node of Ranvier (*). Bar = 50 um.

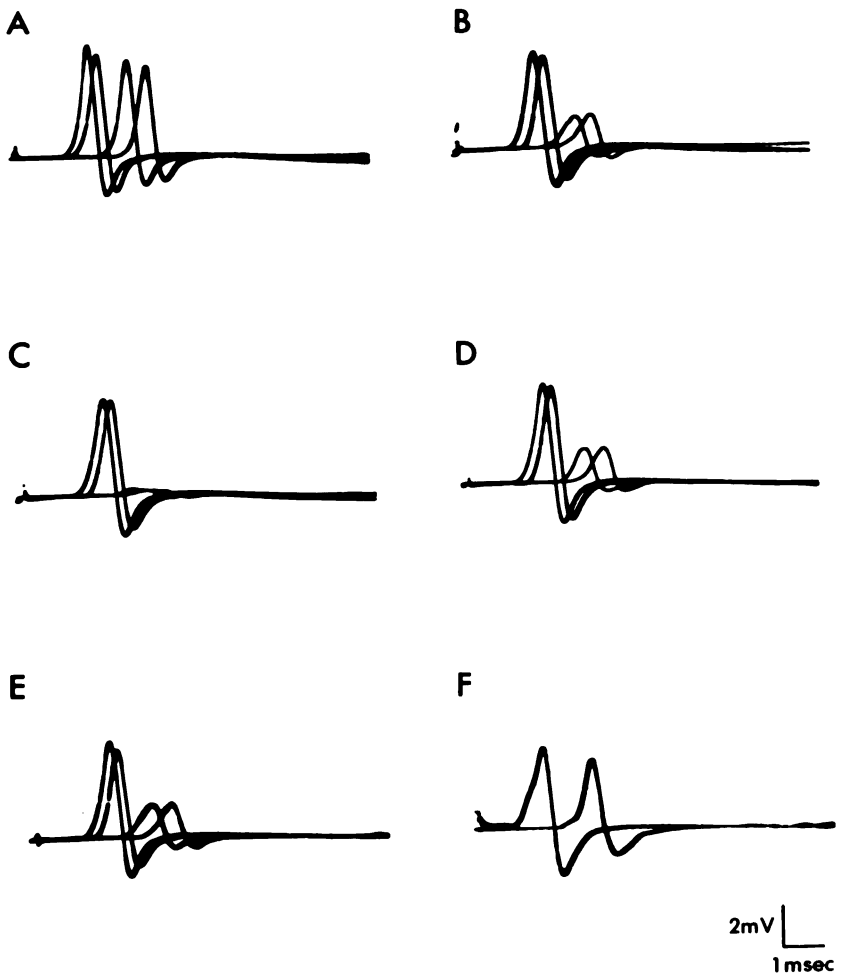


Figure 8.

Sequential CMAP Recordings of a Representative Group A Nerve Studied Before and After Arachidonic Acid Injection

A. Baseline	B. 5 minutes
C. 10 minutes	D. 30 minutes
E. 60 minutes	F. 24 hours

The CMAP after proximal stimulation drops earlier and more profoundly than the distal CMAP. At 24 hours the CMAP amplitudes were slightly reduced, but within the normal range.

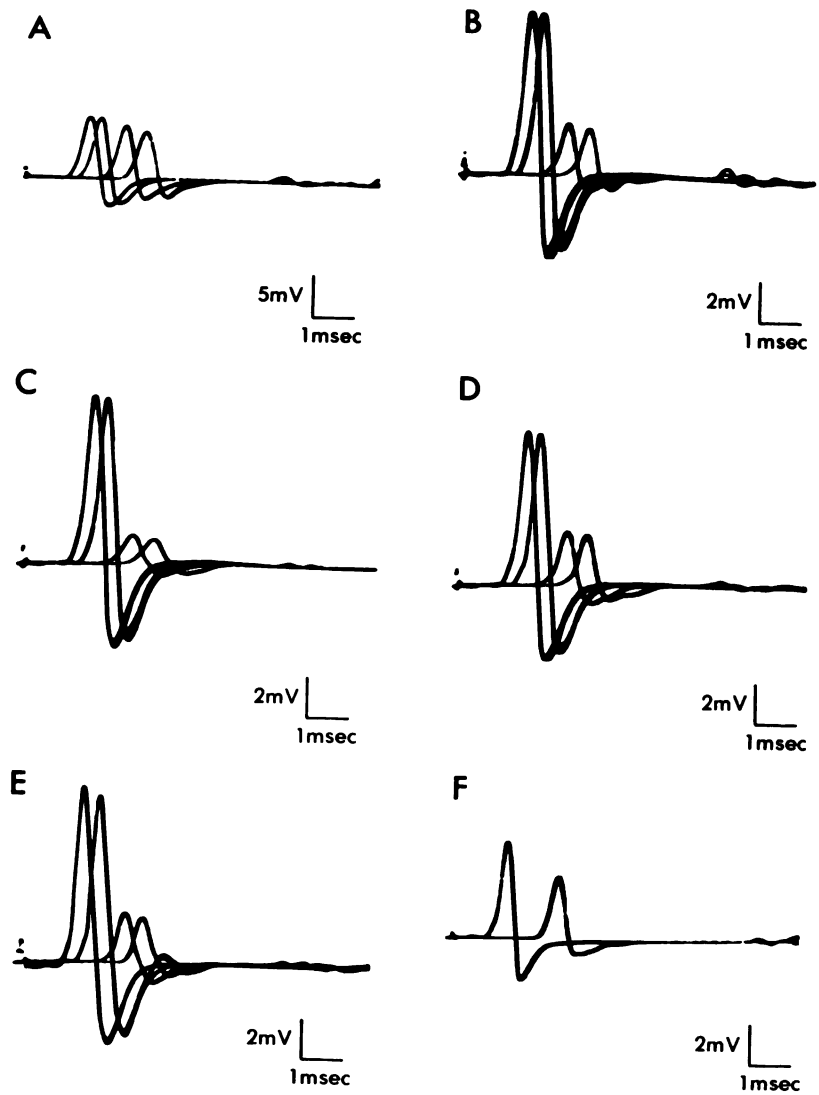


Figure 9.

Sequential CMAP Recordings of a Group A Nerve Studied Before and After Arachidonic Acid Injection

A.	Baseline	B.	15 minutes
C.	30 minutes	D.	60 minutes
E.	150 minutes	F.	24 hours

The proximally elicited CMAP are reduced within 15 minutes of injection and little improvement is seen by 150 minutes. At 24 hours the CMAP amplitudes were slightly diminished and the R value was still reduced. The nerve morphology was normal.

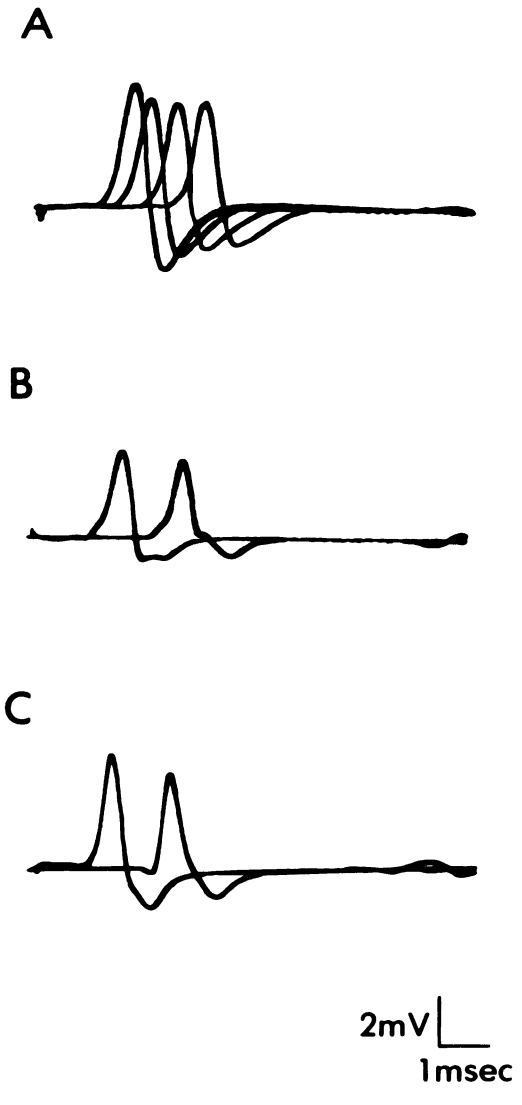


Figure 10.

Sequential CMAP Recordings of a Group B1 Nerve Studied Before and After Arachidonic Acid Injection

A. Baseline
B. 1 day
C. 2 days

Proximal and distal CMAP amplitudes were slightly reduced at 1 and 2 days. The nerve conduction studies correlate well with the occasional degenerating axons in this mild lesion.

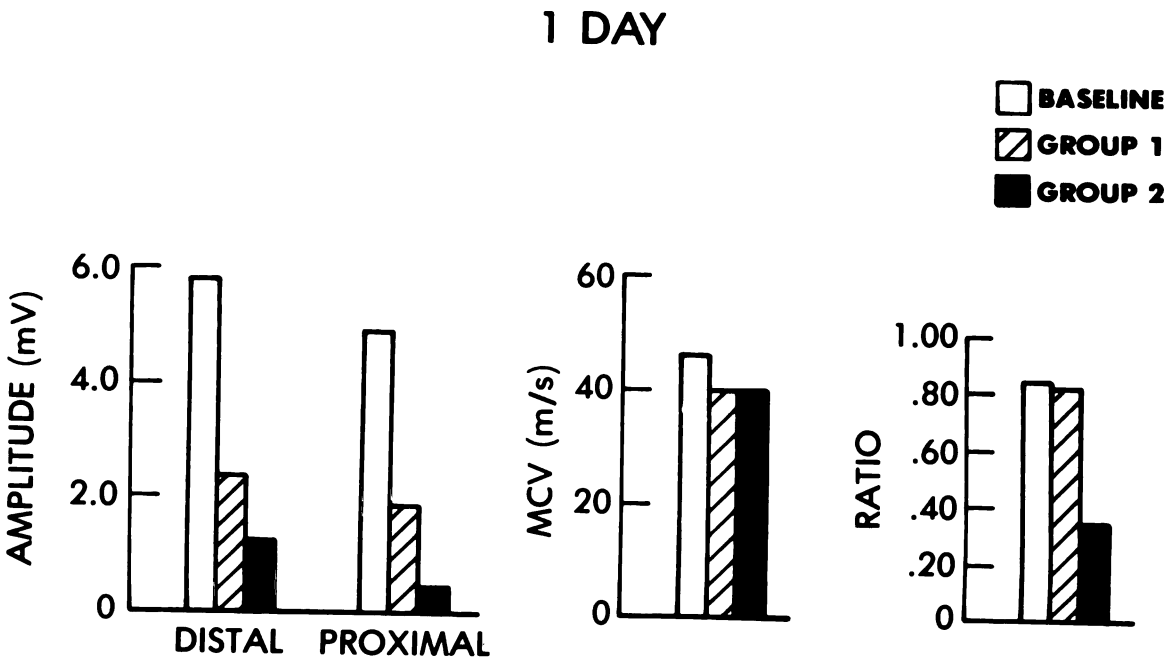


Figure 11.

Mean CMAP Amplitudes, MCV and Ratio 1 Day After Arachidonic Acid Injection

Group 1 nerves showed reduced proximal and distal CMAP amplitudes and minimally slowed MCV at 1 day. Group 2 nerves had evidence of conduction block in some surviving myelinated axons: the proximal CMAP was more severely diminished and the ratio was significantly reduced.

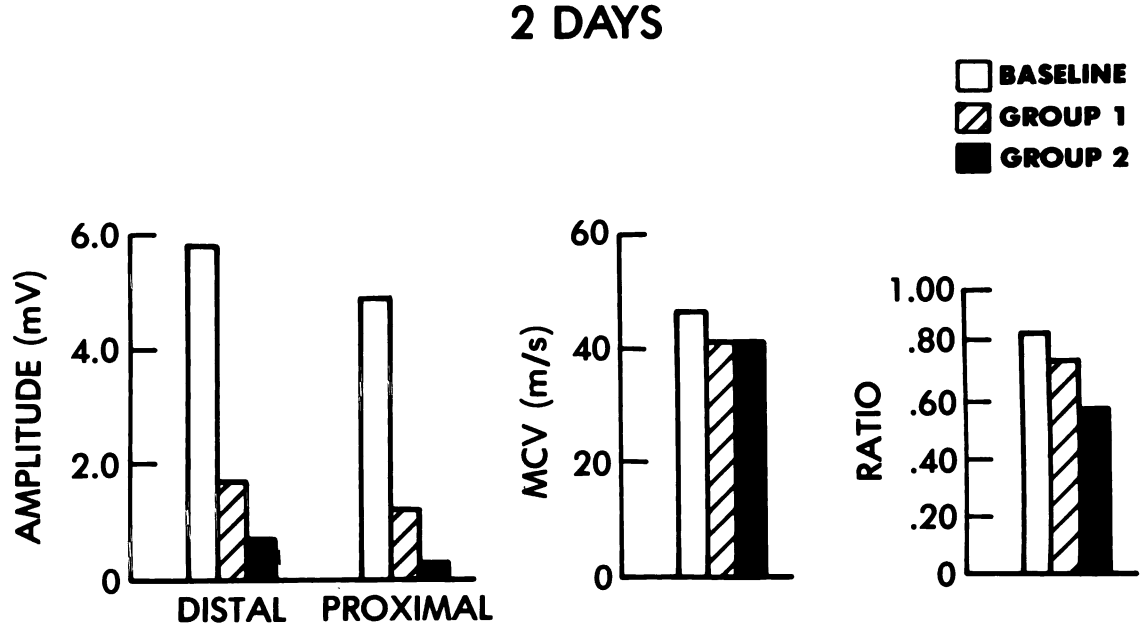


Figure 12.

Mean CMAP Amplitudes, MCV and Ratio 2 Days After Arachidonic Acid Injection

By 2 days the proximal and distal amplitudes were further diminished. The Group 1 ratio fell slightly, reflecting the small number of nerves in this group which developed conduction block. The Group 2 ratio increased due to rectification of the ratio in a majority of these rats.

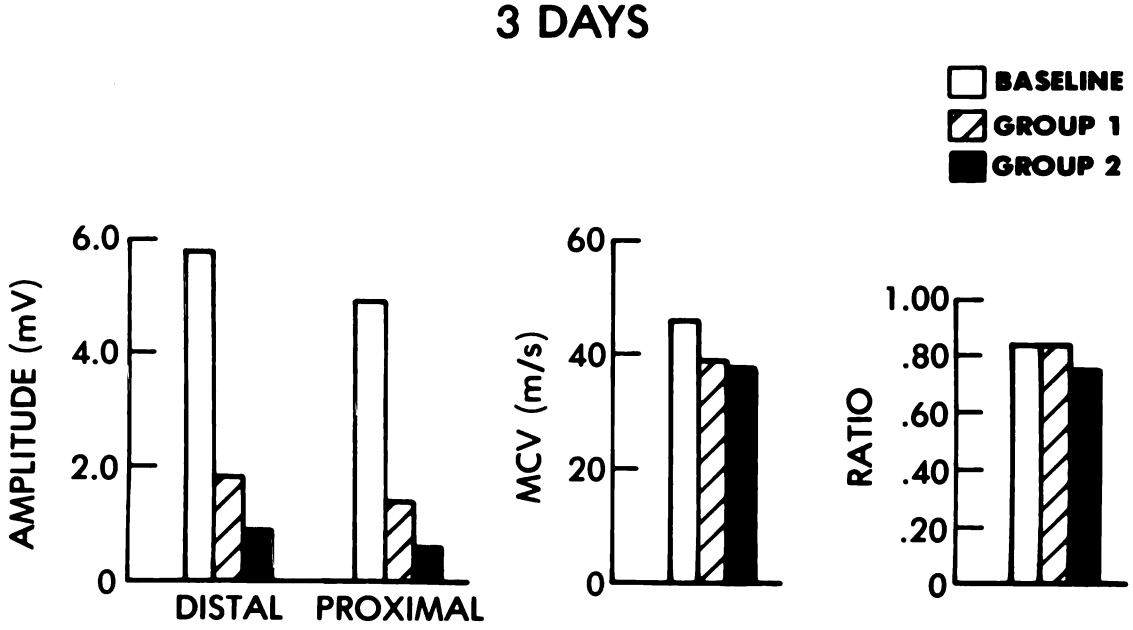


Figure 13.

Mean CMAP Amplitudes, MCV and Ratio 3 Days After Arachidonic Acid Injection

By 3 days the Group 2 ratio had further increased, due to an increase in the proximal CMAP amplitude.

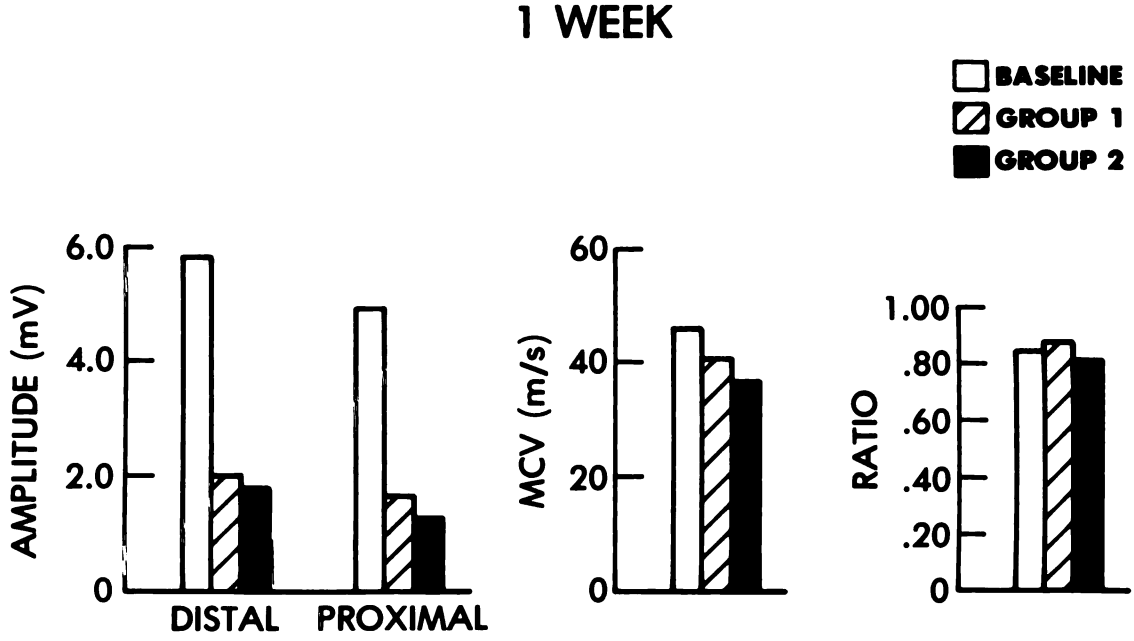


Figure 14.

Mean CMAP Amplitudes, MCV and Ratio 1 Week After Arachidonic Acid Injection

By 1 week conduction block had resolved in the majority of nerves, and the ratios of both groups were within the normal range.

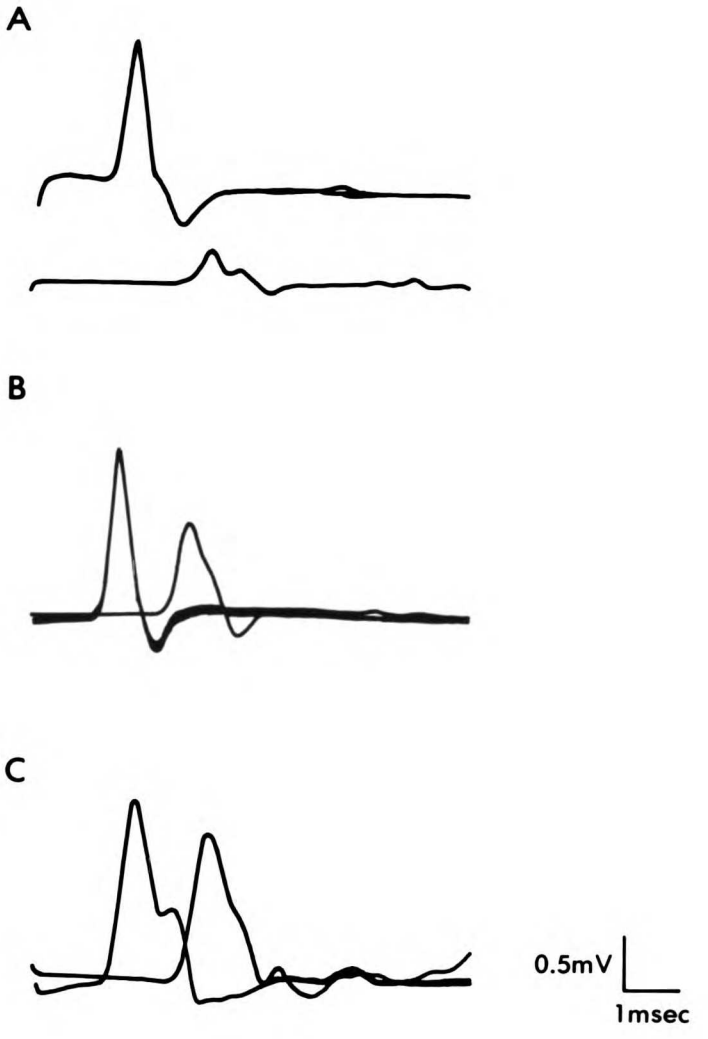


Figure 15.

Sequential CMAP Recordings of a Group B2 Nerve After Arachidonic Acid Injection

A. 1 day
B. 1 week
C. 2 weeks

The proximal CMAP was still severely reduced at 1 day (R = 0.29) but had increased by 1 week (R = 0.59). The ratio was normal by 2 weeks.

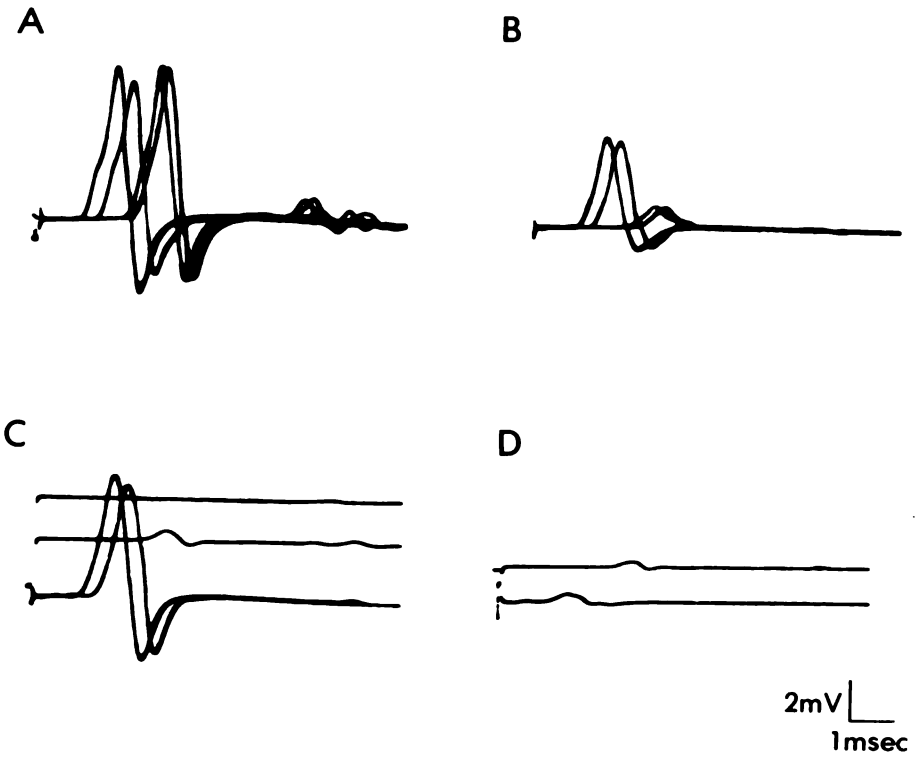


Figure 16.

Sequential CMAP Recordings of a Representative Group B2 Nerve Before and After Arachidonic Acid Injection

A. Baseline B. 20 minutes
C. 30 minutes D. 24 hours

The proximal CMAP was extinguished by 30 minutes while the distal CMAP was only mildly diminished. The ratio was slightly reduced on day 1 and normal by day 2 (not shown). Morphological examination revealed a moderately severe lesion which would account for the low amplitude responses.

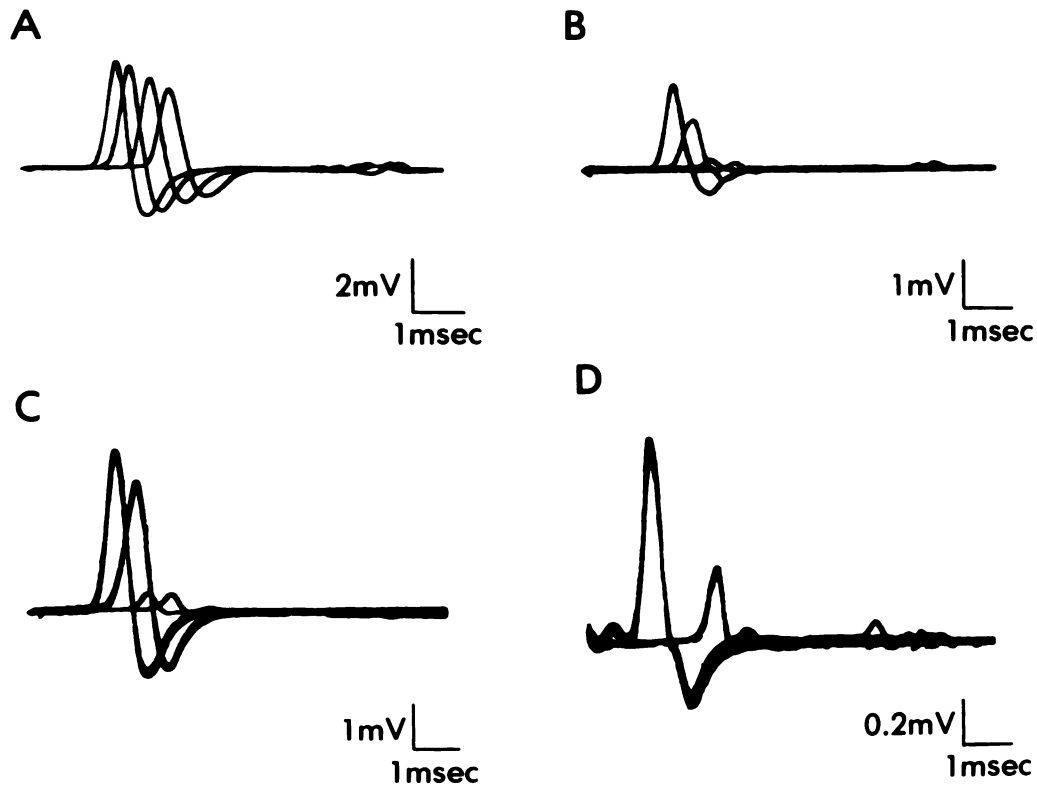


Figure 17.

Sequential CMAP Recordings of a Group B2 Nerve Before and After Arachidonic Acid Injection

A. Baseline B. 15 minutes
C. 60 minutes D. 24 hours

The proximal and distal CMAP amplitudes were severely diminished at 24 hours and the low ratio indicates persistent conduction block.

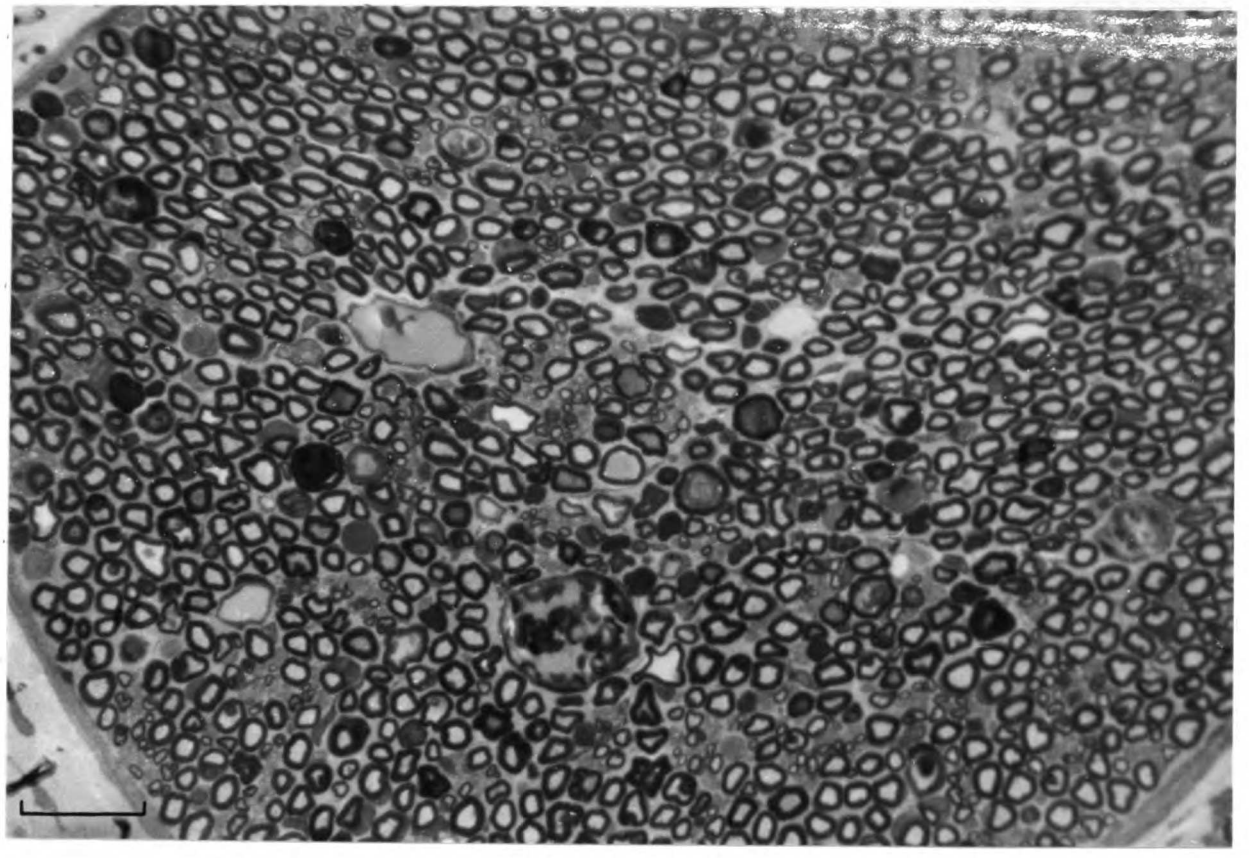


Figure 18.

Transverse Section of the Tibial Nerve,
1 Day After Arachidonic Acid Injection

Bar = 50 um.

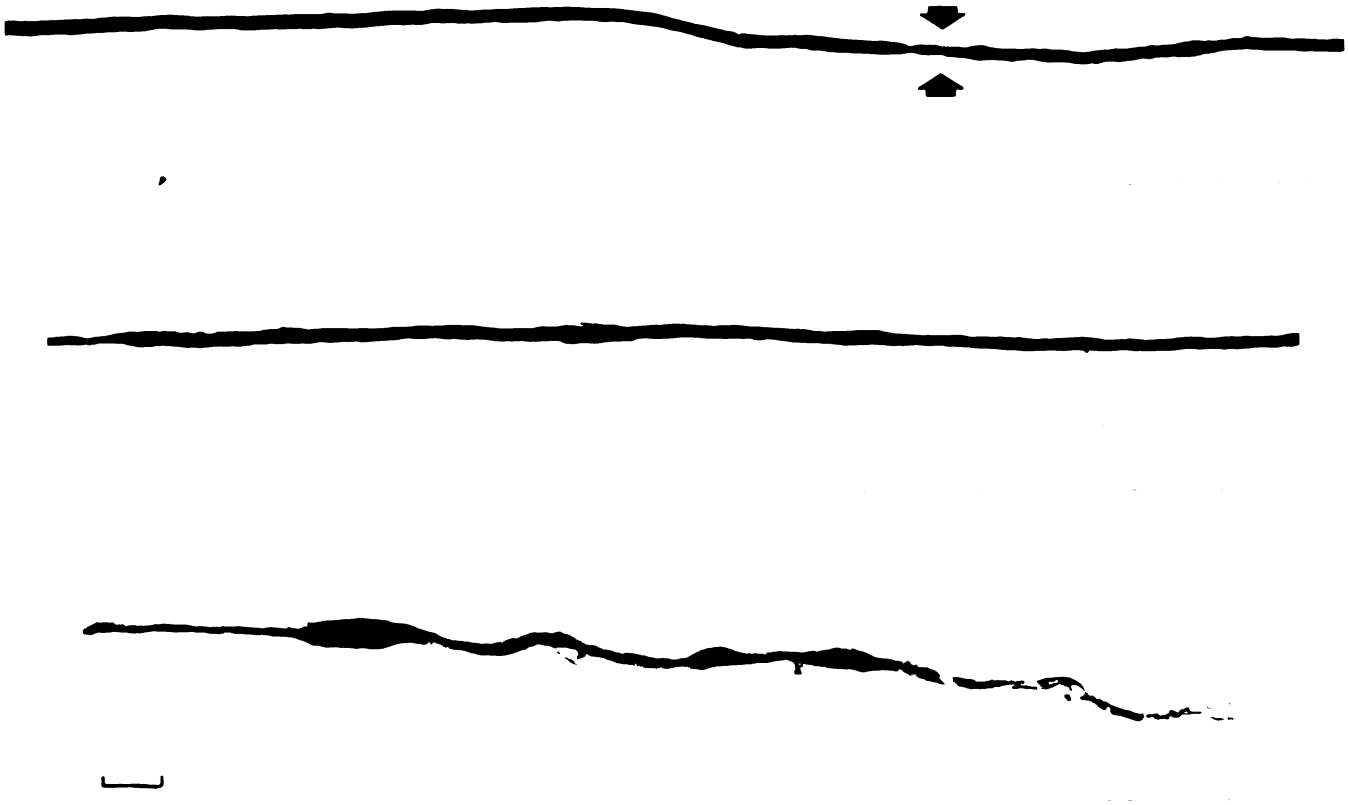


Figure 19.

Single Teased Fiber from the Tibial Nerve,
1 Week After Arachidonic Acid Injection

Consecutive portions are mounted below one another; arrows indicate the presence of paranodal retraction proximal to the segment of Wallerian degeneration. Bar = 50 um.

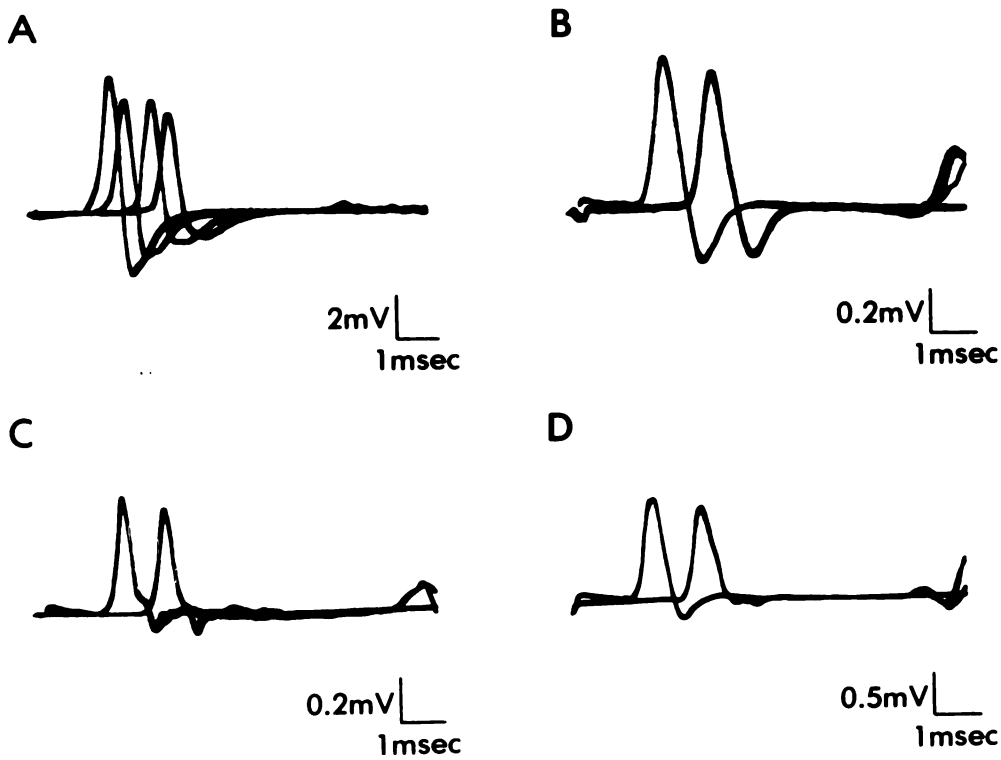


Figure 20.

Sequential CMAP Recordings Before and Up to 3 Weeks After Arachidonic Acid Injection

A. Baseline B. 1 week
C. 2 weeks D. 3 weeks

Proximal and distal CMAP amplitudes were profoundly reduced and only minimal improvement was seen by 3 weeks. This nerve showed a severe central-fascicular infarct with extensive regeneration.

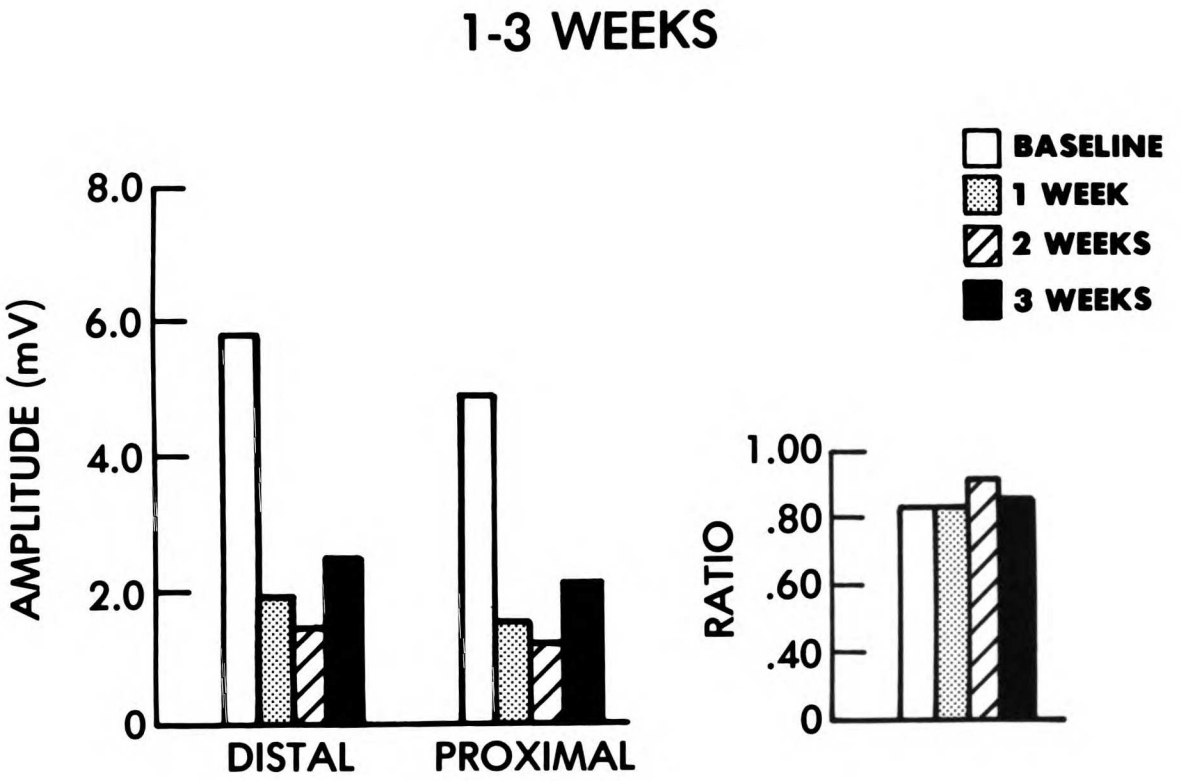


Figure 21.

Mean CMAP Amplitudes and Ratio 1 to 3 Weeks
After Arachidonic Acid Injection

By 3 weeks there was a slight increase in both the proximal and distal CMAP amplitudes. At 1 week the ratio was normal, and no significant changes were seen at 2 or 3 weeks.

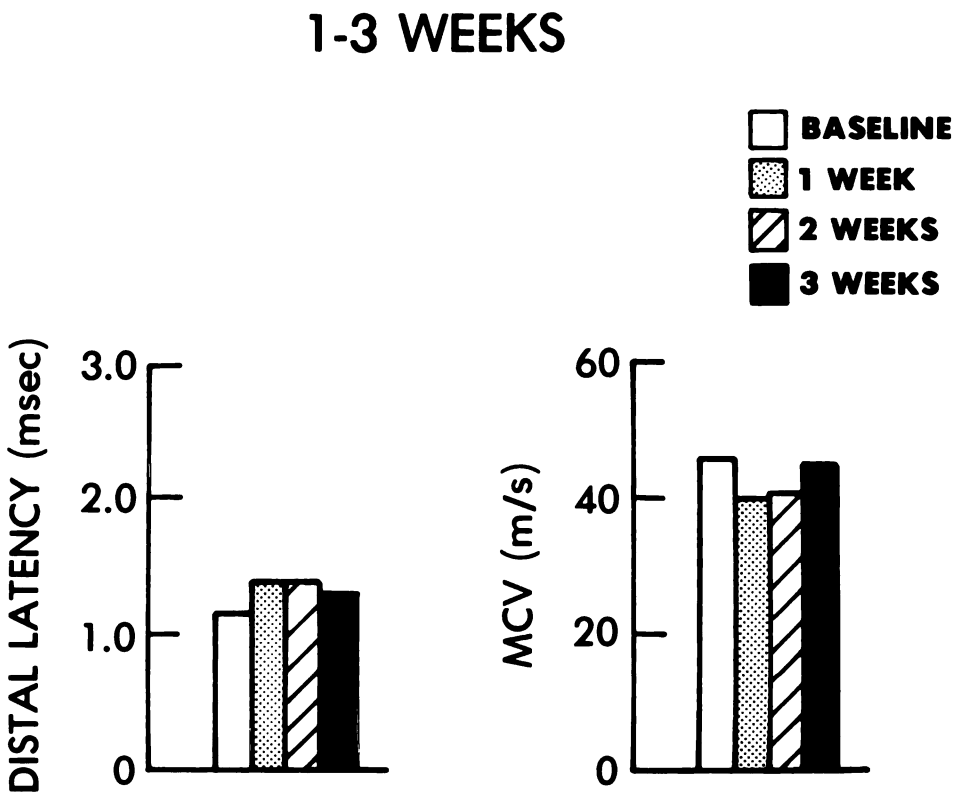


Figure 22.

Mean Distal Motor Latency and MCV 1 to 3 Weeks After Arachidonic Acid Injection

The distal motor latency was prolonged at 1 and 2 weeks. By 3 weeks the DML was mildly reduced, although still above baseline. The 1 and 2 week MCV was slightly slowed from baseline and the 3 week MCV was normal.

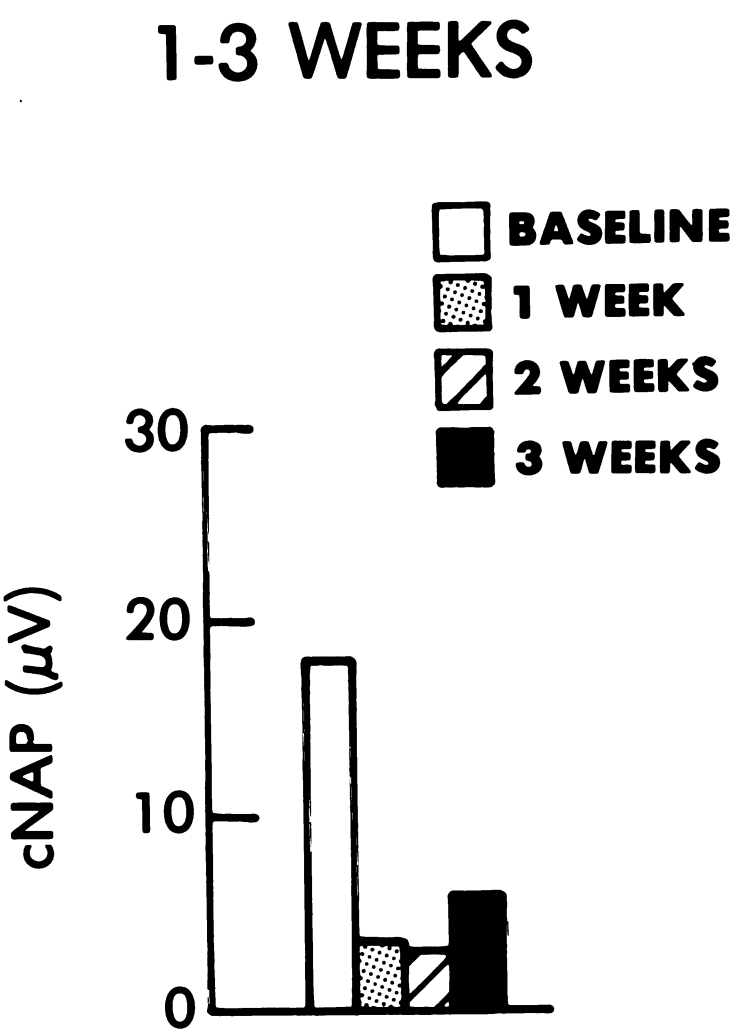


Figure 23.

Mean CNAP Amplitude 1 to 3 Weeks
After Arachidonic Acid Injection

The CNAP was severely diminished from baseline at 1, 2 and 3 weeks with some increase in amplitude by 3 weeks.

Figure 24.

Transverse Section of the Proximal Sciatic Nerve, 1 Week After Arachidonic Acid Injection

Fiber morphology is normal. Bar = 100 um.

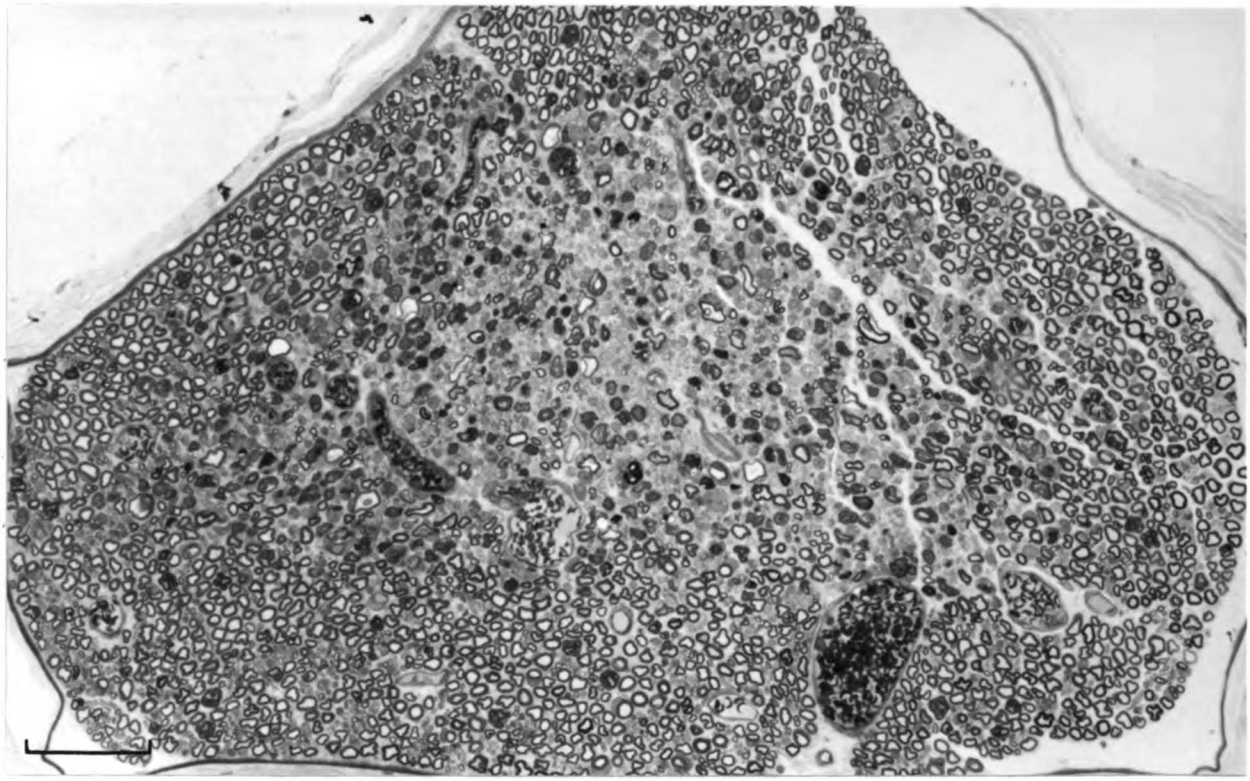


Figure 25.

Transverse Section of the Proximal Tibial Nerve,
1 Week After Arachidonic Acid Injection

Degenerating axons are concentrated in the center of the fascicle and subperineurial axons are relatively spared. Bar = 100 um.

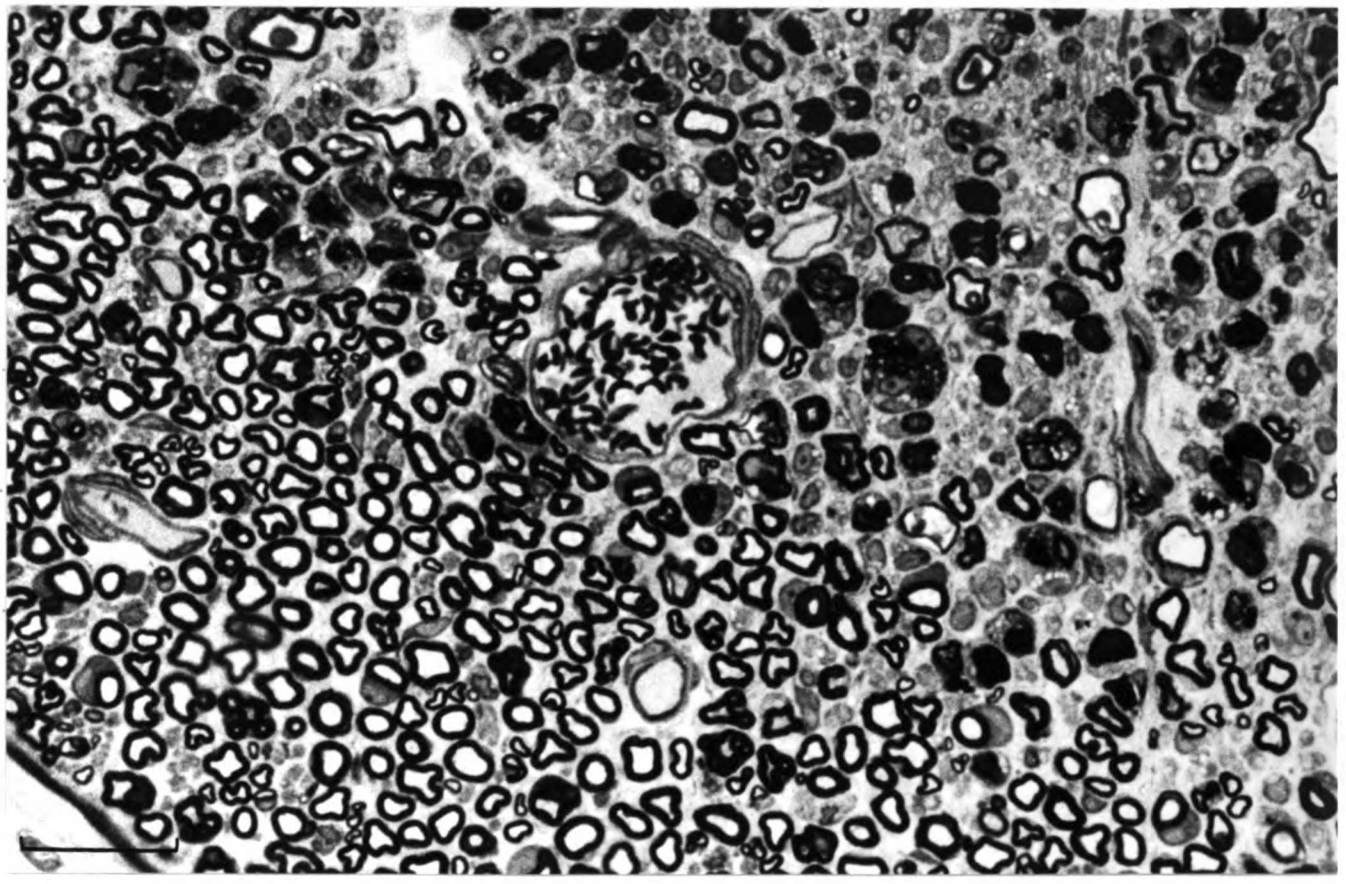


Figure 26.

Transverse Section of the Proximal Tibial Nerve,
1 Week After Arachidonic Acid Injection
(Higher Magnification)

Degenerating axons are concentrated in the center of the fascicle. Bar = 50 um.

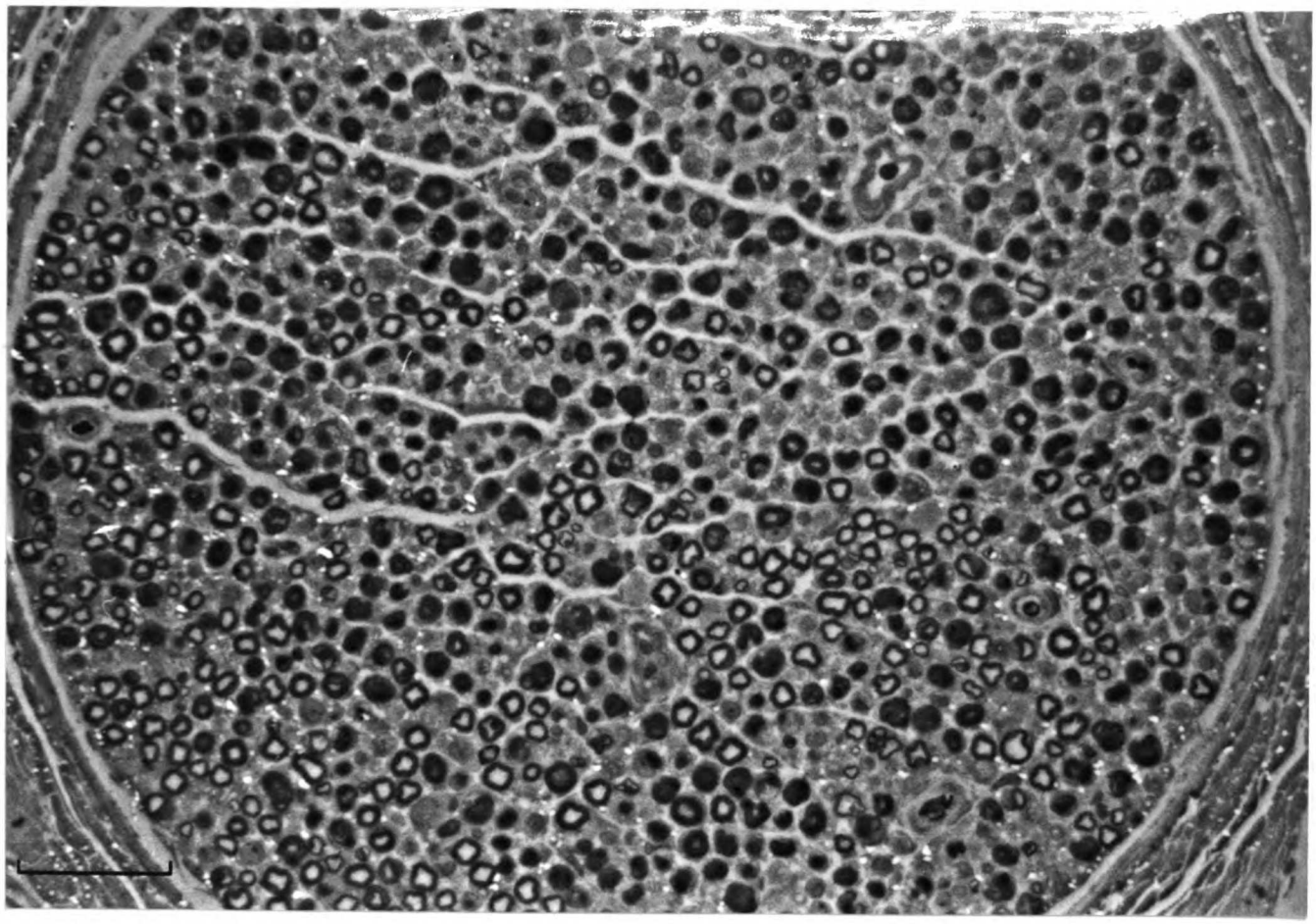


Figure 27.

Transverse Section of the Distal Tibial Nerve,
1 Week After Arachidonic Acid Injection

Degenerating axons are distributed thoughout the fascicle. Bar = 100 um.

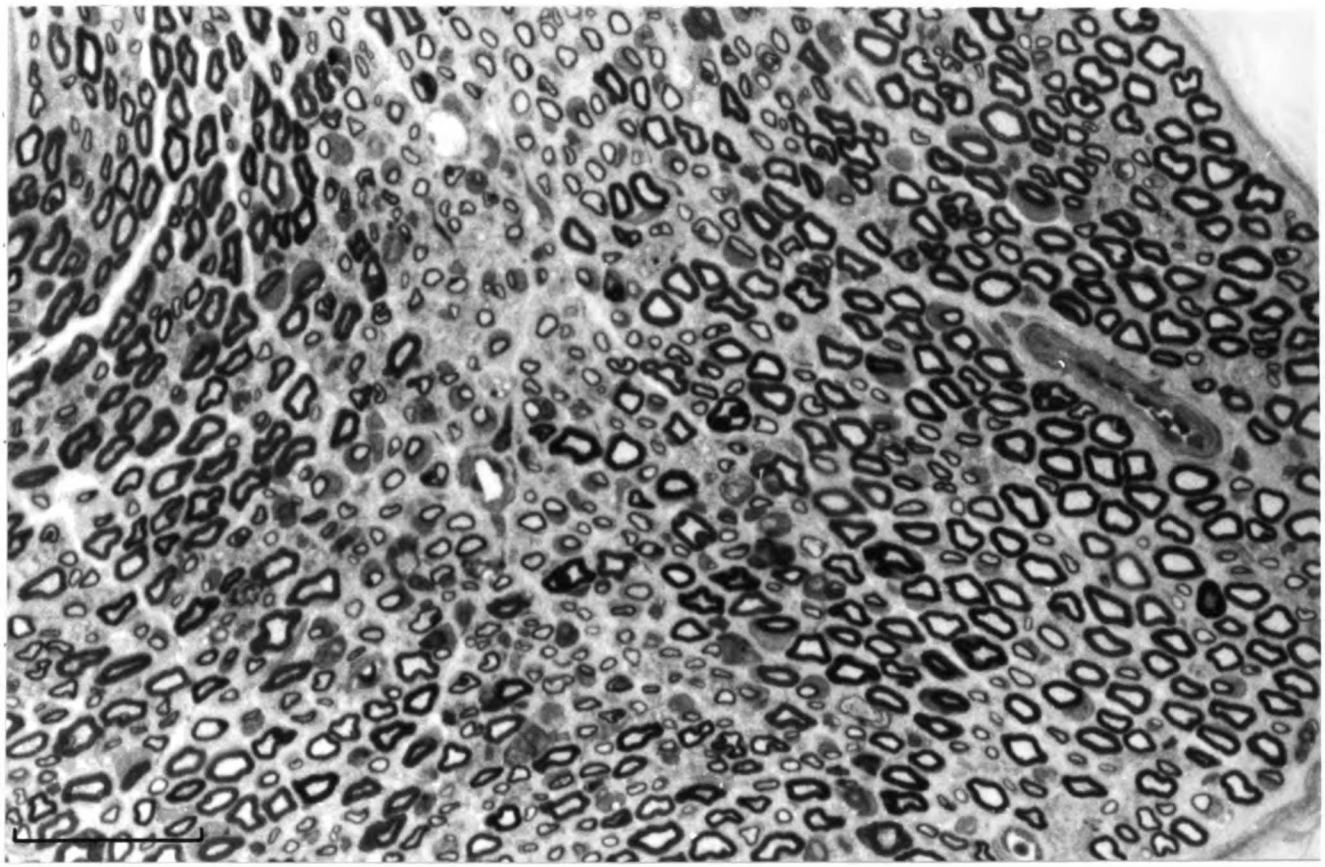


Figure 28.

Transverse Section of the Distal Tibial Nerve,
3 Week After Arachidonic Acid Injection

Many thinly myelinated regenerating axons are apparent. Bar = 100 um.

List of Appendices

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Appendix 1.

Plantar Intrinsic Foot Muscles (Greene, 1955)

M. Flexor digitorum brevis
M. Abductor digiti quinti
M. Quadratus plantae
Mm. Lumbricales
M. Flexor hallucis brevis
M. Flexor digiti quinti brevis
M. Adductor indicis
Mm. Interossei plantares

Appendix 2.

Arachidonic Acid Diluent (Furlow and Bass, 1975 a)

154 mM NaCl, 65.8 mM sodium carbonate,
95% ethanol [10% v/v].

To make 100 ml :

0.9 g	NaCl
0.7 g	Na_2CO_3
10.0 ml	95% Ethanol
90.0 ml	dd H_2O

Appendix 3.

Sample Data Sheet

Tail Mark_____
Rat Number_____

Date ___/___/___

1. Sex: M F Weight_____grams Supplier_____

2. Anesthetic_____ Volume_____ml
   Time_____ Additional doses:_____

3. Leg: L R

4. Arachidonic acid concentration_____mM
   Sigma lot#_____
   Inject_____ml Time_____
   Additional doses:_____

Time	Distance 1		Distance 2		Distance 3								
	D		M1		M2		P		GAIN	MCV			R
5'	____/___		___/___		___/___		___/___		____	___/___/___			____
10'	____/___		___/___		___/___		___/___		____	___/___/___			____
15'	____/___		___/___		___/___		___/___		____	___/___/___			____
20'	____/___		___/___		___/___		___/___		____	___/___/___			____
25'	____/___		___/___		___/___		___/___		____	___/___/___			____
30'	____/___		___/___		___/___		___/___		____	___/___/___			____
40'	____/___		___/___		___/___		___/___		____	___/___/___			____
50'	____/___		___/___		___/___		___/___		____	___/___/___			____
60'	____/___		___/___		___/___		___/___		____	___/___/___			____
90'	____/___		___/___		___/___		___/___		____	___/___/___			____
120'	____/___		___/___		___/___		___/___		____	___/___/___			____

Comments :_____
_____

FOLLOW-UP STUDIES: 1 Day, 2 Days, 3 Days,
1 Week, 2 Weeks, 3 Weeks

Date___/___/___

1. Anesthetic_____ Volume_____ml
   Time_____ Additional doses:_____

D	P	Gain	Distance	MCV	R	CNAP	
___/___	___/___	____	_____	_____	_____	___/___	_____uV

Appendix 4.

Fixative Preparation

3.6% Glutaraldehyde in 0.1M PO_4

To make 100 ml :

7.2 ml	50% glutaraldehyde
50 ml	0.2M PO_4 (pH 7.6)
42.8 ml	dd H_2O

Appendix 5.

Sorenson's Phosphate Buffer (PO_4)

Stock solutions :

A. 0.2M $NaH_2PO_4.H_2O$
27.6g $NaH_2PO_4.H_2O$ in 1000 ml dd H_2O

B. 0.2M Na_2HPO_4
28.4 g Na_2HPO_4 in 1000 ml dd H_2O

To make 100 ml :

ml A	ml B	final pH
43	57	6.9
13	87	7.6

Appendix 6.

Tissue Preparation and Embedding

A. Fix tissue in 3.6% glutaraldehyde in 0.1M PO_4 for a minimum of two hours.

B. Wash twice in 0.1M PO_4 (pH 7.6) for 5 minutes each.

C. Post-fix tissue in 1% OsO_4, 1.5% potassium ferricyanide ($K_3Fe(CN)_6$) for 2-3 hours in the dark. Solution contains 1 part 2% OsO_4 in dd H_2O with 1 part 3% $K_3Fe(CN)_6$ in 0.2M PO_4 (pH 7.6). [Langford and Coggeshall 1980]

D. Wash twice in dd H_2O for 10 minutes each.

E. Dehydrate tissue through a graded series of alcohol:

% Ethanol in dd H_2O	# exchanges	minutes
25	1	5
35	1	5
50	1	5
70	1	5
80	1	5
90	1	5
95	1	5
100	2	10

F. Rinse tissue twice in propylene oxide (PO) for 15 minutes each.

G. Infiltrate tissue with 50% PO / 50% Poly/Bed 812 - Araldite mixture overnight.

To prepare 44.9 ml Poly/Bed 812 - Araldite mixture

10 ml	Poly/Bed 812 Embedding Media, Polysciences, Inc. #8791
10 ml	Araldite Epoxy Resin 6005, Polysciences, Inc. #2116
24 ml	Dodecenylsuccinic Anhydride (hardener), Polysciences, Inc.- #0563
0.9 ml	2, 4, 6 - tri(dimethylaminomethyl)-phenol (accelerator), Polysciences, Inc. #00553

H. Place tissue in 100% Poly/Bed - Araldite in embedding molds for 1-8 hours.

I. Place tissue in fresh 100% Poly/Bed - Araldite in new molds. Orient segments for transverse or longitudinal sectioning.

J. Place molds in 60°C oven for 24 hours.

Appendix 7.

Tissue Preparation and Teasing

A. Fix tissue in 3.6% glutaraldehyde in 0.1M PO_4 for a minimum of two hours.

B. Wash twice in 0.1M PO_4 (pH 7.6) for 5 minutes each.

C. Post-fix tissue in 1% OsO_4, 1.5% potassium ferricyanide for 2-3 hours in the dark.

D. Wash twice in dd H_2O for 10 minutes each.

E. Place segments in 66% glycerol in dd H_2O and leave in the cold overnight.

F. Transfer segments to 100% glycerol and store in the cold.

Appendix 8.

Staining Preparations and Procedures

Methylene Blue - Azure II - Basic Fuchsin (Lillie, 1969)

A. Methylene Blue - Azure II (MB - Azure II)

To make 100 ml :

0.13 g	Methylene Blue
0.02 g	Azure II
10 ml	Glycerol
10 ml	Methanol
30 ml	0.1M PO_4 (pH 6.9)
50 ml	dd H_2O

B. Basic Fuchsin (BF)

To make 10 ml of stock solution :

0.10 g	Basic Fuchsin
10 ml	50% Ethanol

To make 60 ml of working solution :

3 ml	Basic Fuchsin stock
57 ml	dd H_2O

C. Filter staining solutions through Whatman Qualitative 1 filters. Solutions can be kept for 4 months.

D. Staining :

1. Preheat MB - Azure II to 60°C.

2. Immerse sections, mounted on glass slides, in MB - Azure II for 35 minutes at 60°C.

3. Rinse slides three times in dd H_2O, at least 5 minutes per rinse.

4. Immerse slides in BF for 5 minutes at room temperature.

5. Rinse sides three times in dd H_2O, at least 5 minutes per rinse.

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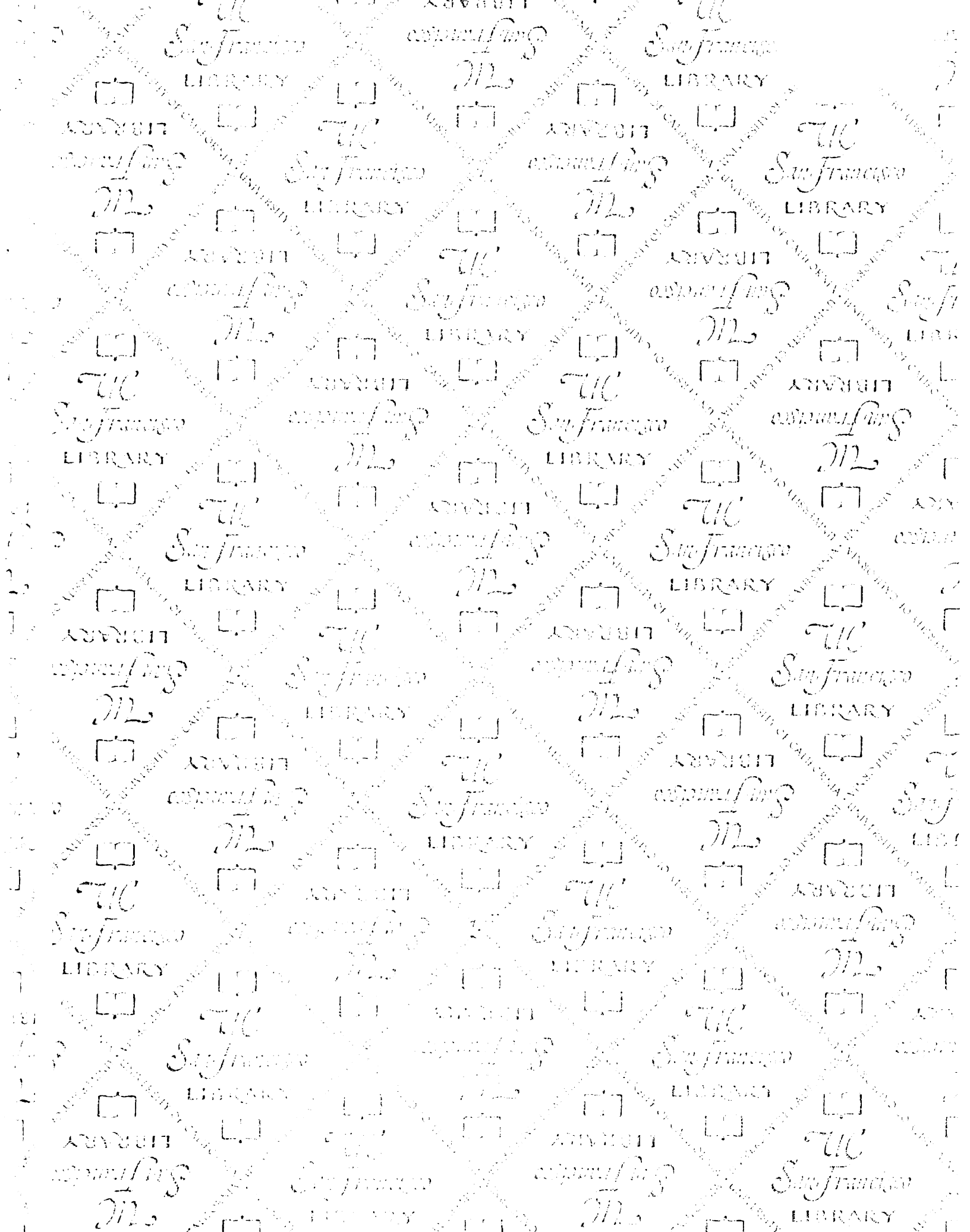
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