AN INVESTIGATION INTO THE RESPONSE OF A SECTIONED MENTAL NERVE IN THE RAT

by

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TABLE OF CONTENTS

PRECIS

The chances of mandibular injury and thus injury to the inferior dental nerve have increased with high speed transport, more contact sport and recent advances in major oral surgery. Clinically most of these nerve injuries resolve themselves with time. However, a review of the literature has not revealed any experimental study on the response of the mental nerve to injury.

This investigation was to determine the mental nerve (branch of the inferior dental nerve distal to the mandible) response to a transverse section. The mental nerve in the Sprague Dawley rat was sectioned and the subsequent response was observed histologically. An attempt was also made to use an electrophysiological technique.

This histological investigation demonstrated that degeneration and regeneration of the mental nerve occurred, in general, in a similar fashion to other nerves. However, because of the mental nerve's composition, peripheral position and the fact that it is in a restricted compartment (i.e. the lip) the relative displacement of the sectioned trunks is reduced. Because of this reduced displacement, successful regeneration is enhanced. During the regenerative phase, most of the axon branches entered the distal trunk. Those that did not enter the distal trunk moved off peripherally via minifuniculi or degenerated. However a large proportion of axons did regenerate. Myelin maturation was observed in all the distal segments but not in all the minifuniculi.

Unfortunately the electrophysiological technique failed to demonstrate the physiological response of the sectioned mental nerve. The exact reason was not established. However, this failure could be due to afferent nerve fibres and/or leakage of current to the digastric neuromuscular apparatus. Thus there were no physiological responses recorded for the sectioned mental nerve.

The histological results concurred with the clinical observation that surgical repair is not required for establishment of mental nerve function after sectioning. However, the quality of the return of sensation could not be determined in this experimental model.

DECLARATION

This thesis is submitted in part fulfilment of the requirements for the Degree of Master of Dental Surgery in the University of Adelaide. Candidature for the Degree was satisfied by a Qualifying Examination in 1976.

This thesis contains no material which, except where due mention is made, has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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CHAPTER I

INTRODUCTION

Peripheral sensory nerve impairment affects the patient both physiologically and psychologically to the extent that the latter can cause the patient to exaggerate an already serious injury. In some cases it becomes more than an annoying factor and becomes a seriously disturbing problem. Even so, the sensory nerve damage is not usually accompanied by the physical handicaps found in motor nerve injuries. Fortunately, however, permanent nerve damage to either motor or sensory nerves does not occur very often.

Loss of sensory nerve function in the perioral area creates a great social problem because of effluence of saliva or involuntary outflow of liquids while drinking, pressure ulcerations and lip biting which are not only noticed by the patients but also people around them. With other regions that have lost sensation, the sensory loss is less obvious to an observer.

The loss of sensory perception of the oral mucosa that is most noticed by the patient is when the inferior alveolar, mental, lingual and infra-orbital nerves are injured. Of the oral nerves, the inferior alveolar, mental and mylohyoid nerves are most commonly injured either through accidents or by surgical trauma.

In the dental literature there has been a relatively recent increase of interest in the study of nerve injuries and surgical

correction of inferior alveolar nerve damage.

The reason for previous lack of interest was because of the difficulty of testing the damaged nerve physiologically. In addition surgical repair is very delicate and very destructive to adjacent structures, especially bone. Also this type of surgery is preferably carried out on young patients and with non-malignant lesions.

The chances of mandibular nerve injury have increased with high speed transport, more contact sport and recent developments in major oral surgery. There is a widely held, but untested, belief that the injured mandibular nerve will regenerate within "18 months" and if this does not occur the patient will have to learn to live with the condition. Obviously this empirical assumption creates an unacceptable state of affairs and basic research in this area, to obtain more insight, is imperative.

Until now, no study has been conducted upon the injuries to the mental nerve, (inferior dental nerve distal to the mental foramen). The present investigation attempts to study the neural response of a transverse section of the mental nerve in the rat,

(a) physiologically,

(b) histologically.

Totally severed nerves with gross loss of continuity present the greatest problem in the return of normal function. The present investigation delves into the area where a mental nerve is sectioned with minimal disruption to adjacent structures to study the nature of the regenerative process.

The clinical impression at present is that the mental nerve, once severed, does not have to be re-aligned and sutured for recovery to take place. The reasons for this recovery could be due to:-

(a) nerve segments re-align when adjacent structures are not grossly disrupted,

(b) the axonal growth in the connective tissue has an affinity for the distal segment even though the proximal and distal segments are not aligned,

(c) the axons do not enter the distal segment and either unite with the old sensory receptors or facilitate in the formation of new receptors,

(d) the denervated area is innervated by adjacent sensory nerves.

To determine whether any or all of these combinations actually occur, histological and physiological methods will be used to study the sequence of events in regeneration of the mental nerve after transverse sectioning.

Before this aspect of research can be conducted in a valid scientific manner, the following areas must be investigated:

1. the best histological technique for studying peripheral nerves,
2. if physiological tests can be conducted on the rat's mental nerve.

CHAPTER II

LITERATURE REVIEW

2.10 CLASSIFICATION OF PERIPHERAL NERVE LESIONS

2.20 NEURAPRAXIA

2.30 DEGENERATION OF NERVE AND ASSOCIATED CHANGES

2.40 REGENERATION OF NERVE AND ASSOCIATED CHANGES

2.50 NEUROMAS

2.60 REVIEW OF ELECTRODIAGNOSTIC TECHNIQUES

CHAPTER II

LITERATURE REVIEW

Peripheral nerve degeneration and regeneration have been reviewed extensively, the most prominent reviews being Ranson (1912), Ramon y Cajal (1928), Young (1942), Guth (1956) and Sunderland (1968). From these reviews it was evident that a considerable amount of insight has been gathered on nerve responses to injuries. However, it was evident also that large voids and conflicts exist in this knowledge.

The reasons for these conflicts are multiple and very little work has been instigated to resolve these discrepancies. Inability to isolate and examine the variant factors independently has been the greatest difficulty. The reasons for these discrepancies are obvious at times (but not necessarily easily proven), while in other instances the reasons are not apparent at all.

Thus the results of neural responses should not be separated from their experimental setting. For example, Kline et al (1964)[ab] demonstrated a species variation in the rate and mode of neural regeneration. Numerous experiments have also demonstrated that type and extent of injury affect the mode and rate of neural regeneration. The level of injury also affects the rate and quality of regeneration. Clinical research and reports have indicated that younger patients have a more satisfactory neural regeneration.

The anatomical composition affects the quality and maturation of nerves. Pure (unmixed sensory or motor) nerves have a better prognosis than mixed nerves, Brown (1972); but which of these pure nerves has the best prognosis is conflicting.

2.10 CLASSIFICATION OF PERIPHERAL NERVE LESIONS

The importance of having a classification of peripheral nerve injuries is so that a simple and exact term can be used to describe a particular nerve injury and convey prognosis. At present there are two classifications to describe peripheral nerve injury. Seddon (1943) used three groups and Sunderland (1951) classified five groups of injuries.

The amalgamation of both classifications is presented below:-

(i) Neurapraxia (Seddon) or First Degree Injury (Sunderland)

This is the blockage of nerve conduction without loss of continuity of the axon. This conduction blockage is only transient.

(ii) Axonotmesis (Seddon) or Second Degree Injury (Sunderland)

This is where there is damage to the axon with subsequent Wallerian degeneration; the connective tissue including the Schwann cell and basement membrane remains intact.

Regeneration then occurs at approximately 1-2 mm/day and is usually effective unless the lesion is very close to the central nervous system.

The subsequent types of injuries are all classified as Neurotmesis by Seddon.

(iii) Neurotmesis (Seddon) or Third Degree Injury (Sunderland)

This is where there is damage to the axon and connective tissue, but preservation of the perineurium and fascicular architecture of the nerve.

Regeneration is less complete than (ii), but still relatively effective.

(iv) Neurotmesis (Seddon) or Fourth Degree Injury (Sunderland)

This is where there is damage to axon, connective tissue and perineurium, though the nerve remains macroscopically intact.

Regeneration is poorly orientated and less effective.

(v) Neurotmesis (Seddon) or Fifth Degree Injury (Sunderland)

This is where there is complete anatomical sectioning of the nerve. Regeneration is poorly orientated, least effective and usually requires surgical correction.

Unfortunately, peripheral nerve injuries cannot at all times be readily placed into the appropriate classification. Varying parameters associated with injuries in general and in particular with nerves, will affect diagnosis and prognosis. For example:-

(a) at times it is very difficult to diagnose the type of nerve injury on clinical grounds (except by direct vision) and even electrophysiological methods are of limited value and can only be utilized at appropriate times (this will be expanded later, vide infra 2.60),

(b) the extent of neural injury dictates the rate and extent of recovery and can prevent accurate diagnosis,

(c) the proximity of the injury to the nucleus affects diagnosis and prognosis,

(d) infections delay or even prevent nerve regeneration,

(e) multiple injuries of the same or different types to the same nerve complicate diagnosis and prognosis.

2.20 NEURAPRAXIA

Neurapraxic injury to the nerve fibre is when there is only a conduction block, no axon interruption and no Wallerian degeneration. Being a fully reversible blockage, there is complete and rapid restoration of function but this is after an inactive period. It is also noted that the conduction block is restricted to the segment damaged, the nerve fibre above and below this site continues to respond to electrical stimulation.

These types of injuries are caused by a comparatively mild trauma, moderate compression, slight stretching, or the passage of a missile near a nerve trunk. Neurapraxia is the most common type of neural injury but is the least studied because of technical difficulties and because it recovers spontaneously.

In experiments to examine these lesions direct pressure and/or ischaemia are used on the nerve. Technically it is very difficult to separate pressure and ischaemic effects from each other to study neurapraxia, so the relative roles of pressure deformation and ischaemia in the production of compression nerve lesions are controversial.

The return to normality has been studied on the electrical properties of these nerves and on the return to function of these nerves.

2.21 TIME TAKEN TO BLOCK CONDUCTION

The physiological tests of blocking nerve conduction to produce paralysis and sensory impairment after direct compression of the nerve or after the limb is rendered ischaemic, vary from 15 to 45 minutes depending on the experiment.

The electrical excitability of nerves subjected to mechanical deformation and ischaemia first increases and then declines, Thompson and Kimball (1936). This decline continues until the nerves cease to respond to electrical stimulation.

Some researchers even found a variation with nerve blocks using pressure or ischaemia. For example, the onset of paralysis and anaesthesia is delayed for longer periods after direct pressure than after cuff compression, Lewis et al (1931). However, Sinclair (1948) demonstrated that following direct pressure, anaesthesia occasionally appeared earlier than in the case of cuff compression.

2.22 VARIATION IN SUSCEPTIBILITY TO NEURAPRAXIA

At present the variation of nerve susceptibility to neurapraxia is very poorly understood because of the conflicting results and the conflicting conclusions. Thus an outline is presented of the various experiments and hypotheses.

The function of the nerve was considered (namely sensory nerves or motor nerves) to have a differing vulnerability to susceptibility to neurapraxic lesions. Allen (1938) concluded sensory fibres were more resistant to pressure than motor nerves but when considering ischaemic effects Magladery et al (1950) found the reverse. However, when the practical aspect is considered all factors indicate a greater susceptibility of motor fibres to compression than sensory fibres e.g. "crutch palsy", "Saturday night paralysis". In these cases, only some sensory modification occurs, if any at all.

The pattern of sensory failure to compression was considered by Sinclair and Hinshaw (1951). They concluded,

"It appears unlikely that it will ever be possible to say, in absolute terms and without numerous qualifications, that in nerve blocks in man one modality fails before another".

Although it can be generalized, these sensory modalities are lost in the following order:

proprioception

tactile

thermal

pain sensibility.

Next the fibre size variation was considered to be involved in the variation of nerve susceptibility to neurapraxia. Gelfan and Tarlov (1956) concluded that, based on electrophysiological studies, the largest fibres were relatively the most resistant. However, when an anoxic block was applied to the small "A" fibres it was found that they were more susceptible than the larger fibres.

Experimentally it has been shown that the larger diameter nerve fibres are compressed disproportionately higher than the smaller diameter fibres, Strain and Olson (1975). When Gasser (1943) carried out his experiments, he concluded that, in general, the larger the fibres the less susceptible they were to neurapraxia, although the size order was not rigidly followed.

At present the balance of evidence is against a size relationship of susceptibility to neurapraxia.

Generally, the interpretation of Lewis et al (1931) has been accepted, in that the rate of sensory and motor failure is dependent upon the distance

from the neural nucleus at which the ischaemia is applied. That is, at the proximal levels the nerves are more susceptible than at the distal levels. Also long nerve fibres are affected earlier than short nerves.

2.23 NEURAPRAXIC RECOVERY FOLLOWING RELEASE FROM COMPRESSION

The isolated nerve trunks constricted and blocked for 30 minutes by arterial sleeves recovered their conduction properties about 40 minutes after release from compression, Weiss and Davis (1943).

Transient but intense paraesthesia is felt in the limb and fasciculation of recovering muscles may be observed within seconds after restoration of circulation. These post-ischaemic effects are the clinical manifestation of abnormal activity in sensory and motor nerves in the form of repetitive discharges. Opinions differ as to the origin of these impulses; Weddell and Sinclair (1947) reported this activity in the peripheral sensory end organs and Lewis et al (1931) noted it in the segment of the nerve recovering from ischaemia.

The nature of the post-ischaemic response is age related, that is, the paraesthesiae decreases with age, Poole (1956). During ischaemic and post-ischaemic paraesthesiae the touch, pressure and pain stimuli, when applied to the area innervated by the nerve fibres which are discharging spontaneously, are either not felt or are felt in a diminished degree, Nathan (1958).

2.24 PROLONGED CONDUCTION NERVE BLOCK

The mildest compression lesion can produce transient effects on nerves that last for seconds, minutes or even hours. However, with progressively increased compression a point is reached where recovery

does not rapidly follow release of pressure but is delayed for days or weeks. This prolonged conduction nerve block can be produced by acute or chronic compression but since the condition is fully reversible there is very little human material available for histological examination, so most of the information is from laboratory experiments on animals. Denny-Brown and Brenner (1944[ab]) found, on anatomical grounds,that this prolonged conduction blockage was due to demyelination with the preservation of axon continuity at the site of compression.

Gelfan and Tarlov's (1956) demonstration suggested electrophysiologically that the effects of compression to produce a conduction block were due to mechanical deformation and not to the associated ischaemia of the compressed segment. Also the recovery from anoxia when it occurred was rapid in comparison with the mechanical deformation which had a relatively long recovery latency. This was in agreement with the clinical results of Bentley and Schlapp (1943) who found the development of a nerve block due to pressure was much slower and that persistence of the block was in marked contrast to the rapid recovery that followed relief of ischaemia.

Weiss and Davis (1943) went further to explain the basic component and suggested that consideration should be given to embarrassment of the "centrifugal flow" inside the axon of substance, or factors vital for the maintenance of axon integrity as a contributing factor to the conduction block.

The acute compression lesions involved in producing prolonged conduction nerve blocks will not be considered further because the

role of mechanical deformation in nerve block production has been convincingly demonstrated. It is the chronic compression lesion mechanism which remains in doubt. Aguayo et al (1971) reported segmental demyelination and a reduction in the number of thick myelinated fibres, but were unable to exclude ischaemia as a possible aetiological factor.

Despite the importance and significance of these findings, they should not be divorced from their experimental setting, for it is possible that, under different conditions, the pathogenesis of these lesions might well follow a different pattern.

2.30 DEGENERATION OF AXONS AND ASSOCIATED CHANGES

When a mild distorting force is applied to a nerve there is only physiological alteration, but as this distorting force is increased in intensity, eventually anatomical alteration can be observed microscopically. These nerve reactions are studied under the degenerative and regenerative responses. It is not possible to make a sharp distinction between these two processes since almost from the onset the degenerative response is a preparation for the regenerative response.

The degenerative aspect considered pertinent to this investigation is the nerve's responses distal to the lesion "Wallerian Degeneration", at the lesion site and the retrograde fibre reaction proximal to the lesion.

2.31 RETROGRADE FIBRE REACTIONS

The pattern of reaction to the injury in the proximal and distal nerve segment is the same, but in the proximal nerve segment the Schwann cell proliferation is not as great, Logan et al (1953). The changes that occur in the nerve segment proximal to the injury are directed centrally and the extent varies with the severity of the injury.

In severe injuries, with the rupture of the endoneurium, the degeneration may extend proximally for several centimeters. While in injuries where the endoneurium has not been ruptured, the retrograde degeneration is usually confined to the adjacent few millimeters of the fibre. Lubinska (1959) reported that the retrograde effects were usually limited to the immediate neighbourhood of the lesion with careful crush injuries. The limit of proximal degeneration of Schwann cells is dependent upon the preservation of the nuclear region.

Physiologically, the nerve segment proximal to the injury has a reduced conduction velocity.

2.32 DEGENERATION OF AXONS AND MYELIN

Axon changes are evident within 24 hours of the injury and take the form of initial swelling, followed by clumping together and fragmentation of the neurotubules and neurofilaments. The axon soon develops varicosities and 48-72 hours after the injury, breaks up into twisted fragments which are dispersed along the fibre.

Nodal and paranodal accumulation of mitochondria, multi-vesicular bodies, lamella bodies and small vesicular and tubular profiles are seen at a proportion of nodes, Ballin and Thomas (1969). This degeneration is believed to develop in a centrifugal pattern in at least some of the fibre systems according to the conclusion of Joseph's (1973) review. However, others, like Sugar (1938), believed the degeneration was uniform along the length of the fibre and somewhat delayed at the nodes of Ranvier, Weddell and Glees (1941).

Conduction of impulses in the distal segment ceases with the loss of axon continuity. So conduction failure occurs after a period of 3-5 days after the injury in humans, Landau (1953) and this is in agreement with numerous experiments on animals.

While these axon changes are occurring a space is developing between the axons and the myelin sheath. There is early widening of the gap between adjacent myelin segments at the nodes of Ranvier. The changes in the myelin sheath become evident 28-96 hours after the injury, as by this time the axon is in advanced degeneration. Myelin sheath degeneration can be observed first physically and then chemically.

Physical Degeneration

The myelin sheath develops irregularities exhibited as folding lamella, splitting and fracturing, eventually forming droplets that ultimately enclose the axon debris. These physical changes are completed in about 8 days, at which time the chemical degeneration commences, Johnson et al (1950).

Chemical Degeneration

The exact nature of the chemical events in the critical early stages of nerve degeneration is not clear. This chemical degeneration is a rapid breakdown of complex myelin fats into simpler fats. There is preliminary evidence that these lipid changes may be preceded by some breakdown of the myelin proteins, Adams et al (1972). It is not known if protein breakdown simply precedes or actually allows the initiation of lipid breakdown.

As the myelin and axon debris is removed by phagocytosis the products of chemical disintegration disappear from the scene.

It is generally accepted that myelin degeneration in most cases is completed by the fourteenth day, but can take longer.

2.33 SCHWANN CELL PROPERTIES

After injury the axon and myelin changes are followed by the Schwann cell reaction. Even though there is a delay the reaction is evident 28 hours after the injury. The Schwann cell reaction is of a hyperactive type, as the changes are consistent with increase of protein synthesis - nucleus enlarges, more cytoplasmic granules. This process continues for the next 48 hours when Schwann cell proliferation is thought to commence.

The initiator of Schwann cell proliferation is thought to be a chemical mediator liberated when the myelin and axon disintegrate or by the physical factors introduced when they collapse, Sunderland (1968).

Controlling factors of Schwann cell proliferation are not understood, but it has been observed that there are species differences and diameter of nerve differences. Thomas (1948) found that the larger the nerve

fibre the greater the cellular proliferation.

In recent research there is reasonable agreement as to when the maximal proliferation occurs. The range extends from 3 days after injury, Bradley and Asbury (1970) to Friede and Johnstone (1967) who found it to be 4-11 days after injury. However, Lee (1963) maintained that no mitotic figures were seen in the Schwann cells.

Schwann cell proliferation declines rapidly when the cell columns originating from the proximal and distal trunks contact each other. This would seem to indicate the duration of the proliferation is dependent upon the size defect resulting from the sectioned nerve, Jurecka et al (1975). There is some conflict on the degeneration of myelinated nerves and unmyelinated nerves. The Wallerian degenerative changes of unmyelinated axons are similar to those of myelinated axons, but follow a slower time course. In addition the compact syncytium of Schwann cells in the endoneurial tube (the bands of Bunger) observed in established degeneration of myelinated axons are not observed in unmyelinated axons, instead there are multiple flattened processes disposed in an irregular manner when viewed on a transverse section, Thomas and King (1974).

The function of Schwann cells in Wallerian degeneration is not entirely understood, but it has been suggested they may:

(a) provide enzymes to destroy the myelin

(b) act as macrophages.

2.34 MACROPHAGES

Macrophages are prominent in the endoneurial tubes at about the third day after injury. The removal of neural debris is continuous into the fourteenth day. Then there is a slow down of activity and it usually finishes by the forty-eighth day after injury. Once they complete their

phagocytic activity they move out of the endoneurial tubes leaving only the Schwann cells.

The origin of the phagocytes in degenerating peripheral nerves has been a controversy for many decades. Most research maintained that the origin of phagocytes in neural degeneration was mainly from the Schwann cells. The most serious challenge to this concept came from the autoradiographic studies of Olsson and Sjostrand (1969). They proved that macrophages appearing in the endoneurium of the distal nerve trunk are derived also from blood leucocytes.

Then Stenwig (1972) suggested a dual source of phagocytes, depending upon the type of injury. In sectioned lesions, a substantial number of the phagocytes are of haematogenous origin, whilst in pure Wallerian degeneration or retrograde degeneration the phagocytes are only of endogenous cells.

This was further modified by Berner et al (1973) where their results suggested that in sectioned nerves some of the macrophages were of haematogenous origin.

The source of the macrophages in pure Wallerian degeneration can be from the Schwann cells or may conceivably be derived from other endogenous cells, Williams and Hall (1971).

These hypotheses were tested by Mei Liu (1974) who showed that degeneration of Schwann cells occurs with the onset of myelin breakdown and that the degeneration products of myelin, axon and Schwann cells are removed by macrophages. While most of the macrophages were originally blood monocytes, some were derived from vascular pericytes. They penetrated the neurilemmal tubes on the third post injury day and began engulfing first the Schwann cells and then the myelin and axons.

2.35 DEGENERATION OF AXON AND OTHER STRUCTURES WITH THE LOSS OF CONTINUITY OF THE NERVE FIBRE

With the severance of the nerve fibre, additional features are superimposed on the simple pattern of Wallerian degeneration already discussed. The areas of interest are:-

(i) Retrograde changes in the nerve fibre.

(ii) The reaction at the severed ends of the nerve fibre.

(iii) The effects of prolonged denervation on the distal nerve segment.

(i) Retrograde Changes in the Nerve Fibre

The fibres in the proximal stump are reduced in diameter progressively after sectioning if peripheral contact is not re-established. Cragg and Thomas (1961) showed that this reduction occurs in the axon diameter and in myelin sheath thickness. In addition there was an associated progressive reduction in conduction velocity. The retrograde diameter reduction has been attributed to the outflow of axoplasm that occurs during regeneration, to the failure of the regenerating axon to establish appropriate connections with the periphery and to disuse.

(ii) The Reaction at the Severed Nerve Ends

When the nerve fibres are sectioned, the segments are separated to a variable degree and the gap is filled with a blood clot. The proximal ends of the nerve swell because of the oedema and the non-specific cellular response that occurs in all injured tissue. There is also no longer a restriction to the regenerating axons and the proliferating Schwann cells to remain within the confines of the endoneurial tubes. The Schwann cell activity at and between the ends are at their height when those within the endoneurial tubes are at their peak. In addition to the Schwann cell proliferation there is a fibroblast proliferation.

Collagen is deposited in the gap between the severed ends and its extent and density greatly influences axon regeneration.

(iii) The Effects of Prolonged Denervation on the Distal Nerve Segment

When the nerve is sectioned, the regenerating axons have to traverse varying distances of connective tissue before entering the distal endoneurial tubes. This means the regenerating axon can be delayed in moving down the endoneurial tube or may not reach the endoneurial tube.

The denervated endoneurial tubes are swollen initially, but by the twelfth day the lumen is becoming smaller. This reduction is proportional to the diameter and is a continuous progressive time dependent process. Over the first three months the reduction is rapid and after this there is only a gradual reduction, Sunderland and Bradley (1950[ab]).

The mechanism of the endoneurial tube reduction is thought to be mainly due to folding and not to the progressive deposition of collagen at the expense of the lumen. This folding is due to the loss of intracellular pressure from the degenerating axons and myelin and their removal. Because of this reduction in pressure the endoneurium loses tension and so collapses. Deposition of the new endoneurial collagen is on the external surface of the basement membrane of the Schwann cell and this causes a sheath thickening, Thomas (1964).

Sunderland (1949) found that despite the narrowing of the endoneurial tube, it will still receive and guide regenerating axons

to the periphery. The collagen will hinder expansion of the endoneurial tubes on re-innervation and so prevent the restoration of normal axon diameters, Abercrombie and Johnson (1946).

As a result of endoneurial tube shrinkage in general there is a reduction of the funiculi, in a similar pattern to that of the endoneurial tubes, Sunderland and Bradley (1950[ab]). In comparison the epineurial tissues do not react in the same way and undergo little change.

2.40 REGENERATION OF NERVE AND ASSOCIATED CHANGES

After the nerve has been injured, before any regeneration can take place, the neuron must recover from the insult. The metabolism has to be re-organised, from that of just functioning and conducting normal maintenance, to that of establishing a new equilibrium and of manufacturing new cytoplasmic material.

This is a physio-chemical process that cannot be directly visualized but the organelles involved in this process can be observed because of their altered morphological features. The organelles involved are particularly those in the soma and especially those in the nucleus. The cell body response will not be dealt with because it is not directly related to the investigation.

2.41 REGENERATION IN UNDAMAGED ENDONEURIUM

Retrograde degeneration results in the axon tip being some distance proximal to the initial injury site. So the regenerative process commences as the axon tip starts to move to the injured site. Axon branching can occur in this region, but is not easily observed until it reaches the injured site. Shawe (1955) observed axon branching 6mm above a crush injury in a motor nerve.

Quantitative estimates of terminal branching are difficult to obtain as it is not an important feature with this type of injury and the results vary with each experiment. Shawe (1955) found, on an average, each axon gave three terminal branches and also suggested that larger axons formed more sprouts than smaller axons.

If branching does occur it is short lived, only one branch survives, and is usually the one that has moved further peripherally down the endoneurial tube and the other branches are resorbed, Denny-Brown and Brenner (1944[c]).

The progress of the axon tip along the endoneurial tube is determined by the resultant action of three forces:

(a) The central force from the soma that propels the axon tip distally, Young (1945).

(b) The axon tip is a specially organized growth cone which, as it moves distally, elongates the axoplasm, Sunderland (1968).

(c) These two forces must act in overcoming the tissue resistance, to allow the axon to progress peripherally.

No mention is made in the literature of the relative importance of the central force versus the growth cone force.

Watrous's (1940) laboratory results suggest that the terminal branches are capable of transmitting the axon reflex.

The axon regeneration within its own intact endoneurial tube is not greatly impeded and is only temporarily slowed down at the injury site.

The presence or absence of debris does not seem to significantly affect axon growth, although Lubinska (1952) concluded that degeneration must reach a certain stage before axon branches will grow into the denervated tubes.

2.42 REGENERATION COMPLICATED BY SEVERANCE OF THE ENDONEURIAL TUBE

The regenerating pattern is complicated with the severance of nerves. This is because whenever a nerve is sectioned a scar develops at the site. The regenerating axons have to cross this scar which is never a suitable medium to propagate axon growth.

Loss of the endoneural integrity affects the regenerating axons so that there is a more extensive and more variable branching than when the endoneurium remains intact. A single regenerating axon may give rise to as many as 50 sprouting axons, Weddell (1942). As these axon branches enter the scar tissue they follow the path of the least resistance and so this medium directs the axons and also impedes their progress. Even when some of the regenerating axons have traversed the scar tissue and are moving down the endoneurial tube they can be deprived of essential factors that are required for their maturation, Weiss and Taylor (1944).

Choice of which endoneurial tube is entered by the axon branches is not understood. Experimental evidence has failed to demonstrate any preference of regenerating axons in entering their original endoneurial tubes. The original axons of a motor nerve do not have any advantage over any other motor axon in re-innervating their muscle, Bernstein and Guth (1961). Also the axons of unmyelinated fibres grow into and along the endoneurial tubes of myelinated fibres in preference to their original pathway, Evans and Murray (1954).

Sunderland (1953) was the only researcher to show any specificity. He found that regenerating axons may have an affinity not only for a distal stump but the distal stump of the same nerve.

Due to the apparent lack of axon specificity for their original endoneurial tubes, the following possibilities can occur:

(a) Single axon develops in an endoneurial tube. The success will depend upon function of the axon and what type of receptors are at the end of the endoneurial tube.

(b) Branches of a single axon enter more than one endoneurial tube. This could result in complicated, reduced or even improved

chances of useful regeneration.

(c) Several axon branches may reach, enter and move down the endoneurial tube.

Davenport et al (1937) reported that as many as five unmyelinated axons can be found in one endoneurial tube and one to three, rarely four, of these eventually become myelinated; but Weddell (1942) observed that ultimately one dominates and the others fail to survive. However, Holmes and Young (1942) reported that two, three or even more fibres may become myelinated within a single tube, and it may be that each develops its own endoneurium.

The mode of regeneration under ideal conditions in the distal segment is similar to when the endoneurium is intact, but is slower and normal dimensions are rarely obtained. Regeneration has been clinically demonstrated even with prolonged denervation, over 12 months, Sunderland (1949).

Myelination of the regenerating axons that have traversed the scar and are moving down the endoneurial tubes proceeds in a centrifuge pattern, but is somewhat delayed when compared to cases where the endoneurium is undamaged. There is lack of agreement as to when myelination commences although the diameter of the axons seems to influence the onset of myelination.

Myelination of the bare axon follows the progress of the axon tip as it moves down the endoneurial tube. The mechanism is that a fine myelin layer is deposited between the Schwann cell and the axon, and it proceeds in the same manner as it does in neurogenesis.

Commencement of myelination varies with type of injury and animal, the range being from 6-21 days after injury.

The Schwann cells become re-oriented so that there is one Schwann cell to each myelin segment, thus restoring the normal arrangement, but now unlike the normal nerve fibre the internode lengths are all approximately the same length, Hiscoe (1947). The axon diameter continues to enlarge after the appearance of myelin until the original dimensions are restored. That the number, size and pattern of fibres return to the normal state was demonstrated experimentally by Gutmann and Sanders (1943). Final development was not reached until end organ connection and functional relationships were re-established. If both criteria were not met a permanent reduction in size was observed, Weiss et al (1945).

(i) Clinical

Only physiological tests can be and have been carried out clinically, as biopsies would destroy many of the regenerating fibres. Therefore insufficient histological material is available and there is inadequate data to establish a definite correlation between the morphological and physiological responses.

Functional recovery in the system requires more than just a re-establishment of anatomical continuity between the neuron and the end organ because there is a delay between the re-establishment of anatomical continuity and the onset of voluntary control of the effector organ.

Sunderland (1968) considered that at least three possibilities could explain this delay phenomenon.

1. Changes in the structure of the nerve fibre leading to functional maturation. The diameter and degree of myelination of the fibre are two known morphological features that influence conduction properties but there are probably others.

2. Analogous changes at the end organ leading to the effective union with muscle fibres.

3. A minimal number of mature nerve fibres must be present before the muscle will respond to voluntary effort.

The relative importance of these factors has not yet been determined.

(ii) Laboratory

Animal experiments have been conducted on the histological investigation correlated with the conduction velocity investigation. One of the earliest was by Berry et al (1944). The changes in the threshold of excitability, and the maximum conduction velocity with regeneration time, did follow the change in diameter of the nerve fibre, i.e. slow initially and then increased in conduction velocity as regeneration progressed.

Since then Cragg and Thomas (1964) concluded that there are other factors than fibre diameter that contribute to the reduction in conduction velocity. These factors are yet to be determined. The consensus of opinion is that the histological appearance (axon diameter and degree of myelination), conduction velocity and action potentials rarely if ever return to normality after the nerve has been severed.

2.43 RATES OF REGENERATION

Various values are given for rates of regeneration, this variation is due to both biological and technical reasons. Another problem is lack of agreement on what constitutes recovery.

The rates of motor and sensory nerve regeneration are basically studied by the following methods:

(a) Histological Methods

- axon regeneration rate,
- myelin advancement rate.

(b) Electrophysiological Methods

(c) Function Recovery Assessment.

Each of these are very important in understanding the rate of regeneration. All these methods have intrinsic limitations and also advantages and disadvantages over each other. From the researcher's point of view, all these broad categories are important, but from the clinican's point of view, the electrophysiological and functional return rates are important. However, to the patient, the rate of functional return is of utmost importance.

The underlying limitation to all these methods is that they are based on the detection of the fastest growing group of fibres. In addition, the rates of nerve regeneration are not uniform and apparently the rate of regeneration de-accelerates as the axon tip progresses distally. This means that the rate of regeneration of the axon tip does not have a uniform velocity. This is the case for humans, as has been demonstrated on numerous occasions, but the regeneration rate in animals is constant, Gutmann et al (1942) and others. The reason for this difference is thought to be due to the small length of nerves in the experimental animals, so the variation in rates of regeneration is not so apparent.

Use of the above methods has shown that histological regeneration is the fastest. Within this method the rate of axon regeneration is faster than the myelination rate. This is followed by the electrophysiological method. The slowest rate is that of functional return. Seddon (1972) summarized

it best by stating,

> "The melancholy conclusion after all the work that has been done on rates of regeneration, although illuminating in itself, is that for practical purposes the old rule of a millimetre a day....."

2.50 NEUROMAS

Traumatic neuromas arise with all total severance injuries and can form bulbar neuromas or amputation neuromas where the distal neural segment is removed, but they can also arise where some of the funiculi lose their perineurial integrity and form lateral neuromas. Spindle type neuromas occur when the perineurium has not lost its integrity.

The size and symptoms of all these neuromas vary considerably. Only the large or symptomatic lesions are clinically termed traumatic neuromas, while the small and asymptomatic neuromas tend to be overlooked.

2.51 REACTION AT THE PROXIMAL END OF THE STUMP

The progress of the regenerating axons is dependent upon the barriers encountered, because the axon branches follow the path of least resistance.

The neuroma is composed of axons, Schwann cells, endoneurial cells, perineurial cells and epineurial cells. Proliferation of these various cells follows a different temporal pattern. The epineurial cells start to proliferate on the first day and continue for a long time. Proliferation of the perineurial fibroblasts commences on the third day and then declines, but the endoneurial fibroblasts do not seem to play an important role during regeneration, Jurecka et al (1975).

The cells involved in the formation of minifunicules that develop from the neuromas are Schwann cells, epineurial fibroblasts and perineural fibroblasts. The connective tissue cells that precede the outgrowing Schwann cells are possibly of epineural origin, Jurecka et al. (1975).

Regenerating axons that do not enter the endoneurial tubes are found to:

(a) become entangled within the scar tissue.

Some of these axons will degenerate.

(b) move off into connective tissue. They can wander over long distances into surrounding tissues along fascial planes. These axons appear to influence the direction of growth of adjacent axons so that closely bunched groups or minifuniculi are formed. It should be stressed that most of the axons manage to penetrate the scar tissue.

Within limits myelination keeps pace with the regenerating axons both inside and outside the neuroma. Axons near the proximal nerve trunk are often myelinated, but in the distal zones the myelinated axons are rare or non-existent. There is also a predominance of unmyelinated axons in the neuroma when compared to normal nerve trunks.

Once the neuroma is formed, there seems to be little change, though there is maturation of the connective tissue, some loss of neural elements and a relative increase in the Schwann cell population.

2.52 REACTION AT THE DISTAL STUMP

The reaction is similar to that of the proximal stump but any axons that are present have reached the area from the proximal stump. In most cases the Schwann cell-fibroblastic reaction in the distal site is insufficient to form any enlargement or is too ill-defined for observation, but it does enlarge if the regenerating axons do reach this distal site. Even so this enlargement remains smaller than the proximal enlargement.

2.60 REVIEW OF ELECTRODIAGNOSTIC TECHNIQUES

The most widely used clinical and laboratory technique to study nerve injury is to use clinical observation in conjunction with electrophysiological investigation and histological examination in appropriate instances.

The reasons for the use of electrodiagnostic techniques are that the degree of impairment and recovery can be quantitated and only reasonable co-operation of the subject under investigation is needed.

The aim of this chapter is to present the fundamental aspects of a complex and expanding subject. The technical aspects will not be considered in any detail, except to present certain principles.

Electrodiagnostic procedures fall into three major categories in which there are multiple variations that will not be discussed.

2.61 EXCITABILITY OF NERVES AND MUSCLES

The principle of this technique is that nerves and muscles are excitable at different levels. That is, the minimal stimulus that will activate an innervated muscle is insufficient to activate a denervated muscle. At present the technique that has proven most satisfactory and is in widest use using this principle is the "strength-duration test".

The stimulator used for this test has a variable milli-voltage and a variable current pulse in milli-seconds. Only one of these is varied at a particular test, usually the duration. This means for a particular voltage the current pulse is increased until there is an initial response. Then the voltage is changed for the next variation of current pulse duration. When sufficient tests have been carried out the summation of these results produces a characteristic strength duration curve, Figure 2.61.

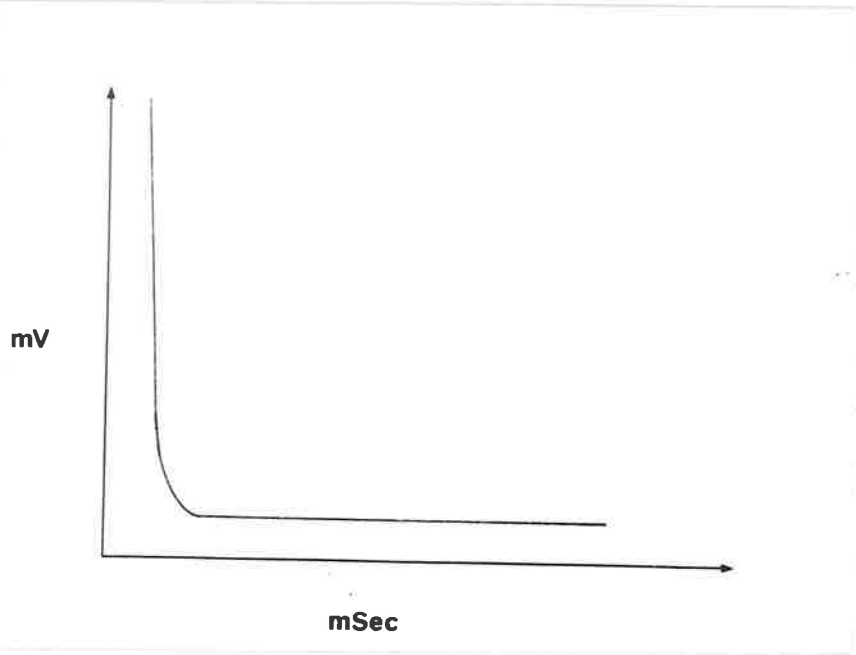


Fig. 2.61

To ensure uniformity of these results:

(a) a square wave form is used,

(b) tested area is marked,

(c) electrodes are in light contact with clean moist skin,

(d) frequency of the stimulus is maintained at a constant level (usually 2 per second).

The minimal milli voltage used to stimulate the nerve is termed the "rheobase". "Chronaxic" is the time duration which is twice the rheobase voltage, figure 2.62.

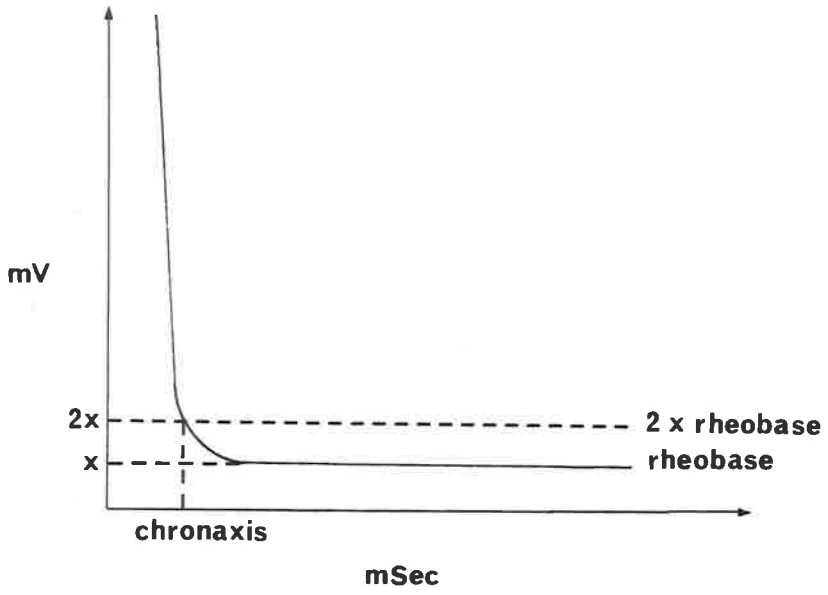


Fig. 2.62

When a muscle is completely denervated the graph takes a different form. The curve has a shallower gradient and the chronaxic is much longer, i.e. the curves move up and to the right, figure 2.63.

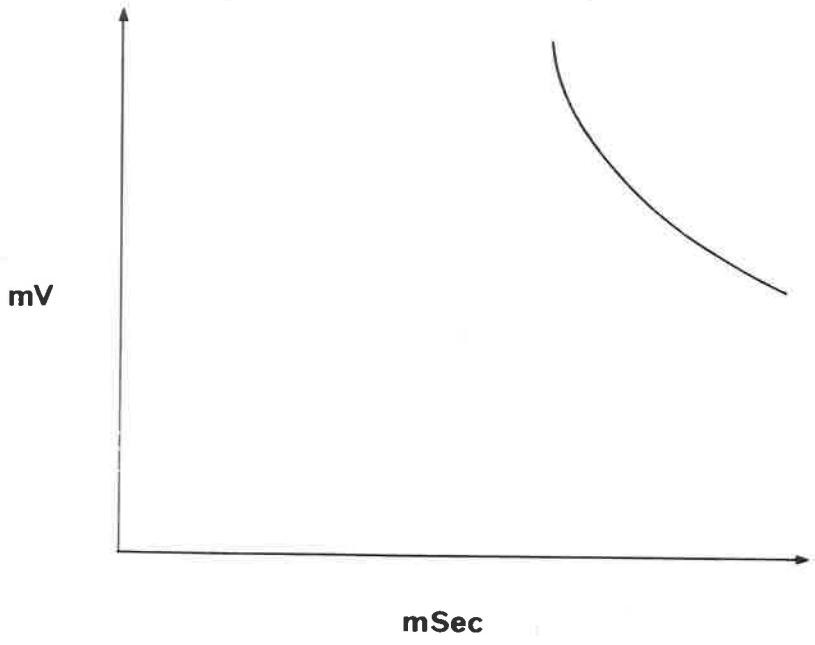


Fig. 2.63

As the nerve becomes re-innervated, a kink is found in the strength duration curve, figure 2.64.

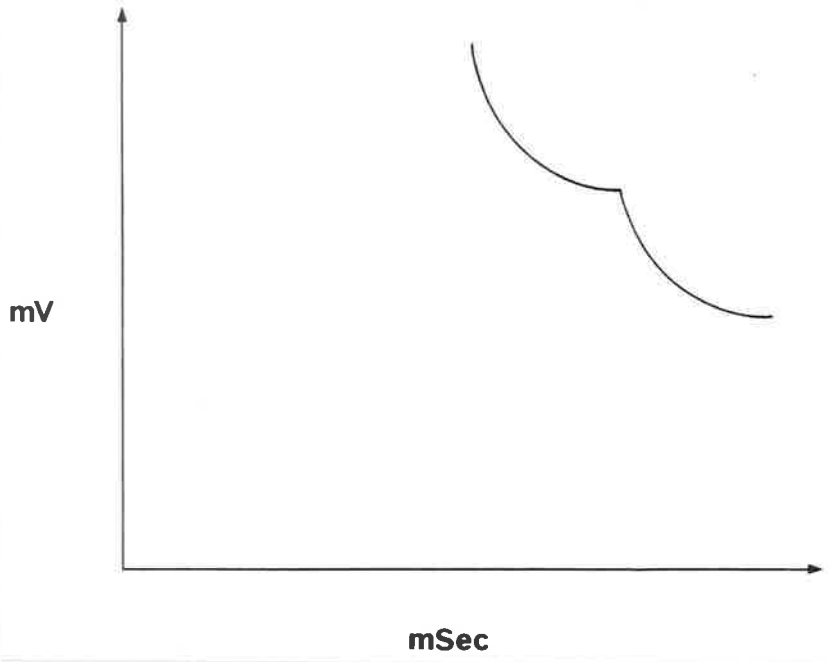


Fig. 2.64

With the progressive re-innervation and maturation of nerve fibres, the curve takes a more flattened out and downward shift, figure 2.65.

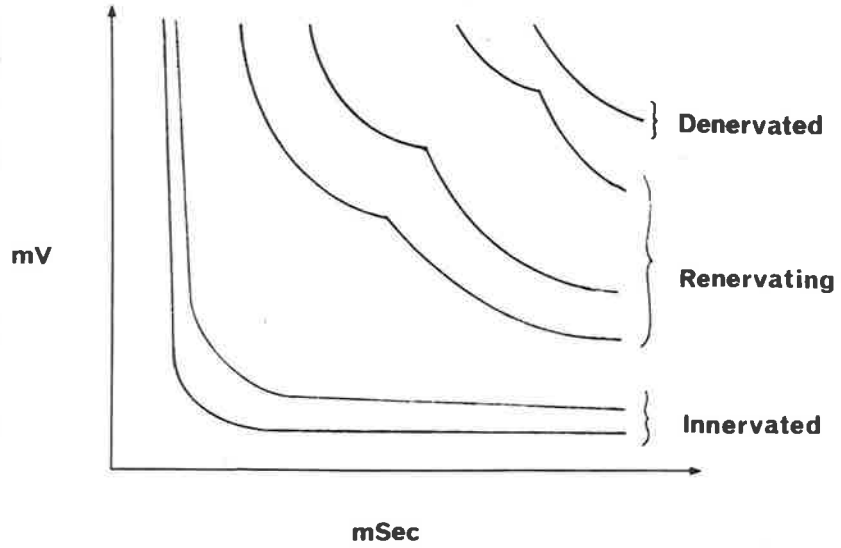


Fig. 2.65

The strength duration curve is really the nerve response to stimulation and so is not useful until seven days after the injury has occurred, i.e. when axons have undergone Wallerian degeneration. The changes in the strength duration curve usually appear before there is clinical evidence of neural recovery.

2.62 NERVE CONDUCTION MEASUREMENTS

The principle of this technique is to record the conduction velocities, conduction latency and nerve action potentials of stimulated nerves, as these physiological measurements are affected with neuropathy. Changes of these parameters are evident 3-7 days after neural injury, or until Wallerian degeneration reaches a point that conduction along the degenerating nerve is no longer possible. This failure of response after 3-7 days is the earliest evidence of severity of an injury and excludes from further consideration the neurapraxic type of injury.

This technique is of value in the recognition of lesions due to local pressure, demyelinations and total sectioning of nerves. It is of relatively limited value in the recognition of partial denervation. Since unsectioned nerves conduct at normal rates, these then block the observation of other impulses.

It should be noted that each nerve has its own conduction velocity. The value of these conduction velocities varies with different recording devices and stimuli, age of patient, Thomas and Lambert (1960), temperature, Abramson et al (1966) and region of the nerve to be tested.
The technique can be used on:-

(i) Motor Nerve Conduction Velocity.

This is obtained when the motor nerve is stimulated with a short duration pulse and the evoked muscle potential is displayed on a cathoderay tube. When the stimulus is allowed to trigger the oscilloscope sweep, the "latent period" is measured. However, when the nerve is of sufficient length it can be stimulated at different points and the latencies are subtracted and divided into the distances between the distal recording oscilloscope and the stimulator, the "conduction velocity" is calculated. This technique is simple in theory, the problem is to apply it clinically.

(ii) Sensory Conduction

A similar principle applies to sensory conduction studies but they are harder to record. Abnormalities in amplitude or latency provide a sign of peripheral nerve lesion, Gilliatt and Sears (1958).

2.63 ELECTROMYOGRAPHY

Electromyography is the technique by which the action potentials of contracting muscle fibres and motor units are monitored.

This monitoring provides information on structure and function of motor units. It may also be possible to localize the site of pathology affecting either muscle or its innervation and, in addition, it may frequently provide evidence regarding the nature of the pathological process.

The principle of this technique lies in the fact that whenever a muscle fibre contracts, the surface membrane undergoes depolarization so that an action potential can be recorded from the fibre. When the fibres of a motor unit are activated, they contract nearly, but not quite synchronously, and their action potentials summate so that a relatively large complex potential, known as the motor unit action potential, can be monitored.

This monitoring can be carried out by use of surface electrodes applied to the overlying skin, but for diagnostic purposes it is preferable to employ concentric needle electrodes. These are inserted into muscles, Adrian and Bronk (1929). They consist of a hollow needle surrounding an insulated wire core which is exposed at the tip. Such electrodes record muscle potentials in their vicinity. This information is displayed on a cathode-ray oscilloscope and frequently is also reproduced through a loud speaker.

The amplitude recording on the cathode ray oscilloscope is roughly proportional to the number of motor units activated in voluntary contraction. Between contractions the relaxed muscle is electrically inactive, but denervated muscle starts to fibrillate 14-28 days after a nerve injury. This is due to contraction of individual muscle fibres, which is most easily detected by electromyography. These action potentials have an amplitude of only 100 microvolts, compared with the 2 millivolts of a motor-unit action potential. The duration of a motor unit action potential is much longer than that of the fibrillation action potential.

With muscle potentials, the duration of the potential is of interest, because slowing of conduction in a few fibres of the motor nerve may result in late activation of part of the muscle and the potential may be both prolonged and polyphasic. When nerve action potentials are recorded temporal dispersion due to slowed conduction in some of the fibres may lead to a significant decrease in amplitude.

CHAPTER III

MATERIAL AND METHOD

3.10	CHOICE OF ANIMAL
3.20	CHOICE OF RATS
3.30	TYPE OF INJURY
3.40	SURGICAL APPROACH
3.50	POST OPERATIVE CARE
3.60	BASIC HISTOLOGICAL METHOD
3.70	PROTOCOL FOR HISTOLOGICAL INVESTIGATION
3.80	PROTOCOL FOR ELECTROPHYSIOLOGICAL TESTING

CHAPTER III

MATERIAL AND METHOD

3.10 CHOICE OF ANIMAL

The most readily available laboratory animals were considered for this investigation, e.g. rabbit, guinea pig, mouse and rat. These animals were considered because they were easily housed, cheap to buy and easy to handle.

For this investigation the rat was the animal of choice. The reason for this was that it has a mandibular nerve of sufficient size to be operated on, unlike the mouse. It has preference over the rabbit and guinea pig because it occupies less space in the animal house, so more animals can be studied; but the most important reason why the rat was chosen was its ability to withstand surgical manipulation and general anaesthesia with low mortality.

However, the rat is not without limitations, because the mandibular nerve has no large blood vessels accompanying it as in humans and, because of the small area innervated by the nerve, physiological testing is difficult.

3.20 CHOICE OF RATS

To maintain some type of uniformity in the experiment and in the results, arbitrary restrictions were placed on the choice of rats. The variety of rat selected was the Sprague Dawley strain. Healthy young (2½-3 month old) male rats were used in the experiment. Age is important because it affects the healing rate. Males were chosen because they are larger than females. Only rats weighing between 220 and 280 grams at the beginning of the experiment were chosen.

3.30 TYPE OF INJURY

The type of injury studied was selected from the most common types of mandibular nerve injuries that occur or are treated in dentistry. These can be classified under two broad headings "chemical" and "mechanical" injuries.

Chemical injuries occur with injection or introduction of chemicals (e.g. endodontic pastes) close to or into nerves.

Physiological injuries are partial or total sectioning of nerves, crushing of nerves, removing sections of nerves or overstretching nerves. These types of injuries can occur singly or in varying combinations.

The most common type of injuries are those of mechanical origin, so these were the type examined. To obtain an accurate picture only one type of injury was considered in isolation. The choice was based on which injury could give the most predictable and consistent results.

The clean transverse total section of mental nerve was the one chosen because it could be easily reproduced under controlled conditions using the available equipment and animal selected. The importance of this fact cannot be over-emphasized because this injury was going to be studied in continuum with different animals over a varying period of time. Because the animals were fairly well inbred and had to comply with the arbitrary restrictions imposed, their responses should have been similar to identical injuries over the same period of time. So a composite picture could be developed, over a period of time, of tissue response to this type of injury.

3.40 SURGICAL APPROACH

An ideal surgical approach to study mandibular nerve injuries has to have zero mortality with minimal morbidity, if any. The access to the

nerve must be easy and rapid. Physiological testing of the nerve should be possible. From the histological aspects minimal serial sections should be required and there should be sufficient land marks to find easily the injury site and to orientate the tissues in the desired plane for examination.

Various surgical approaches have been described for the sectioning of the mandibular nerve in the rat, Steward (1965), Retief and Dreyer (1969), Torneck and Harnett (1971) and Hoffman and Tade (1972). Because these approaches were not specifically designed to study nerve injuries, they have inherent limitations and lack some or many of the ideal properties; so the author has designed a different surgical approach (Hribar 1978).

The animal was anaesthetised with Sagatal (for dosage see appendix I). Then the rat was placed in a supine position on the operating table and stabilized via elastic bands placed on the limbs and one around the head, (figure 3.1).

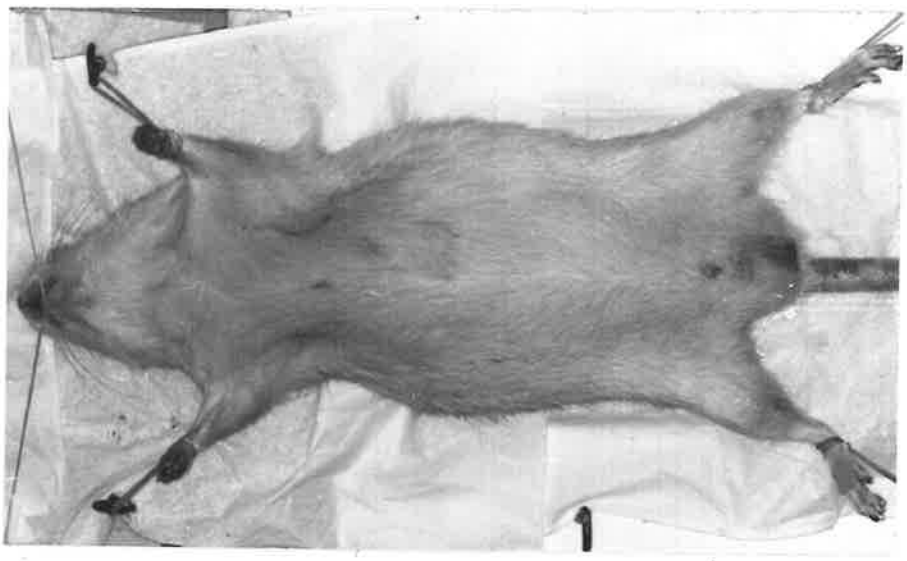


Fig. 3.1 Anaesthetized rat in the operating position on the operating table.

A skin incision was made at the lower border of the body of the mandible. When the skin was elevated and retracted by the mosquito artery forceps the periosteum was incised avoiding the facial artery. Then a blunt dissection was carried out using a wax spatula number 7 to separate the periosteum from the bone. This readily exposed the mental nerve, which was sectioned in a transverse direction approximately 5mm distal from the mental foramen. The skin was then sutured with 3/0 black silk suture.

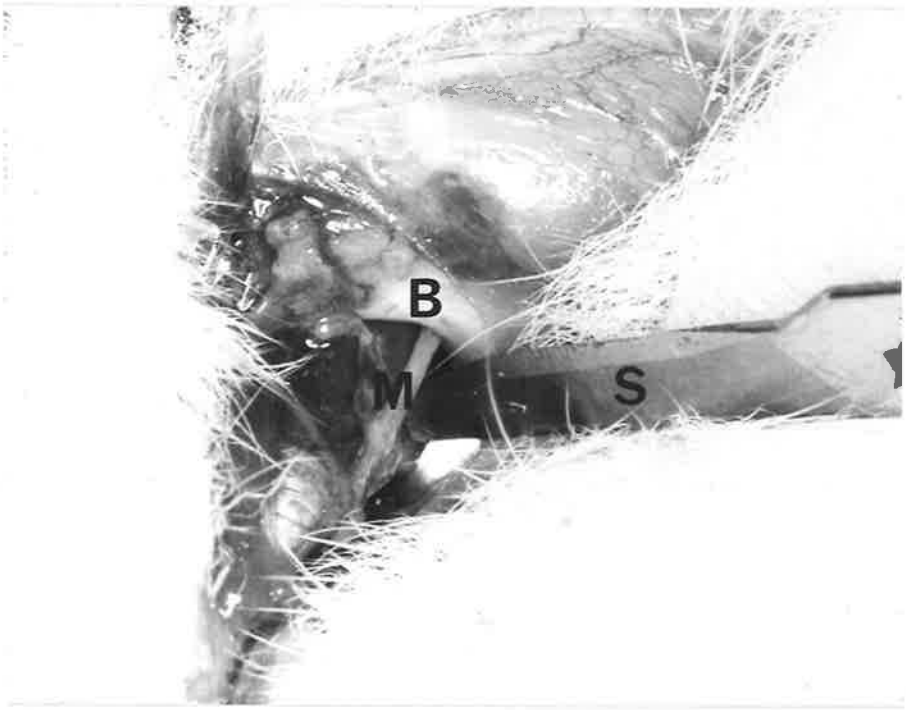


Fig. 3.2 Exposed mental nerve about to be sectioned with scalpel blade(S). Bone (B) and Mental Nerve (M).

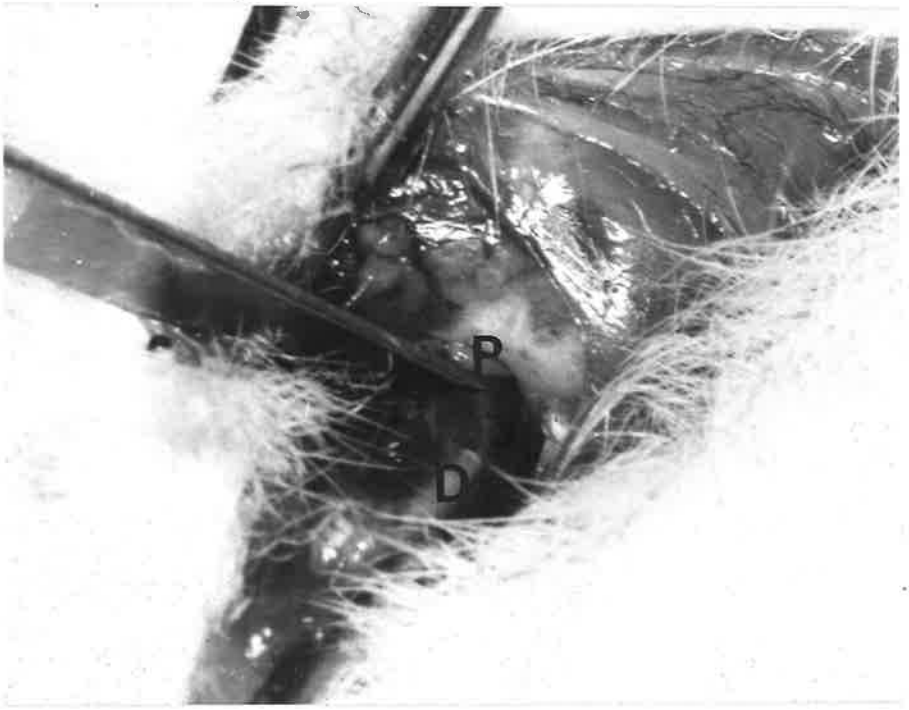


Fig. 3.3 Mental nerve just after sectioning.

Proximal Mental Nerve Trunk (P), and Distal Mental Nerve Trunk (D).

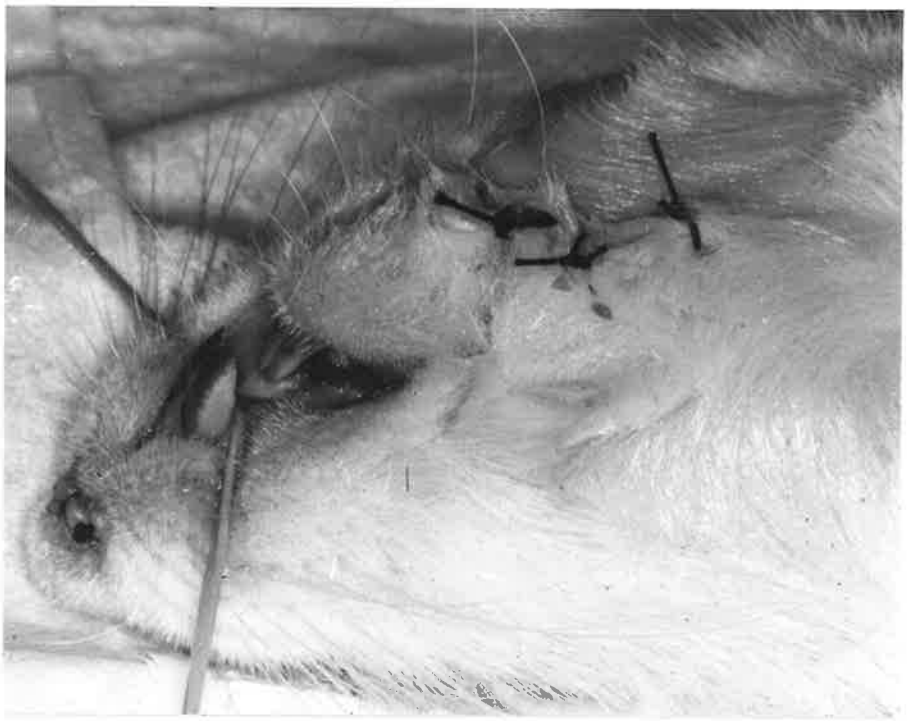


Fig. 3.4 Immediately postoperative after final suturing of skin.

The various techniques were compared and tabulated,(3.1). This demonstrated the suitability of the technique described.

TECHNIQUE	STEWART	RETIEF AND DREYER	TORNECK AND HARNETT	HOFFMAN AND TADE	AUTHOR'S
No. of rats operated	8	8	8	8	200
Mortality	0	1	0	3	0
Operative haemorrhage	1	0	0	4	0
Post Operative Swelling	2	3	3	4	0
Possibility for Physiological testing.	Yes	Yes	Yes	No	Yes
No. of serial sections required	Large	Extremely Large	Extremely Large	Extremely Large	Minimal

Table 3.1 Comparing the various surgical techniques .

3.50 POST OPERATIVE CARE

The animal was positioned with its head down after the operation. This was necessary to reduce the possibility of respiratory obstruction. The rats were under direct supervision until they were fully mobile, and then they were returned to their cages.

No more than four rats were housed in each cage. The storage area was air-conditioned at 65-70^{o}F continuously.

3.60 BASIC HISTOLOGICAL METHOD

Most of the neural fixative techniques, axon impregnation techniques and myelin stains are designed for the central nervous system, but not all of these are suitable for peripheral nerves. The reason for this is the intrinsic difference of the peripheral nerves and also the different surrounding tissues, that is, connective tissue and muscles.

Assessment of common peripheral neural fixatives was conducted, and a new one was devised, F.E.C., which was used exclusively in these experiments, Hribar (1977). Because of expense and being a corneal irritant, osmium tetroxide was not investigated.

In this study decalcification was required. From the assessment of common chemical decalcifiers, Hribar (1976), EDTA at pH 6.5 was used.

From the pilot study it was decided to use, for axon impregnation, (Bodian technique), Luna (1968), myelin stain, (Solochrome Cyanine) Page (1970), Collagen stain (Van Gieson), Drury and Wallington (1967) and Haematoxylin and Eosin, (Lille-Mayer) Lillie (1965).

3.70 PROTOCOL FOR HISTOLOGICAL INVESTIGATION

Histological examination was conducted on the sectioned mental nerve, while the contra-lateral (unoperated) side was used as the physiological control.

Sectioned rats mental nerves were examined post-operatively at the following intervals:

1, 3, 7, 14 days

1, 3, 6, 9, 12 months

The experimental details are summarized in the following table (3.2).

Control	6 rats
Post operative duration	
1 day	6 rats
3 days	6 rats
7 days	6 rats
14 days	6 rats
1 month	6 rats
3 months	5 rats
6 months	5 rats
9 months	6 rats
12 months	6 rats

Table 3.2 Number of rats used in each post operative period.

The rats were sacrificed using a lethal intra-peritoneal injection of 1 ml undiluted 6% Sagatal. Ether was not used to make the animal manageable prior to injecting because Swank and Davenport (1935) found that prolonged etherization (2 hours) produced a marked increase of granular precipitation of myelin. To avoid any chance of this artifact production the

animal was held securely by an assistant wearing heavy duty gloves, while an intra-peritoneal injection was given.

Fig. 3.5 Administering Sagatal to the rat while it was being held by an assistant.

Once the rat died, the whole submandibular region was immediately skinned carefully without disrupting the underlying connective tissue and the mental nerve.

Fig. 3.6 The rats submandibular connective tissue exposed. The artery clip is holding the tissue containing the mental nerve.

Then a hemi-mandibulectomy was performed and during the entire procedure the tissue encompassing the mental nerve was gently held by an artery clip.

Once the mandible was removed, it was pinned to a suitable flat piece of cardboard, with the mental nerve under slight tension. This kept the nerve straight and thus aided orientation of the specimen for embedding and subsequent sectioning.

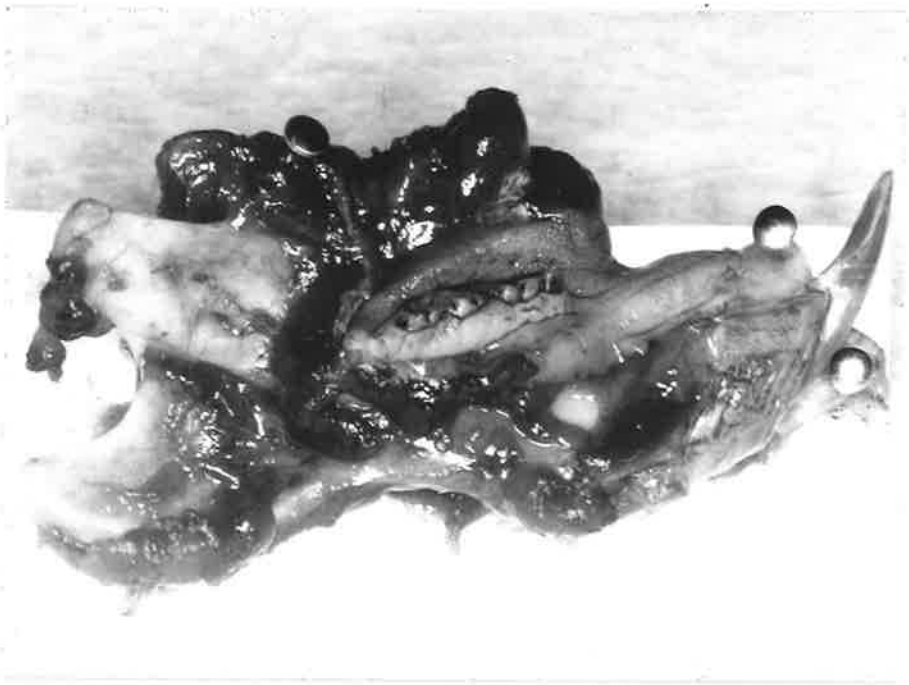


Fig. 3.7 Removed hemi-mandible en bloc and soft tissue pinned to flat cardboard.

The specimen was immediately immersed in the fixative for two weeks and then decalcified. Double embedding was carried out after completion of decalcification. Then the mandible was sectioned just distal to the first molar, parallel to its long axis. Next the specimen was held down to the base of the wax dish (with the mental nerve closest to the base) while molten wax was poured in. This positioning aided orientation for longitudinal sectioning of the mental nerve.

An initial 180 serial sections of 8μ thickness were cut from the embedded tissues as a guide. The sections were placed on 60 slides in groups of three. Every tenth slide was stained with haematoxylin and eosin to determine orientation and depth. If this was insufficient then further sectioning was carried out until the whole nerve was serial sectioned and on numbered slides. Sections that did not contain any nerve pertinent to the investigation were discarded. Every fifth retained slide was impregnated to demonstrate axons. The sixth and seventh slides were then stained for collagen and myelin. The remainder of the slides were then used as a reserve if any particular stain failed to give perfect results.

3.71 METHOD OF HISTOLOGICAL ASSESSMENT

Assessment could be made only on the morphological changes and a quantitative assessment was required that would relate the histological appearance of the sections to time. The optimum features associated with degeneration and regeneration were delineated and it was decided to award a maximum of 2 points for each of the following features:-

1. Absence of inflammatory cells.
2. "Honeycomb" appearance of myelin.
3. Thick struts in the honeycomb appearance of myelin.
4. Deep intensity of myelin staining.
5. Intense axon impregnation.
6. High proportion of large diameter axons.
7. Minimal collagen content in distal trunk.
8. Minimal collagen content at the section site of the nerve trunk.

A normal nerve would rate a maximum score of 16 points and an abnormal nerve would tend towards a lower score.

3.80 PROTOCOL FOR ELECTROPHYSIOLOGICAL TESTING

Physiological investigation could be carried out only if there were a suitable anaesthetic agent and a suitable physiological test. The pilot study reduced the selection of anaesthetic agents to Sagatal. Physiological testing was restricted to electrophysiological methods. Because of the animal size, type of nerve to be tested, area of nerve supply and length of nerve, only the "strength duration test" could be used to initiate the jaw jerk reflex.

The principle of this test was to stimulate the mental nerve electrically (strength-duration technique) to initiate the jaw jerk reflex. Afferent and efferent fibres for the jaw jerk pass through the motor root of the trigeminal nerve. In this experiment the anterior belly of the ipselateral digastric muscle was observed for contractions. Once the contraction was observed this was used to signify a positive jaw jerk response.

The proposed physiological testing was to involve two groups of animals. Physiological testing in both these groups was to be conducted at intervals two and one week before the mental nerve was sectioned on one side. Note, within each group half had the right mental nerve cut while the other half had the left mental nerve cut.

Group one consisted of 15 rats that were to be tested physiologically pre-operatively as mentioned. Testing was to be carried out once a week for three months and then monthly until normal results returned.

Group two consisted of rats that were to be tested histologically (histological protocol for numbers) and also physiologically. These rats were to be tested pre-operatively as in the other group. The next time they were to be tested was just before being sacrificied for histological testing. Thus correlation of histological and physiological response could be made.

The technique was to anaesthetise the rat as for surgical anaesthesia (see appendix I for dose). The anaesthetised rat was placed in a supine position with its head elevated about 30^{o} to the horizontal. This position was chosen so that the mandible was in a state of minimal tension, while the lower lip was everted. This eversion was accomplished by the use of sutures passing through the lower lip and being tied to lateral hooks.

Bipolar electrodes were positioned upon the exposed labial alveolar ridge.

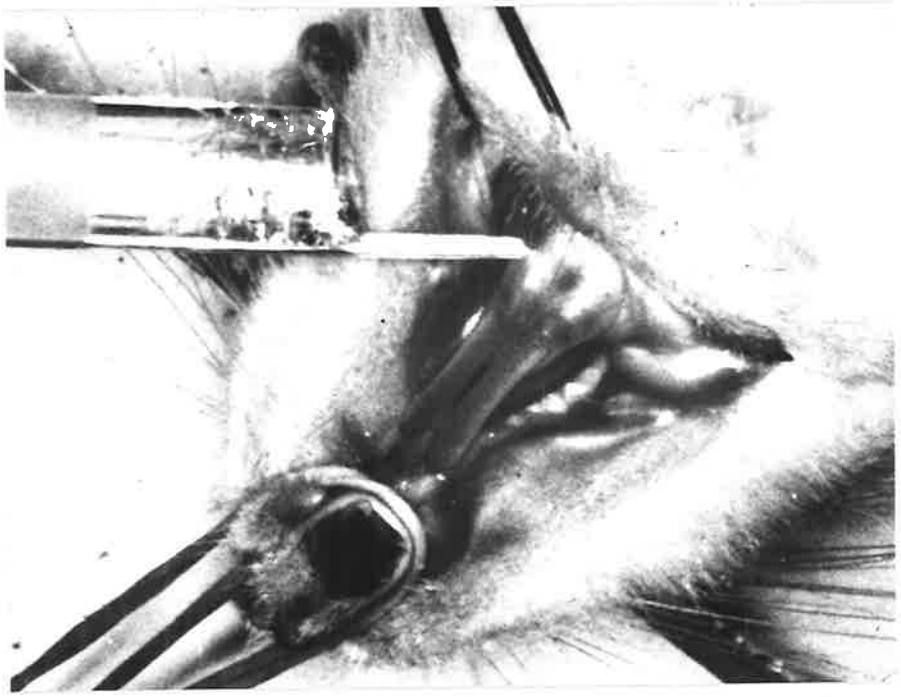


Fig. 3.8 Rat in position with bipolar electrode stimulators in position.

The bipolar electrodes were connected to Grass Instruments Stimulator. The electrodes themselves were covered by plastic tubing except for the tips lightly touching the oral mucosa.

Fig. 3.9 Grass Instruments Stimulator.

The pulse frequency was 2 per second, and the "mode" was set on repeat. This was kept constant throughout the entire experiment. The "delay" setting was on one and was never altered. The "polarity" setting was checked and set in the position that gave the lowest response. This was maintained with each setting. The only variables were the duration of the pulse (milliseconds) and the volts (millivolts).

This technique required two operators, one to palpate the anterior belly of digastric muscle to feel the contraction with their index finger; the other to select pre-determined values of duration and strength of currents in a random manner.

The sequence and values selected were not seen by the person observing the digastric twitch. When the twitch was felt, the operator of the stimulator recorded the values of duration and voltage on graph paper.

CHAPTER IV

HISTOLOGICAL RESULTS

Abbreviations Used

(S.C.) - Solochrome Cyanine

(B) - Bodian

(V.G.) - Van Gieson

(H & E) - Haematoxylin & Eosin

P - Proximal Trunk

D - Distal Trunk

N - Neuroma

M - Minifuniculi

M.F. - Mental Foramen

I - Inflammatory cells

CONTROL

Macroscopically the mental nerve appeared to be a solid trunk. On microscopic examination it demonstrated progressive branching as it extended peripherally. There was no intimate association with large blood vessels to form a neurovascular bundle (figure 4.1).

Fig. 4.1 Control - Mental nerve exhibiting branching,(S.C.) x 40

Axons within the trunk were impregnated uniformly and there was a predominance of large diameter axon fibres (figure 4.2).

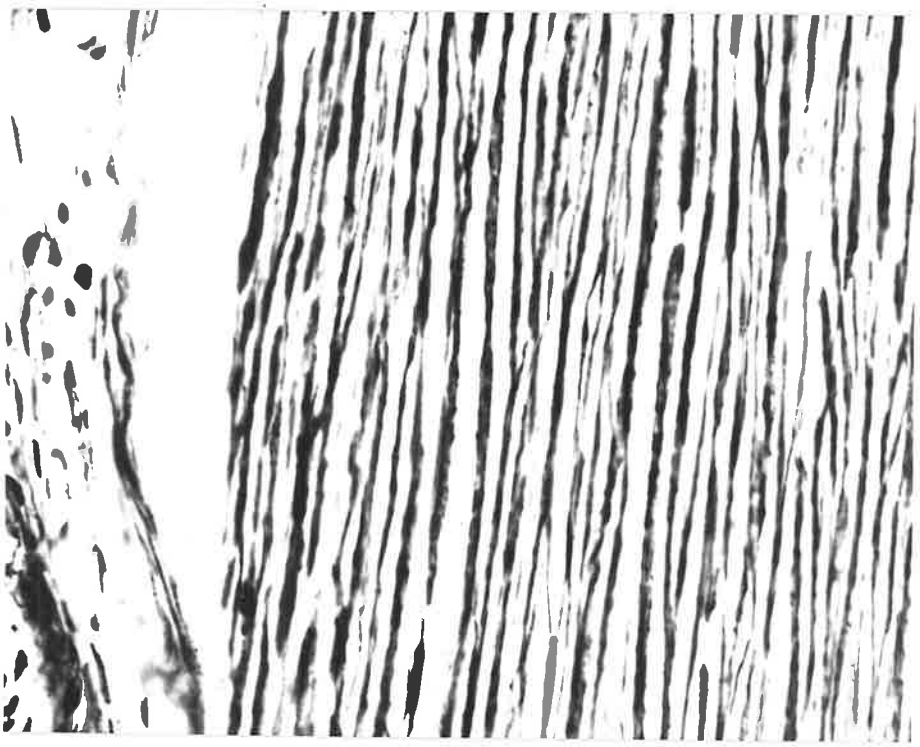


Fig. 4.2 Control - Mental nerve composed predominantly of large diameter axons but there were some small diameter axons. (B) x 40

The myelin has a honeycomb appearance with thick struts of intense blue staining with the solochrome stain (figure 4.3).

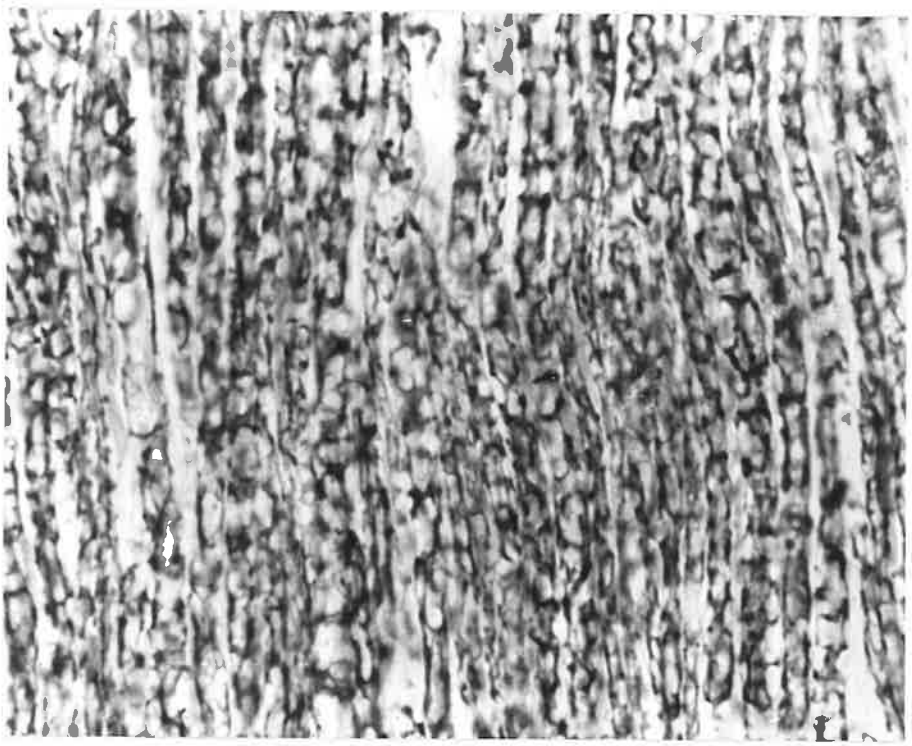


Fig. 4.3 Control - Mental Nerve. The myelin has a honeycomb appearance. (S.C.) x 40

The collagen content within the nerve trunk was minimal and positioned around the myelin. In longitudinal sections of the nerve the content was seen as thin streaks that were parallel to the orientation of the axons (figure 4.4). The extent of collagen was greater around the trunk than in the trunk.

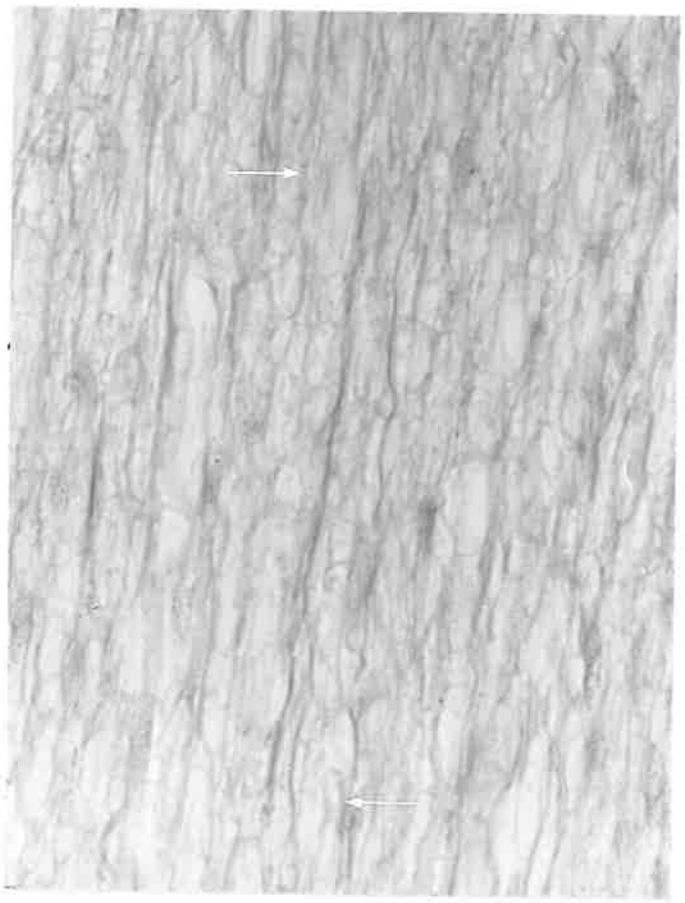


Fig. 4.4. Control Mental Nerve - The collagen appeared as dark streaks (arrowed) in the photomicrographs. (V.G.) x 400

The cell nuclei were not abundant and their distribution was not uniform within the nerve trunk. Their orientation was approximately parallel to the axons. The nuclei of Schwann cells and fibroblasts could not be differentiated (figure 4.5).

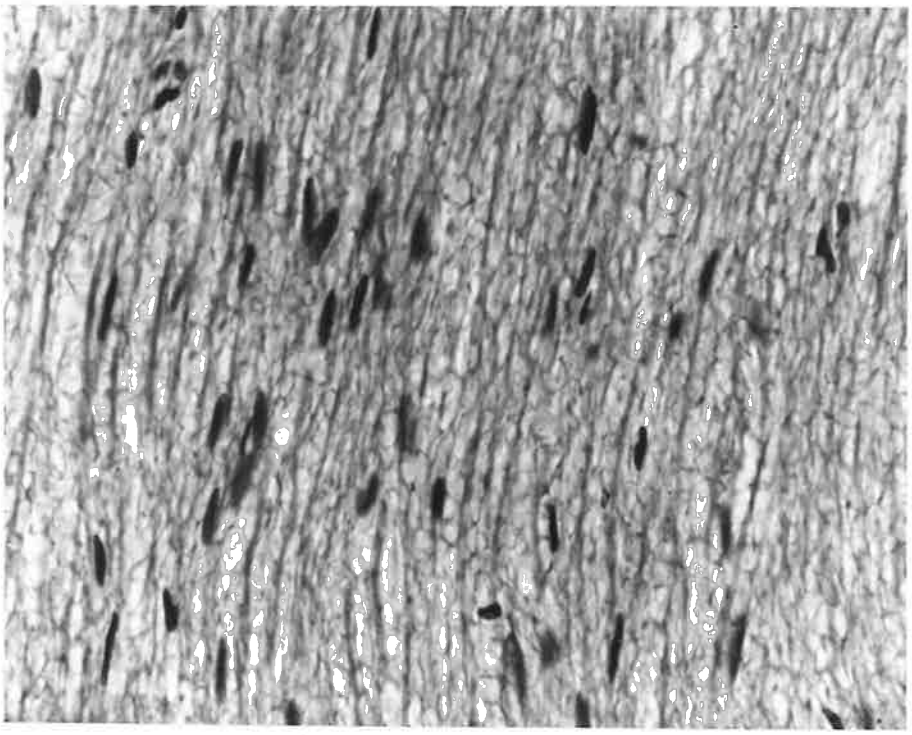


Fig. 4.5 Control Mental Nerve - The nuclear poles were aligned with the myelin trunk. (H & E) x 400

1 DAY

Acute inflammatory cells were evident at the section site and extended into the connective tissue (figure 4.6).

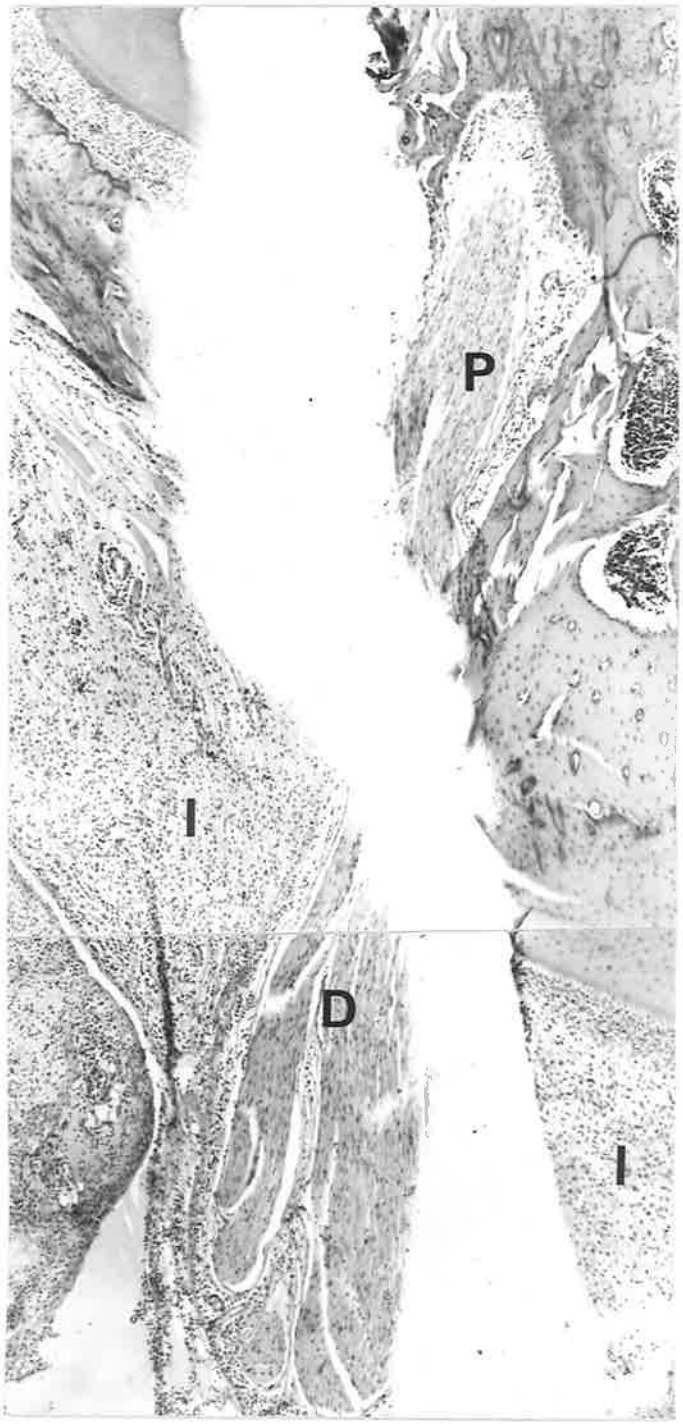


Fig. 4.6 1 day after sectioning of mental nerve - Acute inflammatory cells were present in the connective tissue. Longitudinal split was an artifact at the section site of the mental nerve. (H & E) x 40

The axon continuity was disrupted with the formation of irregular islands of intense impregnation. This was a uniform change throughout the distal trunk (figure 4.7). In the proximal trunk no changes were observed in the axons.

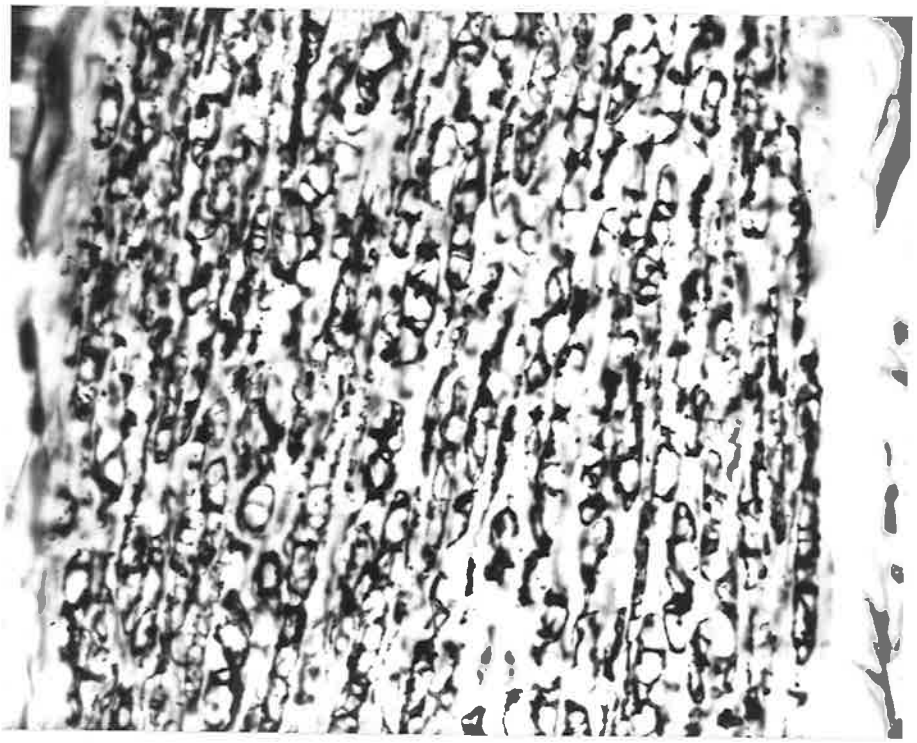


Fig. 4.7 1 day after sectioning. Distal Trunk - Axons were broken down into islands. (B) x 400

Myelin staining was decreased in intensity in the distal trunk and the struts had lost their thickness, but the honeycomb appearance was retained (figure 4.8).

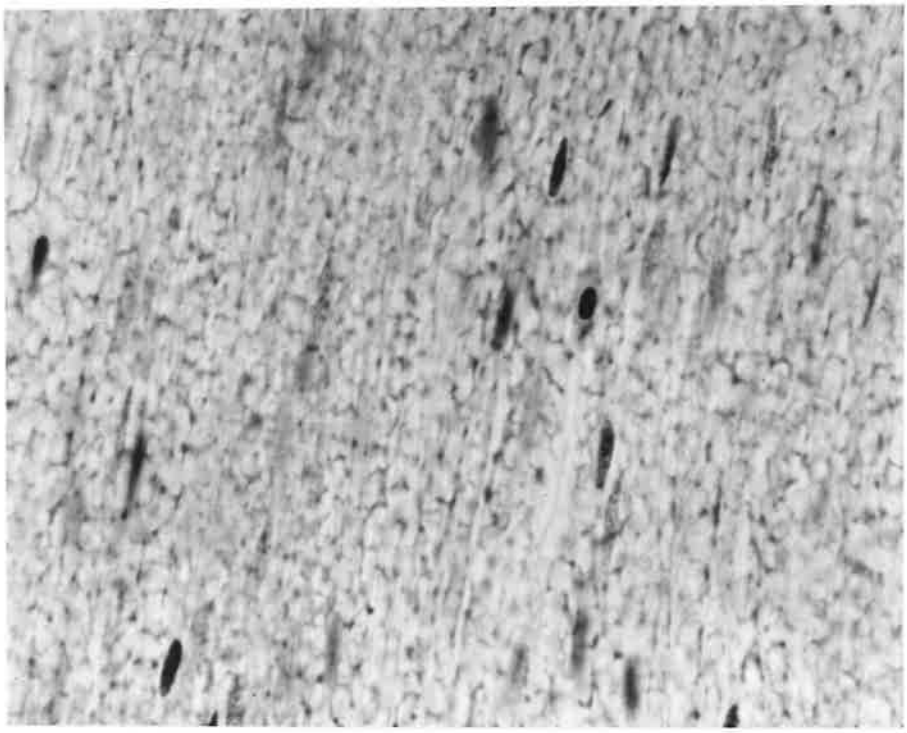


Fig. 4.8 1 day after sectioning. Distal Trunk - Reduction in myelin strut thickness was uniform.(S.C.) x 400

The proximal nerve segment immediately adjacent to the section had a similar appearance. This change was variable between each animal, but in all cases was of the order of several millimeters, although none of the specimens had any myelin change proximal to the mental foramen. This transition was a gradual one to normality in all cases (figure 4.9).

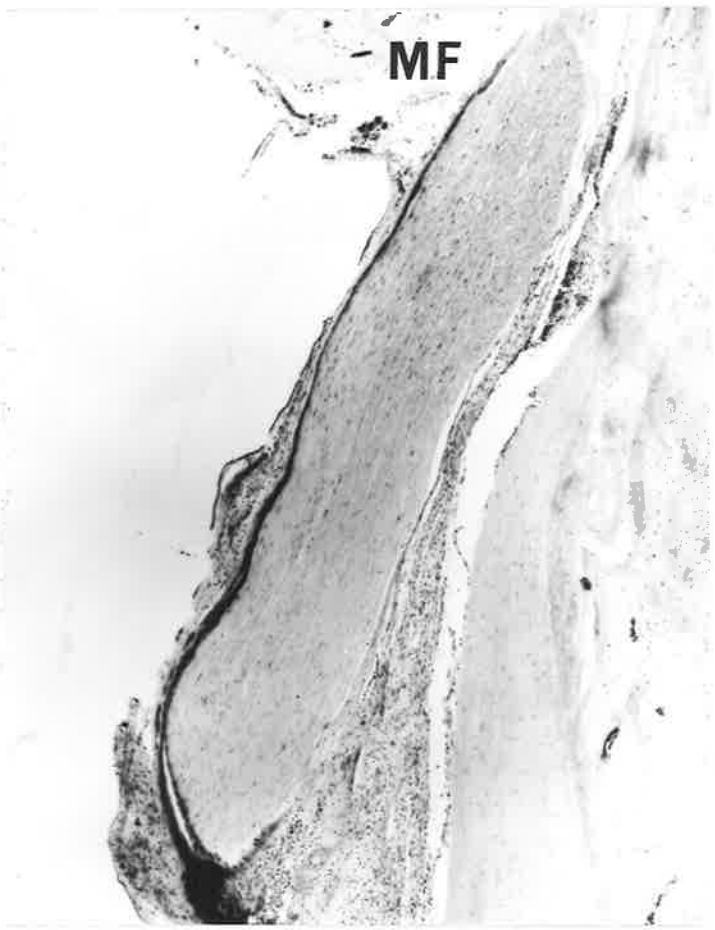


Fig. 4.9 1 day after sectioning. Proximal trunk - The myelin staining was lighter than normal adjacent to section. There was a gradual increase in myelin staining intensity which became normal on approaching the mental foramen. (S.C.) x 40

The collagen content in and around the nerve trunk was unchanged.

3 DAYS

Axon degeneration was uniform in the distal segment; the islands of axons were smaller and were now just twisted strands. The spaces between these remnants were much larger (figure 4.10).

Fig. 4.10 3 days after sectioning Distal Trunk - Axons are undergoing advanced degeneration. Some of the axon fragments are arrowed. (B) x 40

In the proximal trunk adjacent to the section, areas of axon branches were growing out into the connective tissue (figures 4.11 & 4.12).

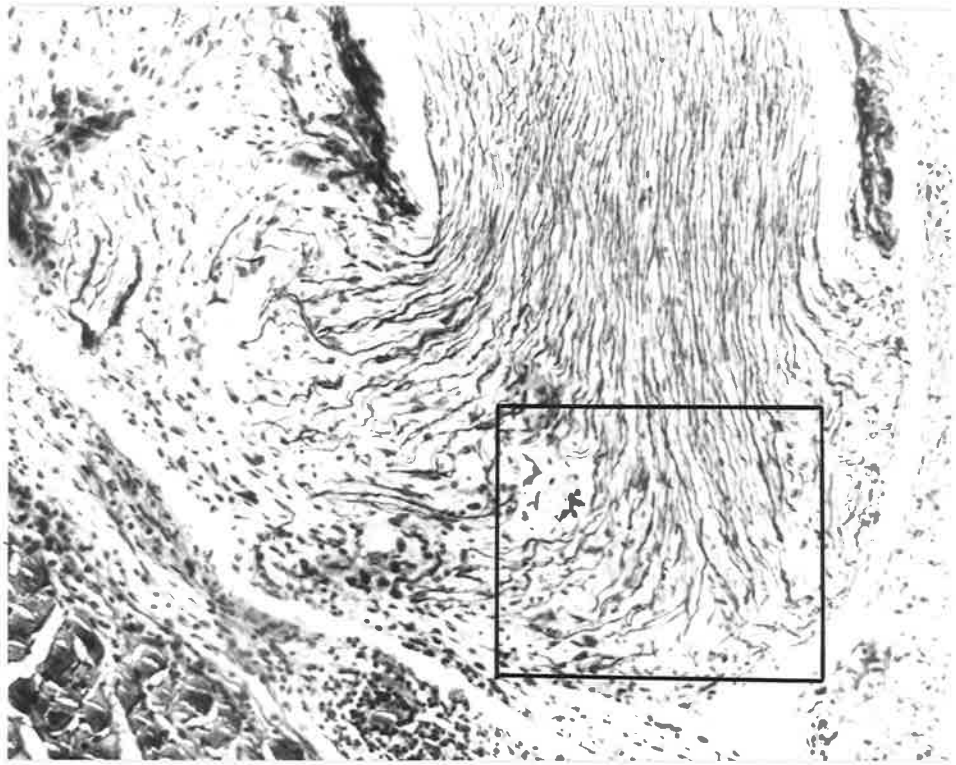


Fig. 4.11 3 days after sectioning. Proximal Trunk - A diverse advancement of axon branches into connective tissue from the site of section. (B) x 40

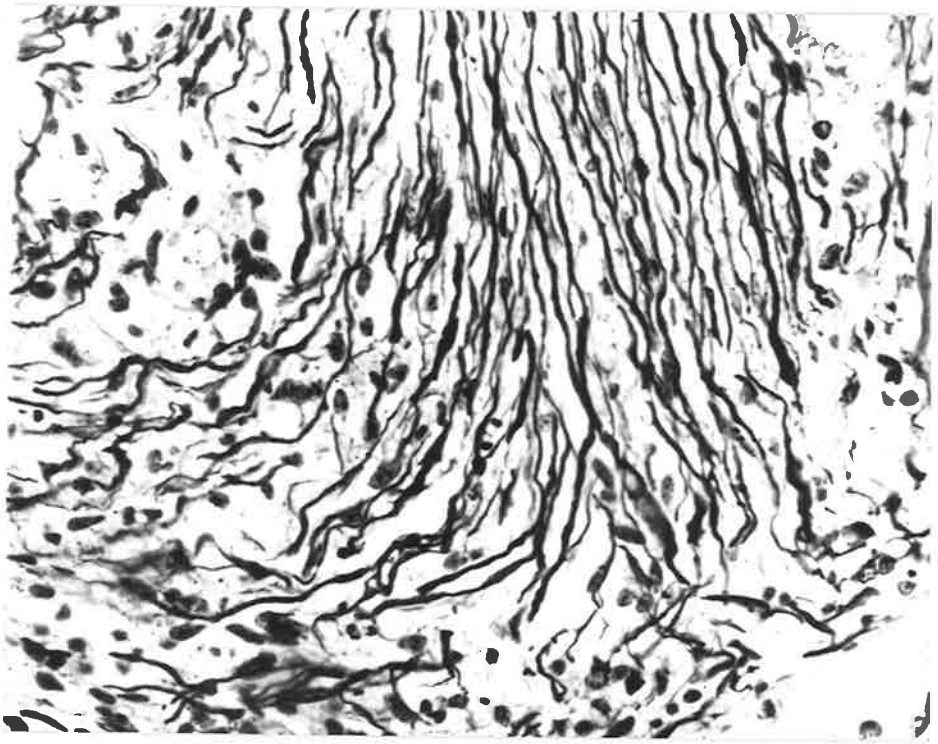


Fig. 4.12 3 days after sectioning. Proximal Trunk - Higher power of box in Fig. 4.11 showing varying size of axon branches. (B) x 250

There was massive destruction of myelin in the distal trunk evident by the loss of the honeycomb appearance and lighter staining (figure 4.13). These changes appeared to be uniform throughout the distal trunk.

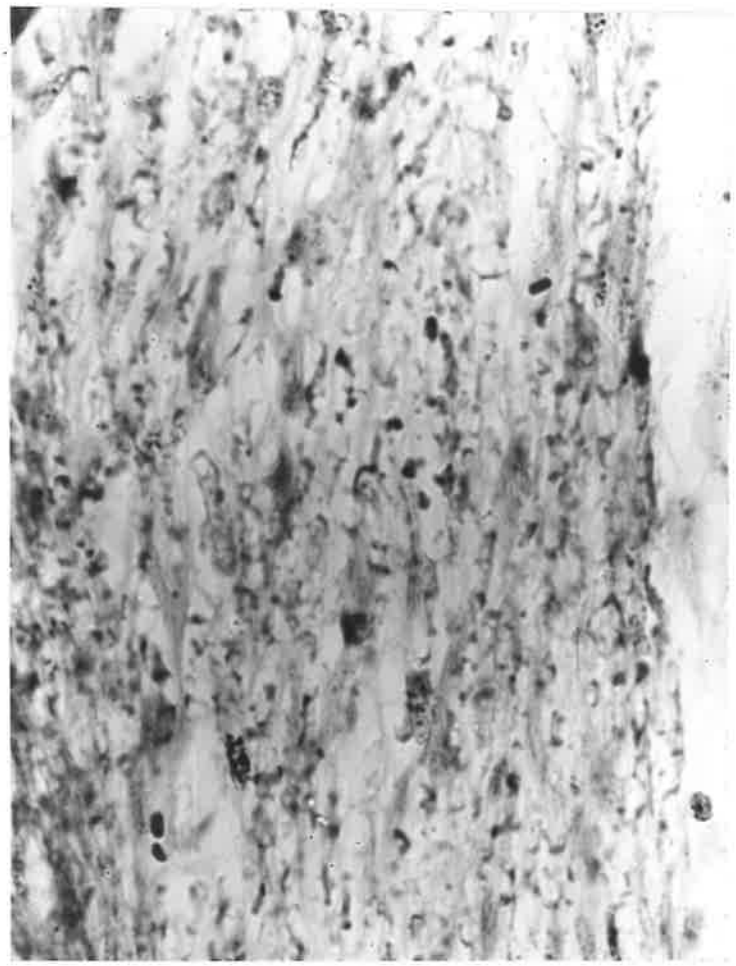


Fig. 4.13 3 days after sectioning. Distal Trunk - There was light myelin staining and no honeycomb appearance, just islands of myelin. (S.C.) x 400

Myelination of the proximal trunk was unchanged from day one. The collagen content within and around the distal trunk had not increased to any significant degree.

7 DAYS

The axon branches from the proximal trunk had progressed further and now they have fanned out into the connective tissue (figure 4.14).

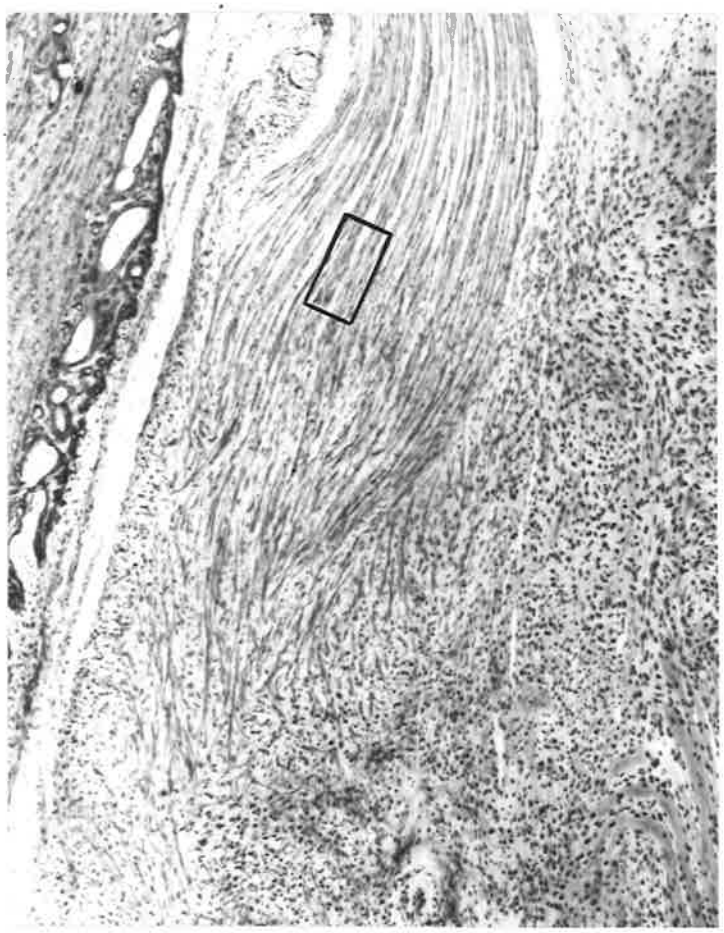


Fig. 4.14 7 days after sectioning. Proximal trunk - Fanning of axon branches into connective tissue from section site. (B) x 40

In the proximal trunk near the section site there were axons and axon branches intermixed with each other, in no particular pattern (figure 4.15).

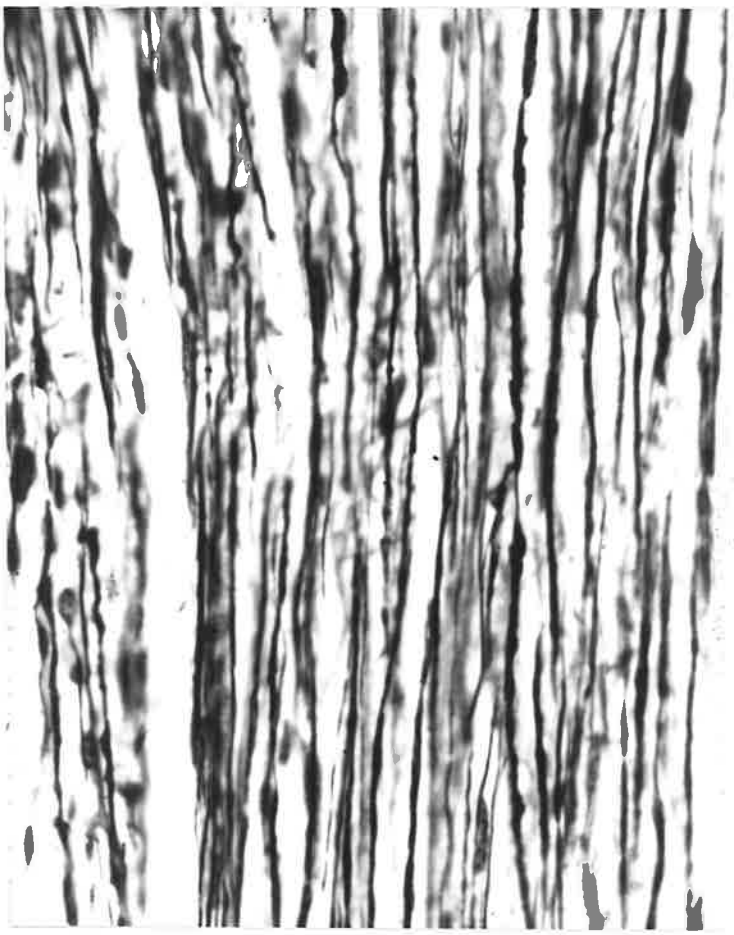


Fig. 4.15 7 days after sectioning. Proximal Trunk - Higher power of box in (Fig. 4.14) demonstrating axons and axon branches appearing side by side. (B) x 400

Axon branches could be observed in the distal segment but had not progressed very far (figure 4.16).

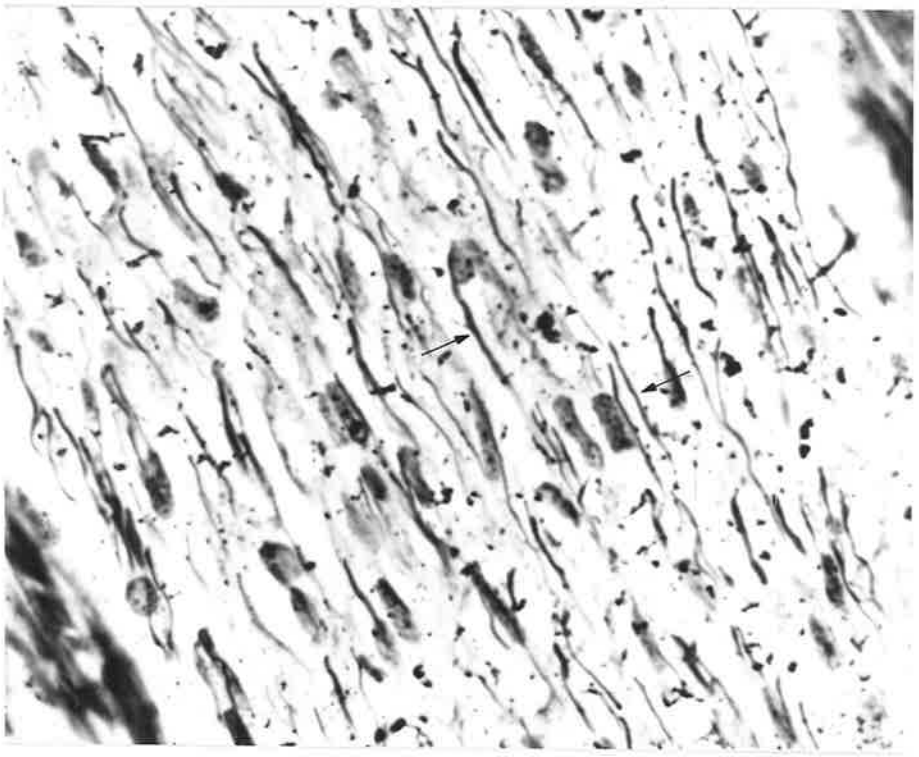


Fig. 4.16 7 days after sectioning. Distal Trunk - Axon branches were present (arrowed) but were not uniform in size. The dark irregular spots were either axon debris or silver precipitate. (B) x 250

The axon branches that apparently had not found their way into the distal trunk (Fig. 4.17) had not progressed as far as those in the trunk (Fig. 4.16).

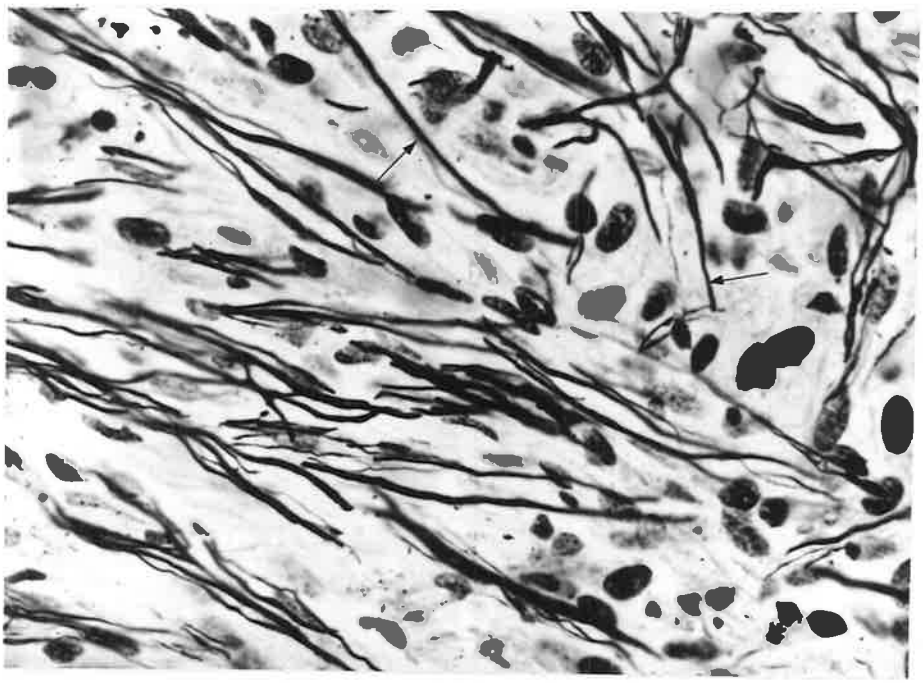


Fig. 4.17 7 days after sectioning. Connective tissue away from the distal trunk - Axon Branches (arrowed) intermingled with cells, (nuclei only stained) were observed. (B) x 400

Obvious axon remnants were still evident in the distal segment (figure 4.18).

Fig. 4.18 7 days after sectioning. Distal Trunk - short distance from axon branches. Axon remnants in endoneurial tubes (arrowed). (B) x 400

Myelin staining was reduced even further in the distal trunk (figure 4.19) than on the third day (figure 4.13).

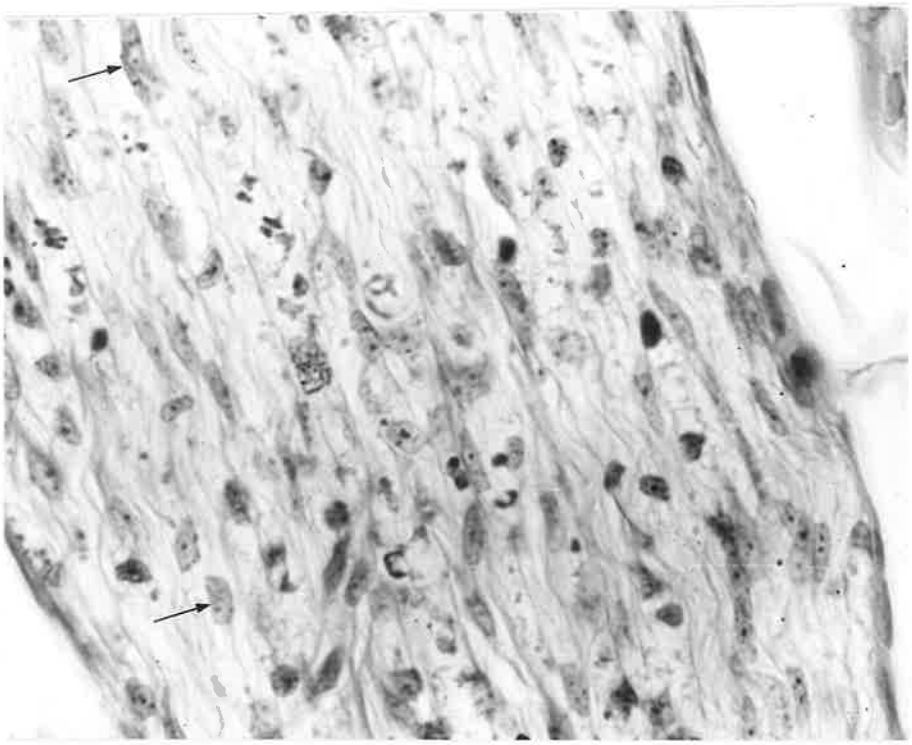


Fig. 4.19 7 days after sectioning. Distal Trunk - Cells with flakes of myelin stained in their cytoplasm are arrowed. (S.C.) x 400

In fact some distal trunks did not show much myelin staining or cells (figure 4.20).

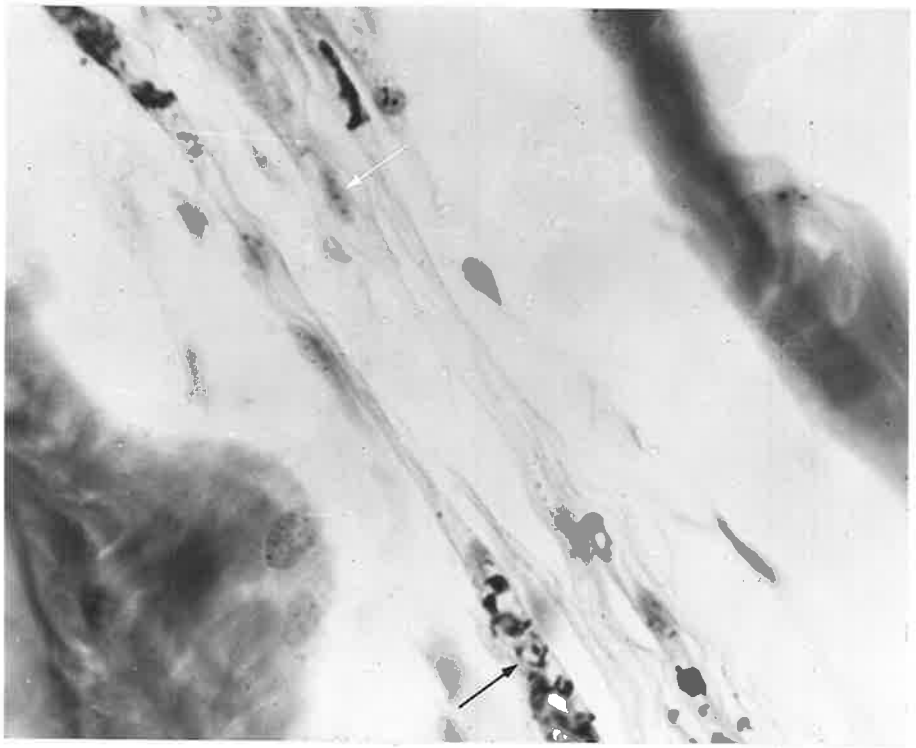


Fig. 4.20 7 days after sectioning. Distal Trunk - Very few cells were present in the trunk. Erythrocytes in the blood vessels are arrowed in black, while cells with myelin stained flakes are arrowed in white. (S.C.) x 400

All the rats in this series had a number of small accessory nerve trunks (comparable to minifuniculi) of normal mature myelin staining and normal mature axon impregnation (figure 4.21 and 4.22).

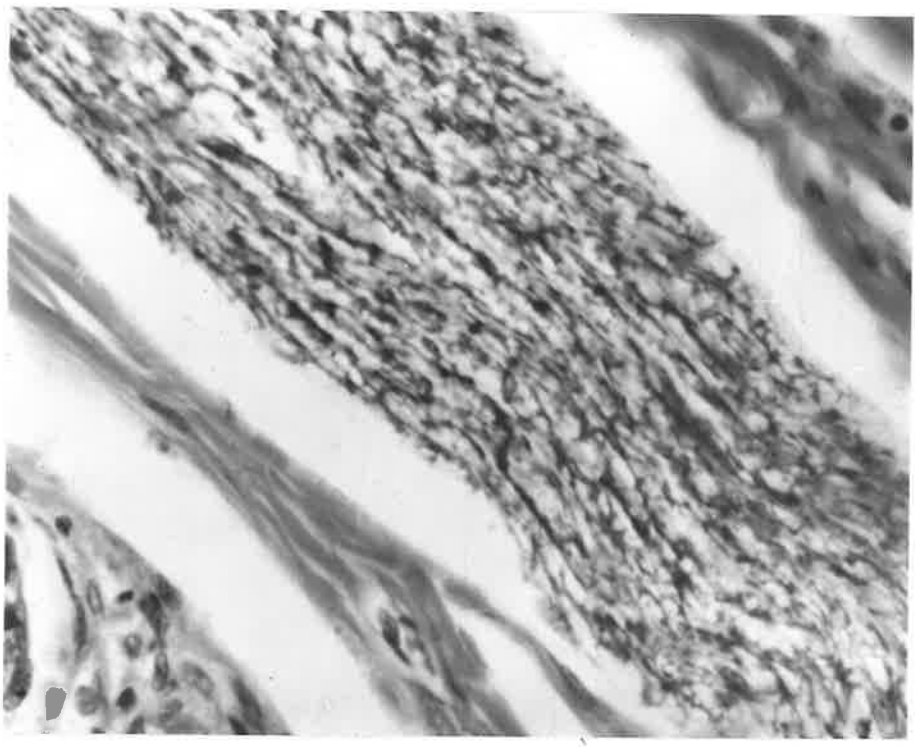


Fig. 4.21 7 days after sectioning. Accessory small nerve trunks. Normal myelin staining and normal architecture were present. (S.C.) x 400

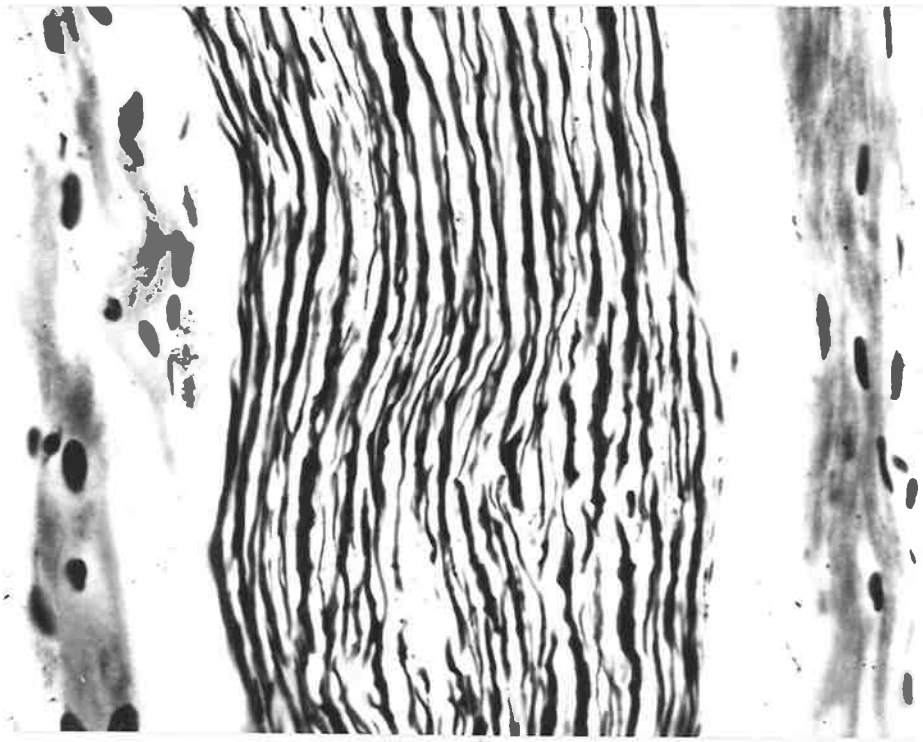


Fig. 4.22 7 days after sectioning. Accessory small nerve trunk - Normal axon staining and normal axon diameter distribution were present. (B) x 400

The collagen content in general had increased. It was mainly concentrated around the section site. This concentration was greater than in the distal trunk, which in turn was greater than the proximal trunk, although in the trunks there was only a marginal increase as compared to the surrounding connective tissue.

There was an increase in the cell population in the proximal dilation, which was greater than the distal nerve trunk that had no obvious dilation (figure 4.23).

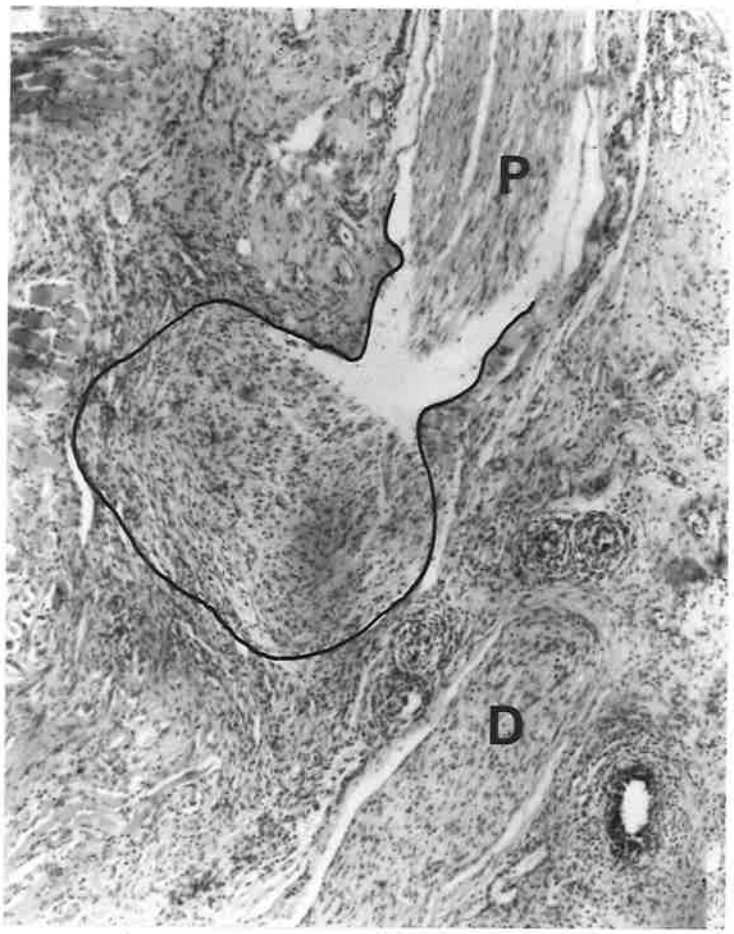


Fig. 4.23 7 days after sectioning. Section site with proximal trunk and distal trunk - There was a swelling on the terminal proximal trunk; the gap between them was an artifact. Note there was no such swelling in the distal segment. (H & E) x 40

The neuroma was in advanced formation now. It consisted mainly of axon branches.

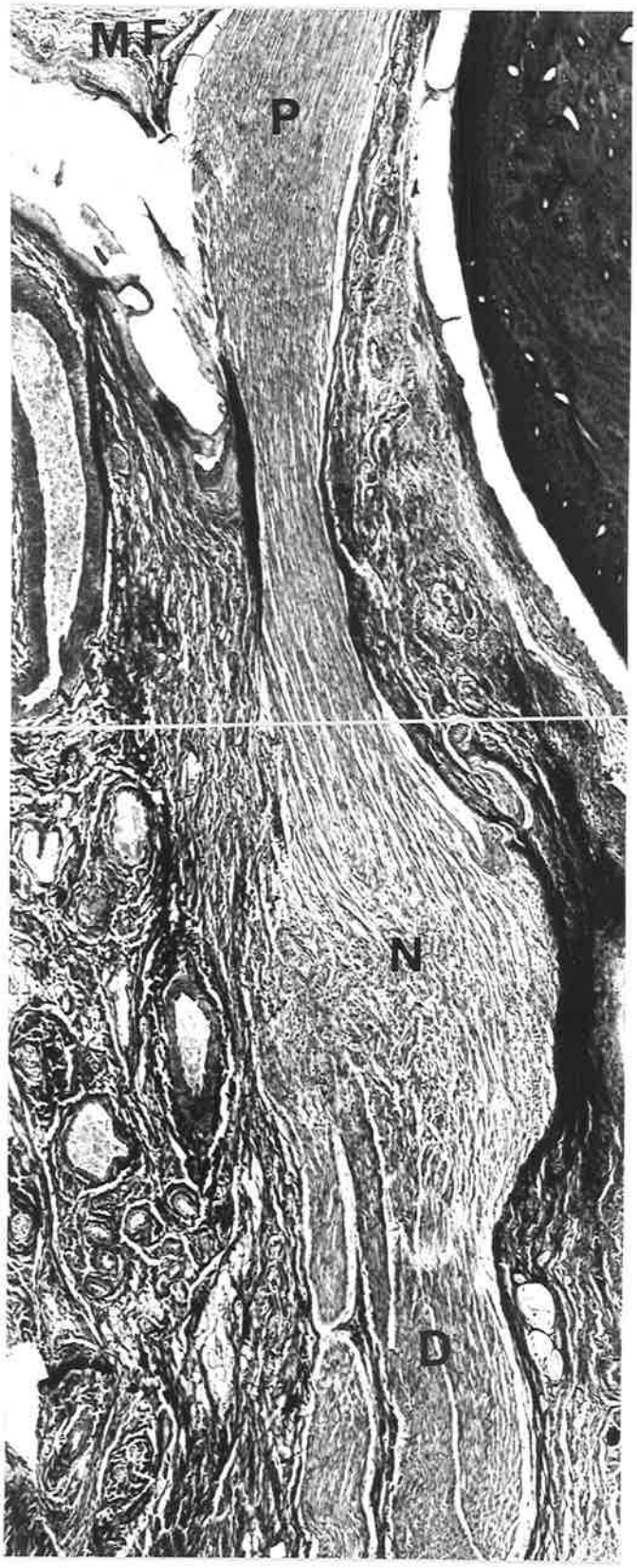


Fig. 4.24 14 days after sectioning. The proximal trunk of mental nerve was observed leaving the mental foramen. The neuroma was at the site of sectioning. The distal trunk had only axon branches. (B) x 40

The axon branches have moved down the distal trunk so far that their ends could not be identified (figure 4.25).

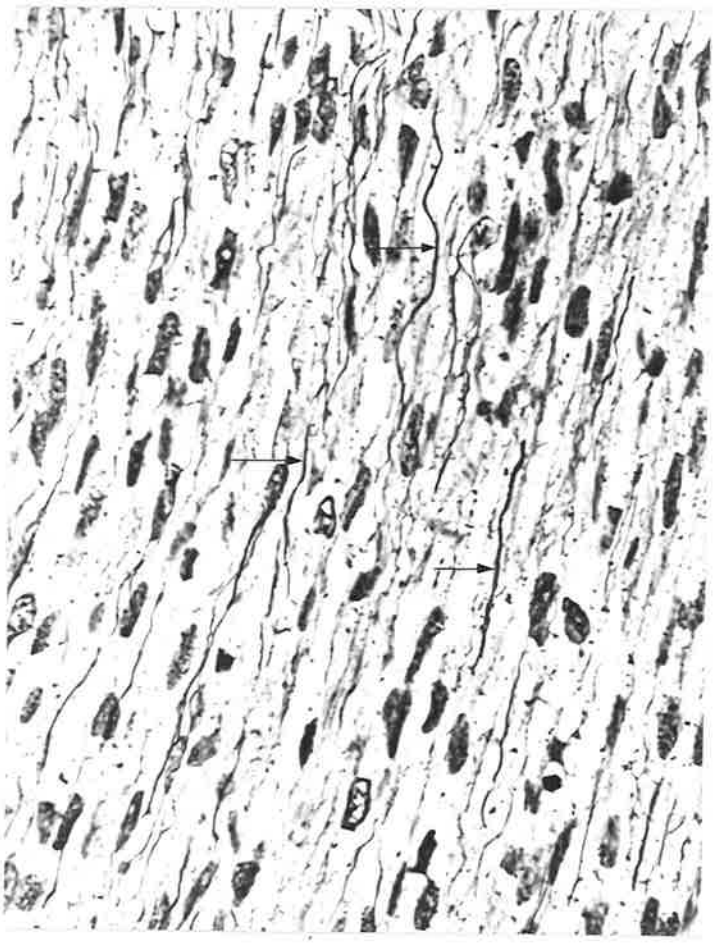


Fig. 4.25 14 days after sectioning. Distal Trunk - This was observed some distance from the section site, in fact further down than seen on Fig. 4.24. Axon branches are arrowed. (B) x 400

There were also numerous axon branches scattered throughout the connective tissue and they all lacked direction, exhibiting a twisting and tortuous course. The most abundant concentration of these axon branches was in the transection site. There were as many axon branches free in the connective tissue (figure 4.26) as on the seventh day.

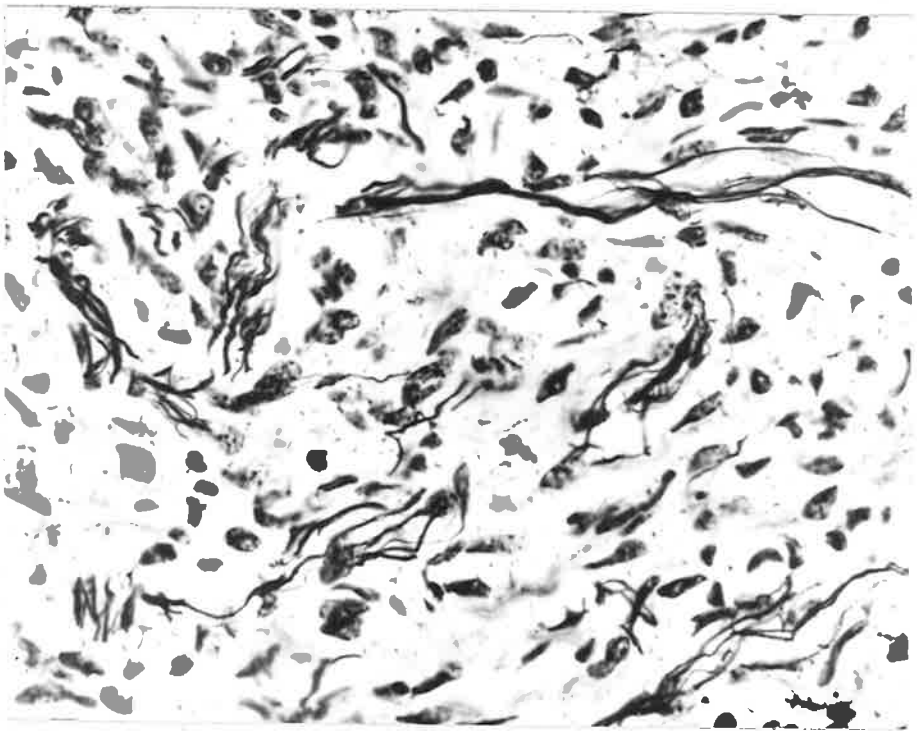


Fig. 4.26 14 days after sectioning. Connective tissue containing axon branches. These axon branches were observed some distance from the neuroma. (B) x 400

In the distal trunk there was only faint myelin staining. This was confined to the flakes in spindle shaped cells (figure 4.27).

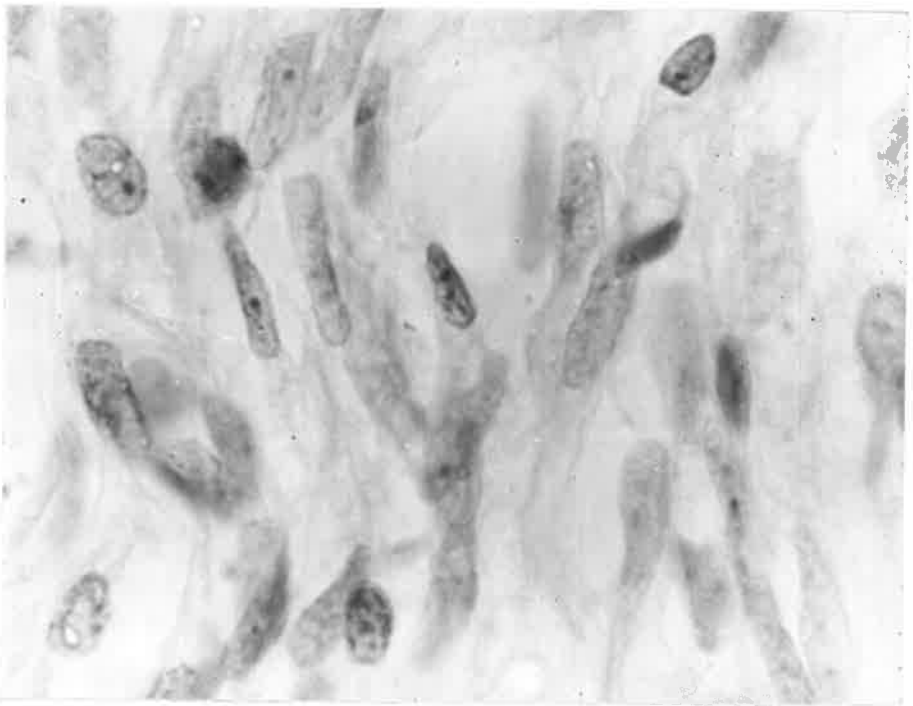


Fig. 4.27 14 days after sectioning. Distal Trunk - Consistent picture through the distal trunk was of spindle shaped cells with myelin flakes. (S.C.) x 1,000

The neuroma still lacked myelin staining as on the seventh day but the proximal trunk adjacent to the neuroma had a mature myelin pattern. As before some normal accessory branches were found in the connective tissue in all the rats. Only occasionally were inflammatory cells found. The collagen content had increased even further in general but especially at the neuroma site (figure 4.28).

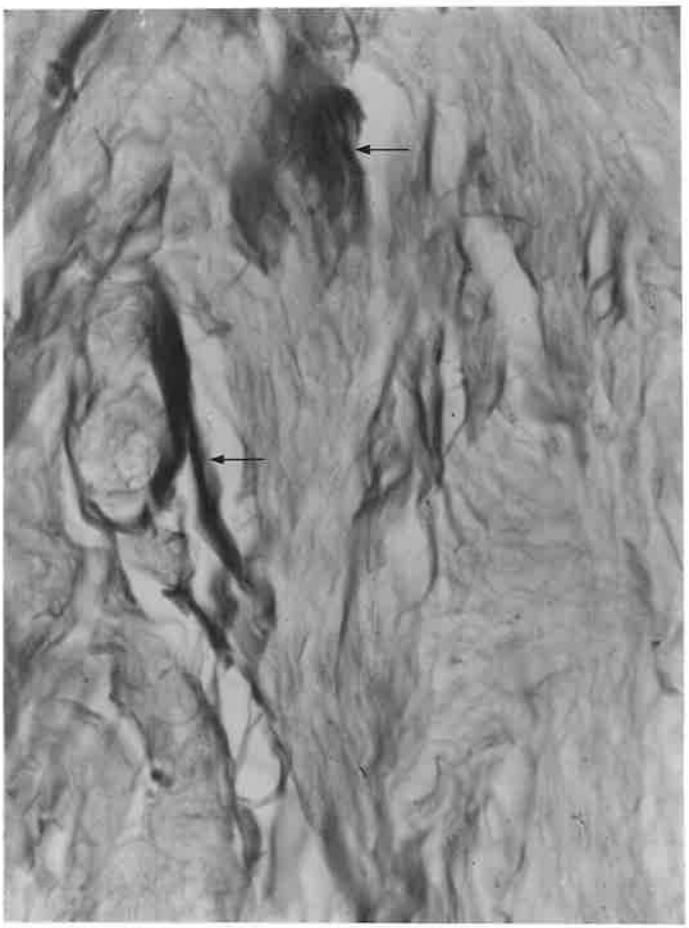


Fig. 4.28 14 days after sectioning. Neuroma - Maximum collagen deposition areas are arrowed. (V.G.) x 400

In addition there was an increase of collagen content in the distal trunk (figure 4.29).

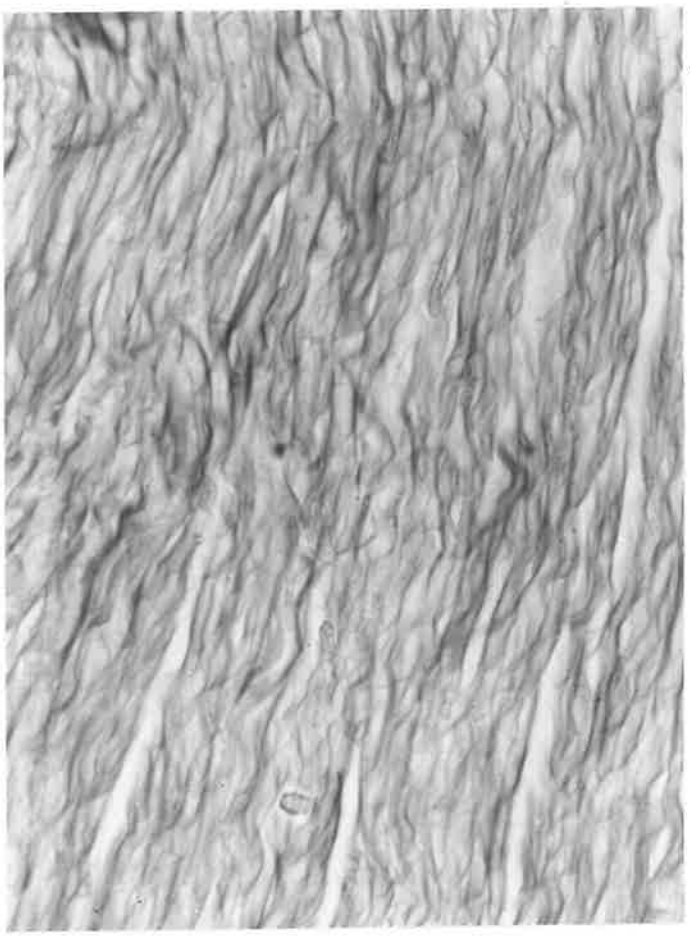


Fig. 4.29 14 days after sectioning. Distal Trunk (Some distance from neuroma), The collagen appears as dark lines which are thicker than in the control (Fig. 4.4). (V.G.) x 400

1 MONTH

The axon pattern in the neuromas varied considerably. Figures 4.30 and 4.31 demonstrate the extensive variations.

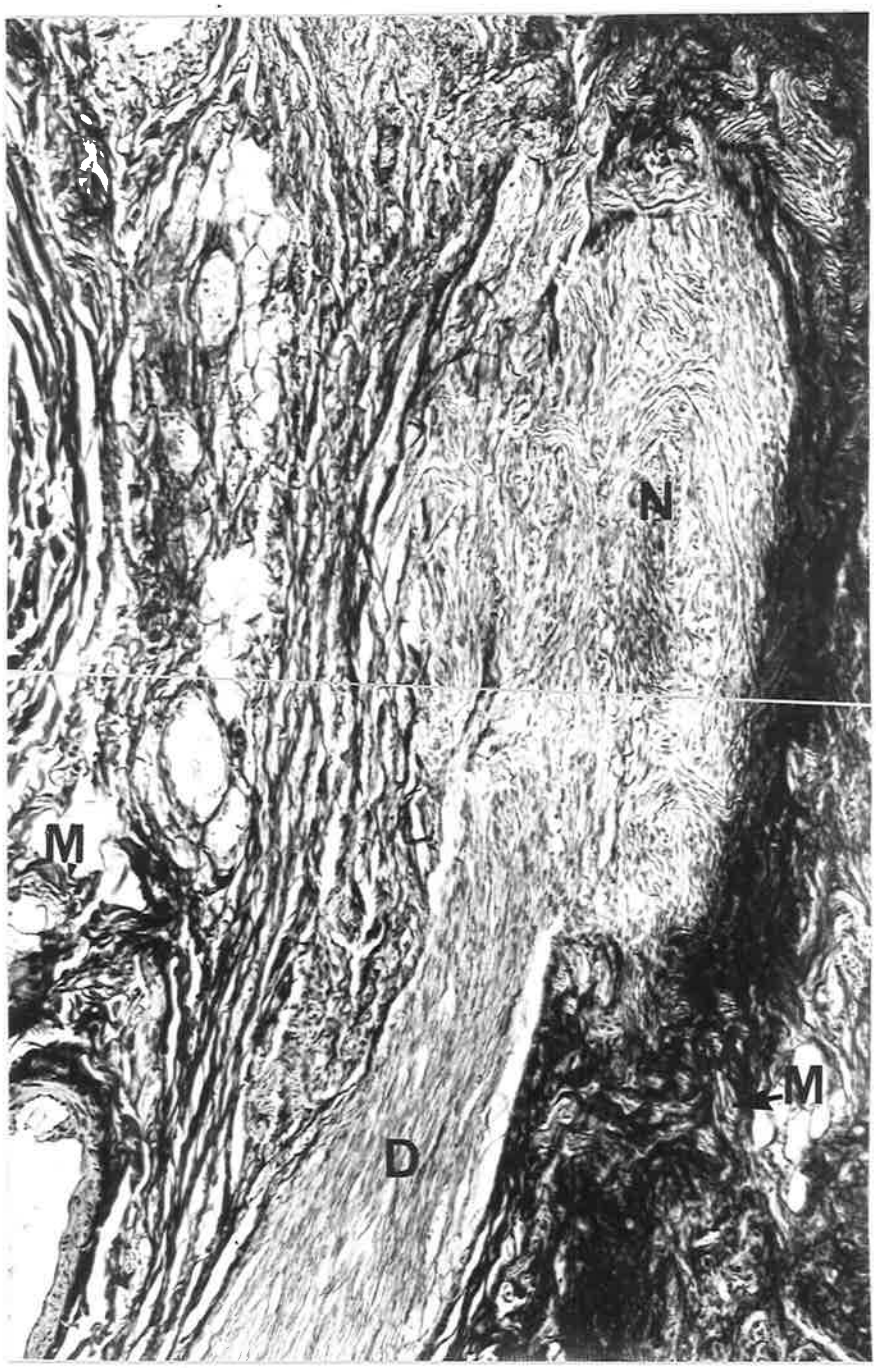


Fig. 4.30 1 month after sectioning. Neuroma and distal trunk - Demonstrating a well organised neuroma, distal trunk and a number of minifuniculi. (B) x 40

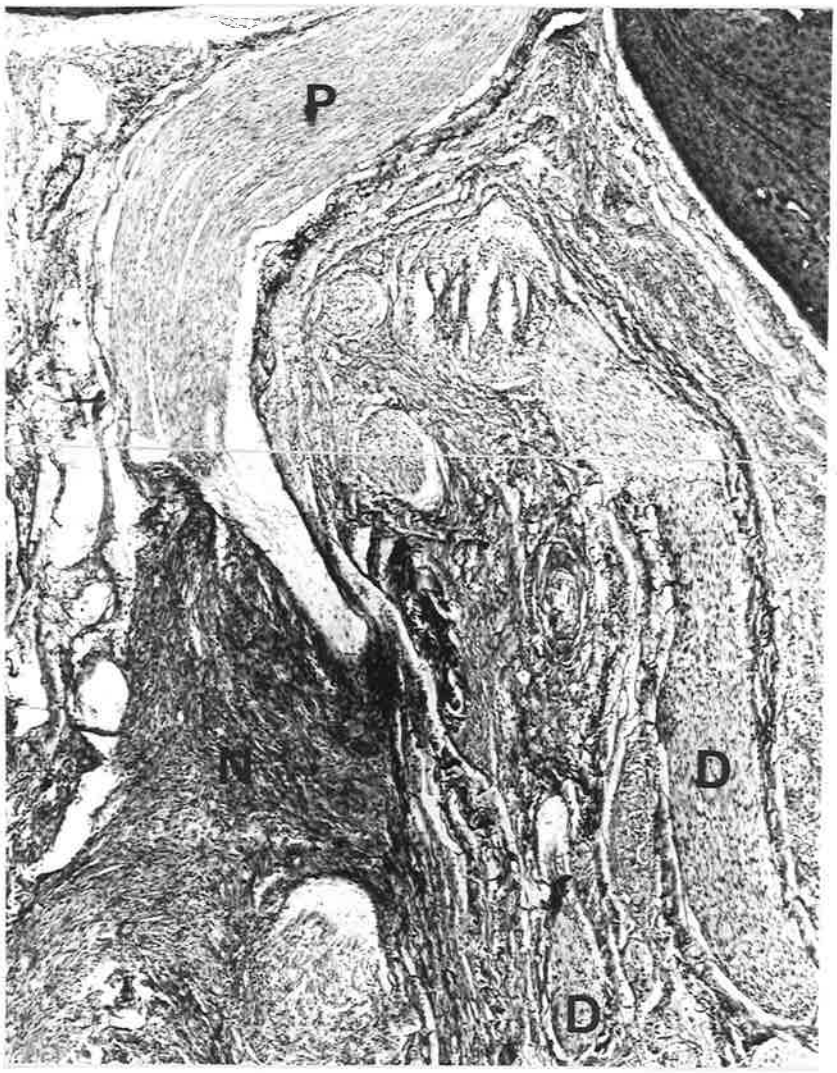


Fig. 4.31 1 month after sectioning . Proximal trunk, distal trunk and neuroma. Demonstrating lack of neuroma organisation and numerous axons and axon branches in connective tissue. (B) x 40

The axon branches had increased in diameter in general when compared with the 14 day series. This increase was not uniform and there was a great variation in diameter size.

There was a reduction in the number of isolated free axon branches in the connective tissue.

Myelin staining was more prominent and could not be seen readily within the cells (figure 4.32).

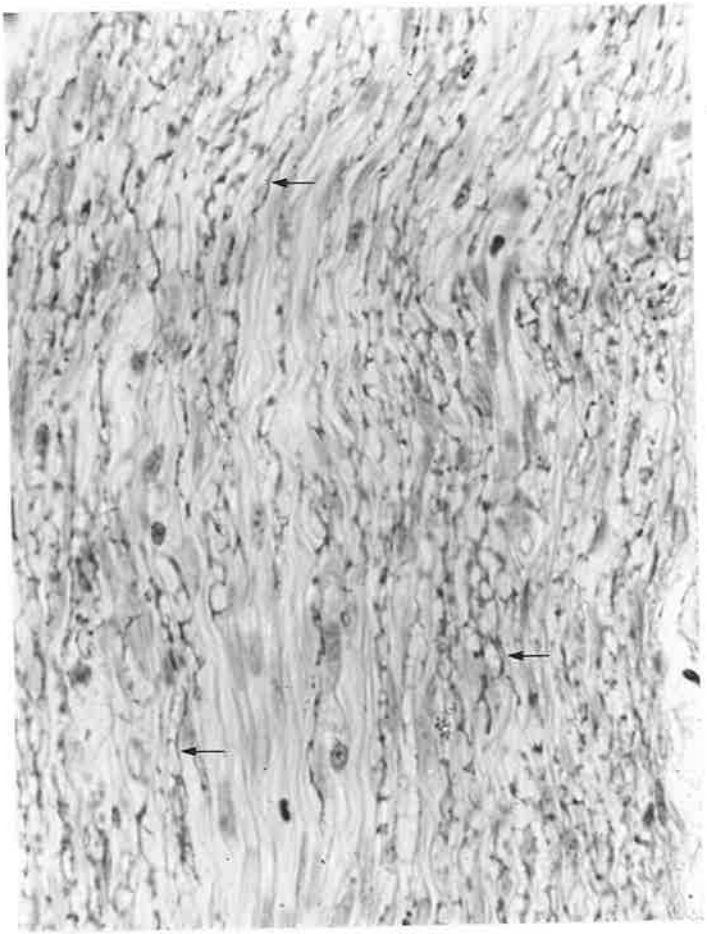


Fig. 4.32 1 month after sectioning. Myelin staining was observed in some cells but mainly as long strands (arrowed). There were some areas of honeycomb appearance.(S.C.) x 400

The extent and degree of myelin staining was not uniform within each rat. In general there was a pattern of more myelin staining in the distal segment followed by the minifuniculi. The minifuniculi had a significant variation in extent of myelin staining but even the least myelin content in the minifuniculi was greater than in the neuroma.

The collagen content appeared to decrease slightly from the previous group. No inflammatory cells were observed in any of the specimens examined.

3 MONTHS

Axon branches have matured significantly but not sufficiently to be considered normal. The axon impregnation was of normal intensity. There was a higher proportion of axons of larger diameter when compared to the one month series. The axon diameter pattern in the numerous minifuniculi and the distal segment were now comparable in appearance (figure 4.33).

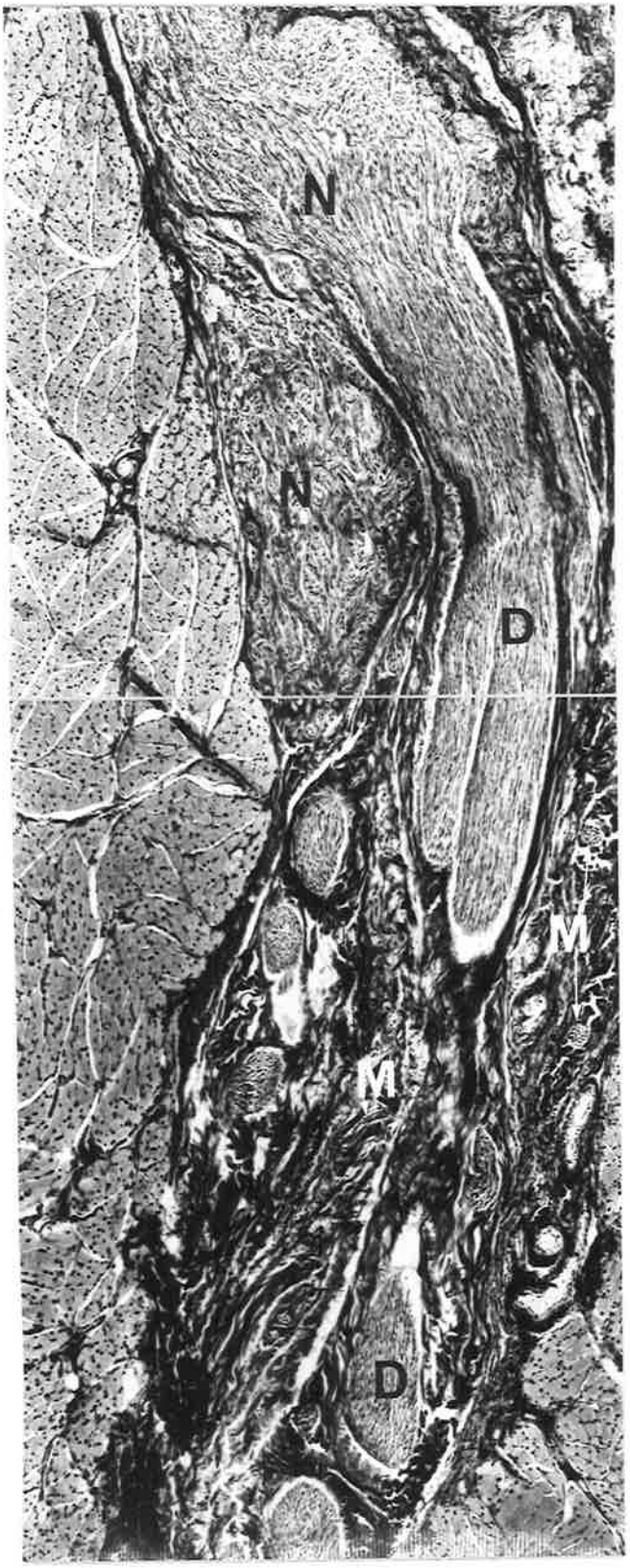


Fig. 4.33

3 months after sectioning. Neuroma, distal trunk and minifuniculi - The neuroma was large and irregular. Evidently there was a reasonable degree of displacement between the proximal trunk and the distal trunk. (B) x 40

There was a further reduction in the free isolated axons in the connective tissue. In fact very few axons could be found free in the connective tissue in this whole group.

The intensity of myelin staining in small patches was within the range of normality, but the characteristic honeycomb pattern was still not a predominant feature. In the neuroma and the minifuniculi there was slightly less myelination than in the distal trunk (figure 4.34).

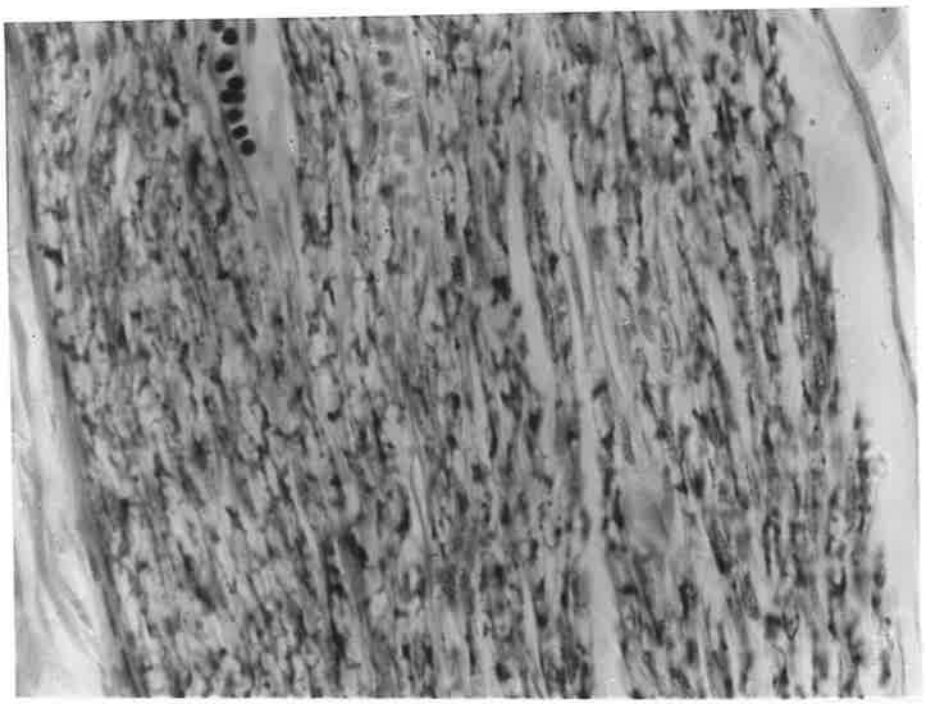


Fig. 4.34 3 months after sectioning. Distal trunk - Myelin content had increased. This was observed as an increase in myelin stain intensity and a reappearance of areas with the honeycomb pattern. (S.C.) x 400

The collagen content was less than in the previous group especially in the neuroma (figure 4.35).

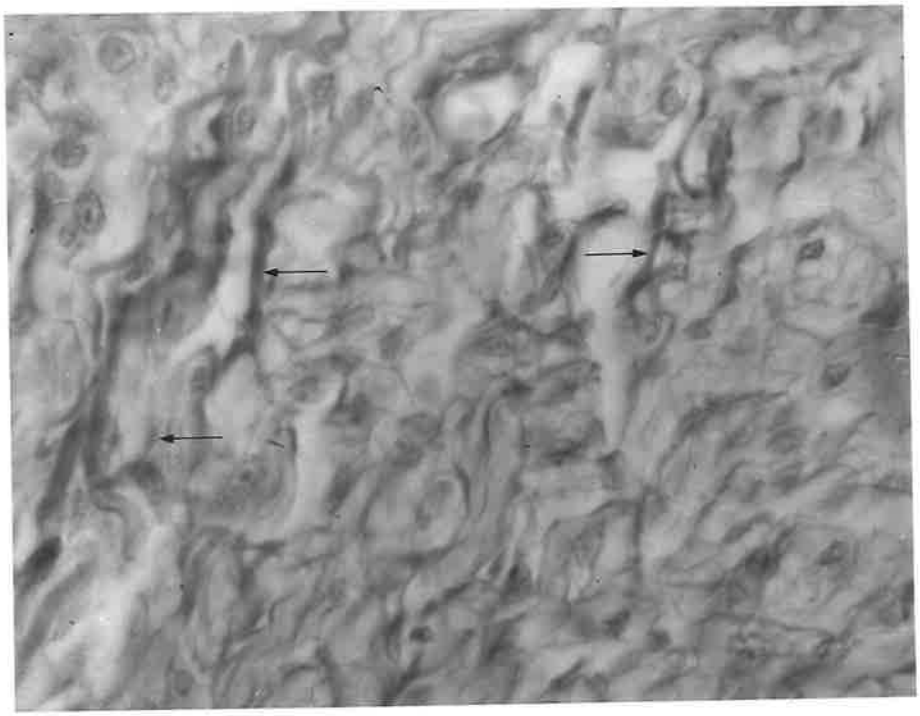


Fig. 4.35 3 months after sectioning. Neuroma - Collagen content was reduced but there were still areas of reasonable deposits (arrowed). (V.G.) x 400

6 MONTHS

The axon features were nearly back to normal in the distal segment (figure 4.36) but could still be distinguished from the control.

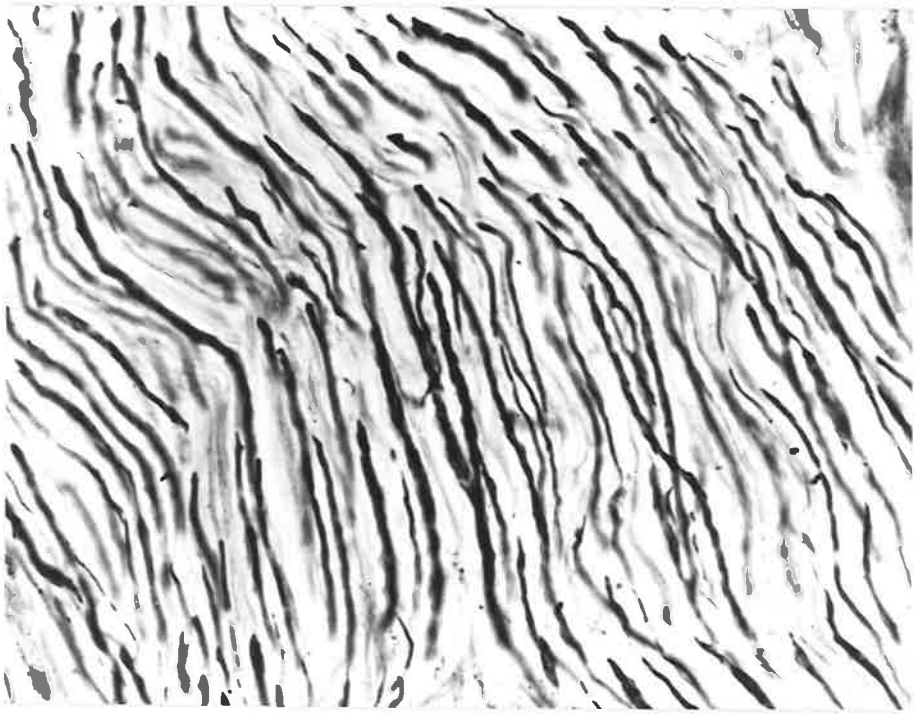


Fig. 4.36 6 months after sectioning. Distal trunk - There was a predominance of small diameter axons, but larger diameter axons were also present. (B) x 400

In the numerous minifuniculi the axon features were comparable to the axons in the distal segment. The axons in the neuroma had all matured to the same degree. No isolated free axons could be found in the connective tissue.

Myelin features in the distal segment and the neuroma had started to show large areas of the honeycomb appearance. The intensity of staining in areas was comparable to the control but the struts still lacked thickness (figures 4.37 and 4.38).

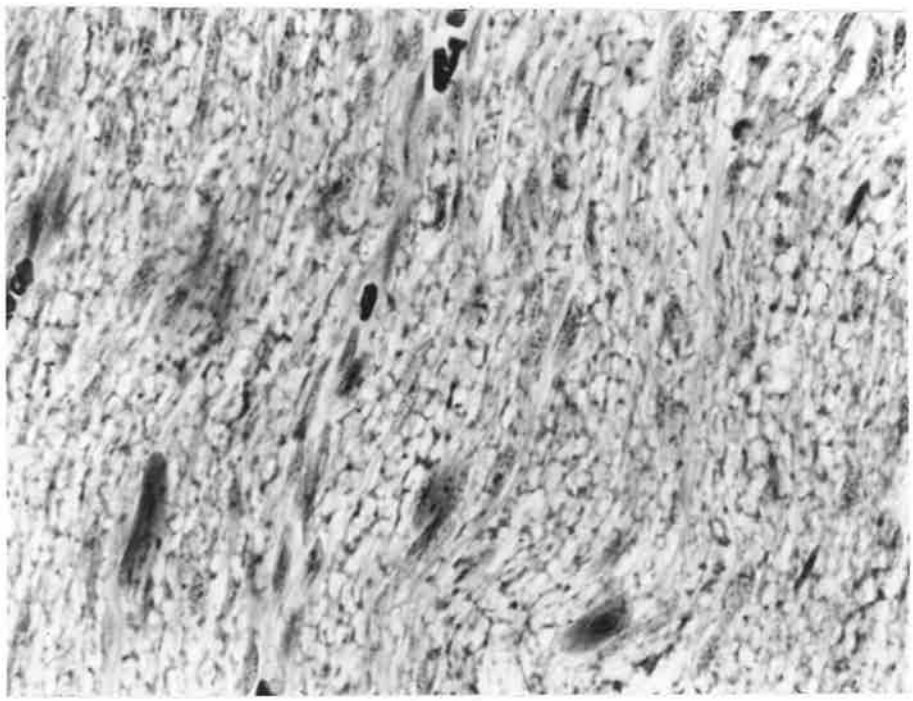


Fig. 4.37 6 months after sectioning. Distal Trunk - Myelin content had increased in general. The honeycomb pattern was prominent, the intensity of staining was normal, but the struts still lacked thickness.(S.C.) x 400

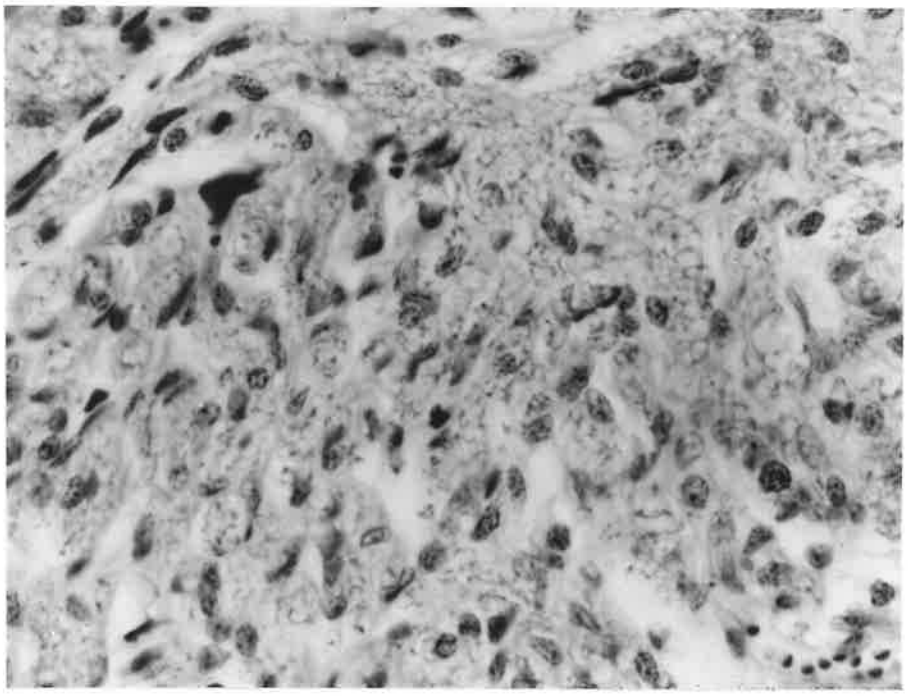


Fig. 4.38 6 months after sectioning. Neuroma - As in the distal trunk, myelin had the honeycomb appearance and the struts lacked thickness, but the intensity of staining was less than that of the distal trunk (figure 4.37).(S.C.) x 400

The collagen content was less than in the previous group, especially in the neuroma (figure 4.39).

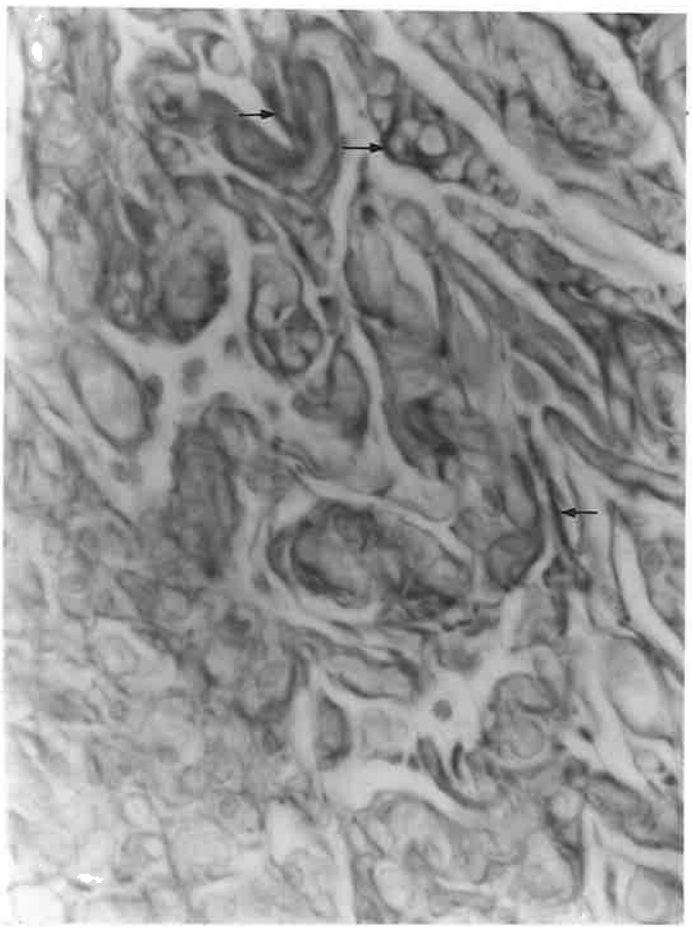


Fig. 4.39 6 months after sectioning. Neuroma - Only small areas of intense collagen deposition now remained (arrowed). (V.G.) x 400

9 MONTHS

The axon features were comparable to the control in the distal segment, in the minifuniculi and in the neuroma (figure 4.40).

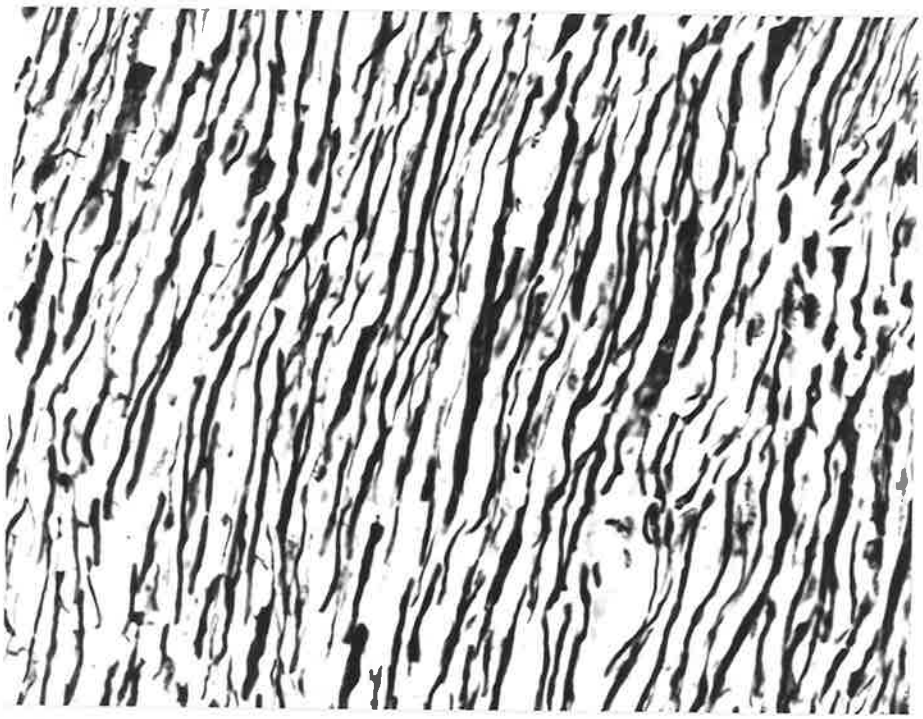


Fig. 4.40 9 months after sectioning. Distal Trunk - There was a predominance of large diameter axons and all had intense impregnation. (B) x 400

Myelin staining in the distal segment and minifuniculi had the typical honeycomb appearance in most areas with a significant number of these having intense staining, but the struts were still thin (figure 4.41). There was a lack of orientation of the nerve fibres in the neuroma, so the myelin pattern was hard to observe (figure 4.42).

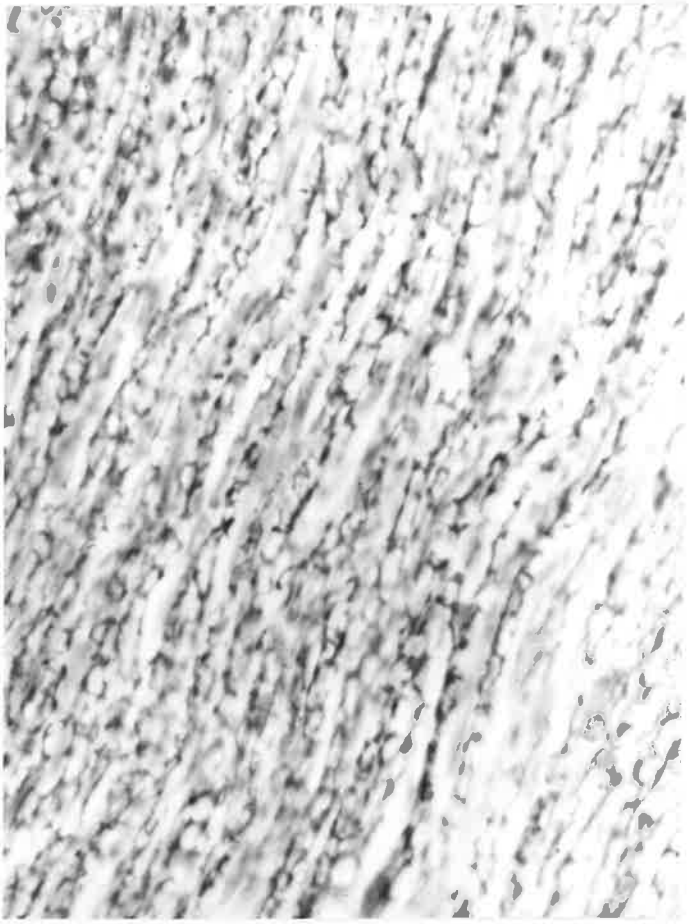


Fig. 4.41 9 months after sectioning. Distal trunk - There was an increase of honeycomb appearance and intensity of myelin staining, but the struts were still thin. (S.C.) x 400

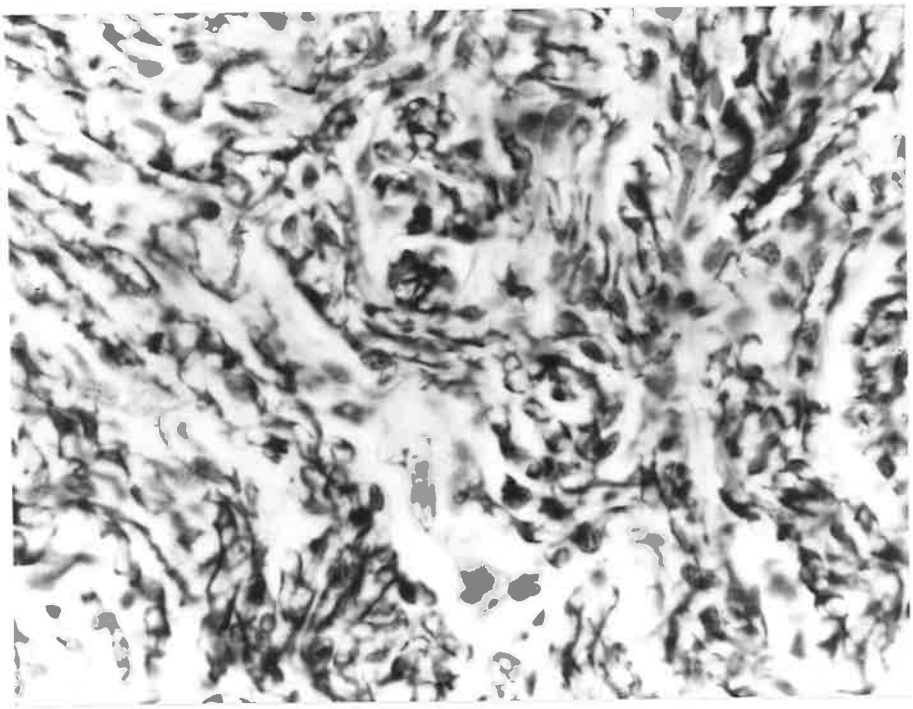


Fig. 4.42 9 months after sectioning. Neuroma - As in the distal segment, the myelin struts were still thin. (S.C.) x 400

The collagen content in the neuroma, distal segment, minifuniculi or connective tissue had not changed from the previous group.

12 MONTHS

The axons were fully matured as in the ninth month series. Myelin had progressed further in that the struts were thicker and there was intense staining (figure 4.43) comparable to the control (figure 4.3).

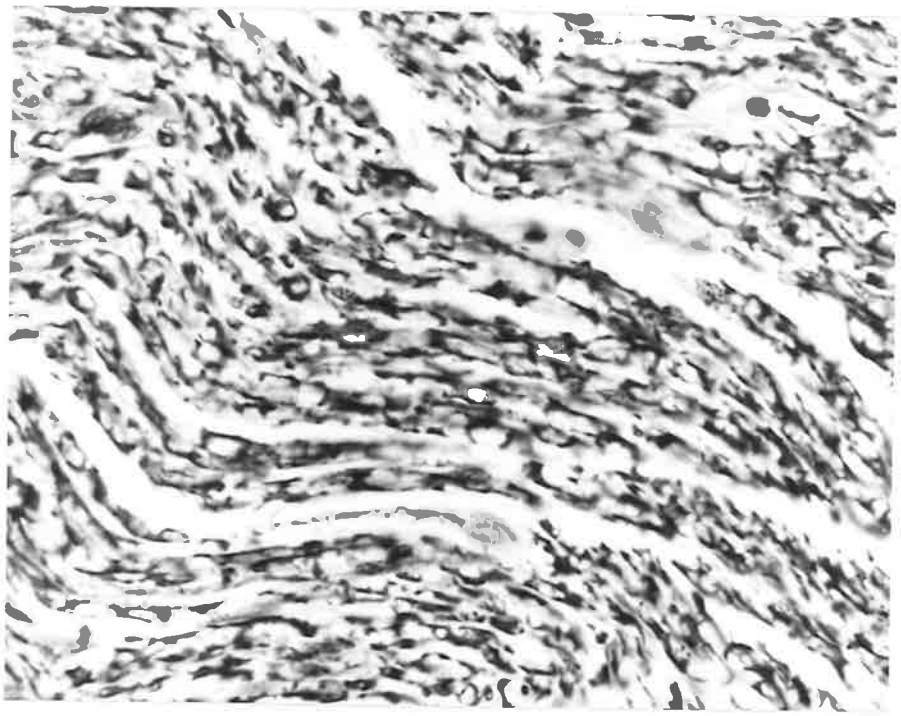


Figure 4.43 12 months after sectioning. Distal Trunk - The myelin had the typical honeycomb appearance, intense staining and thick struts. (S.C.) x 400

The myelin within the neuroma had further matured but was not normal. In most of the minifuniculi there was normal myelination, but in some it was not normal (figure 4.44).

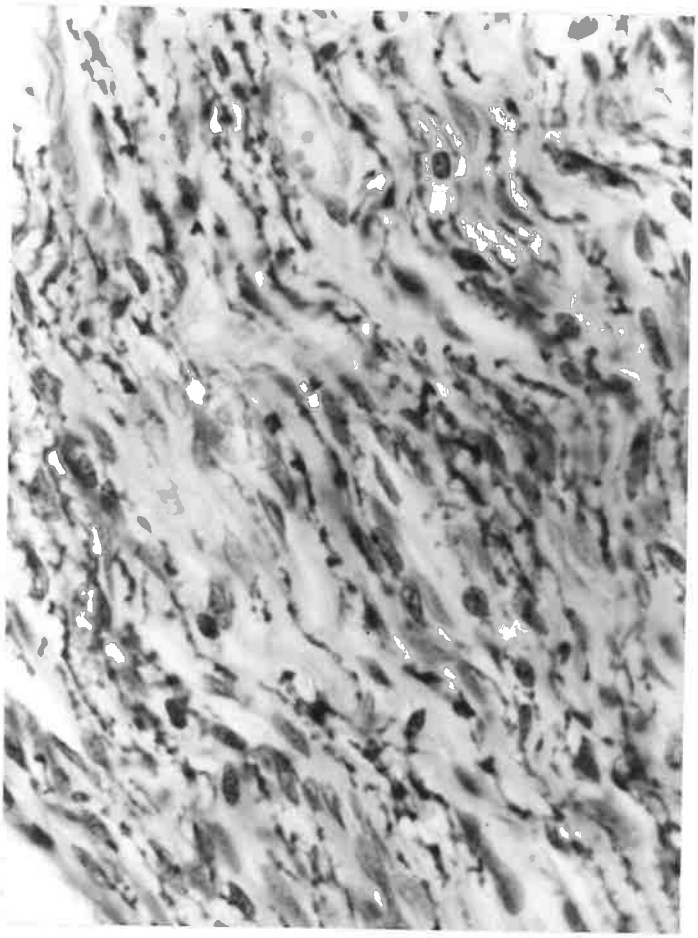


Fig. 4.44 12 months after sectioning. Minifuniculus - The variation of the myelination can be seen. There are also patchy areas of intense staining and honeycomb appearance. (S.C.) x 400

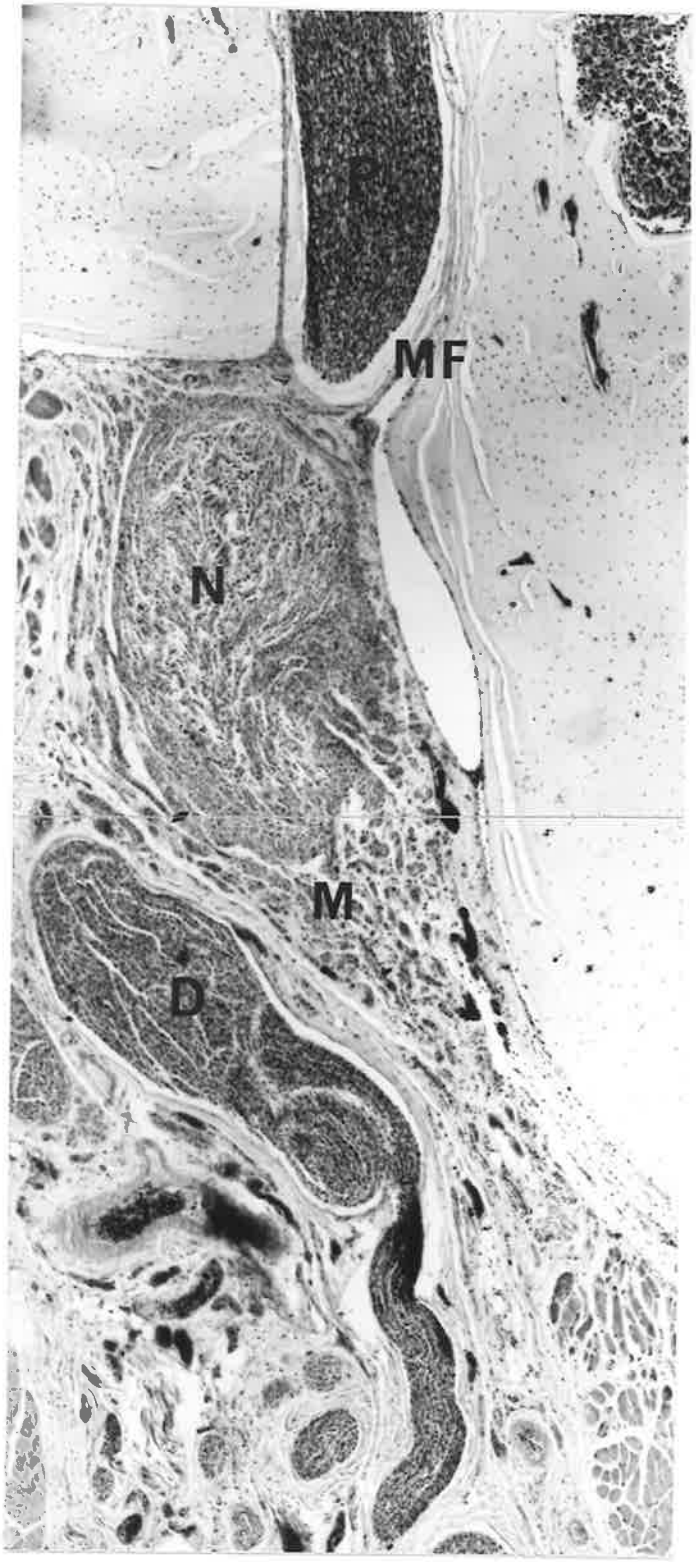


Fig. 4.45 12 months after sectioning. Proximal trunk, neuroma, distal trunk and minifuniculi. Myelin staining was more prominent in the proximal trunk than the distal trunk, followed by the minifuniculi and least was in the neuroma. (S.C.) x 40

Collagen concentration had not decreased any further but its content in the neuroma was greater than in the distal trunk and minifuniculi. These had, in turn, more than the proximal nerve trunk (figure 4.46).

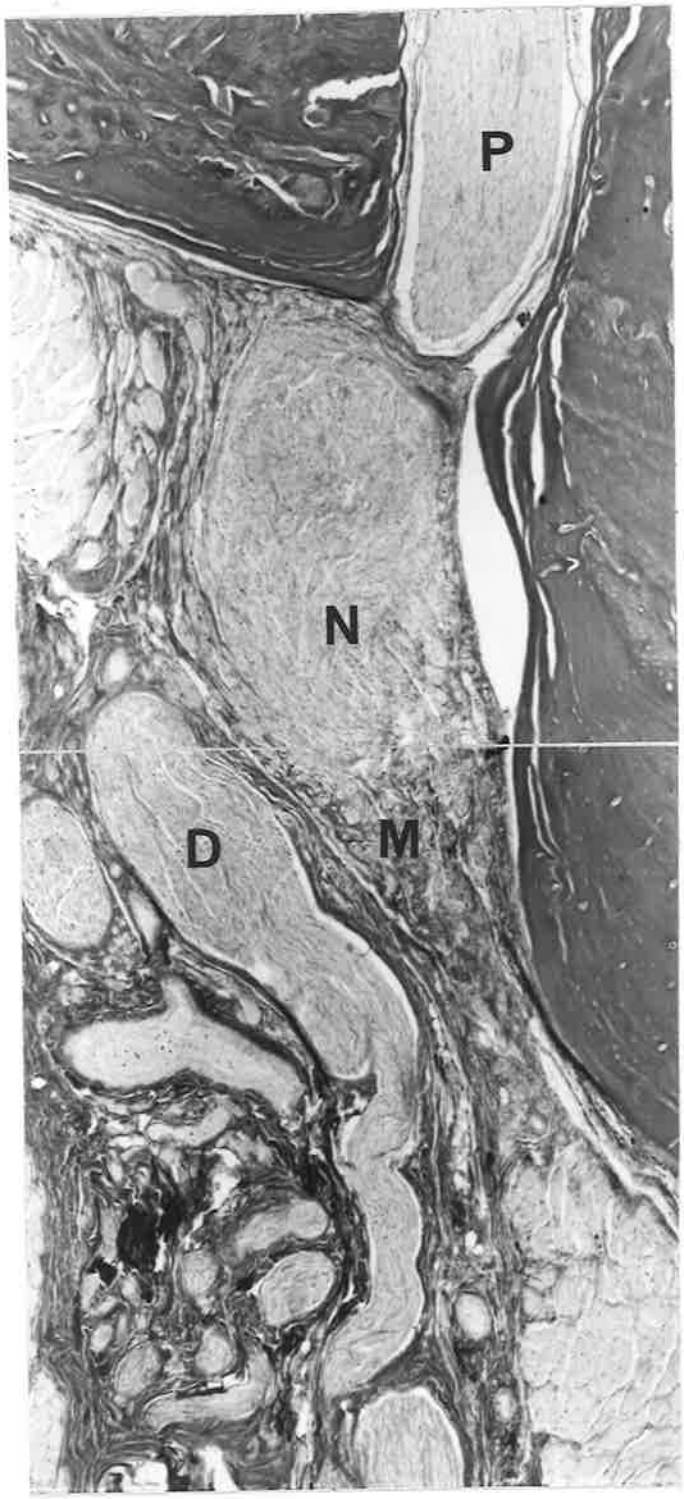


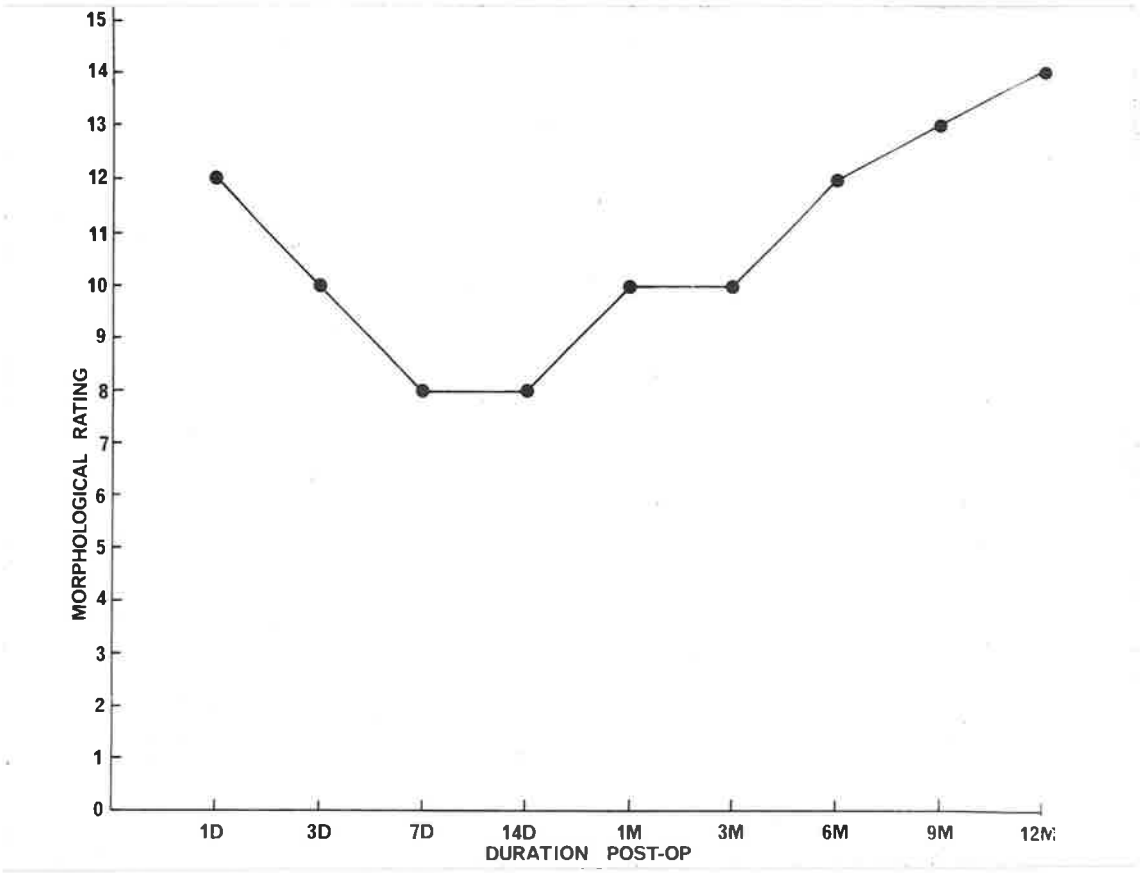
Fig. 4.46 12 months after sectioning. Proximal trunk, neuroma, distal trunk and minifuniculi - The collagen content in the nerve trunks was much less than in the connective tissue. (V.G.) x 40

MORPHOLOGICAL RATING

The summation of the results using the morphological rating is presented in tabulation form (table 4.1) and graph (graph 4.1). Note the abscissa of the graph does not have uniform units of time in the graph. Rating within each group was uniform.

Post operation	Morphological rating
1 day	12
3 days	10
7 days	8
14 days	8
1 month	10
3 months	10
6 months	12
9 months	13
12 months	14
Control	16

Table 4.1 Contents of morphological rating in relation to time.



Graph 4.1 - Morphological rating versus time.

CHAPTER V

DISCUSSION

5.10 ELECTROPHYSIOLOGICAL RESULTS

5.20 HISTOLOGICAL RESULTS

5.30 FURTHER RESEARCH

DISCUSSION

The results of the physiological tests are in Appendix II; the discussion of the problems encountered is presented below. The second part of the discussion relates to the histological results.

5.10 ELECTROPHYSIOLOGICAL RESULTS

The preliminary work on the anaesthetic agent, Sagatal, had shown that it was acceptable but that it exhibited certain problems. Because of these, it was evident that there was going to be a significant mortality and a reasonable number of repeat experiments.

The initial tests using the strength duration technique demonstrated that it was unsuitable for testing the response of the mental nerve to sectioning. This was most obvious on the seventh day after sectioning when the chronaxis of the operated nerve was not consistently and significantly different from the control (non-operated mental nerve). During this period, the greatest difference between these chronaxis should have been evident but this was not so. This implied that there was a leakage of current which could either directly stimulate the digastric muscle or its nerves. Another possibility was that the presence of additional nerves in the area may have been involved in initiating the jaw jerk reflex. It was also possible, that there was a combination of these factors in operation. Thus no electrophysiological tests were conducted in this experiment to determine the physiological response of the sectioned mental nerve.

5.20 HISTOLOGICAL RESULTS

The degenerative and regenerative processes in this experiment were, in general, consistent with the description of neural responses after transverse sectioning given by Ramon y Cajal (1928), Young (1942), Guth (1956) and Sunderland (1968). However, there were some slight differences from the current concepts. Apart from these differences there were significant

variations of neural responses within each group. This was only expressed as degree of response and not by different types of responses e.g. figures 4.19 and 4.20 show the degree of myelin degeneration and figures 4.30 and 4.31 show the different degree of axon regenerating pattern. However, these differences were not reflected in the morphological rating which was uniform within each group.

The morphological rating system was devised to quantitate the optimum histological features of neural response to transverse sectioning. After sectioning the morphological rating should progressively decrease with time as degeneration progresses. As the regenerative process develops the units within the system should counteract the low rating awarded due to the degenerative process. Once the degenerative processes are completed the regenerative process takes over and the rating should increase, which was the case in this instance. The final aspects of the morphological rating were dependent upon the maturation of the regenerated nerve fibres.

The neural responses were reflected in the results obtained; the lowest rating was awarded on the seventh and fourteenth day after sectioning. From the one month period the regenerating and maturity processes have taken over. It should be noted that the morphological rating never returned to normal. At the twelve month period the rating was fourteen out of a maximum of sixteen units.

Myelin response in the proximal trunk was uniform in appearance but varied in the length of involvement. In none of the rats did the myelin response extend more proximally than the mental foramen (figure 4.9). The possible reason for this is that the sectioning was carried out approximately 5mm from the mental foramen. Hudson and Kline (1975) stated the myelin response was known to occupy 2-3mm proximal to the section. Therefore, the myelin response should not have extended to the mental foramen. Actual degree

of myelin response was slight and did not progress any further with time after day one. The response was time limited and progressively decreased, until 14 days after sectioning when normal myelin staining returned approximately up to the site of sectioning.

The axon degeneration in the proximal segment could not be observed at all in any of the sections examined. This was contrary to the opinion held by Mathews and Osterholm (1972) who stated that Wallerian degeneration occurred in the proximal trunk for a distance of 1 or 2 nodes of Ranvier. Neither were any observations made of terminal axon swellings in the proximal trunks in the selected periods of examination. Urich (1976) found these axon swellings which developed as club-like terminal expansions with considerable variations in size and shape. No decrease in axon diameter was observed at any stage [as was reported by Cragg and Thomas (1961)] if peripheral contact was not re-established. This was likely to be due to a large proportion of axons making peripheral contact and also there were the limitations of the experimental model. The axon branches were evident on the third day after sectioning (figure 4.11 and 4.12). This was in agreement with Huber and Lewis (1920) who stated that vigorous regrowth began inside the proximal end within 2-3 days.

On the seventh day after sectioning, in the proximal trunk (figure 4.14) adjacent to the site of section, (figure 4.15) there was a mixture of axons and axon branches. The implication of this was that the axons retracted by varying distances from the site of section even though there was a uniform approach to sectioning of the nerve. Closer examination of all the proximal trunks revealed a random pattern of distribution of axon branches in relation to the axons. This infered that the variation was unlikely to be due to experimental method error.

The myelin degeneration in the distal segment was observed on the first

day after sectioning (figure 4.8), but unlike the proximal trunk the changes were uniform throughout the entire distal trunk and the degeneration progressed with time. On the third day after sectioning, degeneration had progressed further (figure 4.13). In the time span between the seventh day and fourteenth day after sectioning and most probably some days on either side of this period the greatest degree of myelin degeneration occurred (figures 4.19, 4.20 and 4.27). It was unexpected to find that so few cells were observed in some of the distal trunks with the myelin stain. A possible explanation for this phenomenon was that myelin had undergone chemical degeneration within this period as Johnson et al (1950) found. This was supported when the haematoxylin and eosin stain was used in adjacent sections; numerous cell nuclei were stained in the distal trunks (figure 4.23). This implies that there were cells present but very few had sufficient myelin to take up the solochrome cyanine stain.

Axon degeneration in the distal trunk appeared to develop in a uniform manner along the entire length of the distal trunk. On the first day after sectioning there were large islands of broken down axons throughout the distal trunk (figure 4.7). By the third day the degeneration had progressed so far that there were only thin strands of axon debris scattered throughout the distal trunk (figure 4.10). Only isolated remnants of axons were observed by the seventh day throughout the distal trunk (figure 4.18). However, this finding should not be interpreted as support for Sugar's (1938) results which proposed that there was uniform axon degeneration throughout the distal trunk as against Rosenblueth and Del Pozo's (1942) conclusion that the axon degeneration occurred in a centrifugal pattern. The reason why the results from this experiment could not be used to support the process suggested by Sugar was because of the limitations of the present experimental model.

Axon debris was still present as regenerating axons were moving down the distal trunk (figures 4.16 and 4.18). This observation was consistent with Lubinska's (1952) findings that degeneration must reach a certain stage before regenerating axons will grow into the denervated tubes.

An expansion of the proximal trunk was observed at 7 days after sectioning (figure 4.23). This expansion was composed of axons, Schwann cells and fibroblasts (of various origins). However, differentiation between these cells under the light microscope could not be made. This is in accord with the observations of Jurecka et al (1975) who found that under the light microscope this differentiation could not be made easily. No obvious expansion of the distal trunk could be observed in any of the seven day series (figure 4.23). The axons, on leaving the proximal trunk, entered the connective tissue. Their course in the connective tissue was varied (figure 4.11) at 3 days after sectioning demonstrating a flared pattern. With time some of the axons entered the distal trunk. At the seventh day axon branches were observed in the distal trunk (figure 4.16) and by the fourteenth day axon branches had moved further down (figures 4.24 and 4.25), but not all axons entered the distal segment and moved into the connective tissue. The earliest period that this could be observed definitely was at the fourteenth day (figure 4.26). There was a continuous reduction of these free axons with time and at the sixth month none could be found. The reason for this progressive reduction was due to degeneration of some of these axons and more axons followed those that did not degenerate to form minifuniculi. The various proportions of axons that either moved down the distal trunk, formed minifuniculi or degenerated could not accurately be determined with this experimental model.

Small nerve trunks composed of normal mature axons and myelin definitely were observed on the seventh day after sectioning (figures 4.21 and 4.22) and this was within the period of maximum degeneration (see

morphological rating graph). Their size but not their anatomical maturity was comparable to the large minifuniculi that were observed at the third month (figure 4.33). The origin of these small nerve trunks was unknown. Possibly they could have arisen from the inferior dental nerve but left the mandible some distance from the mental foramen and therefore were not sectioned. Another possibility was that they could be related to the digastric neuromuscular apparatus. Sicher and Du Bvul (1975) found that the mylohyoid nerve supplies motor control to the mylohyoid muscle and the anterior belly of the digastric muscle and can also provide, in most humans, a sensory supply to the chin. This implies that the mylohyoid nerve could either stimulate the digastric contraction by direct stimulation or arch reflex. These possibilities could provide the full or partial explanation as to why the electrophysiological results were a failure. On the other hand, the possibility remains that these nerves could be unrelated to the inferior dental nerve or the neuromuscular apparatus of the digastric muscle. Should this be so, their presence would not affect the electrophysiological results.

The severed trunks were not aligned in any of the rats but no matter what the degree of displacement in this series, axon branches entered the distal trunk in reasonable numbers (figure 4.31). This was consistent with the results of Sunderland (1953) who found that axons can travel long distances to find and enter the distal trunk. No correlation could be made in this experimental model with the per cent of axons that enter the distal trunk in relation to the degree of displacement, but it seemed that with greater displacements there were more minifuniculi formed.

These minifuniculi arose from the neuroma (figure 4.45). Most of these minifuniculi had a normal mature myelin and axon form, but some did not

have normal mature myelination (figure 4.44). The size of these minifuniculi varied considerably. There was no problem in differentiating the small minifuniculi from the normal large trunk branches (figure 4.1). The minifuniculi examined close to the neuroma could readily be identified, no matter what size, but the more peripheral the observations made, the greater the problem was to differentiate the minifuniculi from the trunk branches. In addition, the accessory nerve branches with time became increasingly difficult to differentiate. By the twelfth month after sectioning, it was impossible to make any such distinction.

Within the twelve month period most of the nerve fibres had assumed a normal mature pattern in all the distal trunks (figures 4.36 and 4.43). The return of mature morphology of the mental nerve was not in agreement with Bradley's (1975) statement that if the anatomical continuity of the nerve was destroyed, regeneration was likely to be poor or absent. Furthermore, Weiss et al (1945) indicated that normal maturation could only occur if the nerves were in contact with the receptors and functioning. Therefore, from a histological point of view it was apparent that in the rat the mental nerve need not be sutured for anatomical continuity to return.

There was a significant increase in collagen on the seventh day and this reached a maximum on the fourteenth day. The deposition was especially observed in the neuroma (figure 4.28). This was in agreement with Dunphy and Udupa (1955) who found that collagen formation extended from the fifth or sixth day and increased to a maximum on the fifteenth day when they terminated their experiment.

Some of the axon branches on the seventh day had already started to move down the distal trunk (figure 4.16). This meant that the collagen

could not redirect these axons, but it could affect the axon branches that had not entered the distal segment. This could imply that the collagen is not very important in directing the axons when the distal stump is close to the proximal segment. However it can still affect the maturation of the nerves as concluded by Weiss and Taylor (1944).

The collagen content in the distal trunk was never great. This could readily be observed when comparing collagen in the distal trunk at the fourteenth day after sectioning (figure 4.29) and the control (figure 4.4). Both of these represent the extremes i.e. the control was the lowest and the fourteenth day was when the maximum amount of collagen was deposited. In contrast to this, the changes in collagen content in the neuroma were more dramatic. The maximum collagen content was observed at the fourteenth day (figure 4.28), dramatically less at the third month (figure 4.35) and there was a further reduction at the sixth month period (figure 4.39). No further reduction in collagen could be observed over the remaining period of this experiment.

The extrapolation of this experiment to the clinical situation is very limited, but it does have some bearing on the problems encountered in man. When the mental nerve was sectioned and the ends were not approximated by means of suturing, the neuroma interconnected the two segments. In addition the minifuniculi that were formed from axons that did not enter the distal trunk could form useful functioning units. So these two mechanisms could explain why there is a return of sensation to the lip after the mental nerve is sectioned and not sutured. This sensation can be in the form of hyperaesthesia or hypoaesthesia, but it is a return of sensation of some type. The return of sensation is due to a number of factors and their interplay with each other. All these factors are related to the mental nerve's physiology and anatomy. These aspects

can be listed:-

(a) The mental nerve is basically a pure sensory nerve. The premise that a pure nerve either sensory or motor has a better prognosis than a mixed nerve is often supported by clinical observations, Brown (1972).

(b) The mental nerve is distinctly peripheral to the trigeminal ganglion. This aspect is very important as the more peripheral the lesion occurs the better the prognosis. This is a well documented clinical observation, Brown (1972).

(c) The anatomical area containing the mental nerve (the lip) is a restricted compartment of small volume. This implies that the proximal and distal nerve stump cannot be greatly displaced. Brown (1972) has stated that prognosis is influenced by the displacement. Therefore the mental nerve should have a better prognosis after sectioning than most other sensory nerves.

FURTHER RESEARCH

The study was limited to light microscopic investigation of the changes occurring in peripheral nerves after sectioning. More sophisticated techniques using the electron microscope, advanced histochemistry and autoradiography have recently been used, reporting on changes at the cellular level which could not be observed by light microscope. Apart from studying the nerve responses at the anatomical and biochemical levels the electrophysiology techniques have also become more sophisticated recently with the introduction of the electroneuronography tester, Fisch (1974).

It is evident that from this, further profitable research can now be conducted into the neural responses to damage. However, before these advanced techniques are used, there are still research areas where relatively simple experimental techniques should be carried out in the laboratory and in the clinic.

Histological examination should be undertaken of laboratory animals which have had their sectioned mental nerves re-sutured. These results should then be compared with the present investigation.

Another light microscope examination could be made of laboratory animals which have had part of the inferior dental nerve and the whole mental nerve removed. These results could be used to see what effect the inferior dental canal has on regenerating axons with comparatively large distances to cover.

In the clinical sphere the use of physiological techniques to evaluate mental nerve damage and monitor their regeneration may provide a prognostic method for the prediction of complete recovery.

SUMMARY

An experimental project has been carried out on rats to investigate the mental nerve's response to transverse sectioning.

Histological and histochemical methods have provided information regarding the peripheral response of the sectioned nerve. The results obtained from these methods have confirmed the finding of previous investigations into the neural response. Unfortunately the electrophysiological pilot study demonstrated that the strength duration technique could not be used in the rat to evaluate the peripheral neural response.

The present histological and histochemical study has shown:

1. Degeneration of Axons and Myelin.

   (a) Axons in the proximal trunks retracted by varying degrees from the section site.

   (b) No observable axon degeneration took place in the proximal trunk.

   (c) Myelin degeneration was only slight, extending for a few millimetres proximally and was unchanged after day one. The response progressively decreased with time until the fourteenth day after sectioning when the myelin returned to normal up to the section site.

   (d) In the distal trunk there was an apparent uniform axon degeneration throughout the entire segment and a few remnants were observed on the seventh day after sectioning.

   (e) Myelin degeneration in the distal trunk occurred in a uniform manner. However, unlike the myelin response in the proximal trunk, the degeneration progressed with time. On the seventh day after sectioning there was very little myelin staining in some of the distal trunks.

2. Collagen Response.

   (a) Significant collagen deposition was first noticed on the seventh day after sectioning. Progressive deposition of collagen occurred

until the fourteenth day after sectioning when the deposition reached its maximum.

(b) The greatest deposition of collagen occurred at the section site, some deposition occurred in the distal trunk and no change was observed in the proximal trunk.

(c) Significant collagen deposition was observed on the seventh day after sectioning. Simultaneous with this, the axon branches were moving down the distal nerve trunk. This indicated that collagen could not affect significantly the direction of the regenerating axon branches. However, it could still affect the maturation of these fibres.

3. Regeneration of Axons and Myelin.

(a) The regenerating axon branches were observed on the third day after sectioning in the connective tissue.

(b) Seven days after sectioning the axon branches were moving down the endoneurial tubes while there was debris still detectable in the distal trunks.

(c) By the ninth month after sectioning the axons in the distal trunk were comparable to the control in distribution, diameter size and composition.

(d) Regenerating axon branches moved peripherally via the distal trunk and others through the connective tissue. Those moving through the connective tissue could not be found six months after sectioning. This could be due to degeneration of some of these axons or other axons following those that did not degenerate and forming minifuniculi.

(e) It appearedthat the greater the relative displacement of the sectioned trunks the more minifuniculi were observed.

(f) Mature myelination was found in all the distal trunks twelve months after sectioning, but not all the minifuniculi had large proportions of mature myelination.

APPENDIX I

1.0 DIET

All animals used in this investigation were fed on demand with the following stock diet.

Ground wheat	40.0%
Ground barley	18.0%
Bran and pollard	12.0%
Meat and bone meal	9.6%
Extracts of soya meal	6.2%
Fish meal	6.2%
Milk powder	3.0%
Brewers yeast	1.0%
Salt	1.0%
Molasses	3.0%

Vitamin supplement per kilogram of feed:

Vitamin A_3		3928 I.U.
Vitamin D_2		928 I.U.
Vitamin B_6	1.5mgs	
Vitamin B_{12}	0.2mgs	
Vitamin B	3.4mc gms.	
Vitamin E	1.2mgs	
Vitamin K (menadione)	0.5mgs	
Pantothenic acid	0.5mgs	
Choline chloride	25.0mgs	

2.0 SURGICAL ANAESTHESIA

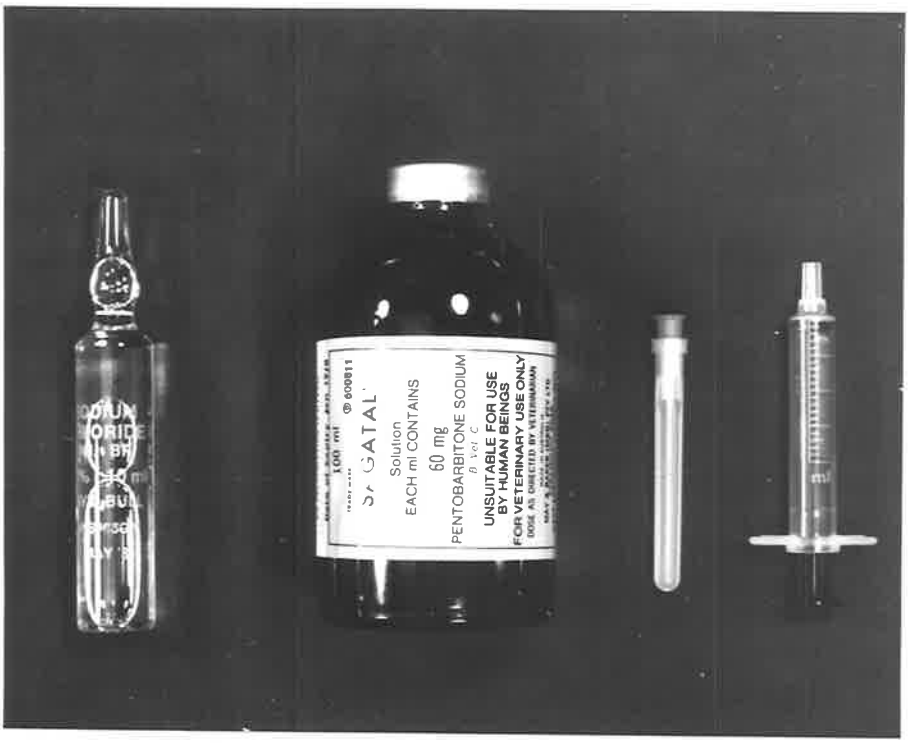
Surgical anaesthesia was induced by using Sagatal, pentobarbitone (Nembutal-Abbot) to the manufacturers' recommendation 1ml of the 6% solution (Veterinary 60mgs/ml) per 5lb body weight (5lb = 2250 gms). The Sagatal was diluted 1:9 with sterile saline in preparation before administering the injection intra-peritoneally.

Therefore the dose required to obtain surgical anaesthesia was calculated upon this formula

$$\text{Dose} = \frac{X \times 9}{2250}$$

(where X = the weight of the rat in grams)

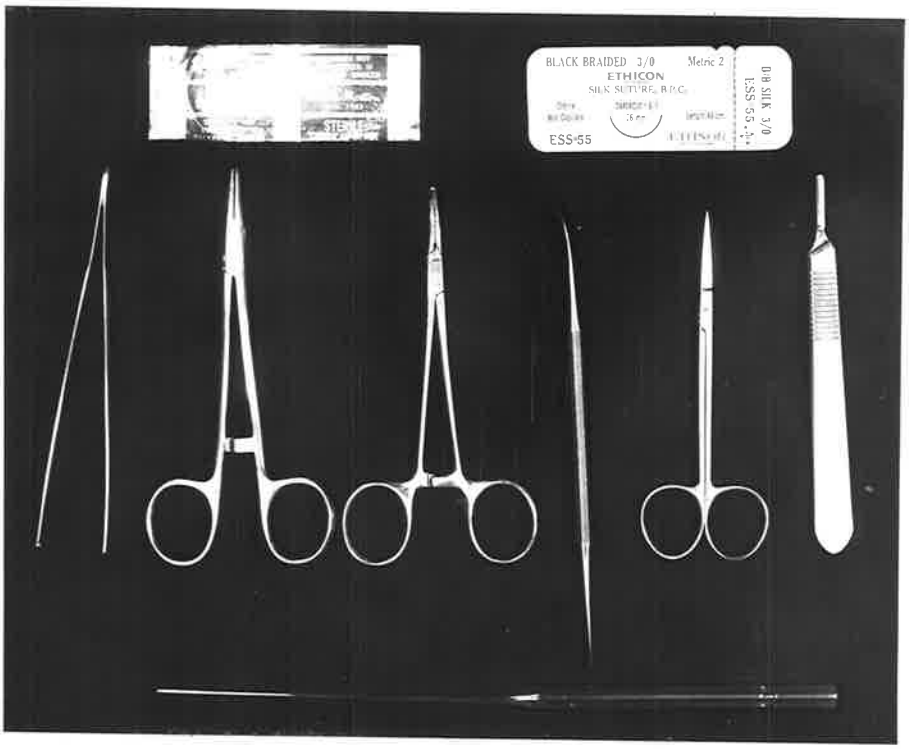
2.1 ANAESTHETIC EQUIPMENT



- 10ml ampule of normal sodium chloride
- bottle of Sagatal
- disposable 5ml syringe
- needle 21 guage

3.0 METHOD OF WOUND PRODUCTION

Instrumentation:



1. Scalpel handle and number 15 scalpel blade
2. Scissors
3. Fine tipped sucker
4. Fine mosquito artery forceps
5. Wax spatula number 7 (periosteal elevator)
6. Kilner needle holder and 3/0 black silk suture
7. Gilles tissue forceps

4.0 TISSUE FIXATIVE

A: The F.E.C. fixative (Hribar 1977)

37-40% formalin, neutral	80mls
50% ethyl alcohol	720mls
Chloral Hydrate	38gms.

Each specimen was placed in a sealed container with 50mls of the fixative for two weeks at room temperature.

B: Decalcification of the Hard Tissue

Decalcification Solution was EDTA at pH 6.5 (Hribar 1976)

Tris Buffer Sigma (7-9)	12.1gms
EDTA	100.0gms
Deionized water	1000mls
KOH pellets	added to adjust pH

The Tris buffer, EDTA and Deionized water were placed in a flask. These contents were magnetically stirred while the KOH pellets were added. Electrodes recorded the changes in pH. The KOH pellets were added until the pH was 6.5.

Tissues undergoing decalcification were provided with fresh decalcifying solutions each day. Determination of end point of decalcification was determined by radiography.

5.0 EMBEDDING PROCEDURE

After decalcification, the specimens were placed in F.E.C. fixative for 24 hours before double embedding. The specimens then went through the following reagents:

37°C

1.	Alcohol	70%	1 hour
2.	"	80%	"
3.	"	90%	"
4.	"	95%	"
5.	"	absolute	"
6.	"	"	"
7.	"	"	"
8.	Absolute alcohol and Methyl Salycylate (ratio 1:1)		2 days
9.	Methyl Salycylate and Celloidin	0.5%	2 days
10.	Celloidin	1%	2 days

60°C

11.	Wax and Methyl Salycylate	(1:2)	1 hour
12.	" "	(1:1)	1 hour
13.	" "	(2:1)	1 hour

56°C

14.	Wax	1 hour
15.	Wax	1 hour
16.	Wax	overnight

The specimens were then placed for one hour in a vacuum to eliminate air bubbles. After this the specimens were blocked in wax at 56°C.

Abstract from International Association for Dental Research
Australian and New Zealand Division - Programme and Abstracts of Papers
17th Annual Meeting (1977) p.11.

Comparison of F.E.C. with Established Neural Fixatives.

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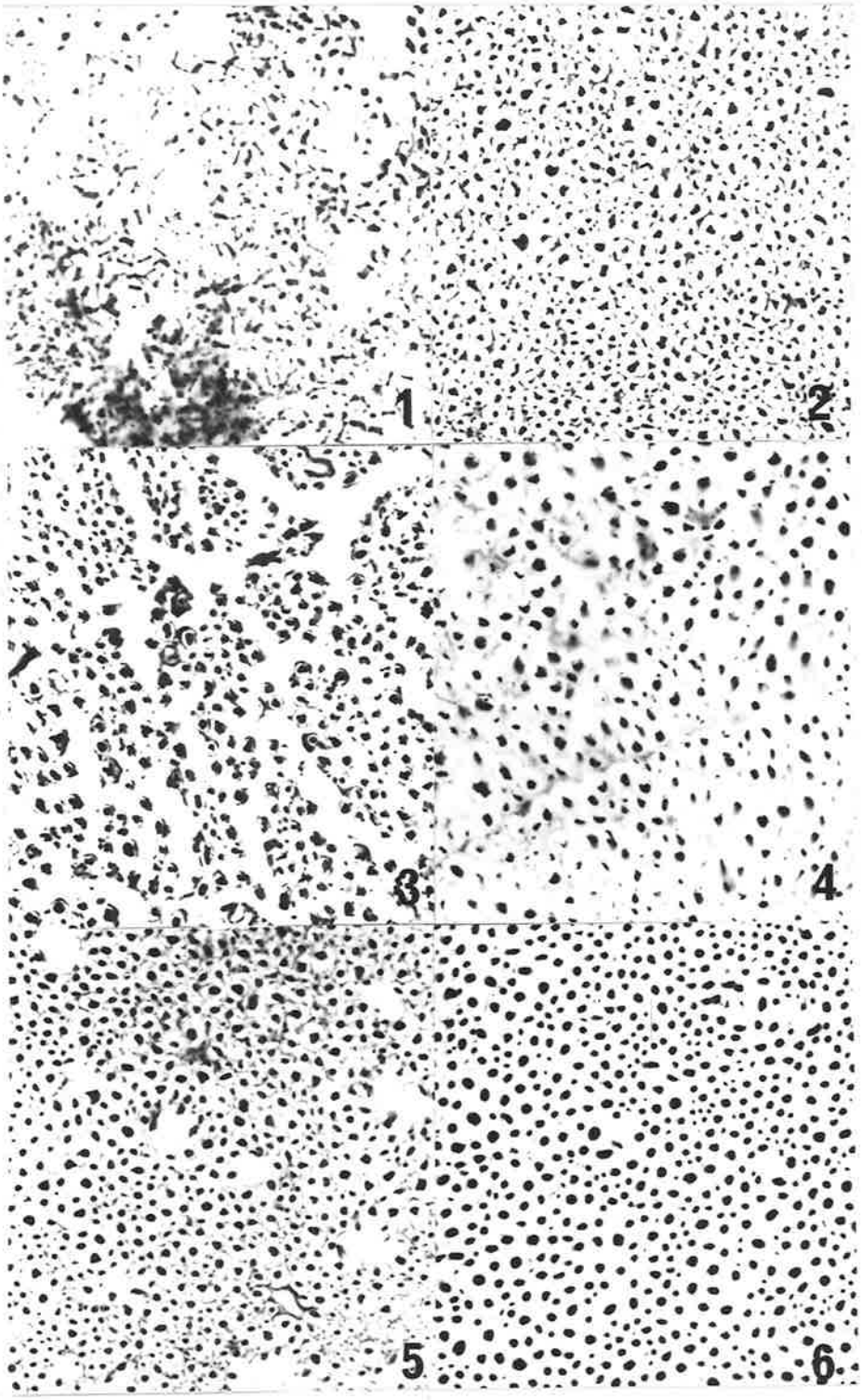
No routine histological fixative gives ideal results with neural tissue which is a considerable technical problem in the evaluation of experimental nerve injury. There are few publications evaluating the various types of neural fixatives. Hence 5 established fixatives and a new fixative were evaluated under standardized conditions. 36 mandibular nerves were obtained from 18 adult albino rats. The nerves were evenly divided into 6 groups and fixed in the following fixtives: Buffered Neutral Formalin, Formalin Ammonium Bromide, Formal Calcium, Bouin's Solution, Foramide Solution and F.E.C. (37% Formalin - 80mls, 50% Ethanol - 720mls, and Chloral Hydrate - 38gms. After 48 hours of fixation, routine standardized histological methods were used to prepare simultaneously 8u thick sections for Bodian's impregnation technique. The sections were then examined, with the experimental number covered, and the results were classified under the following criteria:- Preservation of neural integrity on the slide, adherence of the section to the slide, and impregnation specificity, uniformity and intensity. Using these criteria it was found that, Normal Buffered Formalin and Formal Calcium gave poor results and were totally unacceptable. Bouin's Solution and Formalin Ammonium Bromide gave reasonable results and can be used routinely. Formamide fixative gave better results but had limitations.
F.E.C. gave excellent results and can be strongly recommended for routine fixation.

The effect of fixatives used in the comparison of established neural fixatives.



1. Buffered Neutral Formalin
2. Formalin Ammonium Bromide
3. Formal Calcium
4. Bouins Solution
5. Formamide
6. F.E.C.

APPENDIX II

ELECTROPHYSIOLOGICAL PILOT STUDY

1.0 Dose of Anaesthetic Agent

The dose used was calculated on the formula given in appendix I, using the animals weight. This varied to see what effect the extra anaesthetic agent had on the rat's reponse.

25 non-operated rats were anaesthetised with the following dosage (5 rats in each group)

Group 1 - formula dose

Group 2 - 1¼ x formula dose

Group 3 - 1½ x formula dose

Group 4 - 2 x formula dose

Group 5 - 2¼ x formula dose

The potentially anaesthetised rats were tested using the toe-tug test to determine if anaesthetised. These results were tabulated.

GROUP	DIED	NOT ANAESTHETISED	ANAESTHETISED	AV. DURATION
1	1	1	3	26 min.
2	1	1	3	27 min.
3	1	0	4	30 min.
4	5	0	0	-
5	5	0	0	-

The reason for the variation within each group was unknown as the administration was given by the same operation using a standardized technique. Possible reason for the variation was that the anaesthetic agent was not administered intra-peritoneally. In the rats that died the anaesthetic was partly administered intra-vascularly, even though aspiration tests were negative. In the rats that were not anaesthetised the agent was deposited within the lumen of the gut.

The conclusion was that groups 1, 2 and 3 might be suitable while groups 4 and 5 were unsuitable.

2.0 Long Term Effects

A requirement of the physiological protocol was to anaesthetise the rats once a week for 14 weeks. A long term study on the effects of the anaesthetic agent was conducted.

This experiment used varying anaesthetic dosages to anaesthetise 30 rats. The dosage tested was that of groups 1, 2 and 3. Anaesthetisation was conducted once a week for two months.

The results were tabulated:

Weeks	Group 1			Group 2			Group 3		
	Unaes.	Anaes.	Died	Unaes.	Anaes.	Died.	Unaes.	Anaes.	Died
1	2	8	0	1	9	0	0	8	2
2	3	6	1	1	6	3	0	4	4
3	1	6	2	1	4	2	0	4	0
4	0	7	0	1	4	0	1	3	0
5	1	6	0	0	4	1	0	3	1
6	2	5	0	1	3	0	0	3	0
7	0	6	1	0	4	0	0	3	0
8	1	5	0	1	2	1	1	1	1
ALIVE		6			3			2	

From the above it can be seen that this type of anaesthetic agent is not without its hazards and is not absolutely reliable in producing anaesthesia. There does not appear to be a cumulative effect of the drug on the animals, though with each administration there was a mortality, especially with the higher doses. In Group 1 the mortality was the lowest.

Therefore the Group 1 dosage levels were used in subsequent experiments.

3.0 Duration of Operative Anaesthesia

Even though the Group 1 dosage level presented problems it was safer to use. A further test was carried out using Group 1 dosage on ten new unoperated rats to establish the duration of effective testing.

If the anaesthetic worked, an average of 7 minutes was required (after induction) until the animal could be tested. In five of the animals the right mental nerve was tested first and then the left mental nerve. The alteration of testing was carried out until the rat was unco-operative or until both sides were tested twice.

In the other five rats the only difference was that the left side was tested first instead of the right side. This was done to negate any bias in the electrophysiological testing.

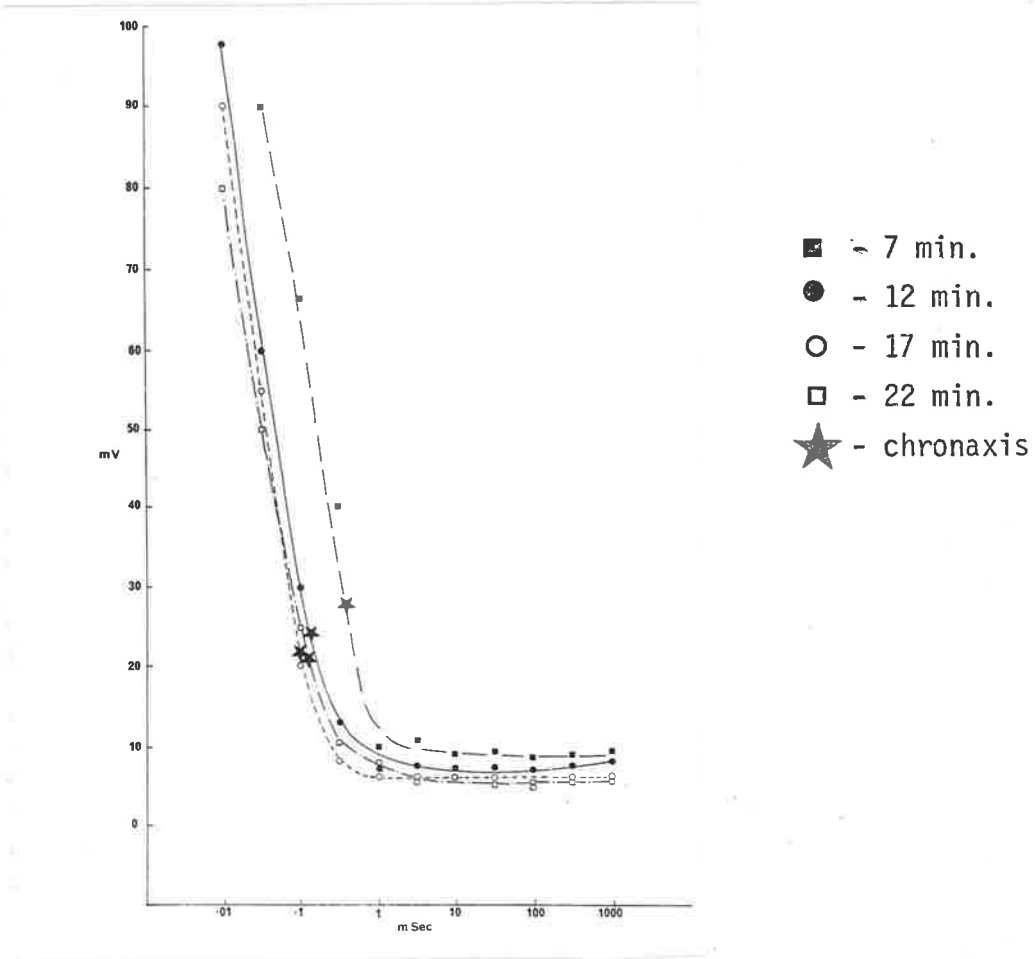
The following table contains individual results of each nerve's rheobase and chronaxis to the corresponding time after injection.

ANAESTHESIA VERSUS TIME

Rat. No.	Left Nerve 7 min.		Right Nerve 12 min.		Left Nerve 17 min.		Right Nerve 22 min.	
	Chr.* m.sec.	Rheo.+ m.V.	Chr* m.sec.	Rheo.+ m.V.	Chr* m.sec.	Rheo.+ m.V.	Chr* m.sec.	Rheo.+ m.V.
1.	.5	10	.4	8	.25	6	.3	5
2	.85	10	.6	4	.4	4	.3	3
3	.6	9	.2	8	.10	6	.15	5
4	.4	9	.45	8	.4	7	.35	6
5	.1	7	.15	6	.09	5		

* Chronaxis
\+ Rheobase

A representative graph was chosen,i.e. rat number 3, graph 2.1



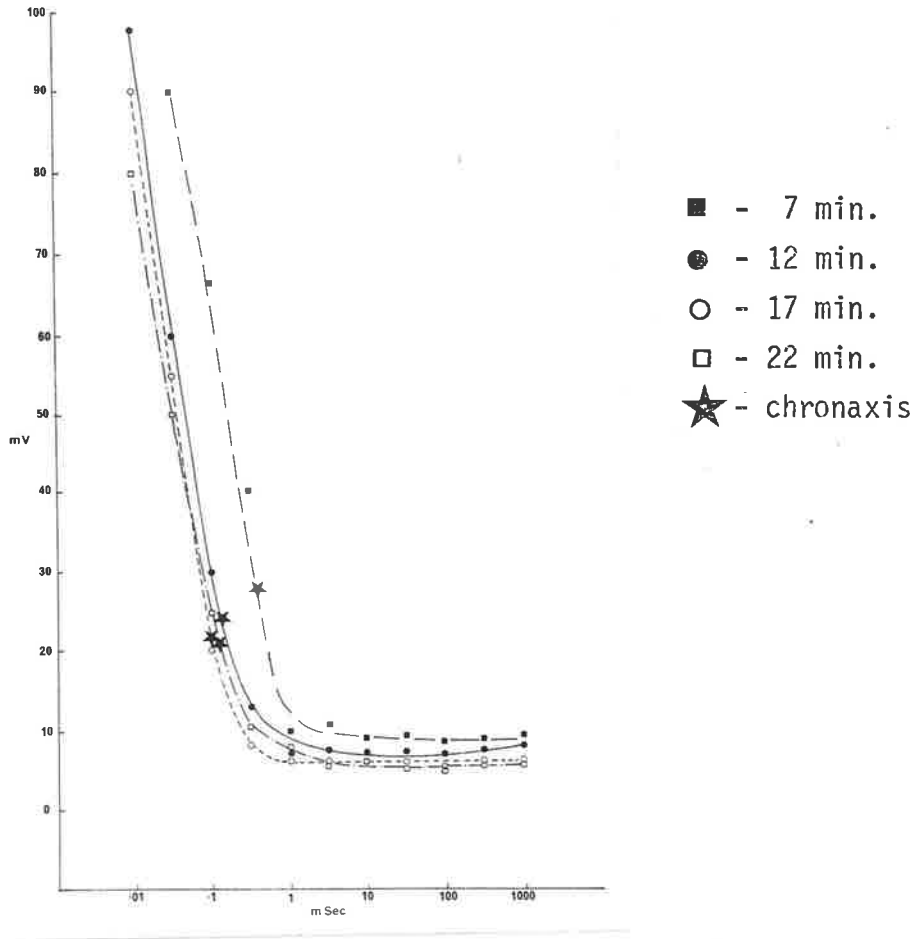
Graph 2.1 - Results of rat number 3.

Rat No.	Right Nerve 7 min.		Left Nerve 12 min.		Right Nerve 17 min.		Left Nerve 22 min.	
	Chr.* m.sec.	Rheo.+ m.V.	Chr.* m.sec.	Rheo.+ m.V.	Chr.* m.sec.	Rheo.+ m.V.	Chr.* m.sec.	Rheo.+ m.V.
6	.5	9	.6	8	.2	4		
7	.85	12	.8	11	.8	10	.3	8
8	.4	12	.2	6	.15	5	.08	4
9	.09	8	.1	6	.35	5		
10	.6	13	.6	10	.7	8	.5	6

* Chronaxis

+ Rheobase

A representative graph was chosen, i.e. rat number 8, graph 2.2



Graph 2.2 - Results of rat number 8.

These results did not show any consistently obvious difference with time in the chronaxis, so the 12 minute interval was selected for further testing.

4.0 Left Mental Nerve Versus Right Mental Nerve Response

Using the previous results the average of the left and right response was compared and no obvious differences were seen.

5.0 Operated Versus Non Operated

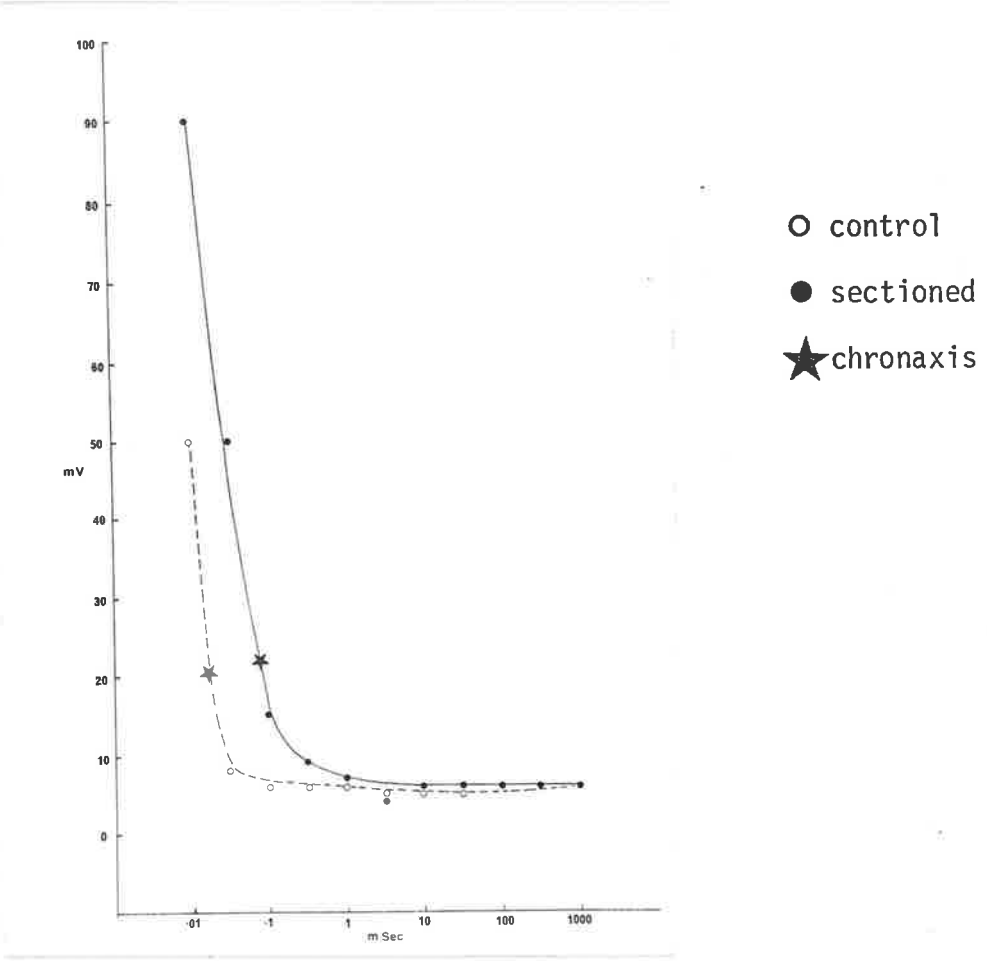
Testing was carried out on 6 rats 7 days post operatively. The right mental nerve was sectioned in three of these rats, while in the others the left mental nerve was sectioned. This meant that in each rat the contralateral side was used as the control.

The results were:

For the sectioned left mental nerve:

Rats	Control (right side)		Sectioned Nerve	
	Chronaxis	Rheobase	Chronaxis	Rheobase
1	.04 m.sec.	6 mV	.09 m.sec.	7 mV
2	.5 "	3 "	.5 "	5 "
3	.6 "	1 "	1.0 "	4 "

A representative graph was chosen, i.e. rat number 1, graph 2.3

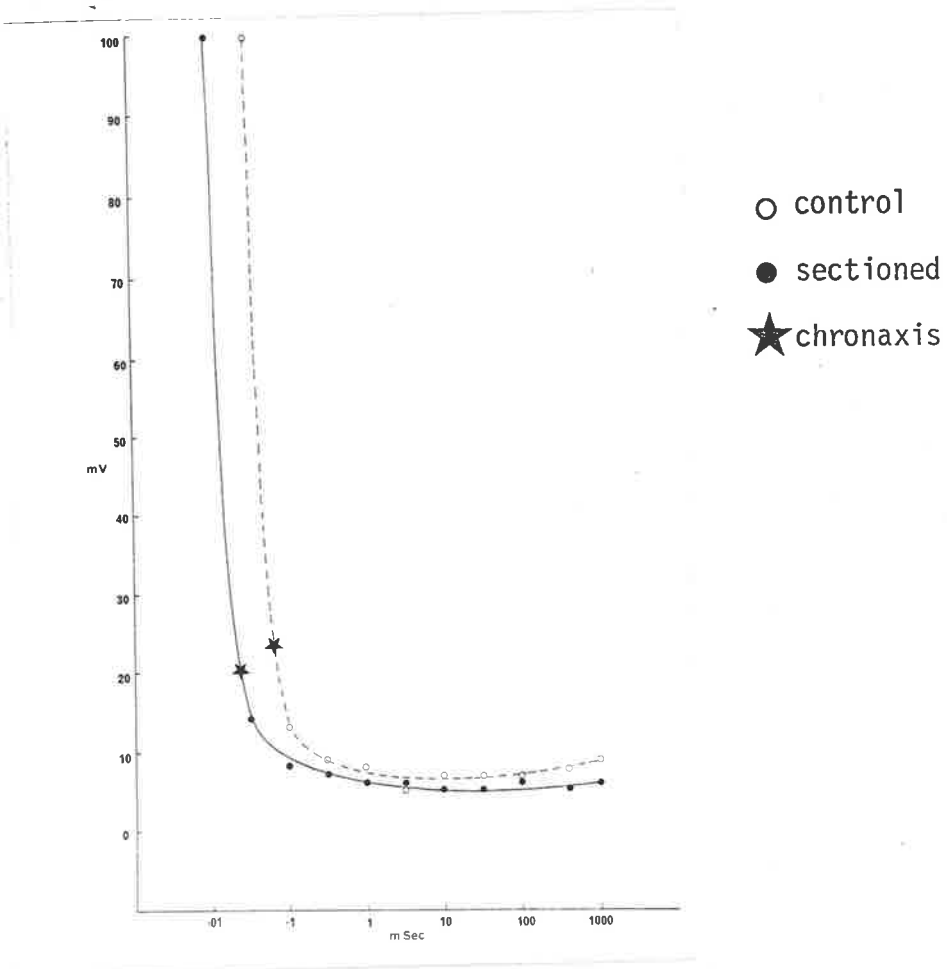


Graph 2.3 - Result of rat number 1.

For the sectioned right mental nerve:

Rats	Control (left side)		Sectioned Nerve	
	Chronaxis	Rheobase	Chronaxis	Rheobase
4	.4 m.sec.	4 mV	.3 m.sec.	6 mV
5	.08 "	5 "	.04 "	8 "
6	.3 "	1 "	.4 "	5 "

A representative graph was chosen, i.e. rat number 5, graph 2.4



Graph 2.4 - Results of rat number 5.

There was not an obvious difference between sectioned and non sectioned mental nerve on the strength duration test.

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