

DEGENERATION STUDIES OF THE FASCICULI IN  
N. ISCHIADICUS IN THE DOG

by

ABDURRAHMAN KASIM GHAJI

D.V.M., Ahmadu Bello University, 1973

---

A MASTER'S THESIS

submitted in partial fulfillment of the  
requirements for the degree

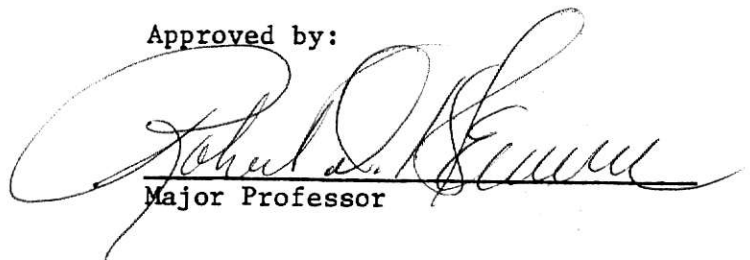
MASTER OF SCIENCE

Department of Anatomy and Physiology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1976

Approved by:



Major Professor

LD  
2668  
J4  
1976  
G52  
C.2  
Document

2.14 11

## TABLE OF CONTENTS

	Page
LIST OF TABLES . . . . .	111
LIST OF FIGURES . . . . .	iv
INTRODUCTION . . . . .	1
LITERATURE REVIEW . . . . .	3
MATERIALS AND METHODS . . . . .	10
Clinical studies	
Electromyographic studies	
Degeneration studies	
RESULTS . . . . .	21
Dissection studies	
Clinical studies	
Electromyographic studies	
Degeneration studies	
DISCUSSION . . . . .	31
ACKNOWLEDGMENTS . . . . .	63
LITERATURE CITED . . . . .	64

## LIST OF TABLES

Table	Page
1. Experimental procedures and postsurgical changes . . . . .	15
2. Results of postsurgical changes and neurological tests on the operated leg . . . . .	24

## LIST OF FIGURES

Figure	Page
1. Schematic diagram showing the levels of resection of the tibial and fibular nerve bundles . . . . .	12
2a. Tibial nerve paralysis . . . . .	14
2b. Fibular nerve paralysis . . . . .	14
3. Preparation of nerve fibers for SEM . . . . .	19
4a. Tibial and fibular nerve bundles at the Foramen ischiadicus majus . . . . .	35b
4b. Tibial and fibular nerve bundles dividing to give rise to the muscular branches . . . . .	35b
5a. Motor unit potential recorded from the normal M. tibialis cranialis . . . . .	36a
5b. Fibrillation potentials recorded from the denervated M. gastrocnemius . . . . .	36a
6a. Normal tibial nerve bundles (Dog B-1) . . . . .	37
6b. Degenerate fibular nerve bundles (Dog B-1). . . . .	37
7a. T.S. of normal tibial nerve fibers . . . . .	39
7b. Degenerate nerve fibers of fibular nerve bundle . . . . .	39
7c. L.S. of the transitional zone of the neuroma . . . . .	41
7d. L.S. of degenerate nerve fibers of the distal stump . . . . .	41
7e. L.S. normal nerve fibers of dog B-1 . . . . .	43
8a. Teased degenerate nerve fibers (L.M.) (Osmic acid stain). . . . .	45
8b. Teased degenerate nerve fibers (L.M.) . . . . .	45
8c. Teased normal nerve fibers (L.M.) (Osmic acid stain). . . . .	47
8d. Teased normal nerve fibers (L.M.) (Silver stain). . . . .	47



Figure	Page
9a. Teased normal nerve fibers (SEM) (Formalin fixed) . . . . .	49
9b. Partially degenerated nerve fibers (SEM) . . . . .	49
10a, b. Degenerate nerve fibers showing myelin swelling (SEM) . . . . .	51
10c, d. Broken myelin bubbles (SEM). . . . .	53
11a. T.S. paraffin section of degenerate nerve bundle (SEM) . . . . .	55
11b. T.S. degenerate nerve bundle (L.M.) . . . . .	55
12a. T.S. paraffin section of normal nerve bundle (SEM) . . . . .	57
12b. T.S. of normal nerve bundle . . . . .	57
13a. L.S. paraffin section of degenerate nerve bundle (SEM) . . . . .	59
13b. L.S. degenerate nerve bundle . . . . .	59

## INTRODUCTION

The N. ischiadicus,<sup>a,b</sup> a plurisegmental nerve, is the largest nerve in the dog. It has been described as the extrapelvic continuation of the Plexus lumbosacralis beyond the Foramen ischiadicus majus<sup>27</sup>. The division between the plexus and the nerve is marked by the contribution of the second sacral spinal nerve root at the Foramen ischiadicus majus<sup>46</sup>. The fibers of N. ischiadicus give out several Rami musculares to the Mm. Biceps femoris, semitendinosus and semimembranosus before turning distad and, at a variable level of the thigh, divides into Nn. fibularis and tibialis<sup>30</sup>. Hoerlein<sup>33</sup> and Sisson<sup>57</sup> described the point of termination of the N. ischiadicus as being in the lower third of the thigh while Miller<sup>46</sup> reported that the point of termination could be as high up as the Articularis coxae. There are some authors<sup>22,46,47,55</sup>, however, who describe the N. ischiadicus as two separate nerve bundles<sup>c</sup> bound together in a common sheath, with each nerve bundle possessing an epineurium. According to the authors, these nerves can be separated to their roots of origin by pulling them apart. Habel<sup>31</sup> omitted the N. ischiadius from his analysis of the pelvic limb nerves of the dog. Degeneration and electrical stimulation studies by McKinley<sup>45</sup> showed that there is no communication between fibers of the two nerve bundles which constitute the N. ischiadicus.

---

<sup>a</sup>The term N. ischiadicus used in this report refers to N. tibialis and N. fibularis communis bound together in a common sheath of connective tissue sheath from the Foramen ischiadicus majus to the point of separation of the nerves.

<sup>b</sup>Anatomical terms used in this report are according to the recommendations of Nomina Anatomica Veterinaria (1973).

<sup>c</sup>Fasiculi and bundles are interchangeable in this report.

It has also been reported that the clinical signs of the paralyses of N. ischiadicus in the dog are similar to the clinical signs observed (in the dog) when both Nn. fibularis and tibialis are traumatized. In spite of the volume of literature that has been reported on degeneration, electrical stimulation, clinical and dissection studies of the N. ischiadicus, there has been no report which correlated the clinical signs of the ischiatic nerve lesions at different levels (lower thigh, middle thigh and upper thigh), to the pattern of degeneration of the fasciculi of the N. ischiadicus. In assessing peripheral nerve injuries<sup>4,35,60</sup>, by exploratory laprotomy and electrodiagnostic techniques it is imperative that the surgeon employing these techniques in the diagnoses of ischiatic nerve injuries knows the functional and structural composition of the nerves or nerve bundles involved. With this in mind, the investigator has undertaken studies on the functional and structural changes in the N. ischiadicus resulting from resection of some of its major nerve bundles. Specific aims are:

1. To dissect the N. ischiadicus and its branches from the Foramen ischiadicus majus to the lower third of the thigh.
2. To study the clinical signs resulting from the resection of the bundles of the N. ischiadicus at the lower third of the thigh, middle of the thigh and at the Foramen ischiadicus majus.
3. To study the electrical patterns in dennervated muscles following the resection.
4. To study the degeneration patterns of the nerve bundles following resection using modified Marchi stain and teased nerve preparations.

## LITERATURE REVIEW

As early as 1894, Sherrington<sup>56</sup> reported that there is no segregation of afferent and efferent fibers within the trunk of nerves. Later, other reports<sup>6,41,43</sup> contradicted Sherrington's findings and they developed a concept of fiber tracts within the peripheral nerve trunks analogous to the fiber tracts found in the spinal cord and the brain. This concept emphasized that in any nerve, the motor fibers to a muscle and the sensory fibers from a particular part of the skin are situated constantly within a definite region of a nerve trunk at any given level. Stoffel<sup>62</sup> developed a similar idea of functional topography of peripheral nerves and observed that it is possible to recognize high up in the trunk the exact fasciculus or group of fasciculi which make up a given peripheral branch. Based on this idea, he set down rules for various operative procedures to be followed in nerve suture or nerve transplants.

General interest in further investigating nerve trunks and their fasciculi declined until the World War I when nerve injuries became a serious problem<sup>45</sup>. Physicians then centered attention on nerve anatomy and physiology with the hope that nerve therapeutics could be furthered by the new facts discovered. A new method of investigation was introduced by Marie<sup>41</sup>, who by faradic stimulation of nerves exposed in operation, with concurrent observations of the muscular contractions, reached essentially the same conclusions as Stoffel.

Sharren<sup>55</sup> challenged the Stoffel theory of tract system within peripheral nerves when she found that transection of more than one-third of the N. tibialis nerve produced no appreciable motor loss or cutaneous desensitization. She also observed that the Nn. fibularis and tibialis

remain separate from their point of origin in the pelvis to their point of separation. Thus, partial injury to the N. ischiadicus could cause complete injury of either the Nn. fibularis or tibialis.

By 1948 Stoffel's theory was disproved by Heinemann, Langley and Hashimoto<sup>40</sup>, using dissection studies; and by O'Connell<sup>50</sup> and McKinley<sup>45</sup> using electrical stimulations and degeneration studies. All of the above authors concluded that in unisegmental nerves, extensive plexuses are found at the junction of the dorsal and ventral roots. Distal to this plexus, unisegmental nerves are composed of a few fasciculi between which are few or no anastomoses<sup>19</sup>. In the pleurisegmental nerves entering the limbs however, there are large interneural plexuses throughout the length of the nerves<sup>50</sup>. These findings agree with Sherrington's findings that there is no segregation of afferent and efferent fibers in a nerve trunk<sup>45,50</sup>.

In degeneration studies carried out by McKinley<sup>45</sup> using the Marchi technique coupled with his electrical stimulation of the fasciculi of N. ischiadicus in the dog are of interest in this study. In his work McKinley partially resected portions of the N. ischiadicus and studied the subsequent degeneration of the distal fibers using the Marchi technique. In this report, similar study was undertaken but instead of partial resection of the fasciculi of the N. ischiadicus, the whole bundle of N. tibialis or N. fibularis was totally resected while these nerves were still in a common sheath. The subsequent degeneration of the distal fibers were studied using modified Marchi technique and teased nerve preparations. Equally important is the report by Worthman<sup>72</sup> who studied the clinical changes following neurectomies of the major nerve trunks of the

dog. In this study, the clinical changes resulting from neurectomies of N. tibialis and N. fibularis at the lower third, middle, and upper third of the thigh were studied.

The study of the nerve degeneration dates back to the days of Waller (1853) who observed that when a peripheral nerve is sectioned, those fibers distal to the cut degenerate--Wallerian degeneration. This degeneration of the distal stump is so universal that it constitutes a law in neuroanatomy<sup>41</sup>. The fibers of the stump proximal to the section will regenerate if the cell bodies are still intact. Since then, the processes of degeneration and regeneration have been of great interest to anatomist, physiologist and clinicians. A considerable volume of literature accumulated on this subject and was compiled by Cajal in his two volumes, *Degeneration and Regeneration in the Peripheral Nervous System*<sup>13</sup>.

It was noted that the changes which occur in the peripheral nerve during degeneration are the same regardless of the type or location of the degeneration under consideration<sup>20,41</sup>. Although the process of degeneration is divided into phases<sup>37</sup> it is a continuous process of progressive liquefaction and absorption of myelin and axon products<sup>13</sup>. Degeneration of the axons is observed a few hours after resection of the nerve, the axon organelles and neurofibrills appear swollen due to loss of function of the membrane. This results in accumulation of fluid in the fibers<sup>37</sup>. Hydrolytic enzymes activated from lysosomal sources subsequently digest cytoplasmic organelles of the axon to a fine granular material<sup>14</sup>. No myelin changes occur within twenty-four hours of injury, but axon destruction is complete forty-eight hours after the nerve has been sectioned<sup>17</sup>. Earlier signs of myelin degeneration include undulations of the surface

membrane, longitudinal retraction of myelin from the nodes of Ranvier and clumping of neurokeratin in the paranodal areas<sup>31,40</sup>. Later, round, circular, oval or slender figures of myelin bubbles and digestion chambers filled with plasma fluids result from myelin breakdown<sup>13,31</sup>. The Schwann cells which hypertrophy and proliferate during the degenerative process play an important role in clearing the axon and myelin debris<sup>12,14,17,21,31,48</sup>. Endoneural macrophages and fibroblasts play a role in the clearing process<sup>21,48</sup>.

The proximal stump of the sectioned nerve degenerates for a few millimeters proximally (retrograde degeneration). The extent of the degeneration depends on the type of the lesion (tear, crush or cut)<sup>17</sup>. Some writers<sup>21,42,64</sup> report the degeneration is extending to the first few nodes of nerve fibers, while Crosby<sup>17</sup> stated that the proximal stump will degenerate to the locus of the first axon collateral branch. The degeneration process of the proximal stump is similar to that of the distal stump except that the latter has an earlier onset<sup>3,38,41</sup>. Several attempts have been made to distinguish the two based on the onset and duration of the degenerative process<sup>26,44,53</sup>.

The cell bodies of the sectioned nerve fibers show signs of degeneration three days after the nerve fibers have been interrupted. The cell bodies become enlarged, the Nissl bodies fragment and disappear and the nucleus moves to the periphery of the cell (cell reaction)<sup>13,17,21,31</sup>. These changes are a result of either nucleoprotein depletion in preparation for the development of new branches<sup>14</sup>, or as a result of loss of functional connection<sup>37</sup>. These changes start early, reach a maximum in a few days and leave traces after 2-3 weeks<sup>21</sup>. The degenerating cells may die or

recover depending on the age and species of the animal<sup>11,63</sup>, distance of injury from the cell body<sup>38,59</sup> and the type of neuron affected with respect to structure and function. In young animals, subsequent to cell body reaction, the axon may show centrifugal degeneration also known as indirect Wallerian degeneration<sup>28</sup>. This centrifugal degeneration was not observed in fibers of the peripheral nervous system (PNS) of the adult animals<sup>8,28,63</sup>.

Various staining techniques for differentiating between normal and degenerating nerve fibers have been developed<sup>1,16,26,49,61</sup>. Some of these are for demonstration of axons while others are for myelin demonstration. The techniques for the demonstration of degenerate axons require pretreatment with a variety of reagents (potassium permanganate, uranyl nitrate, silver nitrate) which suppress the impregnation of the normal fibers. Others<sup>1,2,9</sup> have developed methods for the simultaneous staining of axon and myelin. Other investigators have stained one part of a nerve with silver salts for the axons and the adjacent part of the nerve for myelin sheath<sup>2,18</sup>. The popular paraffin methods for the demonstration of normal and degenerate myelin are the hematoxylin and osmic acid methods<sup>51,54</sup>. The principles of the hematoxylin method is based on the fact that myelin is rendered insoluble to fat solvents by pretreatment with chrome salts. Of the hematoxylin methods, the Weigert-Dals method is the more reliable. Kultschitsky's method is also recommended because it requires little differentiation. But for the demonstration of myelin sheaths as solid rings or tubes, for the measurements of the diameters of myelin sheaths, for tracing new fine fibers in section or simply for dramatic demonstration of myelin, the fixation effect, if not the coloring property of osmic



acid seem a prerequisite<sup>51</sup>. Marchi (1892) developed a technique--the Marchi technique--for the demonstration of degenerate myelin. The Marchi technique has subsequently been modified by Swank and Davenport<sup>61</sup>, Glees<sup>26</sup> and Adams<sup>1</sup> for frozen sections and histochemical staining. Page<sup>51</sup> successfully combined the hematoxylin and the Marchi technique for paraffin sections of the peripheral nervous system. Osmium tetroxide has been frequently used in fixing tissues for teased nerve preparations and electron microscopy.

Another method for the study of degenerating nerve fibers is by teased preparation. The examination by light microscopy of single nerve fibers, isolated by teasing is now an established technique. It was first described by Gambault in 1880. Gutmann and Sanders, Thomas and Spenser, Lubinska<sup>42</sup>, Spenser<sup>58</sup>, Virgilio<sup>64</sup> and several others investigators have employed this method for the studies of degeneration of the proximal stump of a sectioned nerve and for measurements of internodal length and fiber diameter. The phenomenon of segmental demyelination and, more recently a variety of peripheral neuropathies, have been investigated using this technique. Most of the teased preparations have been stained for myelin with osmic acid. Axonal fragmentation in Wallerian degeneration which is not shown in osmic acid preparations has been shown by reduced silver methods<sup>26,64</sup>.

Recently a number of investigators<sup>58,59</sup> have examined teased degenerate nerve fibers under scanning electron microscope (SEM). Arnold<sup>5</sup> successfully observed paraffin section under scanning electron microscope for differentiating certain tumors of the brain.

Electrodiagnostic tests have a long history of usefulness in the clinical evaluation of the peripheral nerve. Erb (1868) first described in detail the response of normal and denervated muscle to electrical stimulation. In the years that followed, continuing improvement in apparatus and technique led to more sensitive quantitative and qualitative methods of electrodiagnosis. The most useful methods employed in the study of peripheral nerves are conduction velocity measurements and electromyography<sup>15,38</sup>. The earliest experimental work in the recording and interpreting of electrical potential by means of electromyography was carried out by Adrian in 1925. Since then considerable improvement in apparatus and technique has led to numerous applications of this tool in research and clinical investigation of neuromuscular diseases of man. Only recently did several authors<sup>10,15,29</sup> employ this technique in the study of peripheral nerve degeneration and repair and in the diagnosis of various types of spinal cord lesions in the dog.

## MATERIALS AND METHODS

Dissection and separation of the bundles of the N. ischiadicus were performed on six dogs obtained from the necropsy laboratory, Dykstra Veterinary Clinic, Kansas State University.

Six other dogs (five males and one female) were used for surgical experimentation (Table 1). Prior to surgery each dog was given a neurological examination, in which the gait, stance and posterior limb reflexes were observed and recorded. Complete histories of these dogs were unavailable, as they were stray dogs. Each dog was anesthetized with pentobarbital sodium (60 mg per ml) at the dose of 30-40 mg per kilogram. The N. ischiadicus was bared under aseptic conditions at the levels indicated in Fig. 1. A one centimeter piece of the tibial nerve bundle on the right hind leg and the fibular nerve bundle on the left hind leg were resected using sharp scissors and without disturbing the rest of the bundles of the nerve.

Between two and four days postoperative, the dogs were examined for posture, gait, placing reflexes, toe pinch reflex, extensor thrust reflex, flexor reflex, and cutaneous desensitization of the affected limbs. The examinations on dogs of Group A were videotaped for record purposes.

Between 8-10 days post neurectomy dogs of Group B were tranquilized with Acepromazine<sup>a</sup> (10 mg per ml) at the dose of 0.5 mg/kg of body weight and EMG recording on denervated muscles was performed. Muscles innervated by the unresected bundle of the N. ischiadicus and the corresponding muscles of the other leg were used as controls. The equipment used for

---

<sup>a</sup>Product of Ayerst Laboratories, Incorporated, N.Y. 10017.



**THIS BOOK  
CONTAINS  
NUMEROUS PAGES  
THAT WERE  
BOUND WITHOUT  
PAGE NUMBERS.**

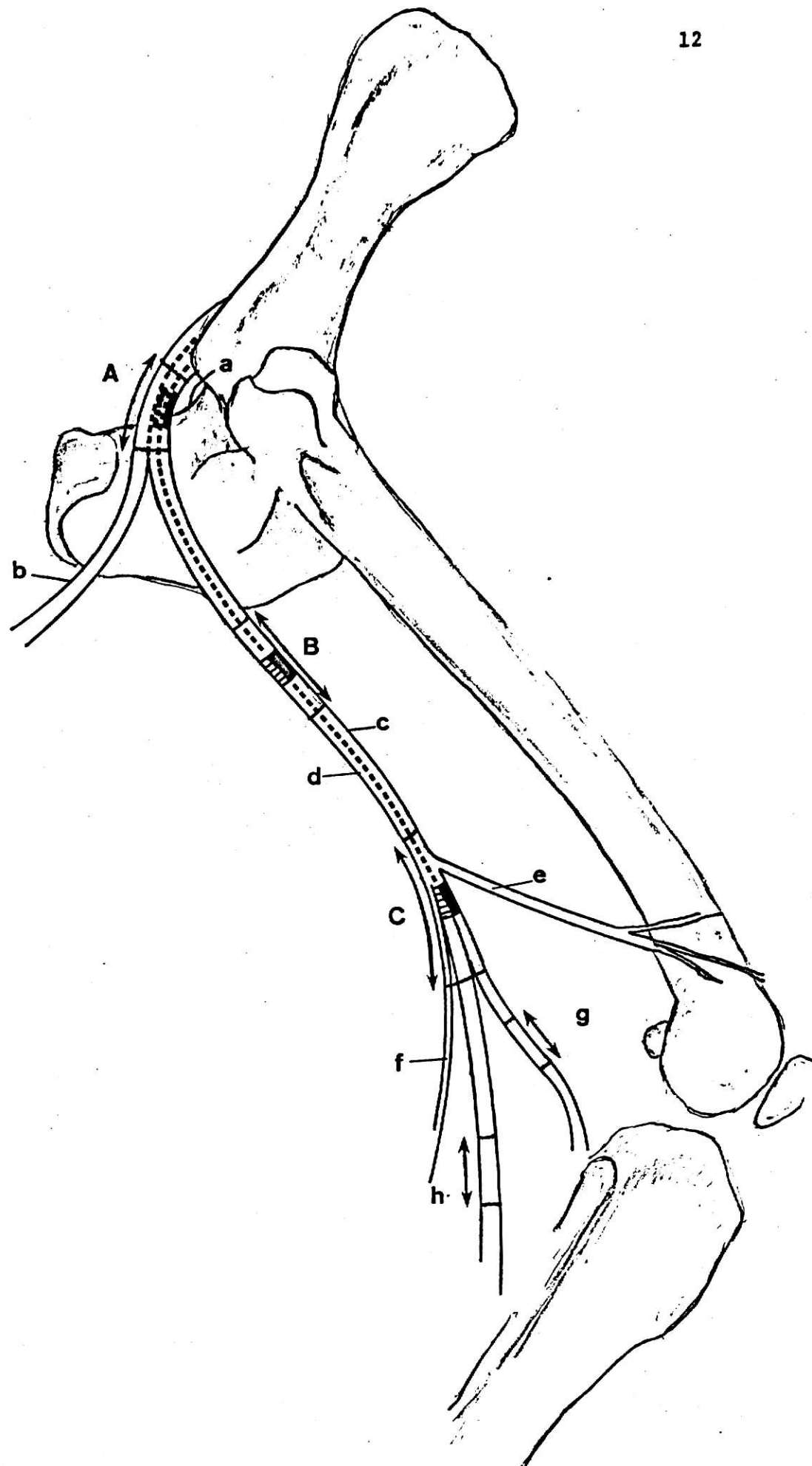
**THIS IS AS  
RECEIVED FROM  
CUSTOMER.**

#### EXPLANATION OF FIGURE

Fig. 1. Schematic diagram showing the levels of resection of tibial and fibular nerve bundles: A, level of resection of the nerves in the dogs of group A; B, level of resection of the nerves in the dogs of group B; and C, level of resection of the nerves of the dogs of group C; a, the nerves exit the Foramen ischiadicus majus; b, Rami muscularis; c, fasciculus of the *N. fibularis communis*; d, fasciculus of the *N. tibialis*; e, *N. lateral cutaneous sural*; f, *N. caudal cutaneous sural*; g, *N. fibularis communis*; h, *N. tibialis*. Arrows show the portions of the nerves that were sectioned.

**THIS BOOK  
CONTAINS  
NUMEROUS  
PAGES THAT ARE  
CUT OFF**

**THIS IS AS  
RECEIVED FROM  
THE CUSTOMER**







#### EXPLANATION OF FIGURES

- Fig. 2a. Tibial nerve paralysis. The metatarsus is almost flat on the ground when the dog was raised.
- Fig. 2b. Fibular nerve paralysis. The hock was permanently extended.

**THIS BOOK  
CONTAINS  
NUMEROUS PAGES  
WITH PICTURES  
THAT ARE CROOKED  
COMPARED TO THE  
REST OF THE  
INFORMATION ON  
THE PAGE.**

**THIS IS AS RECEIVED  
FROM CUSTOMER.**



TABLE 1. Experimental procedures and postsurgical changes

Group	Identification	Age yrs	Sex	wt (lb)	Nerve bundle resected and level	Post surgical changes
A	A-1	1 1/2	M	45	Right fibular at A*	Dog showed signs of fibular nerve paralysis on the right hind leg+
	A-2	2	M	43	Left tibial at A*	Dog showed signs of tibial nerve paralysis on the left hind leg++
	B-1	1	F	40	Right tibial at B**	Dog showed signs of tibial nerve paralysis on the right hind leg
B	B-2	2 1/2	M	48	Left fibular at B**	Dog showed signs of fibular nerve paralysis on left hind leg
	C-1	2	F	57	Right tibial at C***	Dog showed signs of tibial nerve paralysis on right hind leg
	C-2	3	M	52	Left fibular at C***	Dog showed signs of fibular nerve paralysis on left hind leg

\* Nerve bundles resected above the major trochanter of os femoris (Fig. 1).

\*\* Nerve bundles resected at the middle of the thighs (Fig. 1).

\*\*\* Nerve bundles resected at the lower third of the thighs (Fig. 1).

+ Signs of fibular nerve paralysis are straightening of the hock (Fig. 2b) with tendency to "knuckle" over, desensitization of the dorsal surface of hock and loss of flexor reflex.

++ Signs of tibial nerve paralysis are dog walks with flexed hock (Fig. 2a), desensitization of planter hind paw and loss of extensor thrust reflex.

EMG recordings consisted of unipolar electrodes<sup>a</sup> coated with teflon except at the very tip connected to an a.c. amplifier<sup>b</sup> with a range of 5-500 microvolts. The amplified electrical activity of the muscle was displayed on an oscilloscope<sup>c</sup> whose screen was photographed with a polaroid camera<sup>d</sup> attached to the scope photographed the pictures of electrical activities of the muscle. An audio amplifier<sup>e</sup> was used to listen to the EMG activities of the muscles. Recordings were made from denervated M. gastrocnemius and M. tibialis cranialis while the same muscles of the unoperated leg and muscles innervated by the unresected bundle served as control. In addition, the M. pectineus was also used as a control. Recordings were obtained by inserting a clean electrode into the belly of the muscle which was swabbed with 70% alcohol. The ground and reference electrodes were similarly inserted subcutaneously near the muscle being studied. The electrical activities of the various muscles were examined and photographed.

Between 10-21 days postoperative, the dogs were euthanized with an overdose of Pentobarbital sodium (60 mg per ml). Portions of the nerves both proximal and distal to the lesion of the N. ischiadicus were placed into Ringers solution, portions of Nn. tibialis and fibularis after separation were also collected. Corresponding portions of the N.

---

<sup>a</sup>Monopolar electrode, TECA, White Plains, N.Y. 10603.

<sup>b</sup>Grass model P5 low level a.c. preamplifier, Grass Instrument Co., 101 Old Colony Ave., Quincy, Massachusetts.

<sup>c</sup>Teknotrix, Type 5 64 B.

<sup>d</sup>Teknotrix camera model C-12.

<sup>e</sup>An audioamplifier.

ischiodicus of the unoperated leg were collected as controls. Collected tissues were dissected out and placed on cork strips and fixed in formol saline for 5 days. Then 3 mm blocks of these nerves were then cut and placed in Flemming's solution<sup>51</sup> for another 4-5 days. The tissues were then briefly washed in distilled water then under running water for 24 hours, double embedded<sup>16</sup>, sectioned at 8  $\mu$ m and stained in hemaloxyl in using the method described by Page<sup>51</sup>.

Proximal and distal stumps of Dog A-1 were fixed in formol saline for 5 days then post fixed for 4-5 days in 2% Osmium tetroxide pH 7.2 buffered with 0.2 M phosphate buffer, washed in 0.2 M phosphate buffer (pH 7.2) and dissected into nerve bundles under the dissecting microscope. The proximal and the distal nerve bundles of tibial and fibular nerves were teased to individual or group of nerve fibers. Teased nerve fibers were then mounted on a slide, examined and photographed under light microscope. Other teased nerve fibers were passed through graded concentrations of ethanol 50%, 60%, 70%, 80%, 90%, 100% and U.S.1. absolute pure ethyl alcohol U.S.P. reagent quality<sup>a</sup> for ten minutes each. The nerve fibers were mounted on a cover slide and transferred from the absolute pure ethyl alcohol U.S.P. reagent quality critical point dried in a precooled Pelco Model H. critical point dryer, mounted on stubs by scotch tape and coated with carbon and gold before examining under ETEC Autoscan Model U.1. (Fig. 3).

Transverse and longitudinal sections of proximal and distal stumps of tissues prepared for paraffin sections of Dog A-2 were cut at 6, 10 and

---

<sup>a</sup>Product of the U.S. Industrial Chemicals Co., New York, N.Y. 10016.

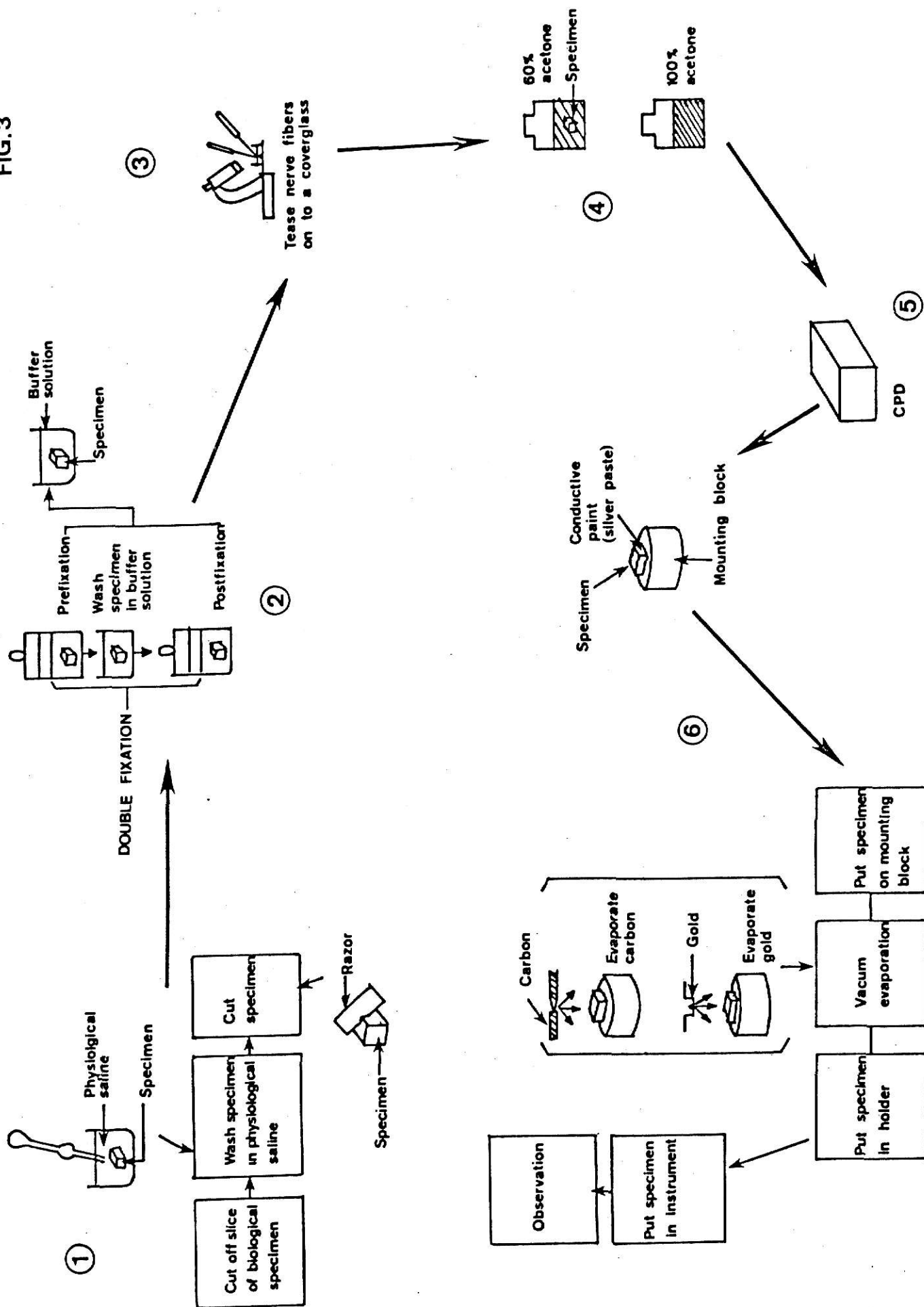




EXPLANATION OF FIGURE

Fig. 3. Preparation of nerve fibers for Scanning Electron Microscope.  
1-6 shows the various steps.

FIG. 3



20  $\mu\text{m}$ ; 6 and 10  $\mu\text{m}$  sections were stained in hematoxylin<sup>51</sup>, while the 20  $\mu\text{m}$  sections were deparaffinized, mounted on stubs and coated with carbon and gold before examining under the scanning electron microscope.

Portions of the proximal and the distal stumps of nerves collected from Dog B-1 were prepared according to the method described by Virgilio<sup>64</sup> for silver staining of teased peripheral nerve fibers. The silver stained nerve fibers were examined under light microscope.

## RESULTS

### Dissection Studies

In all six dogs dissected, it was observed that the fasciculi of the Nn. tibialis and fibularis separated from the common sheath in the lower third of the thigh. The point of separation varied from 4 centimeters to 7 centimeters proximal to the lateral head of M. gastrocnemius. The N. fibularis communis, the smaller and more cranial of the two main nerve fasciculi, ran distocranial across the lateral head of the M. gastrocnemius covered by M. biceps femoris. The N. tibialis, the larger of the two nerves, was about 5 millimeters wide after the point of separation and was flattened transversely between Mm. semitendinosus and biceps femoris. The N. tibialis consisted of a group of nerve bundles which crossed the popliteal space and disappeared between the two heads of M. gastrocnemius after giving off branches to the M. biceps femoris.

Other branches of the N. ischiadicus that were observed in the lower third of the thigh were the N. cutaneous sural lateralis and the N. cutaneous sural caudalis. The N. cutaneous sural lateralis was a branch of the fasciculus of N. fibularis. It branched off about 10 millimeters before the point of separation of Nn. fibularis and tibialis. It ran under M. biceps femoris before piercing this muscle to supply the lateral side of the leg. The N. cutaneous sural caudalis was a slender nerve which branched from the fascicle of N. tibialis near the point of separation, coursed between the Mm. biceps femoris and semitendinosus to the planter surface of M. gastrocnemius to supply the proximal caudal part of the skin of the leg.

The fasciculi of Nn. tibialis and fibularis were bound together in a common sheath of connective tissue, from the point of separation to the Foramen ischiadicus majus. The fasciculus of the N. fibularis was the smaller and cranial to the fasciculus of the N. tibialis from the point of separation to the Foramen ischiadicus majus. It was possible to forcefully separate the two fascicles from the point of separation dorsally up to the Foramen ischiadicus majus. At the gluteal region the fasciculi of the Nn. tibialis and fibularis divided and gave rise to large Rami muscularis which innervated the Mm. biceps femoris, semitendinosus and semimembranosus. A smaller branch was also given off to M. abductor cruris caudalis. Thus the fasciculi of Nn. tibialis and fibularis entered the Foramen ischiadicus majus and hence the Truncus lumbosacralis as two fasciculi bound together in a common sheath of connective tissue.

#### Clinical Studies

A summarization of the results of the clinical studies was presented in Table 2. The extended hock of dog A-1 (Fig. 2a) and the flexed hock of dog A-2 (Fig. 2b) were also shown. All of the dogs (6) examined post operatively showed locomotor disturbances, cutaneous desensitization and lost reflexes depending on which of the nerve bundles were resected. These disturbances were observed regardless of the level of resection of the nerve bundles. Dogs on which the fasciculi of N. tibialis was resected walked with the hock flexed, the planter surface of the leg and paws were desensitized and the extensor thrust reflex of the affected legs was lost. The dogs on which the fasciculi of N. fibularis were resected stood with the affected legs extended at the hock and experienced knuckling over the dorsum of the paws 2-3 days after the nerve resection. The flexor and

placing reflexes were lost and the dorsum of the paws and legs were desensitized. In dog C-2 the knuckling over was observed one week after the nerve resection. This caused the dorsum of the paw to be eroded and sore.

Although the denervated muscles were palpated for consistency, actual measurements of the diameters of the limbs to determine the degree of atrophy of these muscles were not taken. Thermal sensitivities of the denervated areas, temperatures of the denervated areas and the sympathetic functions, e.g. sweating, vasomotor and pilomotor controls were not determined as they were subjective tests.

#### EMG Studies

Various types of electrical activities were observed on the oscilloscope when the needle electrode was inserted into the normal M. gastrocnemius of dog B-2 and M. tibialis cranialis of dog B-1. On insertion of the needle electrode, a brief electrical discharge lasting a little longer than the actual movement of the needle was often observed. This electrical activity is called insertion activity. After insertion of the needle and as long as the muscle was at rest, EMG activity was usually not present. Only occasionally was spontaneous activity recorded in the muscle. During muscle contraction (produced by pinching the toes or bearing weight on the legs) a small number of biphasic and triphasic motor unit potentials with amplitude between 500 microvolts and 0.5 millivolts and duration between 7 milliseconds and 20 milliseconds were observed (Fig. 5a). The number and pattern of discharge of the motor unit potential increases with the increase in the strength of the muscle contraction, until individual motor unit potentials are no longer differentiated,

TABLE 2. Results of the postsurgical changes and neurological tests on the operated leg

Dog ID	Stance	Gait	Flexor			Extensor thrust	Desensitization
			Toepinch reflex	reflex of the hock	Placing reflex		
A-1	Extended hock	Knuckling over 1-3 days	Present	None	None	Present	Dorsal leg and paw**
A-2	Flexed hock	Walks with hock flexed	Present	Present	Present	None	Planter leg and paw
B-1	Flexed hock	Walks with hock flexed	Present	Present	Present	None	Planter leg and paw
B-2	Extended hock	Knuckles over	Present	None	None	Present	Dorsal leg and paw
C-1	Flexed hock	Walks with hock flexed	Present	Present	Present	None	Planter leg and paw
C-2	Extended hock	Knuckles over*	Present	None	None	Present	Dorsal leg and paw

\* Knuckling over observed 1 week later with the dorsum of the paw eroded and sore.

\*\* Only the lateral and planter surfaces of paws were desensitized.

\*\*\* The reflex was elicited by pressing the web between the toes of the dog.

producing interference pattern. Similar electrical activities were recorded from the M. pectineus which was examined.

When the needle electrode was inserted into the denervated Mm. gastrocnemius or tibialis cranialis of dogs B-1 and B-2 respectively, the resting muscle possessed fibrillation potentials characterized by small, sharp, repetitive electrical discharges 10-15 times per second (Fig. 5b). Over the speaker these potentials produced popping sounds like the noise made by frying eggs or rain falling on a tin roof. The fibrillation potentials have two or more phases with a positive initial phase. They have an amplitude between 100 and 350 microvolts and the duration of 1 to 2 milliseconds. The firing was initially rapid (10-15 times per second) but later slowed down to a constant rate (5 times per second). Motor unit potentials were not observed in the denervated muscles.

### Degeneration Studies

#### A. Gross Observations

In all of the six dogs, the resected bundle of the N. ischiadicus showed an enlargement (neuroma) at the point of resection. Similar enlargement was not observed on the unresected nerve bundle nor on the bundles of the N. ischiadicus of the control legs. The gaps between the proximal and distal stumps which were created by resecting a one centimeter block of nerve were closed within one week by a web of connective tissue network. A transverse cut through the proximal stump showed a normal bundle and a swollen edematous neuroma. A similar cut of the distal stump showed a smaller swelling (glioma) at the tip of the resected bundle. The distal portion of the resected bundle appeared



shrunken, grayish in color and without the glistening shine of normal fascicles.

#### B. Stained Sections

The transverse section of the neuroma revealed a heavy wall of epineurial connective tissue around the resected bundle. At the periphery of the bundle and just within the thick walled epineurium (Fig. 6b) was a well demarcated perifascicular fluid space, this was continuous with smaller fluid spaces around the secondary bundles of the fascicle. Within the thick walled epineurium, there was a corresponding increase in the thickness of perineurial and endoneurial walls. This increase in the connective tissue content of the fascicle appeared to scatter the degenerated and regenerated nerve fibers of the bundle. The degenerated nerve fibers, some of which stained deep black while others appeared ballooned, were distributed uniformly among normal and regenerating fibers within the fasciculus (Fig. 7b). Interspersed among the myelin rings were pale staining unmyelinated nerve fibers, endoneural collagen, Schwann cells, and blood capillaries. The endothelium of the capillaries were thickened making the capillaries very distinct.

These degenerative changes of the resected bundle progressed proximally (retrograde degeneration) for 2 to 3 millimeters beyond the neuroma after which only normal nerve fibers were observed (Fig. 7c). When longitudinal sections of the neuroma were cut degenerate nerve fibers appeared as a conglomeration of myelin figures. Round, spherical, oval, and elongated myelin figures alternated with thin strands of neurilemma which had few scattered fat droplets around them (Fig. 7d). This imparted a varicose or moniliform appearance to the degenerate fibers. Degenerate

axons and scavengers of degeneration such as macrophages, Schwann cells and fibroblasts were not easily identified on this section as they do not stain well with osmic acid.

Outside the walls of the neuroma and scattered in the connective tissue were large round and hexagonal black staining degenerated fat globulets, and a section of the unresected normal nerve bundle (Fig. 6a). Sections of smaller nerve bundles which appeared normal were observed in the connective tissue (Fig. 7a). The epineurium, perineurium, endoneurium and capillaries of these bundles showed no evidence of hypertrophy and the neural elements showed no signs of degeneration (Fig. 4c). The myelin rings were more uniform in size, closely packed and uniformly stained with osmic acid. The longitudinal sections showed the normal fibers as cylindrical tubes which were interrupted at the nodes of Ranvier and Schmidt Lantermann's incisures (Fig. 7c).

Stained sections of the distal stump showed features similar to those of the proximal stump. The resected bundle showed thickening of the epineurium and hypertrophy of perineurium and endoneurial collagen. Most of the neural contents of the fascicle were replaced by connective tissue and by large dark stained degenerate myelin products (Fig. 7f). Longitudinal sections showed fewer strands of degenerate fibers with various forms of myelin figures (Fig. 7d). Most of the products of Wallerian degeneration had been phagocytosed by the Schwann cells and tissue macrophages. When transverse and longitudinal sections of the branches of Nn. tibialis and fibularis of the resected bundles (after they separate from the common sheath) were examined similar degenerations were observed.

### C. Teased Nerve Fibers--Light Microscope

#### 1. Osmic acid preparations

a. Distal portion of the neuroma: nerve fibers examined in this region showed typical Wallerian degeneration of myelin. The integrity of the axis cylinder was broken down into multiple myelin bubbles which appeared three times the diameter of normal fibers. These bulbous swellings were interconnected by narrow neck-like constrictions (Fig. 8a). These constrictions were strands of proliferated endoneurial collagen and neurilemma of Schwann cells which held the myelin bubbles together. The myelin swellings were often seen to contain eosinophilic structures, the nature of which could not be determined by light microscopy. Along with the strands of degenerated myelin, fine regenerated nerve fibers which sometimes appeared to run through the neurilemma of the Schwann cells and through the myelin bubbles were observed.

b. At the junction of the neuroma and normal nerve bundle the nerve fibers did not undergo complete Wallerian degeneration. In this region, nerve fibers exhibited denuded internodes, short internodes, segmental demyelinated internodes and numerous sprouts of regenerated nerve fibers.

#### 2. Silver impregnated fibers.

Silver impregnated fibers of the neuroma showed products of degenerated fibers similar to that observed in osmic preparations (Fig. 8b). In addition, remnants of degenerated axons enclosed in a reticulate outline of myelin residue were observed. The regenerated nerve fibers, Schwann nuclei and collagen fibers were better stained with the silver preparation.

Degenerate osmicated and silver impregnated degenerate nerve fibers of the distal stump appeared similar to the degenerate fibers of the neuroma. In addition, there was a noticeable increase in connective tissue support of the degenerated axon and myelin products.

The teased nerve fibers of the unresected nerve bundle showed no sign of degeneration. The tubular structure of the axis cylinders, the nodes of Ranvier and Schmidt Lantermann's incisures were well preserved (Figs. 8c and d). Some fibers which were stained with osmic acid however, showed some dark staining clumps of chromatin around the nodes of Ranvier (Fig. 8e). This was probably a response of the nerve fibers to minor trauma or ischemia caused by surgery. Regenerated fibers, proliferated Schwann cells and an increased amount of endoneurial collagen were not observed.

#### D. Teased Nerve Fibers (SEM)

An examination of teased retrograde degenerated nerve fibers in the region of the resection neuroma under SEM revealed the three dimensional configuration of myelin figures. Some of the fibers examined revealed the presence of unusually swollen portions of myelin (Figs. 10a, b, c and d). Examination of the swelling indicated that the axon ran through the swelling. At the region of the constrictions the regenerated fibers were enclosed in a sheath of connective tissue. Some of the nerve fibers of this region had experienced partial degeneration of their myelin sheath. The fibers showed minor distortion of the surface morphology and the myelin seemed to have retracted from the nodes of Ranvier (Fig. 9b). Fine myelinated nerve fibers were adherent to the outside of the myelin swellings. Fibers of the unresected nerve bundle showed normal fibers with no

distortion of the surface features. The nodes of Ranvier were easily noticeable (Fig. 9a).

Paraffin sections of the neuroma, when observed under SEM, revealed features similar to those observed in stained sections under light microscope (Figs. 11a and b). The epineurium of the neuroma was thickened and the myelin rings of various sizes were easily observed. Endoneurial collagen and Schwann cells which were not stained in osmic sections were easily observed under SEM (Figs. 11 and 12). A longitudinal section (Figs. 13a and b) through the degenerate bundle showed the myelin figures as open cavities of oval, round and barrel like structures. Some of the myelin figures contain remnants of degenerate axon.

## DISCUSSION

By dissection it was observed that after the second sacral spinal root contribution to the Plexus lumbosacralis, the plexus divided into two nerve bundles (tibial and common fibular nerve bundles). These bundles were bound together in a common sheath of connective tissue. These nerve bundles were closely bound together in the gluteal and upper thigh region but were loosely bound together in the lower third of the thigh. As stated by Miller<sup>46</sup> and Shareen<sup>55</sup> it was observed that these two nerve bundles could be forcefully pulled apart to their roots of origin. The controversy over the point of separation of these two nerve bundles was related to the extent and amount of connective tissue binding. In this study it was observed that the two nerve bundles gradually move apart until they reach the popliteal fascia where they pursue different courses. Because these two nerve bundles were separate from the Plexus lumbosacralis, this study did not agree with the description of these two nerve bundles as terminal branches of the N. ischiadicus as reported by Sisson et al.<sup>57</sup>, Evans et al.<sup>22</sup>, Gashal<sup>27</sup> and Hoerlein<sup>33</sup>. The muscular branches to the Mm. biceps femoris, semitendinosus and semimembranosus which have been described by these writers<sup>22,27,57</sup> as branches of the N. ischiadicus were found to originate from the tibial and fibular nerve bundles. The sensory branches lateral cutaneous sural and caudal cutaneous sural nerves were found to be branches of common fibular and tibial nerve bundles respectively.

In the clinical studies, the stance and locomotor disturbance observed as a result of the resection of the nerve bundles in the common sheath were similar to the disturbance observed by Worthmann<sup>66</sup> in dogs

whose tibial and fibular nerve bundles were resected after these nerves separated from the common connective tissue sheath.

The desensitization of the lateral and planter surfaces of the legs which had been observed in the sciatic nerve lesions but not in tibial or fibular nerve resections were also observed when the tibial and fibular nerve bundles were resected at the sciatic foramen. This was because the sensory fibers joined the tibial and fibular nerve bundles proximal to the point of separation and are thus unaffected by the resection of Nn. tibialis and fibularis. The persistence of "knuckling over" observed in one of the dogs was contrary to the report by Hoerlein<sup>33</sup> that knuckling over wears off a few days after the operation. The persistence of knuckling over could not be explained by any of the methods employed in this study. The loss of reflexes observed in these experiments were similar to those reported by Habel<sup>30</sup>, Hoerlein<sup>33</sup> and Worthmann<sup>66</sup>. The presence of toe pinch (flexor reflex) after resection of either tibial or fibular nerves was possibly due to double innervation of the deeper structures of the paws.

Fibrillation potentials observed in electromyographic recordings in this study were similar to those reported in denervated muscles of the dog by Botelho<sup>10</sup> and Chrismann<sup>15</sup>, and to the stimulation studies of McKinley<sup>45</sup> and Huddleston<sup>34</sup>. The observation of fibrillation potential on the 7th day postoperatively was consistent with the finding by Chrismann<sup>15</sup> that fibrillation potentials occur between 7-15 days after the nerve damage. The presence of fibrillation potentials in the muscles which were innervated by the resected nerve bundle and motor unit potentials in the muscles innervated by the unresected bundle agree with the findings of dissection

and clinical studies in this report; that is the two nerve bundles of the sciatic nerve did not exchange fibers along their course in the common sheath.

The pattern of fiber degeneration of the resected nerve bundle was similar to that reported by McKinley<sup>45</sup>. The intraneural plexus that are found in the sciatic nerves of men as reported by Dempsher<sup>19</sup>, McKinley<sup>45</sup> and O'Connell<sup>50</sup> were not observed in the sciatic nerve of the dog. Retrograde degeneration observed in this study extended 2-3 millimeters proximal to the neuroma as reported by Cajal<sup>13</sup>, Elliot<sup>20</sup>, Grant<sup>28</sup> and Jenkins<sup>37</sup>. The presence of short internodes, segmental demyelination, myelin bulbs and numerous sprouts of regenerating fibers in teased nerve preparations of the proximal stump agrees with the reports of Glees<sup>26</sup>, Lubinska et al.<sup>42</sup>, and Virgilio<sup>64</sup>. The close association of the regenerated fibers agree with the report by Ham<sup>31</sup> and Nathaniel<sup>49</sup> that the regenerated fibers grew into the Schwann sheath of the degenerated fibers. Teased nerve preparation showed that all the fibers of the distal stump of the resected bundle degenerated. Fibers of the unresected nerve bundles showed only minor surface changes probably as a result of trauma during surgery or due to post surgical ischemia and edema. The phagocytic role of Schwann cells and tissue macrophages as reported by Weiss<sup>65</sup>, Lee<sup>41</sup>, Nathaniel<sup>48</sup>, and Cajal<sup>13</sup> were not studied as neither the osmic acid nor the silver impregnations were specific for these cells. The proliferation of endoneurial collagen was better observed under the scanning electron microscope. The hollow cavities of the ballooned fibers and their contents were also better demonstrated by the paraffin section observed under the scanning electron microscope.



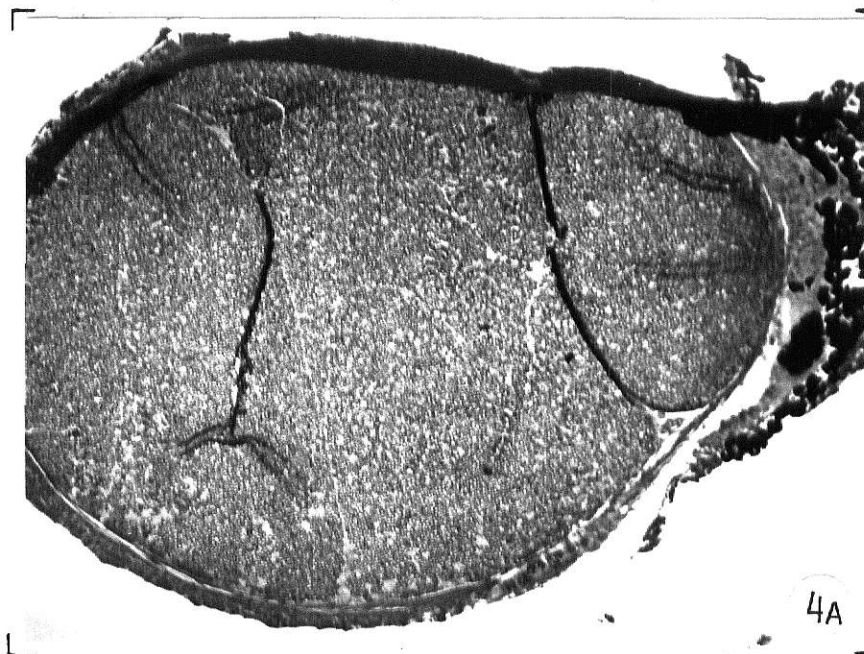
On the basis of the results of the preceding dissection, clinical, electromyographic and degeneration studies, the sciatic nerve in the dog consists of two separate nerve bundles, the tibial and common fibular nerve bundles bound together in a common sheath just as the Nn. ulnar and median formed a nerve trunk in a common sheath from their origin in the Plexus brachialis into the middle of the arm. Because the fibers of these nerves originate directly from the plexus lumbosacralis and because these two nerves are structurally and functionally independent, the description of these two nerves as the terminal branches of the sciatic nerve should be discontinued. Instead the use of the terms tibial and common fibular nerves should be extended to include the portions of the nerves from the Foramen ischiadicus to the popliteal space. Such change will enable clinicians employing exploratory laprotomy to locate the point of lesion in dogs showing signs of tibial or fibular nerve paralysis to include all portions of the nerves that are likely to cause such signs, instead of restricting their examination to the portions of the nerves distal to the point of separation.



# EXPLANATION OF FIGURES.

Fig. 4a. Tibial and fibular nerve bundles at the Foramen ischiadicus majus (Bodian stain, X12.5).

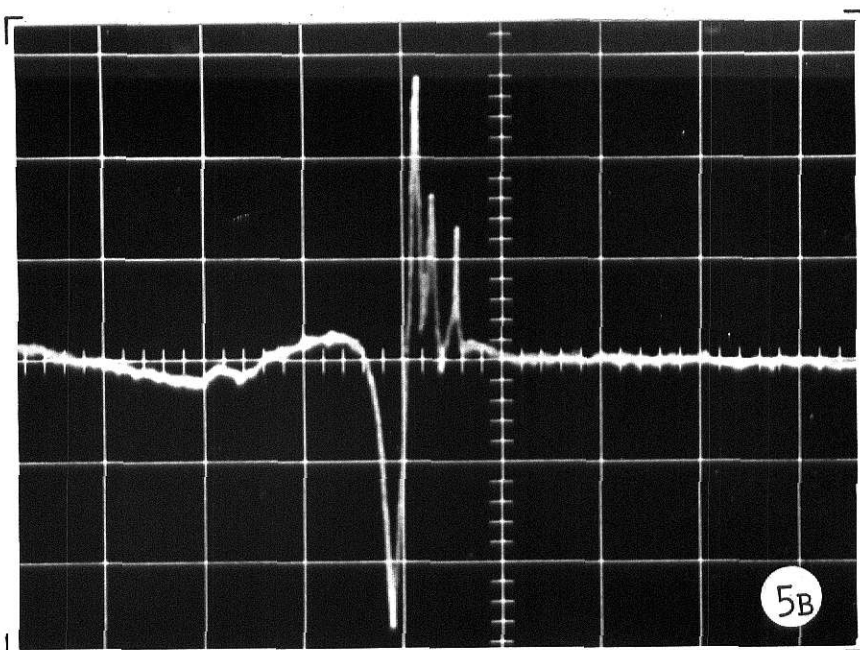
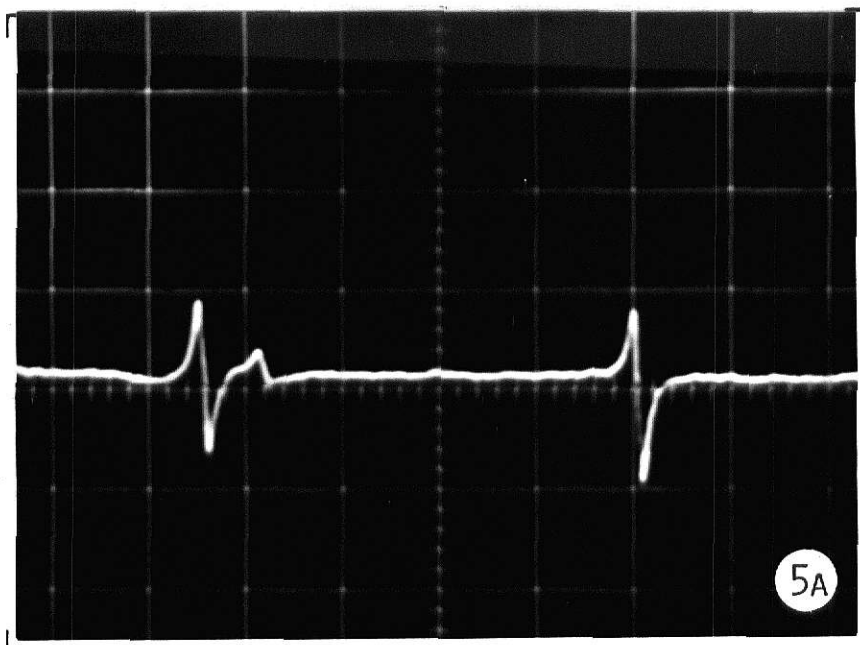
Fig. 4b. Tibial and fibular nerve bundles dividing to give rise to the Rami muscularis: a, tibial nerve bundle; b, fibular nerve bundle; c, Rami muscularis (Bodian stain, X12.5).



## EXPLANATION OF FIGURES

Fig. 5a. Motor Unit Potential recorded from the normal M. tibialis cranialis 200  $\mu$  volts = 1 division sweep = 5 mseconds per division

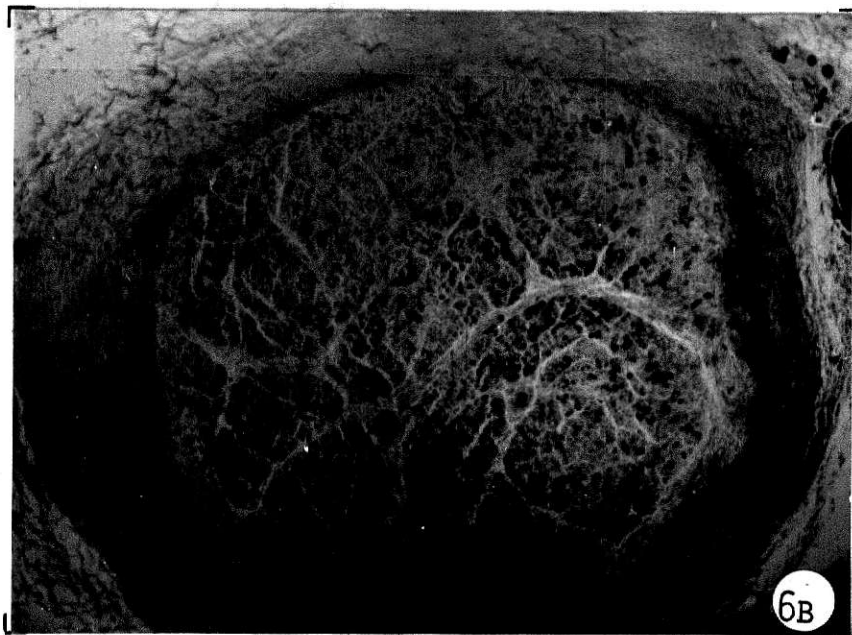
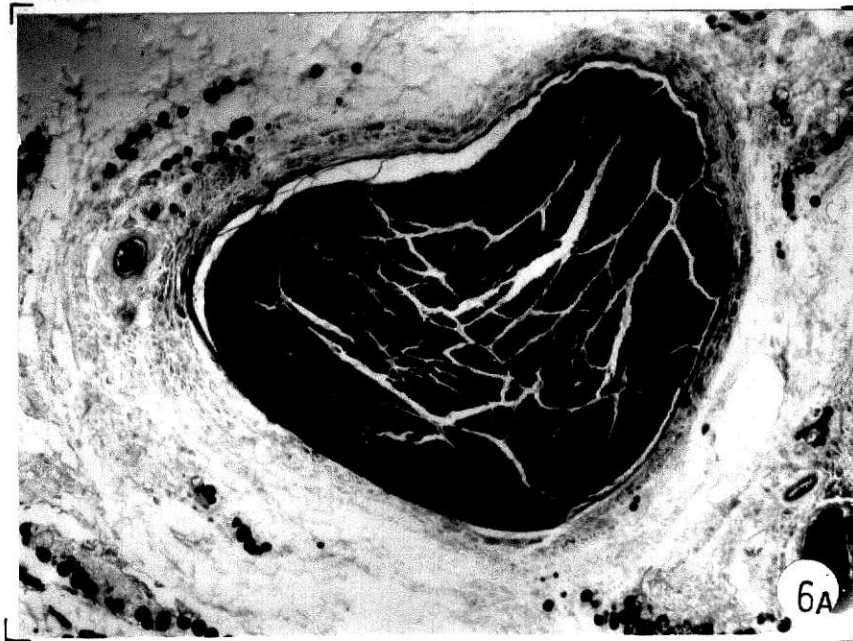
Fig. 5b. Fibrillation potential from the denervated M. gastrocnemius 200  $\mu$  volts = 1 division sweep = 2 mseconds per division



EXPLANATION OF FIGURES

Fig. 6a. Normal tibial nerve bundle (modified Marchi stain X12.5).

Fig. 6b. Degenerate fibular bundle (modified Marchi stain X12.5).



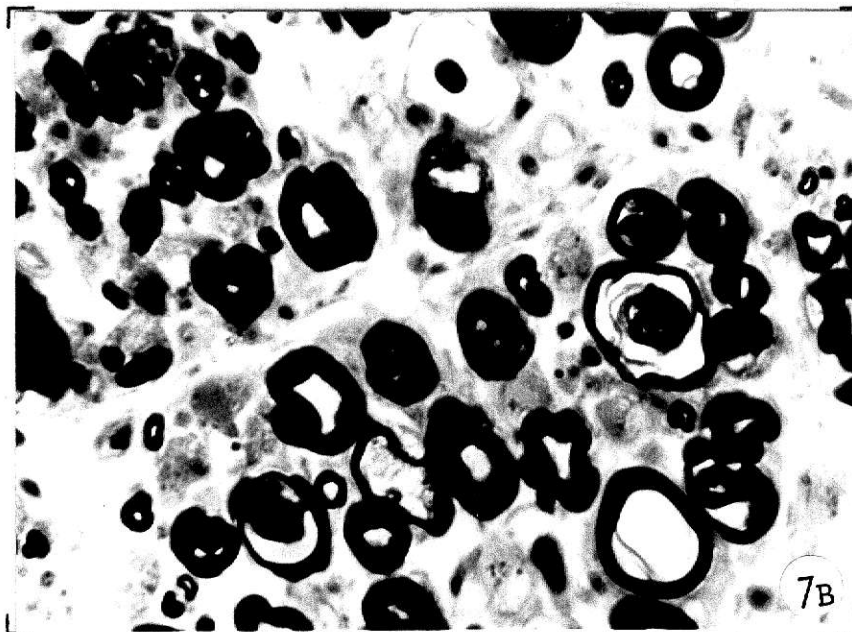


---

#### EXPLANATION OF FIGURES

Fig. 7a. Transverse section of normal fibers of tibial nerve bundle (modified Marchi stain X200).

Fig. 7b. T.S. degenerate nerve fibers of fibular nerve bundle (modified Marchi stain X200).

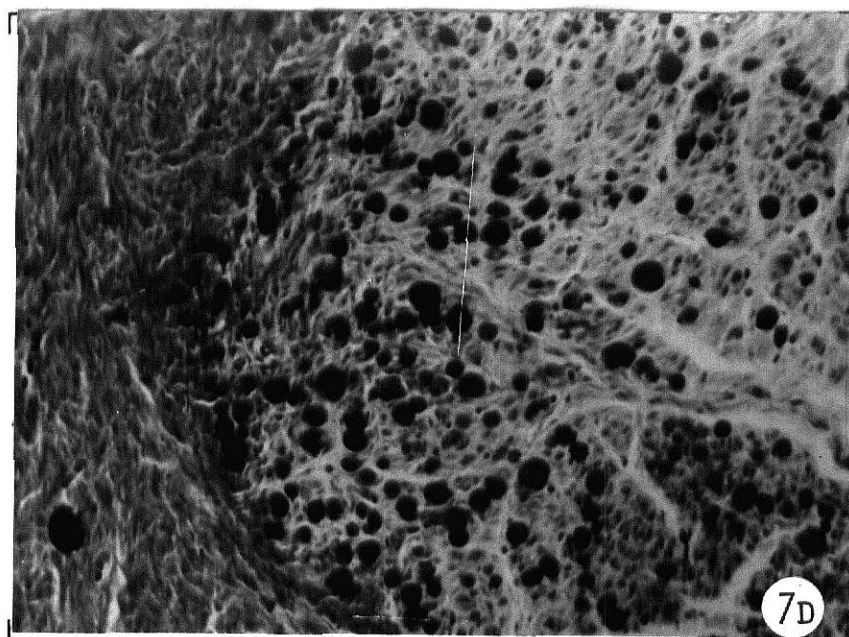
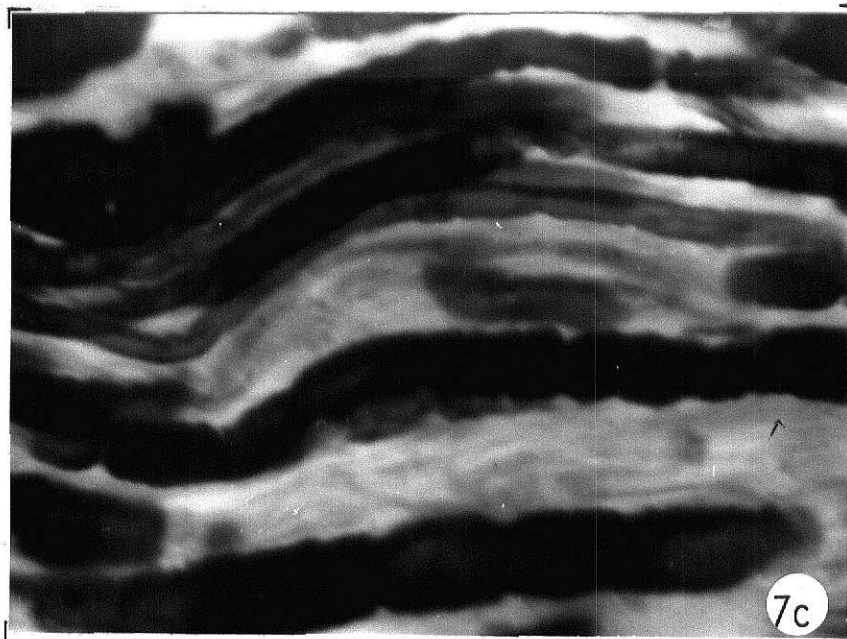


---

EXPLANATION OF FIGURES

Fig. 7c. L.S. transitional zone of the neuroma approximately 2 mm from the neuroma (modified Marchi stain X160).

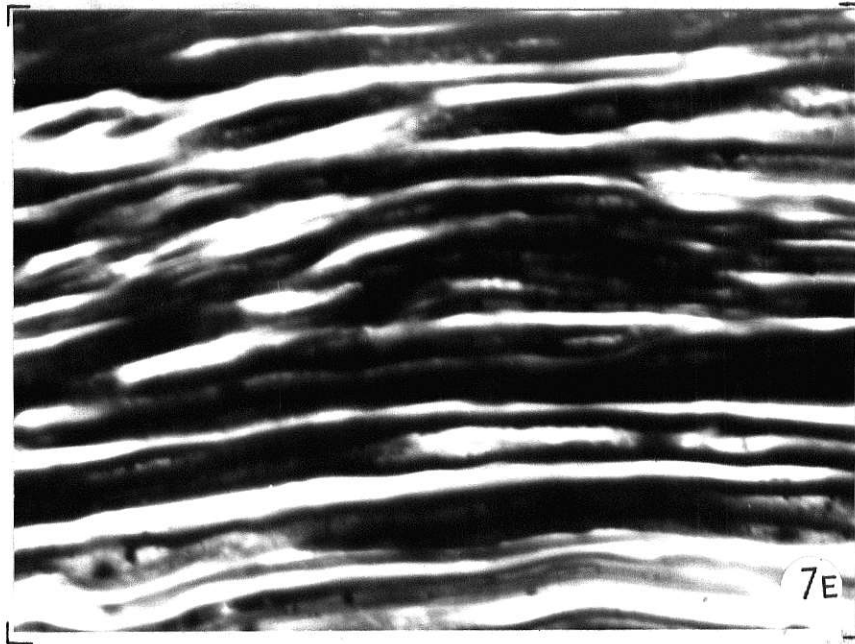
Fig. 7d. L.S. degenerate nerve fibers of the distal stump (modified Marchi stain X160).



---

EXPLANATION OF FIGURE

Fig. 7e. L.S. normal nerve fibers (modified Marchi stain X200).

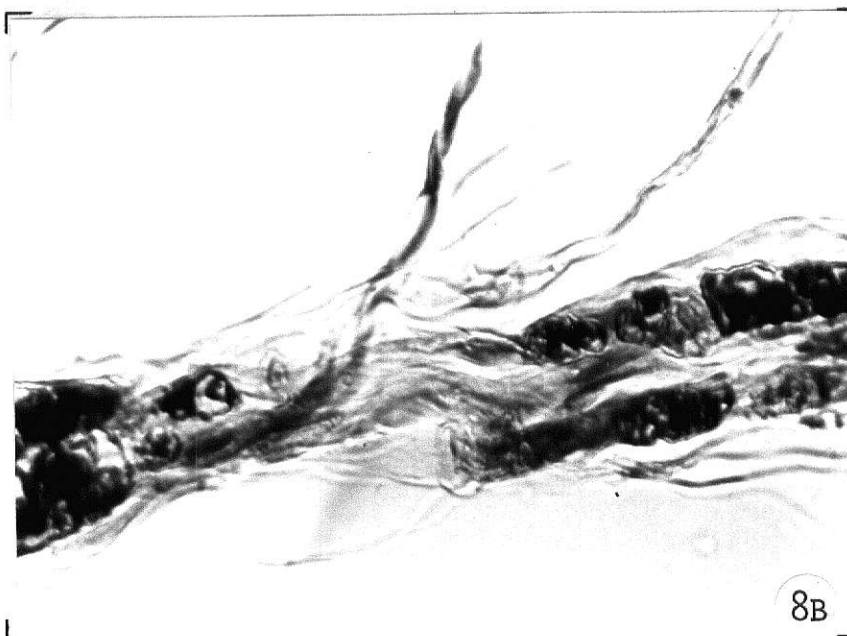
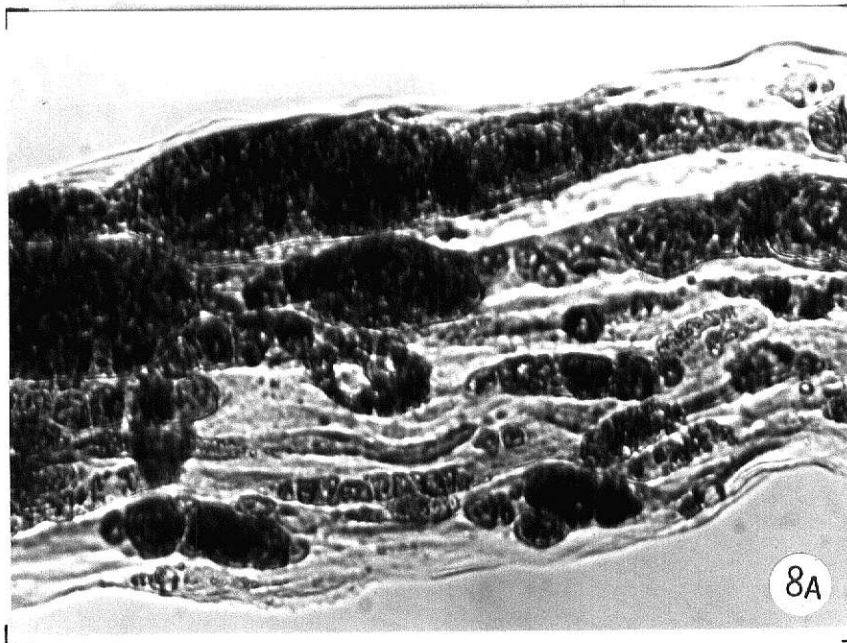


---

EXPLANATION OF FIGURES

Fig. 8a. Teased degenerate nerve fibers (Osmic acid stain X100).

Fig. 8b. Teased degenerate nerve fibers (Silver stain X100).





## EXPLANATION OF FIGURES

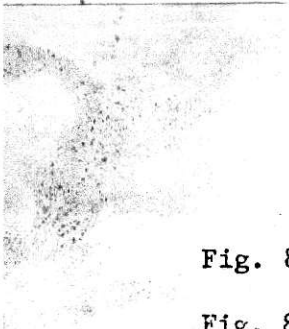


Fig. 8c. Teased normal nerve fibers (Osmic acid stain X100).


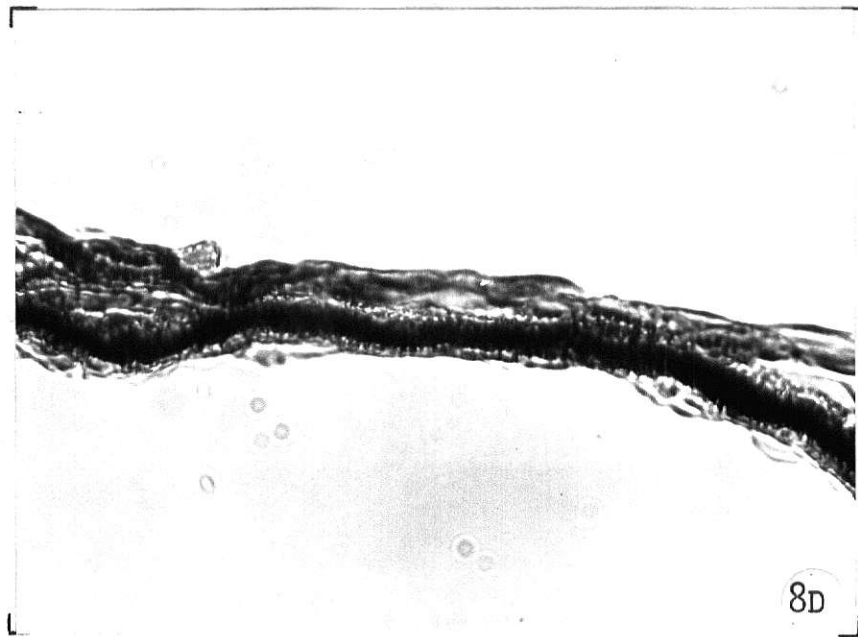
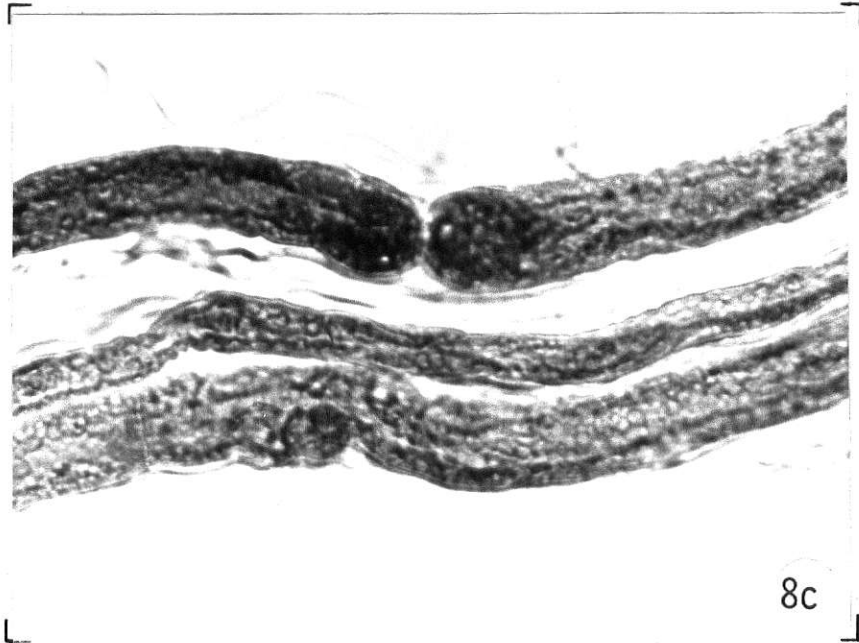


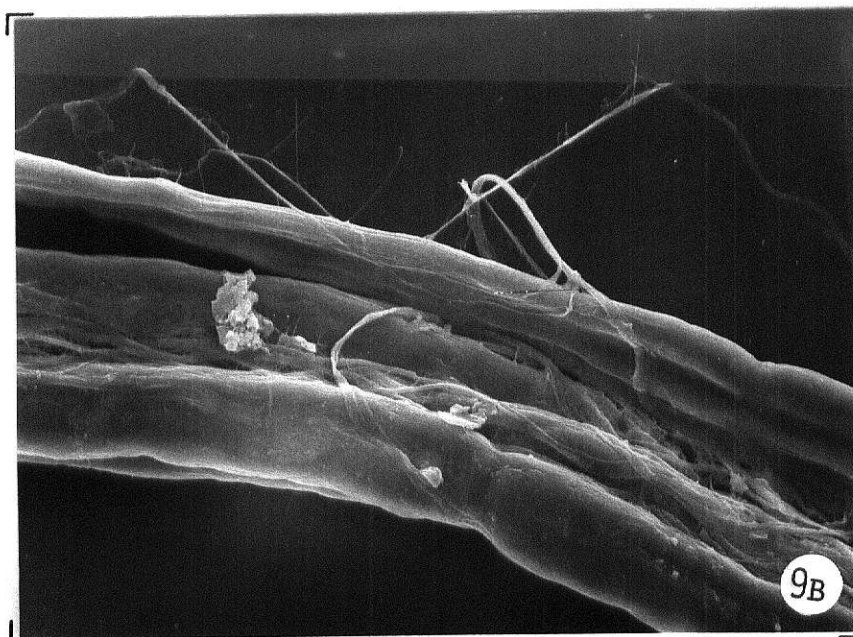
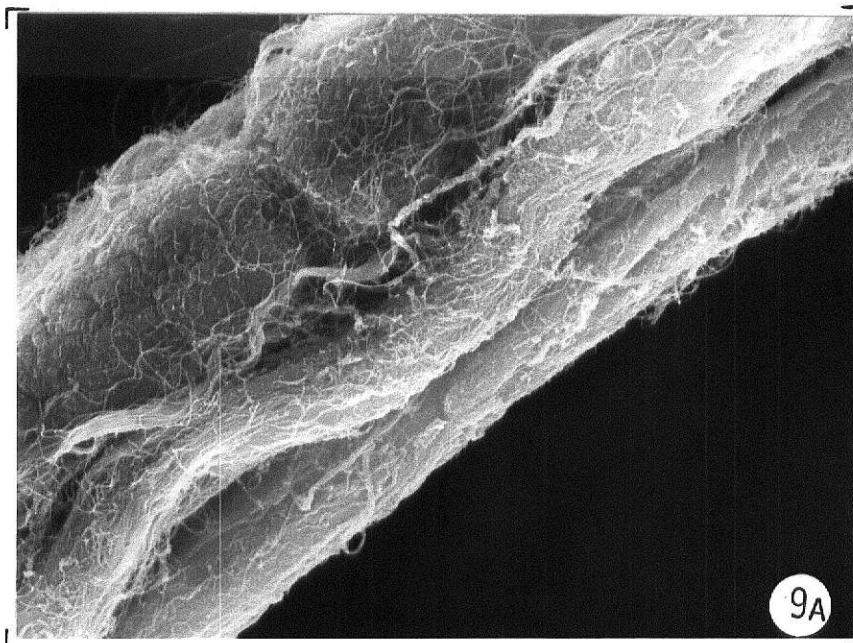
Fig. 8d. Teased normal nerve fibers (Silver stain X50).



#### EXPLANATION OF FIGURES

Fig. 9a. Teased normal nerve fibers (SEM) (Formalin fixed X1300).

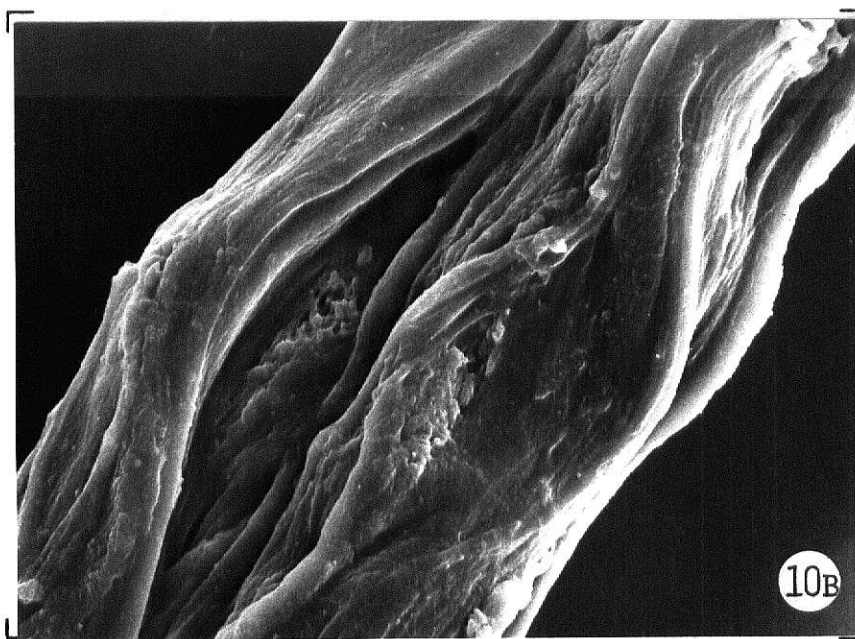
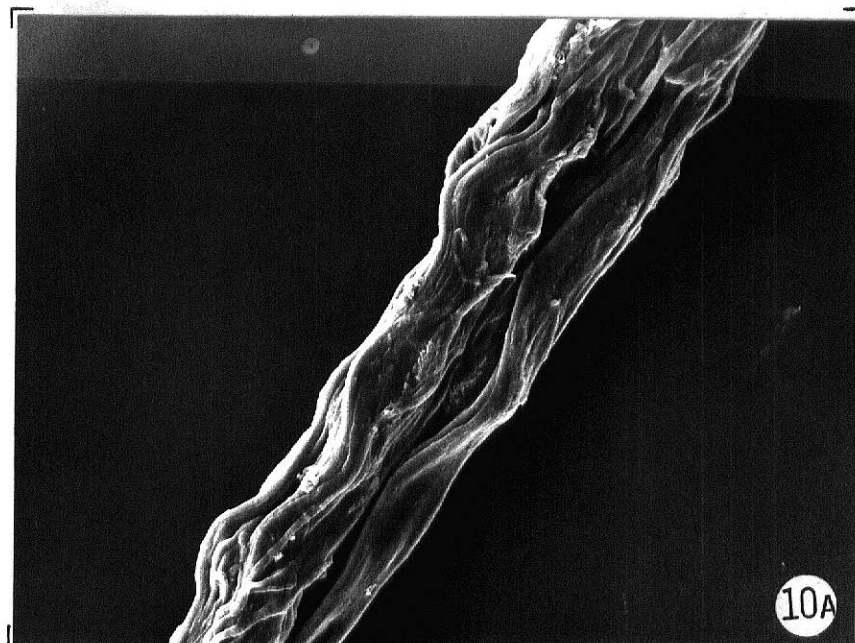
Fig. 9b. Partially degenerated teased nerve fibers. Note the retraction of the node of Ranvier, X600.



## EXPLANATION OF FIGURES

Fig. 10a. Degenerate nerve fibers showing myelin swellings (SEM)  
(Osmic acid fixed X800).

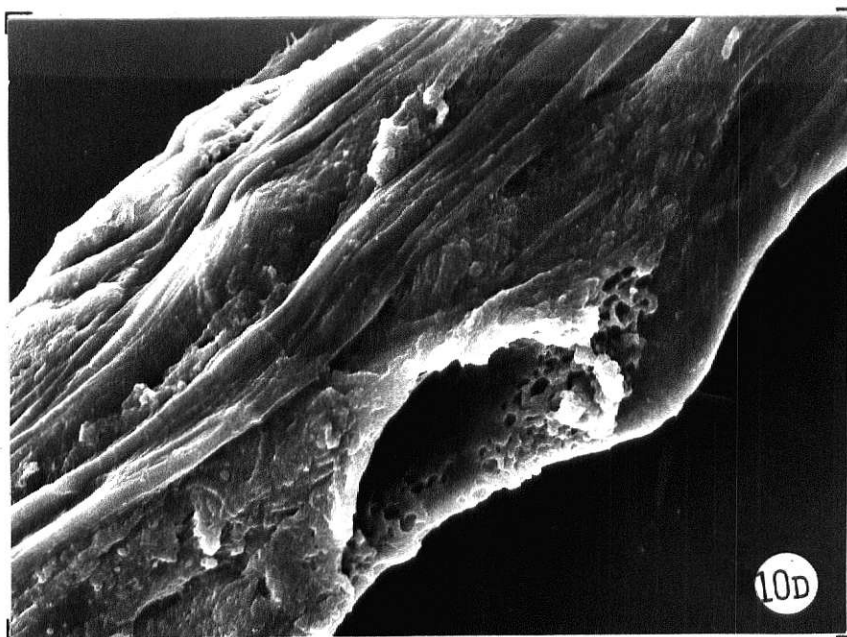
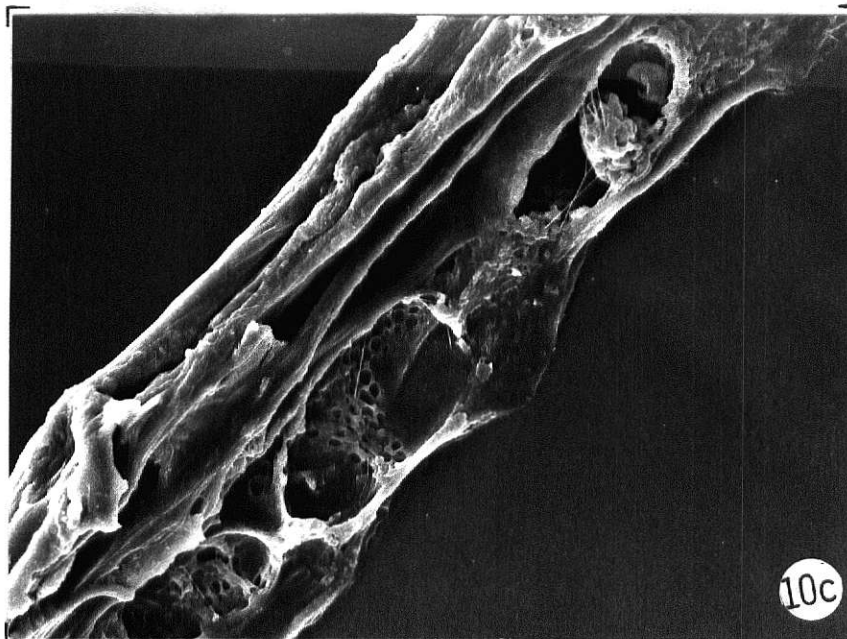
Fig. 10b. Swollen portion of myelin. Note the fine regenerated  
fibers that run close to it (Osmic acid fixed X2600).



---

#### EXPLANATION OF FIGURES

Figs. 10c and d. Broken myelin bubbles (SEM) (Osmic acid fixed X1500 and X2500).



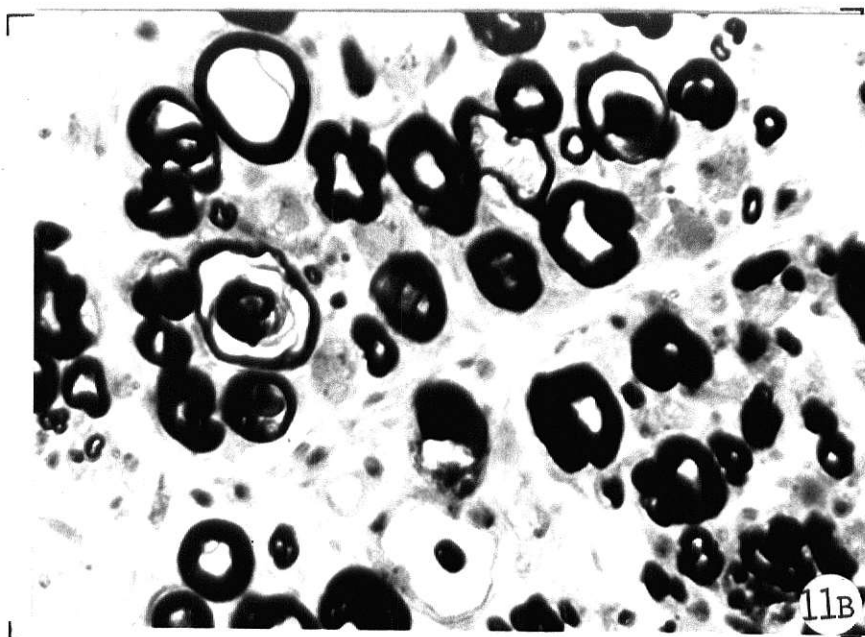
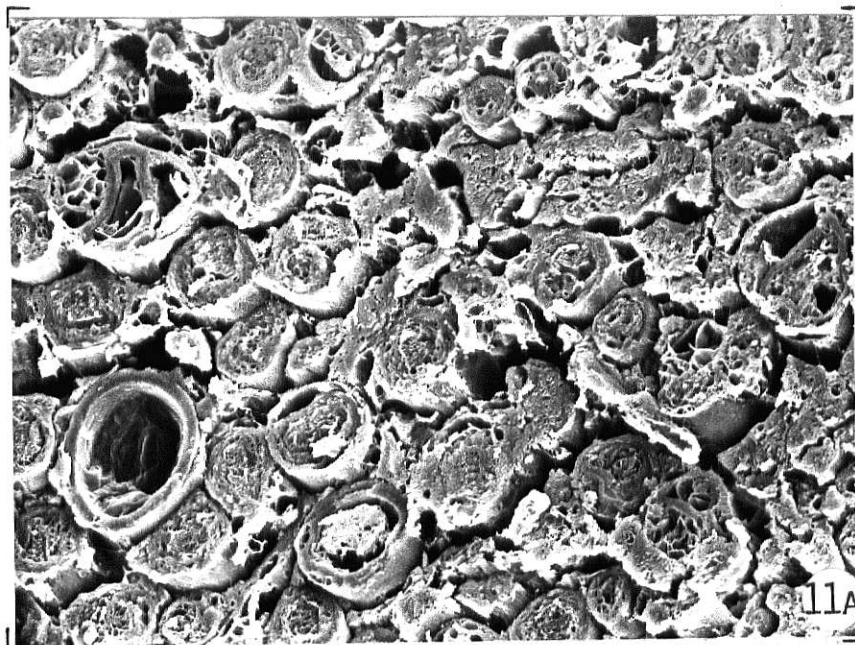


---

EXPLANATION OF FIGURES

Fig. 11a. Scanning electron micrograph of paraffin section of degenerate nerve bundle. Note the increase in the amount of endoneurial collagen.

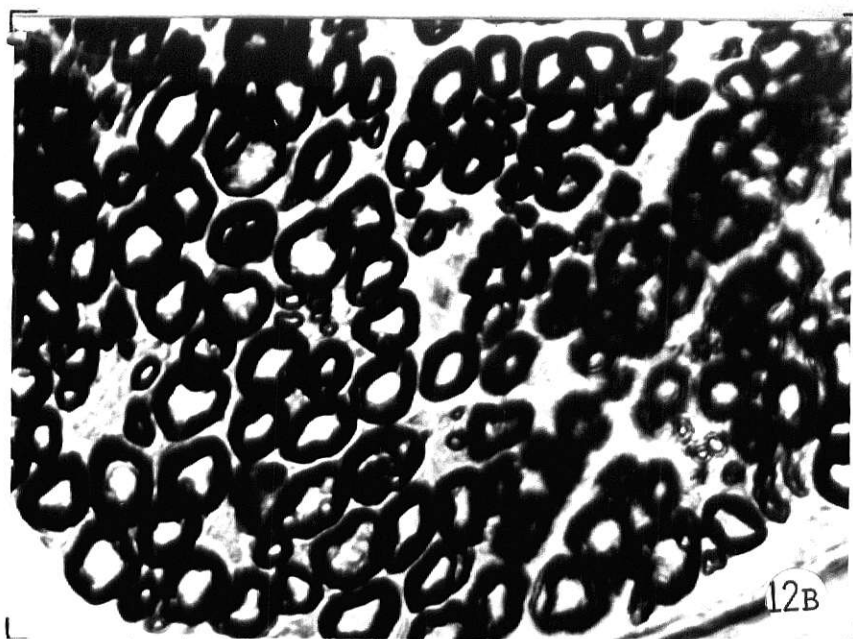
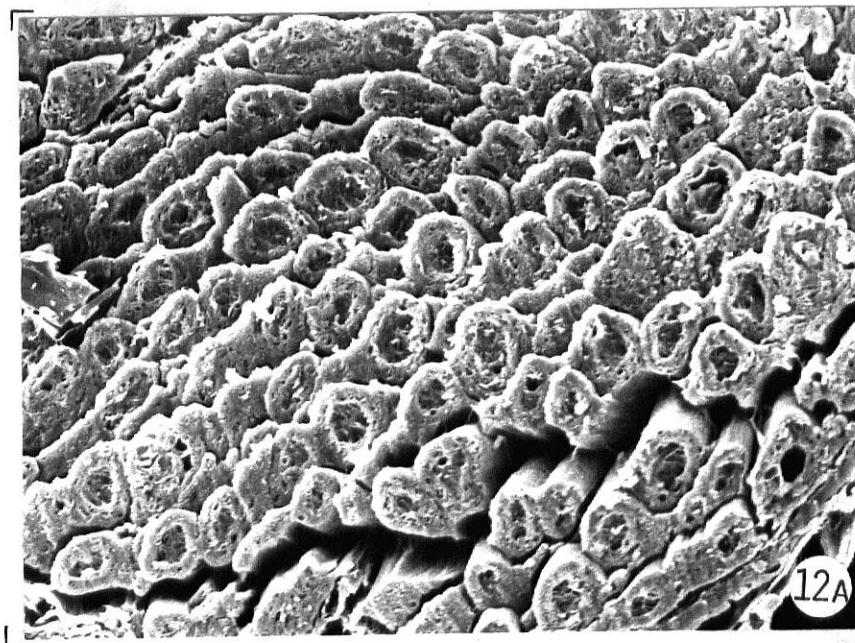
Fig. 11b. T.S. of the degenerate bundle (modified Marchi stain X200).



## EXPLANATION OF FIGURES

Fig. 12a. Scanning electron micrograph of paraffin sections of normal nerve bundle.

Fig. 12b. T.S. of normal nerve bundle (modified Marchi stain (X100)).

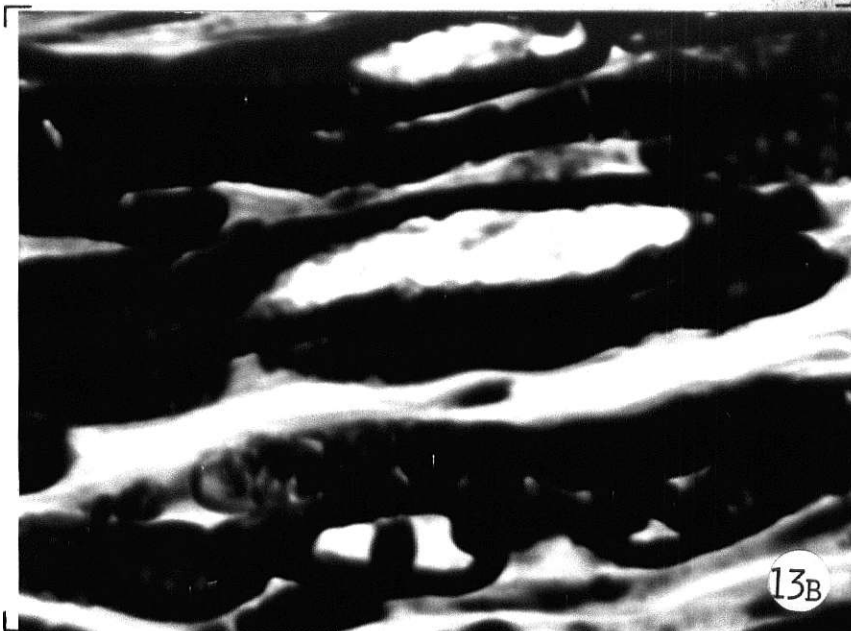
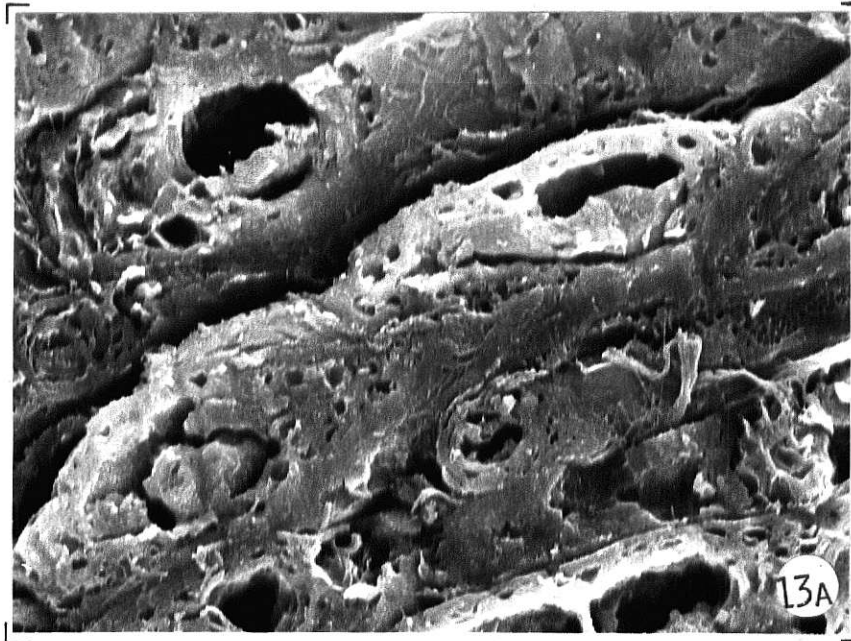


---

#### EXPLANATION OF FIGURES

Fig. 13a. Scanning electron micrograph of longitudinal paraffin section of degenerate nerve bundle. Note that the broken myelin bubbles contain remnants of degenerate axon, X1300.

Fig. 13b. L.S. of degenerate nerve bundle (modified Marchi stain X200).











## ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Robert D. Klemm for his invaluable and patient guidance during this study. I also wish to thank Miss Elaine Oates for her help in the preparation of the manuscript.

## LITERATURE CITED

1. Adams, C.W.M.: A Histochemical Method for the Simultaneous Demonstration of Normal and Degenerate Myelin. *J. Path. Bact.*, 77 (1959): 648-650.
2. Adams, W.T., Robert, W.T., and Davenport, H.A.: Staining Sections of Peripheral Nerves for Axis Cylinder and for Myelin Sheath. *Stain. Tech.*, 23 (1948): 191-196.
3. Aldskogius, H., and Grant, G.: Silver Impregnation of Degenerating Dendrites, Cells and Axons Central to Axonal Resection. A Nauta Study on Hypoglossal Nerve of Kittens. *Exp. Brain Res.*, 3 (1976): 150-162.
4. Archibald, J.: *Canine Surgery*. Second Edition. Am. Vet. Pub. Inc. California. (1974): 900-907.
5. Arnold, A.D.B.: Comparative Study of the Tumors of the Central Nervous System of Man by Scanning Electron Microscope, Phase Contrast and Light Microscopy. *Proc. Scan. Elect. Mic.*, 2 (1976): 15-18.
6. Bardeen, C.R.: Development and Variation of the Nerves of the Musculature of the Inferior Extremity and the Neighbouring Regions of the Trunk in Man. *Am. J. Anat.*, 6 (1906): 259-390.
7. Barr, M.L., and Hamilton, J.D.: A Quantitative Study of Certain Morphological Changes in the Spinal Motor-neurons During Axon Reaction. *J. Comp. Neurol.*, 89 (1948): 93-122.
8. Beresford, W.A.: A Discussion of Retrograde Changes in Nerve Fibers. *Prog. Brain Res.*, 14 (1965): 33-56.
9. Bodian, D.: A New Method for Staining Nerve Fibers and Nerve Endings in Mounted Paraffin Sections. *Anat. Rec.*, 65 (1936): 89-97.
10. Botelho, S.Y., Steinberg, S.A., McGrath, J.T., and Zislis, J.: Electromyography in Dogs with Congenital Spinal Cord Lesions. *Am. J. Vet. Res.*, 28 (1967): 205-212.
11. Brodal, A., and Grant, G.: Morphology and Temporal Course of Degeneration in Cerebellar Mossy Fibers Following Transection of Spinocerebellar Tracts in the Cat. *Exp. Neurol.*, 5 (1962): 67-87.
12. Bunge, R.P., Bunge, M.B., and Ris, H.: Ultrastructural Study of Remyelination in an Experimental Lesion in Adult Cat Spinal Cord. *J. Biophysiol. Biochem. Cytol.*, 10 (1961): 67-76.
13. Cajal, R.Y.S.: *Degeneration and Regeneration of the Peripheral Nervous System*. London, Oxford, Vol. 1 and 2 (1928).

14. Cavanaugh, W.M.: Quantitative Effects of Peripheral Innervation Area on Nerves and Spinal Ganglion Cells. *J. Comp. Neurol.*, 93 (1951): 181-219.
15. Chrisman, C.L., Burt, J.K., Wood, P.K., and Johnson, E.W.: Electromyography in Small Animal Clinical Neurology. *J. Am. Vet. Med. Assoc.*, 160 (1972): 311-318.
16. Clayden, E.C.: Practical Section Cutting and Staining. 4th Ed. London, Churchill. (1962).
17. Crosby, E.C., Humphery, T., and Edward, W.L.: Correlative Anatomy of the Nervous System. The MacMillan Company, New York (1962): 1-44.
18. Foley, J.O.: Protargol Method for Staining Nerve Fibers in Frozen or Celloidin Sections. *Stain. Tech.*, 18 (1943): 27-33.
19. Dempsher, J., and Sprauge, J.M.: A Study of the Distribution of Sensory and Motor Fibers in the Lumbosacral Plexus and the Thoracic Nerves of the Cat. *Anat. Rec.*, 102 (1948): 195-204.
20. Elliot, C. H.: Studies of Motor Cells of the Spinal Cord. *J. Comp. Neurol.*, 81 (1944): 97-103.
21. \_\_\_\_\_: Textbook of Neuroanatomy. J. B. Lippin Co., Philadelphia. (1963): 35-46.
22. Evans, H.E., and deLahunta, A.: Millers Guide to Dissection of the Dog. W.B. Saunders Company, Philadelphia. (1971): 189-210.
23. Fletcher, T.F.: Lumbosacral Plexus and Pelvic Limb Myotomes of the Dog. *Am. J. Vet. Res.*, 31 (1970): 35-41.
24. \_\_\_\_\_, and Kitchell, R.L.: The Lumbar Sacral and Coccygeal Tactile Dermatomes of the Dog. *J. Comp. Neurol.*, 128 (1966): 171-180.
25. Gage, E.D.: Sciatic Nerve Compressions in a Dog. *Southwestern Vet.*, Winter (1971): 99-101.
26. Glees, P.: The Marchi Reaction, Its Use on Frozen Sections and Its Time Limit. *Brain*, 66 (1943): 229-232.
27. Goshal, N.G.: Spinal Nerves. Part One, Dog. Sisson and Grossman's The Anatomy of the Domestic Animals. W.B. Saunders Company, Philadelphia. (1975): 1699-1711.
28. Grant, G.: Neuronal Changes Central to Site of Axonal Transection. In Contemporary Research Methods in Neuroanatomy, Springer Verlag, New York. (1970): 173-185.

29. Griffith, I.R., and Duncan, I.D.: Some Studies of Clinical Neurophysiology of Denervation in the Dog. *Vet. Sc.*, 5 (1974): 377-383.
30. Habel, R.E.: *Applied Veterinary Anatomy*. Edward Brothers Incorporated, Michigan. (1973): 133-143.
31. Ham, A.W.: *Histology*. 7th Ed. J.B. Lippincott Co. (1974): 466-525.
32. Hirano, S.D.: Fine Structural Aspects of Demyelination (Abs.). *J. Neuropath. Exp. Neurol.*, 30 (1961): 571-580.
33. Hoerlein, B.F.: *Canine Neurology, Diagnosis and Treatment*. W.B. Saunders Company, Philadelphia. (1966): 73-92.
34. Huddleston, O.L., and Clayton, S.W.: Segmental Motor Innervation of Tibialis Anterior and Gastrocnemius Planteris Muscles in the Dog. *J. Neurophysiol.*, 138 (1943): 772-775.
35. Hugo, V.R.: Pathology of the Peripheral Nerve Injuries. In *Neurosurgical Surgery of Trauma*. Office of the Surgeon General, Department of the Army, Washington, D.C. (1965): 537-545.
36. Hugo, R.V.: Examination and Clinical Manifestations of Peripheral Nerves Injuries. In *Neurosurgical Surgery of Trauma*. Dep. Army, Washington, D.C. (1965): 553-563.
37. Jenkins, W.T.: *Functional Mammalian Neuroanatomy with Emphasis on Dog and Cat*. Lee and Febiger, Philadelphia. (1972): 118-128.
38. Lagos, J.C.: Current Concepts in Diagnosis. Electromyography in the Diagnosis of Neuromuscular Diseases. *South. Med. J.*, 66 (1973): 823-829.
39. Lakomy, M.: Changes in Myelin Sheath of Peripheral Nerves in Sheep (Abs.). *Polkie Archiwum Weterinaryjne*, 15 (1972): 2.
40. Langley, J.N., and Hashimoto, M.: On the Suture of Separate Nerve Bundles in Nerve Trunk and on Internal Nerve Plexuses. *J. Physiol.*, 51 (1906): 318-346.
41. Lee, J.C.: Electron Microscopy of Wallerian Degeneration. *J. Comp. Neurol.*, 120 (1963): 1-27.
42. Lubinska, L.: Demyelination and Regeneration in the Proximal Parts of Regenerated Nerve Fibers. *J. Comp. Neurol.*, 117 (1961): 275-289.
43. Marie, P.: Des Resultats Fourms par L'elecrlisation Directe des troncs Nerveux la place operatoire chez les blesses atteints de traumatismes des Nerfs (Abs.). *Bull. de L'Acade de Med.*, 73 (1915): 798-810.

44. Marion, C.S.: The Recognition and Prevention of Artefacts on the Marchi Methods. *J. Neurol. Neurosurg. Psychiat.*, 19 (1956): 74-83.
45. McKinley, J.C.: The Interneural Plexus of Fasciculi and Fibers in the Sciatic Nerve. *Arch. Neurol. Psych.*, 6 (1921): 377-399.
46. Miller, M.E.: Guide to Dissection of the Dog. Edward Bros., Michigan. (1962): 247-255.
47. \_\_\_\_\_, Christensen, J.C., and Evans, H.E.: Anatomy of the Dog. W.B. Saunders Company, Philadelphia. (1968): 572-625.
48. Nathaniel, E.J.H., and Pearse, D.C.: Degeneration Changes in the Rat Dorsal Roots (Abs.). *J. Ultrast. Res.*, 9 (1963): 511.
49. Nauta, W.J.H., and Gygyax, P.A.: Silver Impregnation of Degenerating Axon Terminals in the Central Nervous System. *Stain Tech.*, 26 (1951): 5-11.
50. O'Connell, J.E.A.: The Intraneural Plexus and Its Significance. *J. Anat.*, 70 (1936): 468-497.
51. Page, K.M.: Histological Methods for Peripheral Nerves. *J. Med. Lab. Tech.*, 27 (1970): 1-16.
52. Pearse, A.G.E.: Histochemistry, Theoretical and Applied. 3rd Edition. Little Brown Company, Boston. (1968): 398-446.
53. Probst, M.: Zur Anatomie und Physiologies des Kleinhirns (Abs.). *Arch. Psychiat. Newenter*, 35 (1902): 1-48.
54. Ralis, H.M., Beeslay, R.A., and Ralis, Z.A.: Techniques in Neurohistology. Butterworth, London. (1973): 105-135.
55. Shareen, J.: Injuries of Nerves and Their Treatment. William Wood and Company, New York. (1907).
56. Sherrington, C.S.: On the Anatomical Constitution of Nerves of the Skeletal Muscle. *J. Physiol.*, 17 (1894-5): 211-258.
57. Sisson, S., and Grossman, J.D.: Anatomy of the Domestic Animals, 4th Edition. W.B. Saunders, Philadelphia. (1953): 869-878.
58. Spenser, P.S., and Lieberman, A.R.: Scanning Electron Microscopy of Isolated Peripheral Nerves. *Acta. Neuropath., Berl.*, 16 (1970): 535-511.
59. Sunderland, S.: Classification of Peripheral Nerves Injuries Producing Loss of Function. *Brain*, 74 (1951): 491-516.

60. Swaim, S.F.: Peripheral Nerve Surgery in the Dog. J. Am. Vet. Med. Assoc., 161 (1972): 905-911.
61. Swank, R.L., and Davenport, H.A.: Chlorate Osmic Formation Method for Staining Degenerate Myelin. Stain. Tech., 10 (1935): 87-90.
62. Stoffel, A.: Vorschlage zur Behandlung der Glutanslahmungen Mittels Nerven Plastik (Abs.). Dtsch. Z. Chir., 107 (1910): 240-262.
63. Van Gehuchten, A.: La Degenerescence dite Retrograde on degenerescence Wallerienne Indirecte (Abs.). Nevrxax, 5 (1903): 1-107.
64. Virgilio, E.S., and Gerald, V.M.: Staining Axons of Normal and Abnormal Teased Peripheral Nerves. J. Neuropath. Exp. Neurol., 27 (1968): 677-683.
65. Weiss, P., and Hiscoe, H.B.: Experiment on Mechanism of Nerve Growth. J. Exp. Zool., 107 (1928): 315-396.
66. Worthman, R.P.: Demonstration of Specific Nerve Paralyzes in the Dog. J. Am. Vet. Med. Assoc., 131 (1957): 174-178.

DEGENERATION STUDIES OF THE FASCICULI OF  
N. ISCHIADICUS IN THE DOG

by

ABDURRAHMAN KASIM GHAJI

D.V.M., Ahmadu Bello University, 1973

---

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Anatomy and Physiology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1976



# ABSTRACT

Tibial and fibular nerve bundles of the N. ischiadicus were individually resected at the hip, middle and lower third of the thighs. Clinical signs resulting from the resection of the nerve bundle were recorded. Electromyograms were recorded from muscles which were innervated by the branches of the resected nerve bundle. Proximal and distal portions of the nerves were collected 9-20 days after the operation. Marchi stain and teased nerve preparations were employed to study the pattern of degeneration of the resected nerve bundle. It was observed that: degenerate nerve fibers were limited to the fibers of the resected nerve bundle and its branches; resection of the fasciculi of the N. ischiadicus give rise to clinical signs similar to the clinical signs of resected tibial and fibular nerves. Fibrillation potentials were recorded from the muscles that were innervated by the branches of the resected bundle only. It was therefore concluded that in the dog, the Plexus lumbosacralis divided into Nn. tibialis and fibularis which remained in the common sheath from the foramen ischiadicus to popliteal space. It was recommended that the terms Nn. fibularis and tibialis be extended to include the portions of these nerves from the foramen ischiadicus to popliteal space, and that the description of these nerves as branches of the N. ischiadicus should be discontinued.