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Sympathetic Innervation at the Apical Level of the Cat's Canine Tooth- A Quantitative Study

by

Brian R. Noga

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

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Department of Anatomy

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SYMPATHETIC INNERVATION AT THE APICAL LEVEL OF THE CAT'S CANINE TOOTH- A QUANTITATIVE STUDY

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Abstract

Matthews (´76) demonstrated that the pattern of intradental nervous response to the chemical stimulation of dentine could be modified by concurrent sympathetic activity. Since pain is the predominant and probably the only sensation arising from the tooth pulp there may exist a system in which nociception is modulated by efferent autonomic activity. Fried and Hildebrand (´78) demonstrated the possible coexistance of sympathetic and sensory axons in nerve fibers of the dental pulp. The modulation may be a result of the close approximation of the somatic and sensory elements. Examination of this hypothesis is handicapped by the fragmentary knowledge we have of the anatomy of pulpal sympathetic innervation. The sympathetic axons at the apex of the cat´s canine were examined with respect to their proportion and relationship to sensory axons.

The superior cervical ganglion (SCG) was removed unilaterally from six adult cats, male and female (2.3-3.5 kg) under general anaesthesia using a mixture of alphaxolone-alphadolone (Alfathesin, Glaxo Laboratories). The operation was performed on the right side in three animals and on the left side in the remaining three. Two animals were sacrificed at 2, 4 and 7 days post-operatively. With the same anaesthetic the head was perfused via the common carotid arteries: initially with a cold (4^{o} C) prewash solution containing 1% procaine hydrochloride and 0.12% heparin in normal saline and subsequently with a 4^{o} C fixative mixture of 3% glutaraldehyde, 0.1% sucrose

and 0.5% dextrose in a 0.1M Sorensen's phosphate buffer at pH 7.4. The mandibular canines were decalcified in cold EDTA according to the technique of Warshawsky and Moore ('67), cut transversely into 1mm sections, washed and postfixed in cold 2% osmium tetroxide. The sections were stained "en bloc" with uranyl acetate, dehydrated and embedded in Araldite. Blocks were sectioned with glass knives, stained with uranyl acetate and lead citrate and examined with a Hitachi HU12 electron microscope. The innervation of the canine apices was studied quantitatively.

The majority of the axons entering the pulpal apices were of non-myelinated nature (75.9 to 85.6%). From 4.8 to 14.9% of the total number of axons on the operated side were degenerating non-myelinated axons. In contrast, only a small proportion (0.8 to 5.6%) of degenerating non-myelinated axons were found on the control side. Dense-cored vesicles were observed within some intact axons in the operated apices. Although suggestive of a contralateral sympathetic innervation other explanations are more plausable. A significant ($p<0.01$) decrease in the proportion of degenerating axons in the operated side occured as survival time increased. In addition, an increase in the amount of axonal loss (as a result of complete degeneration) was observed concurrently. The degenerating axons were found to be significantly larger in size than normal non-myelinated axons. No extrusion of degenerating axons from the Schwann cells was detected.

8.1$\pm$ 4.0% of the total number of axons in the cat mandibular canine were lost or degenerate as a result of the sympathectomy-all were non-myelinated. Degenerating axons were often seen

within the same Schwann cells as intact axons and were sometimes in intimate contact with them. No membrane specializations were seen. It was proposed that this contact may be the anatomical basis for the direct modulation of nociceptive activity by sympathetic neurons although other explanations (Matthews '76) are plausible.

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Dedication

This thesis is dedicated to
my parents for their support and
encouragement throughout
my years in university,
and to Faith Jacyk
for her patience, support
and understanding.

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List of Abbreviations:

Å - angstroms

C - celcius

DCVs - dense-cored vesicles

EDTA - disodium ethylenediamine tetracetate

HRP - horseradish peroxidase

IAN - inferior alveolar nerve

IM - intramuscular

IV - intravenous

MN - mandibular nerve

NA - noradrenaline

PO - propylene oxide

SCG - superior cervical ganglion

UA - uranyl acetate

µm - micron

5-OHDA - 5-hydroxydopamine

6-OHDA - 6-hydroxydopamine

CM - centimeter

Deg - degenerative

D.I. - degenerative-intact

NM - non-myelinated

Introduction- A Statement of the Problem

Recently, Matthews (´76) described modifications in the pattern of nervous response, recorded from stimulated dentine, as a result of concurrent sympathetic activity. He suggested several mechanisms which may be responsible for this effect, including direct action of the sympathetic system on the receptors.

That the sympathetic nervous system is capable of modulating sensory activity from receptors is not a novel concept. It has been shown to modulate the activity of muscle spindles (Eldred et al., ´60; Hunt, ´60), Pacinian corpuscles (Loewenstein and Altimirano-Orrego, ´56; Schiff, ´74) and aortic and carotid-body chemoreceptors (Biscoe and Sampson, ´68; Mills, ´68).

Non-myelinated postganglionic sympathetic nerve fibers have been identified within the dental pulps of various animals including cats, monkeys and humans by means of histological (Anneroth and Norberg, ´68; Avery et al., ´80; Chiego et al., ´80; Kukletová et al., ´68; Larsson and Linde, ´71; Pohto, ´72; Pohto and Antila, ´68a,b,´72; Sheinin and Light, ´69; Waterson, ´67,´69) surgical, chemical denervation (Avery et al., ´71; Christensen, ´40; Cox and Avery, ´75; Fehér et al., ´77; Fried and Hildebrand, ´78) and physiological techniques (Avery et al., ´71; Beer et al., ´74; Bolme and Edwall, ´71; Edwall and Kindlová, ´71; Edwall and Scott, ´71; Kroeger, ´68; Matthews, ´76; Matthews and Robinson, ´79,´80; Ogilvie et al., ´66; Taylor, ´50; Tønder, ´75; Tønder and Naess, ´78; Scott et al., ´72; Weatherred et al., ´63; Weiss et al., ´70).

Since pain is the predominant and probably the only sensation arising from the tooth pulp (Anderson et al., '70) there may exist a system in which nociception is modulated by efferent autonomic activity. Examination of this intriguing hypothesis is handicapped by the fragmentary knowledge we have of the anatomy of the pulpal sympathetic innervation. Histochemical techniques have established that the pulp does have a sympathetic innervation but the limited resolution of these techniques has precluded a thorough investigation of the extent of the innervation in terms of both distribution and number of axons. Only recently has the distribution of the adrenergic nerve endings within the pulp been quantitatively described (Avery et al., '80). Although various nerve section experiments have been conducted (Avery et al., '71; Cox and Avery, '75; Fehér et al., '77) the reports fail to give any detailed quantitative evaluation of the sympathetic fibers.

A recent application of the chemical sympathectomy technique to the dental pulp (Fried and Hildebrand, '78) resulted in micrographs of Schwann cells containing degenerate sympathetic as well as intact, presumably sensory, axons. It may be that the close approximations of somatic and autonomic elements may allow the sympathetic axons to modify the activity of the sensory axons.

This thesis reports attempts to estimate the number of sympathetic axons entering the apex of the cat's canine and to detect whether or not they are in contact with sensory axons. One cannot distinguish non-myelinated sympathetic and sensory axons on the basis of morphology unless adrenergic terminals are

visualized. Therefore, the technique employed was unilateral surgical sympathectomy comparing, after allowing adequate time for degeneration, apical fiber counts on operated and control sides.

Literature Review

Sympathetic Nervous System:

Postganglionic Fibers:

Postganglionic axons leave the sympathetic ganglia directed to the periphery to reach the effector organs. In the rat, the fibers are usually small non-myelinated axons with a diameter of 0.1 to 0.8 μm (Matthews, '73). Myelinated fibers make up approximately 1% of the total number of nerve fibers in the cervical sympathetic trunk (Dyck and Hopkins, '72) but are known only to project to the nictitating membrane in the cat (Thompson, '61).

The great majority of the postganglionic axons originating from the sympathetic ganglia are adrenergic. The cholinergic axons project to sweat glands and skeletal muscle (Uvnäs, '54).

Although adrenergic axons usually travel together, cholinergic axons are occasionally found in close contact with them. Both types have been observed within the same Schwann cell in intramuscular nerve bundles (Gabella, '76) and axo-axonic synapses between adrenergic and cholinergic terminals have been found (Ehinger et al., '70; Manber and Gershon, '79). In addition, Fried and Hildebrand ('78) have illustrated degenerating sympathetics in apposition with intact, presumably sensory, axons within the same nerve fiber. However, the nature of the intact axons is still unknown.

The Structure of Adrenergic Axons and Terminal Plexus:

The typical sympathetic neuron has a single axon of about 1µm in diameter which continues for distances of up to 10 cm before branching into an extensive terminal network (Livett, ´70). This network has a characteristically beaded appearance (Gabella, ´76). The beaded dilatations or varicosities are 1 to 2 µm in diameter and are separated from each other by about 3µm (Dahlström et al., ´66). The varicosities contain aggregates of mitochondria, clear and dense-cored vesicles and release the neurotransmitter noradrenaline (Burnstock and Holman, ´61; Hamberger et al., ´63; Norberg, ´67). This is consistant with the concept that the terminal varicosities constitute the autonomic "ground plexus" (Hillarp, ´59).

Nerve Terminal Membranes:

The terminal areas of the axon are only partially enveloped by Schwann cells and the distal areas may lack a sheath or even a basement membrane (Thaemert, ´63). The limiting membranes are not specialized: they do not exhibit thickened zones as seen in other synapses and they are separated from the likewise unspecialized plasma membranes of the effector cells by variably large gaps. Accordingly, autonomic nerve terminals were named endings "en passant".

Vesicle Types in Adrenergic Fibers:

There are three types of "synaptic vesicles" commonly found in the varicosities of adrenergic fibers (Grillo, ´66; Tranzer

and Thoenen, '68a,b): small clear and small dense-cored vesicles of 300 to 600Å in diameter, and large dense-cored vesicles of 600 to 1500Å in diameter. The small dense-cored vesicles are the most predominant type although the proportions are variable (Hökfelt, '67a,b; Machado, '67). The dense-cored vesicles can sometimes be seen in the axonal region between the varicosities (Tranzer and Thoenen, '68a). The dense-cored vesicles contain noradrenalin (von Euler, '66; Hökfelt, '67b) whereas the clear vesicles may contain acetylcholine (Whittaker, '59).

Dense-Cored Vesicles Have Been Reported in Other Neuron Types:

In general, fibers that contain dense-cored vesicles typify adrenergic neurons. However, large dense-cored vesicles have also been described in cholinergic neurons (Burnstock and Robinson, '67; Grillo, '66; Tranzer et al., '69) and in the growth cones of developing and regenerating peripheral axons (Ochoa, 76).

Sympathetic Innervation of Organ Systems:

Virtually all organ systems are innervated by sympathetic nerves (see review by Norberg, '67 and Gabella, '76). Virtually all arteries and some veins are innervated. In general, non-myelinated adrenergic fibers produce vasoconstriction. However, vasodilatory cholinergic sympathetic fibers have been found projecting to skeletal muscle and facial blood vessels.

Sympathetic Innervation of Sensory Organs and Receptors:

The sympathetic nervous system may have a role in modulating

the activity of a variety of sensory receptors. In most cases it is not known whether the effects are due to a direct innervation of the receptors or whether they are due to secondary influences.

Inner Ear:

The blood vessels supplying the eighth cranial nerve and inner ear structures are richly innervated by adrenergic neurons (Densert, '75; Spoendlin and Lichtensteiger, '66; Ylikoski et al., '79). In addition, adrenergic fibers, independant of the blood vasculature, have been described underneath the vestibular sensory epithelium (Spoendlin and Lichtensteiger, '66; Ylikoski et al., '79) in a position to influence the threshold of the initial segments. Similarly blood vessel-independant fibers have been found in the eighth nerve where a direct influence on the myelinated sensory fibers could occur (Ylikoski et al., '79).

Physiological studies of the influence of sympathetic discharge on sensory receptor activity have yielded conflicting evidence. Seymor and Tappin ('51) observed a rise in cochlear microphones following sympathetic stimulation while Rambo et al. ('53) found no change. Whether the influence could be the result of altered blood flow is unclear. Cochlear blood flow change with sympathetic stimulation has been observed by Todd et al. ('74) but not by Suga ('76).

Chemoreceptors:

Carotid Body:

The cat carotid body Type 1 cells are innervated by efferent fibers that run in the carotid sinus nerve (Biscoe et al., '70). The efferent fibers are capable of inhibiting the discharge of the chemosensory nerve fibers (Biscoe and Sampson, '68; Fidone and Sato, '70; Neil and O'Regan, '71; Sampson and Biscoe, '70). In addition, Sampson ('72) demonstrated that catecholamines mimic the inhibition and that both catecholamine depression and efferent inhibition are blocked by alpha-adrenergic blocking agents. He suggested that sympathetic discharge caused catecholamine release from Type 1 cells which would then depress receptor discharge.

The issue has become somewhat clouded, however, by the finding that sympathetic stimulation results in an increase in chemoreceptor activity (Biscoe and Purves, '67). It was suggested that this was due to a modulation in the vasomotor tone and blood supply.

Aortic Body:

Postganglionic sympathetic fibers are distributed along with afferent fibers within the aortic nerves (Belmonte et al., '72; Mills, '68). Lee et al. ('64) reported that carotid occlusion increased the discharge of aortic chemoreceptors and attributed this to reflex activation of sympathetic efferents since this effect was never observed following sympathectomy and adrenalectomy. In addition, sympathetic stimulation increases the discharge rate of aortic chemoreceptors recorded in the ipsilateral aortic nerve (Mills, '68).

Mechanoreceptors:

Pacinian Corpuscles:

An increased mechanoreceptor responsiveness along with increased amplitude and rate of generator potential development occurs upon application of NA to the cat's Pacinian corpuscle (Loewenstein and Altimirano-Orrego, '56) or following close arterial injection of adrenaline (Leitner and Perl, '64). Intracorpuscular catecholamines (Choukov, '68) and non-vascular adrenergic fibers (Santini, '69) have been reported in the avascular inner core region of the mesenteric corpuscle of the cat in close relation to the main axon. However, the adrenergic fibers may only play an accessory role in modulating the sensitivity of the end organ. The mechanoelectric transduction probably takes place entirely on the afferent nerve terminal (Schiff, '74). Freeman and Rowe ('81) have suggested that the increased responsiveness of cutaneous corpuscles is most likely due to local changes in blood flow since only perivascular fibers have been found in the vicinity of the inner core (Spencer and Schaumberg, '73).

Baroreceptors:

Whether there is a direct sympathetic influence on the baroreceptor responses of the carotid sinus is still unknown. The activity of the baroreceptor recorded from the aortic nerve of the cat increases with intravenous NA (Belmonte et al., '72) and

is suppressed by adrenergic blocking agents (Heymans, ´55). However, the effect of adrenaline may be primary (Paintal, ´64) or secondary to the contraction of smooth muscle (Heymans, ´55).

Cutaneous Mechanoreceptors:

Sympathetic discharge and adrenaline infusion can elicit and enhance mechanoreceptor activity recorded from cutaneous nerves in isolated frog skin (Chernetski, ´64; Loewenstein, ´56). The adrenergic terminals have no certain relation to smooth muscle cells (Fuxe and Nilsson, ´65) and it is not known whether the facilitatory effect is due to the direct action of catecholamines on the receptors. In mammals, the excitatory effect is secondary to contraction of the smooth muscles of the skin (Douglas and Gray, ´53).

The carpal tactile hairs of the cat, which possess slowly adapting mechanoreceptors, are also affected by catecholamines or sympathetic stimulation (Fuxe and Nilsson, ´65). Sympathetic activity also results in increased sensitivity of 3 of 4 classes of frog skin mechanoreceptors (Calof et al., ´81). This effect was not due to blood flow or tissue compliance changes and most likely reflected a direct action of the neurotransmitter on the receptor membrane.

Muscle Stretch Receptors:

The afferent endings of the muscle spindle (Bhoola et al., ´62; Calma and Kidd, ´62; Eldred et al., ´60; Hunt, ´60; Paintal,

´59) and Golgi tendon organ (Eldred et al., ´60) are excited by adrenaline and sympathetic stimulation. In addition, they are subsequently depressed and may then exhibit brief periods of stimulation (Bhoola et al., ´62; Paintal, ´59). These latter effects are most likely due to asphyxia produced by vasoconstriction (Calma and Kidd, ´62; Eldred et al., ´60; Paintal,'59) although some doubt exists (Hunt, ´60). The initial excitation of the endings is probably due to decreased threshold of the sensory nerve fiber (Paintal, ´64). Both vascular and non-vascular adrenergic nerve fibers have been found near the intrafusal muscle bundle (Ballard, ´78; Barker and Saito, ´80).

Taste and Olfactory Receptors:

Sympathetic stimulation has been shown to enhance gustatory activity in the rat and frog (Chernetski, ´64; Kimura, ´61). In mammals, increased olfactory nerve response to odour occurs upon cervical sympathetic stimulation, possibly due to vasomotor alterations (Beidler, ´61).

Dental Pulp Nociceptors:

An increase in the response of intradental nerves to the chemical stimulation of dentine has been found as a result of concurrent sympathetic stimulation (Edwall and Scott, ´71; Matthews, ´76). Furthermore, this response was obtained with sympathetic stimulation that was well within the physiological range (Matthews, ´76). The possible mechanisms responsible for the sympathetic modulation of nociceptor activity are discussed

in the section dealing with pulpal sympathetic function (see later).

The Innervation of the Tooth:

Pain from the Tooth:

It is generally considered that the only sensation felt from the tooth is that of pain (Anderson et al., '70; Edwall and Olgart, '77; Närhi and Antila, '73). The general assumption of the "pure" nociceptive sensibility of the pulp is largely based upon clinical experience and observations. Few controlled experiments have been conducted. This is important since suggestion, distraction and past experience can affect the subjective description of pain (Gracely et al., '78).

The sensation felt is usually described as painful and is not related to the type of stimulus used (Anderson, '63; Anderson et al., '70; Edwall and Olgart, '77; Hensel and Mann, '56; Naylor, '64). In addition, tooth pulp stimulation did not elicit pain or cerebral cortical potentials in a man suffering from congenital insensitivity to noxious stimuli (Chatrian et al., '75). This is suggestive of an exclusively nociceptive sensibility of the pulp. However, the threshold sensation evoked by electrical stimulation of dentine in humans is not always described as pain (Gracely et al., '78; Hunt et al., '77; Matthews et al., '76; Mumford, '65,'76). Only with an increase in stimulus and intensity, does the sensation become painful (Hunt et al., '77; Shimuzu, '64). This has led to the view that

"pre-pain" sensations may be present at low threshold stimulation (Shimuzu, ´64) and that they may be important in reflex suppression of pain in the brain stem (Dubner et al., ´78; Hu et al., ´78; Sessle and Greenwood, ´76).

Nerve fibers which respond specifically to heat and cold have been found in the dental pulps of experimental animals (Funakoshi and Zotterman, ´63; Matthews, ´68,´72,´77a). This however does not mean that different sensations may be distinguishable. The responses may be due to the direct excitation of axons and not necessarily to the stimulation of specialized receptors (Matthews, ´77a).

The studies of human threshold sensory experiences do not provide conclusive evidence for or against the exclusive role of the pulp in nociception since the results are open to various interpretations. Thus, categorically, the predominant sensation of the tooth pulp is one of pain but further work is needed before a definite answer can be obtained.

Nerve Supply to the Tooth:

Basic Nerve Types:

The dental pulp has a rich innervation (Engström and Ohman, ´60). Histologically both small diameter myelinated Aδ-type and non-myelinated C-type nerve fibers exist in the teeth of man (Dahl and Mjör, ´73; Engström and Ohman, ´60; Frank, ´66a; Graf and Björlin, ´51; Harris and Griffin, ´68; Johnsen et al., ´76; Uchizono and Homma, ´59), monkey (Bueltman et al., ´72), dog

(Windle, ´27) and cat (Beasley and Holland, ´78; Holland and Robinson, ´82,´83; Johnsen and Karlsson, ´74,´77).

The trigeminal nerve has been shown to be the major source of innervation for teeth (Windle, ´27). Sympathetic axons have been inferred to account for only a small part of the pulpal innervation (Christensen, ´40; Ogilvie, ´69). The presence of parasympathetics (Armenio and LaForgia, ´55; Chiego et al., ´80; Kukletová et al., ´68) or cholinergic sympathetics (Nelson et al., ´69; Weiss et al., ´69,´72) is still uncertain. Recent experiments have also demonstrated the existance of substance P-containing neurons (Olgart et al., ´77a,b,c) and nerves containing vasoactive intestinal polypeptide (Uddman et al., ´80) in the dental pulps.

The Number and Proportion of Nerve Types:

The number and proportion of myelinated and non-myelinated axons depends upon the pulpal level examined as myelinated nerve fibers branch and lose their sheath as they ascend the pulp chamber (Christensen, ´40). The proportions also vary in different animals and teeth.

With the exception of the light microscopic study by Graf and Björlin (´51), which did not provide adequate resolution of non-myelinated axons, the majority of the axons found in the pulps of human (Johnsen and Johns, ´78), marmoset (Bueltman et al., ´72) and cat teeth (Beasley and Holland, ´78; Holland and Robinson, ´82,´83; Johnsen and Karlsson, ´74; Fried and

Hildebrand, '81a; Mumford and Bowsher, '76) are non-myelinated. Non-myelinated axons account for 66 to 90% of the pulpal nerve supply which may contain as many as 3470 axons (Beasley and Holland, '78) at the pulpal cornua.

Holland and Robinson ('82,'83) recently demonstrated that the total number of axons and the proportion of non-myelinated axons at the apical region of the cat's canine can vary considerably between animals. This variation did not appear to be related to age, weight or sex. A high variation in the number of non-myelinated axons between sides in the same animal is also found. However, the proportion of non-myelinated axons does not vary by much more than 6% between left and right sides of the same animal.

Pulpal Axon Size:

Since the cross-sectional shape of axons is frequently irregular (Beasley and Holland, '78) circumference and/or axonal area is usually measured. The diameter of each axon is then determined as if the profile were perfectly circular.

Bimodal axon diameter or circumference distributions are reported for pulpal axons, one group being myelinated and the other non-myelinated (Beasley and Holland, '78; Bueltman et al., '72; Johnsen and Johns, '78; Johnsen and Karlsson, '74) with overlaps of 3.2% (Beasley and Holland, '78) and 5% (Bueltman et al., '72). The larger myelinated axons fall into the Aδ category (although some outside of this range have been described by Holland and Robinson, '82,'83) and the non-myelinated axons into

the C-fiber category (Beasley and Holland, ´78; Bueltman et al., ´72; Johnsen and Johns, ´78; Johnsen and Karlsson, ´74). The modal circumference for myelinated axons varies from 4.8 to 8.5 µm, with larger values (9.8 to 15.0 um) found in pulpal apices (Holland and Robinson, ´83). The modal circumference for non-myelinated axons is around 1.0 µm. The modal diameter varies between animal and tooth type as well as the pulpal level examined (Beasley and Holland, ´78; Bueltman et al., ´72).

Although the predominant proportion of pulpal axons is non-myelinated, C-fiber activity has rarely been observed in neurophysiological experiments following tooth pulp stimulation (Funakoshi and Zotterman, ´63; Greenwood et al., ´72). Whether this is a reflection of the difficulty in recording non-myelinated axons is not known. It is possible that the high proportion of non-myelinated axons is purely a reflection of branching and unsheathing of larger myelinated axons within the tooth (Beasley and Holland, ´78). This may be the case as only 40% of the fibers of the trigeminal sensory root (Young and King, ´73) and approximately 50% of the fibers of the inferior alveolar nerve (Fried and Hildebrand, ´82; Holland, ´78) are non-myelinated. The small amplitude responses of C-fibers have only recently been recorded from the tooth pulp by a number of investigators (Anderson and Pearl, ´74a,´75; Matthews, ´77a; Matthews and Robinson, ´79,´80) and an efferent function has been ascribed to at least some of them (Matthews and Robinson, ´79,´80).

Nerve Distribution in the Pulp:

In the root pulp the sensory nerves run in bundles, often together with pulpal blood vessels and divide into cuspal nerves (Almedia and Bozzo, ´73; Engström and Ohman, ´60; Graf and Björlin, ´51; Langeland and Yagi, ´72; Rapp et al., ´57). These divide further into smaller branches and finally into single axons forming a network of small nerve fibers, the plexus of Raschkow, in the subodontoblastic region (Fearnhead, ´63). The degree of branching (Harris and Griffin, ´68; Holland and Robinson, ´82,´83) suggests an enormous overlap in the receptive fields of individual fibers.

The fibers in the plexus of Raschkow appear to be sensory in origin since no changes in this area were found after superior cervical ganglionectomy (Arwill et al., ´73; Christensen, ´40) and none of the axons or terminals observed by Holland (´80a) showed vesicular contents that would suggest a sympathetic origin. In addition, this area has been heavily labelled after injection of radioactive amino acids into the trigeminal ganglion (Byers and Kish, ´76; Menke et al., ´77; Pimendis and Hinds, ´77a,b; Weil et al., ´75).

Harris and Griffin (´68) described three basic types of nerve endings on the basis oftheir morphology and position in the pulp: fine diameter non-myelinated endings; non-myelinated perivascular endings; and beaded nerve fibers with axonal expansions. The beaded fibers, most likely of sympathetic origin, have been described in three zones in the pulp (Avery et al., ´80): odontogenic, free-lying and vascular-related.

No specialized receptor structures, except those reported by Pimendis and Hinds (´77a,b) have been found in the dental pulp. The nerve endings appear to lie freely (Harris and Griffin, ´68) with a high degree of axonal exposure to the extracellular space (Holland, ´80a).

The Innervation of Dentine:

Structures resembling nerve axons have been described within the dentinal tubules of a variety of animals including mice (Corpron and Avery, ´73), rats (Byers and Kish, ´76), cats (Frank et al., ´72; Holland, ´75a,´76c,´81a) monkeys and humans (Arwill, ´67; Fearnhead, ´57,´61,´63; Frank, ´68a). Studies utilizing nerve degeneration techniques (Arwill et al., ´73; Fearnhead, ´57) and radioactive tracers (Byers, ´77,´79,´80; Byers and Kish, ´76; Byers and Matthews, ´81; Fink et al., ´75,´76; Menke et al., ´77; Pimendis and Hinds, ´77a,b; Weil et al., ´75) indicate a predominant if not exclusive sensory innervation of the dentine. These studies support the view that the structures described in dentine by Frank (´66a,b,´68a,b) and Arwill (´63,´68) are in fact, sensory.

Not all areas of the dentine appear to be innervated. The heaviest concentration of possible nerve endings is found in the dentinal tubules of the crown (Arwill, ´67; Arwill et al., ´73; Byers, ´77,´79,´80; Byers and Matthews, ´81; Corpron and Avery, ´73; Fearnhead, ´57,´63; Holland, ´75a,´76c,´81a). In addition, the innervation appears to be limited to the inner third of the dentine (Byers, ´77; Byers and Matthews, ´81; Fearnhead, ´57;

Holland, '75a,'76c,'81a; Thomas, '79).

Sensory Mechanisms Involved in Pulpal Nociception:

A variety of hypotheses have been proposed to explain the mechanism of sensory transduction within the tooth. The odontoblast process has been implicated in the transmission of stimuli across the dentine (Yamada, '63). However, it may not be directly responsible since it does not extend to the sensitive dentine-enamel junction (Brännström and Garberoglio, '72; Garant, '72; Holland, '75c,'76b,'81a; Thomas, '79; Tsastas and Frank, '72). In addition, it seems unlikely that the odontoblast would function as an elaborate receptor considering its transmembrane potential (Winter et al., '63) and functional role (Weinstock and Leblond, '74).

The relationship of the nerve fibers within the dentinal tubules to pulpal nociception is unclear. Whether the fibers have any independant functional role or whether they are linked to odontoblasts is not known. Specialized junctions have not been observed between the processes within the tubules (Holland, '81a). Physiological experiments in cats and humans have yielded differing results (Anderson and Matthews, '66; Anderson and Naylor, '62; Dellow and Roberts, '66; Edwall et al., '73; Haegerstram et al., '75; Scott, '66) suggesting that structural differences in dentine may be important.

It is possible that the mechanism responsible for dentine sensitivity may involve fluid movement within the dentinal

tubules as postulated in the hydrodynamic hypothesis (Brännström, ´63,´66). Whether the odontoblast would be acting as a mechanoreceptor or whether the nerve endings would be excited directly is not known. Recent studies on cat dentine (Horiuchi and Matthews, ´74) suggest that the nerves are excited by changes in the ionic composition of the extracellular fluid rather than by displacement of the tubule contents.

Recent studies of Raschkow's plexus by Holland (´80a,´81b) have shown two structural features which may be related to their role in nociception. One is the relatively high degree of axonal exposure compared to that seen in the pulpal apex (Holland and Robinson, ´82,´83) and peripheral nerves (Holland, ´82) together with the absence of any organized endothelium. This could leave the axon in an ideal position to sense both chemical and physical changes in the extracellular space. The other is the close contact between many of the axons, a feature that increases as the nerve fibers ascend through the pulp (Holland and Robinson, ´83). It has been suggested that afferent nerve fibers may be coupled to one another in such a manner as to form a complex sensory unit in which stimulation of one fiber may cause near synchronous discharge in many others (Matthews, ´77a,b; Matthews and Holland, ´75). Whether gap junctions (Holland, ´75b,´76a,´77,´80b) and/or axonal membrane apposition in the same nerve fiber (Holland, ´80a) is responsible for the coupling is unresolved. The difficulty in identifying axons after they lose their sheath (Corpron and Avery, ´73; Dahl and Mjör, ´73; Frank, ´68a; Holland, ´75b,´76a,´80b) has precluded the identification of the processes connected by gap junctions. In addition if

Gasser's (´55) computations are correct a high degree of membrane-to-membrane apposition would be required to allow cross-excitation: this being more than that observed by Holland (´80a). The problem could be overcome if higher degrees of contact were present or if the apposed membranes were of low resistivity (Bennet, ´77).

Pulpal sympathetics have also been implicated in the modulation of pulpal nociception. Matthews (´76) found that physiological discharge of sympathetic nerves can increase the response to the chemical stimulation of dentine. A more detailed summary of sympathetic involvement in dental pain is provided later.

Sympathetic Nerves in the Dental Pulp: Overview:

Postganglionic sympathetic fibers have been described qualitatively in the proximity of the blood vasculature within the dental pulps of cats (Christensen, ´40; Pohto and Antila, ´68b,´72), rats (Larsson and Linde, ´71), rabbits (Pohto and Antila, ´68b,´72; Waterson, ´67,´69), monkeys (Pohto, ´72; Pohto and Antila, ´72) and humans (Anneroth and Norberg, ´68; Kukletová et al., ´68; Pohto and Antila, ´68a,b; Waterson, ´69). Only a few studies have suggested that the sympathetic fibers may occur peripherally in the plexus of Raschkow (Avery and Cox, ´77; Avery et al., ´74,´80; Cox and Avery, ´75,´78; Pohto and Antila, 68b) and some of the nerve fibers in the micrographs published by Frank (´66b,´68b; Frank et al., ´72) do show occasional dense-cored vesicles. However, peripherally located adrenergic

fibers have not been found in other studies (Arwill et al., '73; Christensen, '40; Holland, '80a; Kukletová et al., '68; Pohto and Antila, '68a) suggesting that sympathetics are essentially vascular-related.

The failure to describe pulpal sympathetics quantitatively has been due to the problem of identifying sympathetic axons from sensory axons and the poor resolution of the techniques employed.

The Origin of Pulpal Sympathetics:

The first description of the origin of pulpal sympathetics was obtained using the method of retrograde neuronal degeneration (Christensen, '40). According to Christensen non-myelinated sympathetic nerve fibers arising from the SCG are distributed cranially by way of the carotid arteries and their branches (fig. 1). The sympathetic fibers are derived from the plexus on the external carotid where they are distributed by way of the external and internal maxillary arteries to the dental tissues. Offsets of this plexus extend along the inferior alveolar artery and are joined by the inferior alveolar nerve (IAN) near the mandibular foramen. Some of the rami become incorporated in the IAN where they are distributed to the mandibular teeth through their apical foramina. Sympathetic fibers are distributed to the maxillary teeth via complementary arteries and nerves.

Studies of the stimulation of the mandibular nerve (MN) and IAN and recording the vascular responses within the teeth have given conflicting results. Stimulation of the MN (Neidle and Liebman, '64a,b) and the IAN (Taylor, '50) had no effect on

pulpal blood flow. Intrapulpal vasodilatation was produced with IAN stimulation (Gazelius and Olgart, ´80; Kroeger, ´68) although this may be due to a sensory nerve axon reflex. Vasoconstriction has been reported with stimulation of the MN (Bishop and Dorman, ´68) and the IAN (Anderson and Linden, ´77; Tønder and Naess, ´78). In addition, Ogilvie (´69) showed that vasoconstriction produced by SCG stimulation was blocked with IAN or MN sectioning, indicating that both nerves carry sympathetics.

In an attempt to clarify the conflicting results, Matthews and Robinson (´79,´80) observed the effects of nerve sections on the compound action potentials recorded from dental nerves and pulp. They established that sympathetic fibers were present in the IAN and MN. The fibers travel from the SCG via the internal carotid plexus, enter the cranial cavity through the foramen lacerum and join the trigeminal nerve at its ganglion (fig. 2). A few fibers may also cross under the base of the skull and join the MN without entering the cranial cavity. The majority of the fibers are distributed to the teeth with the mandibular, inferior alveolar, maxillary and infra-orbital nerves. Contrary to that found by Christensen (´40), no fibers were found leaving the external carotid plexus to join the IAN.

Few Sympathetic Axons Are Present in the Dental Pulp:

Using light microscopy Christensen (´40) demonstrated that few sympathetic nerve fibers enter the dental pulp of cats. Little change in the distribution of nerve fibers in the pulp was observed after unilateral removal of the SCG. In addition,

unilateral extirpation of the pulps from all teeth resulted in only slight chromatolysis in the ipsilateral SCG. Christensen attributed this scarcity of sympathetic nerve fibers to the paucity of smooth muscle in the tunica media of the pulpal blood vessels. However, light microscopy does not provide adequate resolution of non-myelinated nerve fibers which could make up the bulk of the pulpal supply.

Other nerve sectioning experiments support the idea that few pulpal sympathetics are present. Fearnhead (´61) found that all myelinated nerves degenerated one month after IAN resection: only a few non-myelinated axons were present. Sectioning of the IAN at the level of its entrance into the mandibular foramen resulted in the loss of all myelinated and most non-myelinated fibers (Fried and Hildebrand, ´80b) except those around the blood vasculature (Avery et al., ´71; Cox and Avery, ´75; Fehér et al., ´77). Transection of the SCG also resulted in degeneration of only a few non-myelinated fibers, whereas all the myelinated fibers remained intact. Avery et al. (´71) and Fehér et al. (´77) also found that transection of the SCG and IAN resulted in complete loss of nerve fibers.

Although Arwill et al. (´73) were in agreement with studies of IAN transections, they found no loss of non-myelinated fibers after SCG transection and only incomplete loss after IAN and SCG transection. They concluded that there may be differences in degeneration and regeneration times for pulpal nerves or that the dental pulp may receive a partial innervation along other nerves, as has been observed by others (Fried and Hildebrand, ´80b;

Robinson, ´79,´80; Rood, ´77).

All of the nerve sectioning experiments can be criticised in two respects: a) no studies were quantitative or complete; b) none of the studies took into consideration that pulpal sympathetics have been found in the IAN (Anderson and Linden, ´77; Christensen, ´40; Matthews and Robinson, ´79,´80; Ogilvie, ´69; Tønder and Naess, ´78) and therefore sectioning of this nerve would destroy part if not all of the pulpal sympathetic population. In addition, nerve degeneration and regeneration times were not considered. This is important since sensory and sympathetic axons may degenerate and regenerate at different rates (Dyck and Hopkins, ´72; Wakade, ´78). Only Fehér et al. (´77) seemed to consider this.

Fried and Hildebrand (´78) used 6-hydroxydopamine (6-OHDA) to specifically induce degeneration of sympathetic axons in the dental pulps of cat incisors. Within 24 hours 2 to 4 degenerate non-myelinated axons were observed. By 48 hours marked alterations were found in "several" non-myelinated axons. No quantitative observations were provided. In addition, since the extent of degeneration with 6-OHDA is dose-dependant (Jonnson and Sachs, ´71) a complete degeneration of sympathetic axons may not have occurred.

Few adrenergic nerve fibers have been demonstrated within the pulps of man and various animals using histochemical fluorescence techniques (Anneroth and Norberg, ´68; Kukletová et al., ´68; Larsson and Linde, ´71; Pohto, ´72; Pohto and Antila, ´68a,b,´72; Waterson, ´67,´69). Because of the poor resolution of

this technique few quantitative observations have been made. It was observed that 6 to 16 arterioles were innervated by sympathetic terminals in the midroot level in the monkey (Pohto, ´72; Pohto and Antila, ´72).

Physiological experiments (Matthews and Robinson, ´80) also support the idea that few pulpal sympathetics are present. Responses from non-myelinated sympathetic fibers were recorded in only 6 of 27 teeth after ipsilateral stimulation of the SCG.

The Sympathetic Innervation Varies With Tooth Type, Age and Level:

Using histochemical fluorescence techniques, Pohto (´72) and Pohto and Antila (´72) observed a differential degree of innervation according to tooth type and age. They found higher numbers of sympathetic fibers in anterior teeth and few, if any, in premolars and molars. Furthermore, older animals exhibited less innervation within the pulp, indicating a decrease in sympathetic tone with the onset of age. These changes may reflect the retrogressive vascular and neural changes in the pulp with the progression of age and may be expected to occur bilaterally (Bennet et al., ´64; Bernick, ´67; Fried and Hildebrand, ´80a,´81a,b).

A differential innervation at different levels within the pulp has also been reported. Cox and Avery (´78) and Avery et al. (´80) reported adrenergic terminals throughout the pulps of mouse mandibular molars using light and electron microscopy following 5-OHDA enhancement. They noted that the majority of the endings

(581 within 8 molars) were in the coronal and central pulp (84.9%) with fewer endings in the bifurcation and root pulp (15.1%), contrary to that found in the rat incisor (Larsson and Linde, '71). These values represent an average of 35.5, 26.1, 5.4 and 5.6 endings in each area respectively, or approximately 70 endings per tooth.

Dental Pulp Sympathetics are Non-myelinated:

A variety of studies have shown that the sympathetic postganglionic fibers projecting to the dental pulp are non-myelinated. After resection of the SCG, only degenerating non-myelinated nerve fibers were found in the pulps of rabbit, monkey and cat teeth (Arwill et al., '73; Avery et al., '71; Cox and Avery, '75; Fehér et al., '77). Fried and Hildebrand ('78) observed only degenerating non-myelinated axons after chemical sympathectomy in cat incisors. In addition, Matthews and Robinson ('80) could only measure conduction velocities attributable to non-myelinated fibers from pulpal nerves after stimulation of the ipsilateral SCG.

Ground Plexus Structure of Pulpal Sympathetics:

With histochemical fluorescence techniques, an intensely fluorescent ground plexus of fine adrenergic neurons has been demonstrated around the smooth muscle of some arterioles and metarterioles (figs. 3,4) in humans (Anneroth and Norberg, '68; Kukletová et al., '68; Pohto and Antila, '68a,b; Waterson, '69), monkeys (Pohto, '72; Pohto and Antila, '72), rabbits (Pohto and

Antila, ´68b,´72; Waterson, ´67,´69), cats (Pohto and Antila, ´68b,´72) and rats (Larsson and Linde, ´71; Scheinin and Light, ´69). Venules are usually devoid of adrenergic nerve plexuses but may contain a few anastomosing fibers (Pohto, ´72; Pohto and Antila, ´68a,b).

Although most adrenergic nerve endings (39.6%) are vascular-related, some (29.8%) freely-lying endings have been described (Avery et al., ´80). Independant adrenergic axons were also found in the peripheral pulp of the rabbit and monkey incisors (Pohto and Antila, ´68a,b,´72). These fibers were not in association with blood vessels, possessed no varicosities and were coiled to a great extent.

Adrenergic fibers have also been described in the odontogenic zone (Avery and Cox, ´77; Avery et al., ´80; Cox and Avery, ´75,´78) and dentinal tubules (Avery and Cox, ´77). However, peripherally located sympathetics have not been observed in a number of other studies. Arwill et al. (´73) found no change in intratubular nerves after SCG transection. No adrenergic fibers have been found in the subodontoblastic capillary plexus (Kukletová et al., ´68; Pohto and Antila, ´68b; Provenza, ´68). In fact, adrenergic neurons have not been observed past the cell-free zone of Weil in some species (Pohto and Antila, ´68a). In an electron microscopic examination of the subodontoblastic plexus in the cat, Holland (´80a) failed to find any sympathetic terminals. In adition, Raschkow´s plexus appears to consist mainly, if not entirely, of trigeminal neurons since removal of the SCG does not affect this area (Arwill et al., ´73;

Christensen, '40) and heavy labelling occurs after radioactive amino acid injection into the trigeminal ganglion (Byers and Kish, '76; Menke et al., '77; Pimendis and Hinds, '77b; Weil et al., '75).

The Structure of Dental Pulp Sympathetic Terminals:

Dental pulp adrenergic nerve endings show features typical of sympathetic nerve endings (Avery et al., '80). Both clear and dense-cored vesicles have been found in varicosities of 3 to 14μm in diameter. The dense-cored vesicles consist of small (100 to 400Å) and large (500 to 1,500Å) varieties.

A Contralateral Sympathetic Innervation has not been Detected:

All available evidence indicates that a contralateral sympathetic innervation of the dental pulp is unlikely. Christensen ('40) found no degeneration in the SCG after total tooth pulp extirpation on the contralateral side. Cox et al. ('77a,b) failed to report a contralateral extension in anterior teeth after HRP injection in the monkey. Avery and Cox ('77) found only ipsilateral labelling of the SCG after HRP injection into the molar, premolar and cuspid (canine) teeth of primates. Chiego et al. ('80) also reported a lack of contralateral innervation in posterior teeth of the monkey after tritiated HRP injection.

Physiological studies also indicate a lack of crossover. Contralateral stimulation of the cervical sympathetic trunk of

the cat produces no change in blood flow of the opposite teeth (Ogilvie, ´67,´69). In addition, contralateral stimulation of the cat SCG only produces responses in ipsilateral canines (Matthews and Robinson, ´80). However, it is possible that a crossover, smaller than the normal ipsilateral projection, could go undetected with this recording technique (Wallin, ´78).

The difficulty in demonstrating a sympathetic crossover to the teeth is similar to that seen for the sensory innervation. Although a transmedian trigeminal innervation has been reported for premolars (Anderson and Pearl, ´74b; Anderson et al., ´77) other studies utilizing peroxidase and tritiated proline have failed to demonstrate a contralateral extension (Arvidsson, ´75; Byers and Kish, ´76; Fuller et al., ´79; Furstman et al., ´75; Pimendis and Hinds, ´77b; Wilson et al., ´80) beyond the first incisor (Byers and Matthews, ´81). This conclusion has been supported by comprehensive electrophysiological experiments (Lisney, ´78; Matthews and Lisney, ´78; Nord and Rolice, ´80; Robinson, ´80,´81).

Functions of Pulpal Sympathetics:

Vasomotor Control:

A decrease in blood flow and generalized vasoconstriction has been observed directly in the dental pulp during stimulation of the cervical sympathetic trunk (Ogilvie et al. , ´66; Taylor, ´50). As a result of generalized vasoconstriction (Bishop and Dorman, ´63; Ogilvie, ´67; Ogilvie et al., ´66) a decrease in

pulp tissue pressure (Kroeger, ´68; Weatherred et al., ´63; Weiss et al., ´70), pulpal blood flow (Beer et al., ´74; Edwall and Scott, ´71; Miura and Kendo, ´69; Neidle and Liebman, ´64a,b; Ogilvie, ´67,´69; Ogilvie et al., ´66; Pohto and Scheinin, ´58,´62; Scott et al., ´72; Tønder, ´75; Tønder and Naess, ´78; Weiss et al., ´70) and rate of tracer molecule disappearance (Bolme and Edwall, ´71; Edwall, ´72; Edwall and Kindlová, ´71; Edwall and Scott, ´71) has been observed. In addition, resection of the SCG results in vasodilatation of the pulpal vessels (Avery et al., ´71).

Intravenous or local application of NA results in a decrease in pulpal blood flow (Beer et al., ´74; Bolme and Edwall, ´71; Edwall and Kindlová, ´71; Meyer and Path, ´79; Ogilvie, ´69; Ogilvie et al., ´66; Taylor, ´50; Tønder and Naess, ´78), pulpal tissue pressure (Beveridge et al., ´64; Simard-Savoie et al., ´79; Weatherred et al., ´63) and an increase in pulpal blood pressure (Matthews, ´76; Sticht, ´67) suggesting vasoconstriction. This response can be eliminated by alpha-receptor blockers (Ogilvie, ´67; Pohto and Antila, ´72; Sticht, ´67). In addition, adrenergic alpha-receptors have been found in the dental pulp by a variety of authors (Edwall, ´72; Edwall and Kindlová, ´71; Matthews, ´76; Neidle and Liebman, ´64a; Ogilvie, ´69; Pohto and Scheinin, ´58; Tønder and Naess, ´78).

A number of neuronal mechanisms have been proposed to account for pulpal vasodilatation. A cholinergic vasodilatory component has been postulated since acetylcholinesterase-positive

neurons associated with pulpal blood vessels have been found in a number of species (Avery and Rapp, '58; Avery et al., '71; Kukletová, '69; Kukletová et al., '68; Nelson et al., '69; Pohto and Antila, '68b,'72; Rapp et al., '67,'68; Sticht, '67; TenCate and Shelton, '66; Weiss et al., '69,'72). Whether these neurons are sympathetic (Nelson et al., '69; Weiss et al., '69,'70,'72), parasympathetic (Armenio and LaForgia, '55; Chiego et al., '80; Kukletová et al., '68) or sensory (Avery et al., '71; Pohto and Antila, '68b,'72) is unclear. Other pulpal vasodilator components may include nerve fibers containing substance P (Gazelius and Olgart, '80; Gazelius et al., '77; Olgart et al., '77a,b,c) or vasoactive intestinal polypeptide (Uddman et al., '80).

Dentinogenesis:

A temporary acceleration of tooth growth occurs after resection of the SCG (Edwards and Kitchen, '38; King, '37). A 20 to 30% increase in the rate of eruption of rat incisors following IAN resection has been observed by Taylor and Butcher ('51) but not by Edwards and Kitchen ('38). An irregular (Avery et al., '71; Isotupa and Ronning, '77) and hyperactive (Avery and Cox, '77; Cox and Avery, '75) phase of dentinogenesis has been described with total neural isolation of the tooth.

Thus a loss of rate control may result with sympathetic and/or sensory isolation. However, it is not known to what extent each contributes to the normal process. In IAN resection experiments the presence of pulpal sympathetics within this nerve

(Matthews and Robinson, ´79,´80) was not taken into account. In addition, it is not known whether sympathetics have a direct influence upon odontoblasts or whether the changes are secondary to changes in blood supply.

Modulation of Dental Pain:

Pulpal sympathetics have been implicated in the modulation of sensory activity within the dental pulp (Edwall and Scott, ´71; Matthews, ´76) but the mechanisms responsible for this phenomenon have not been found.

Edwall and Scott (´71) observed that sympathetic stimulation produced an increase, followed by a decrease, in the frequency of spikes recorded from dentine during the application of isotonic sodium citrate. They suggested that pulpal vasoconstriction could decrease the availability of metabolites resulting in a transient increase in excitability (Paintal, ´64) followed by a decrease as the electrolyte concentration gradient, across the excitable membrane, changed.

In a more recent experiment, it was found that physiological discharge of sympathetic nerves could increase the response of intradental nerves to the chemical stimulation of dentine (Matthews, ´76). Matthews did not, however, find a marked depression in impulse discharge with sympathetic stimulation as described by Edwall and Scott (´71).

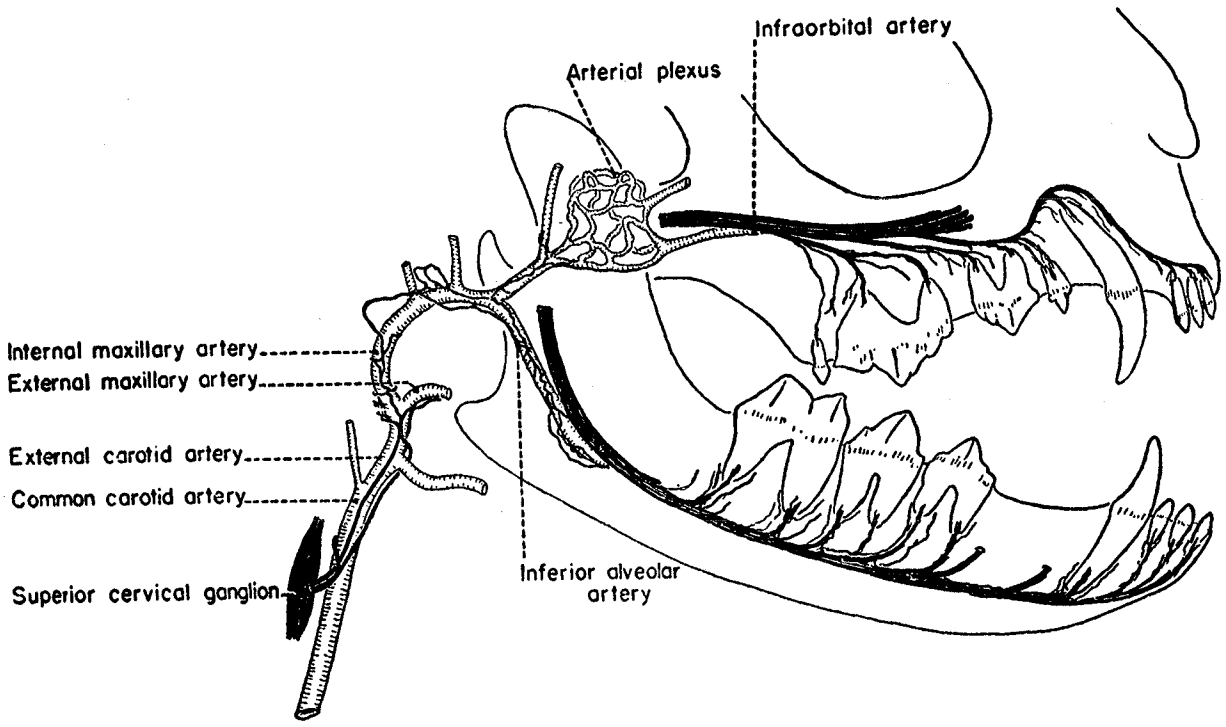
Matthews concluded that the sympathetic nervous system could exert a modulating effect on the sensitivity of the nerve endings

involved in pain sensation from teeth. He postulated the following mechanisms for the sympathetic modulation of sensory excitability: a) the increase in receptor excitability occurs secondarily to vasoconstriction and ischemia; b) changes in pulpal tissue pressure result in a response change in mechanosensitive endings which respond to osmotic effects through dentine; c) a decrease in tissue pressure results in a decreased flow of fluid through the dentinal tubules and hence a more rapid diffusion of ions from the exposed dentine; d) a decrease in pulpal blood flow allows the chemical stimulus to produce a greater change in the composition of the extracellular fluid around the nerve endings, thus resulting in excitability; and e) a specific or direct mechanism exists whereby sympathetic fibers regulate the receptor excitability.

The alterations in sensory unit excitability may be produced through an indirect mechanism. An alteration in pulp tissue pressure (Kroeger, '68; Weatherred et al., '63) could produce deformation of the pulp in localized areas (Närhi, '78) or fluid movement within the dentinal tubules (Brännström, '60; Weatherred et al., '63) resulting in an activation of nerve fibers as in the "hydrodynamic" hypothesis of Brännström. It has been observed that inflammation, which elevates pulp tissue pressure (Stenvik et al., '72; Van Hassel, '71), may be responsible for the severe throbbing pain experienced with pulpitis (Funakoshi and Zotterman, '63; Mumford, '76; Närhi, '78; Närhi and Antila, '73). It is possible, however, that some of the perivascular fibers may be afferent and thus account for this type of toothache (Pohto and Antila, '72).

The initial rise in impulse frequency (Edwall and Scott, ´71) may be due to a direct effect of NA on the sensory units (Olgart and Gazelius, ´77; Paintal, ´64). In addition, the odontoblast may be acting as a receptor (Anderson et al., ´70; Matthews, 72; Matthews and Holland, ´75) which could be controlled by sympathetics. Adrenergic terminals have been reported near odontoblasts although this is contestable (Arwill et al., ´73; Byers and Kish, ´76; Christensen, ´40; Holland, ´80a; Kukletová et al., ´68; Pohto and Antila, ´68a,b).

In a recent application of the chemical sympathectomy technique to the dental pulp (Fried and Hildebrand, ´78), micrographs of nerve fibers containing degenerate sympathetic and intact, possibly sensory, axons were presented (fig. 5). The possibility exists that the close approximations of somatic and autonomic elements may account for the sensory modulation during sympathetic discharge. However, since destruction of sympathetic axons with 6-OHDA is dose dependant (Jonsson and Sachs, ´71) the normal axons may have been unaffected sympathetic axons. Thus the mechanism of the sympathetic modulation of sensory activity is as yet unknown.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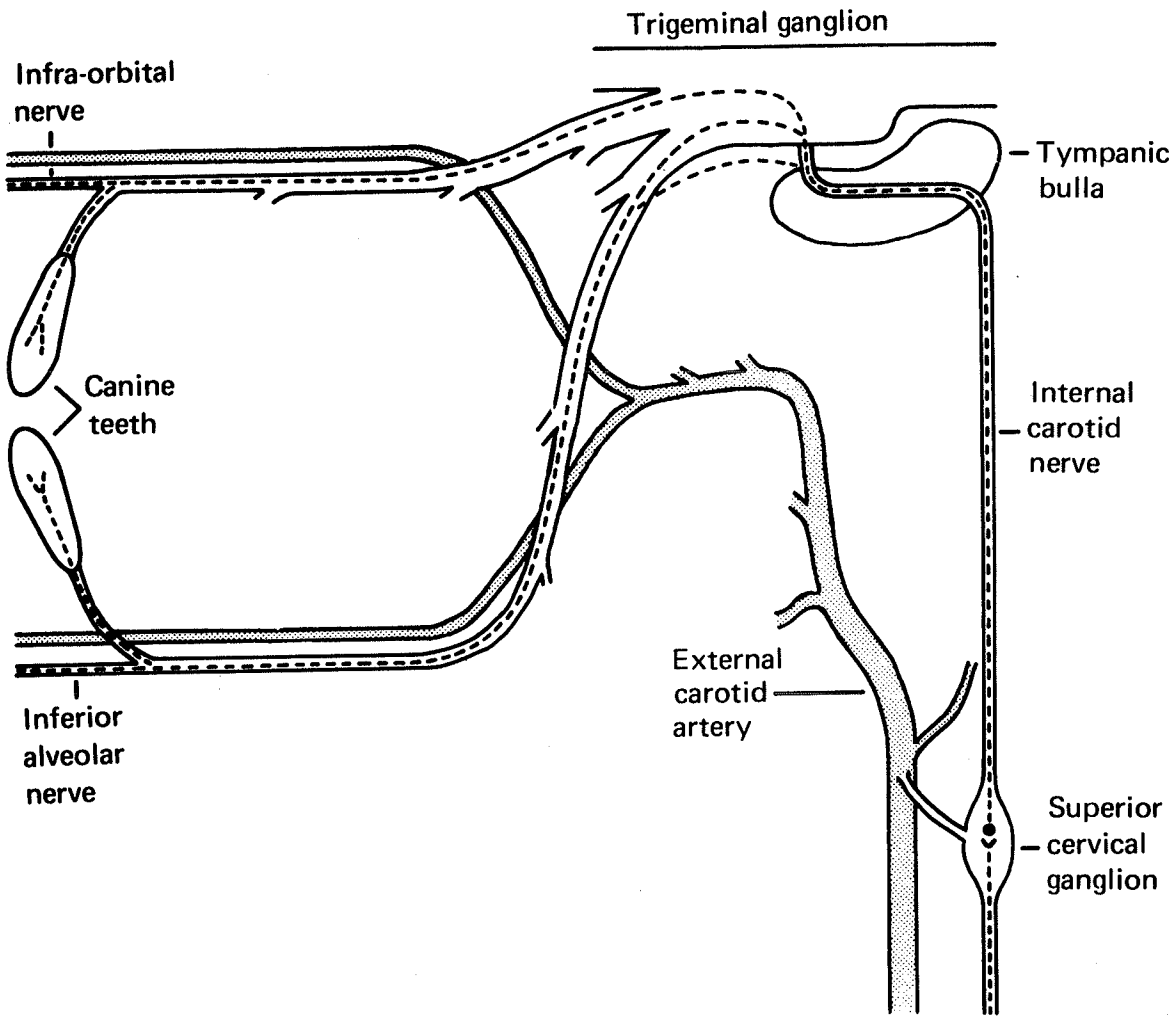
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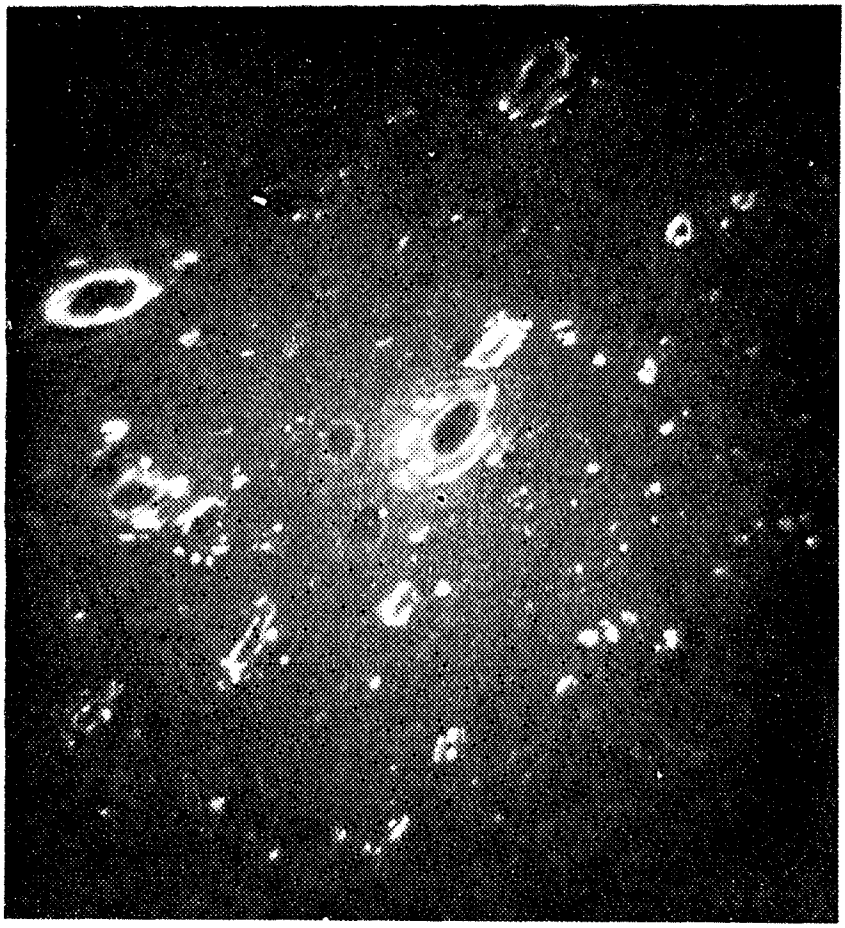
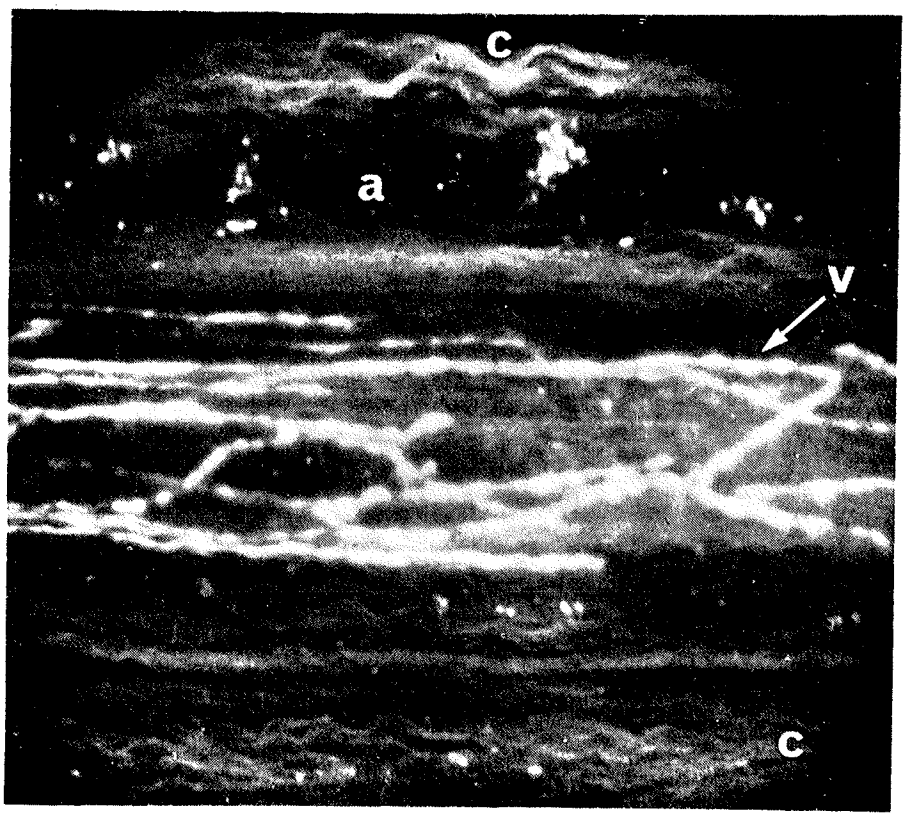


Fig. 4

Longitudinal section of cat lower cuspid pulp showing a sympathetic adrenergic ground plexus covering an arteriole: a, paraxial arteriole without sympathetic innervation; v, varicosities of individual fluorescent fibers; c, collagenous fibers illustrating auto-fluorescence. Formaldehyde-induced fluorescence technique (after Pohto and Antila, ´68b).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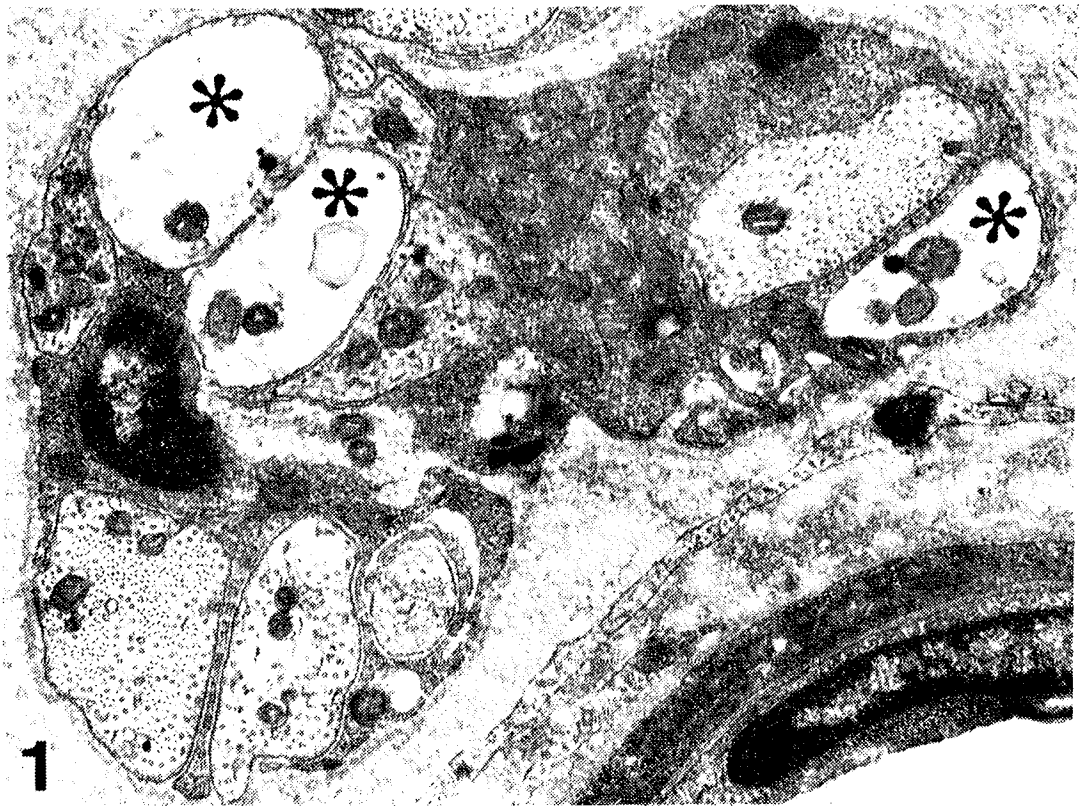
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Materials and Methods:

Examination of the sensory-sympathetic relationship in the dental pulp is hampered by the present knowledge we have of its sympathetic innervation. No quantitative information concerning the proportion of sympathetics entering the dental pulp is available. In addition, the relationship between the sympathetic fibers and terminals to pulpal afferents is not known. A complete investigation of these matters is beyond the scope of the present study since it would involve examination of the dental pulp at multiple levels in a large number of animals. However, the examination of the proportion of sympathetic axons and their relationship to sensory axons at the pulpal apex, is feasible.

Of major importance in the design of this experiment is the choice of a suitable technique for the investigation of the morphological relationship of sympathetic and sensory axons entering the tooth. The technique must have adequate fixation and resolution at the electron microscopic level. In addition, since all sympathetic and some sensory axons in the dental pulp are non-myelinated the technique must allow for a distinction of the two.

Choice of the Technique:

A variety of techniques are available for the study of the distribution and structure of the sympathetic innervation apparatus.

Fluorescence techniques such as the Falck-Hillarp (Falck,

´62; Falck et al., ´62) and glyoxylic acid method (Axelsson et al., ´73; Björklund et al., ´72) for the visualization of primary catecholamines can only be used at the light microscopic level and thus have limited resolution capabilities. In addition, demonstration of preterminal axons is often difficult with these methods. Although adrenergic fluoresence can be enhanced with 5-OHDA (Norberg and Hamberger, ´64) the intervaricose regions often remain unchanged.

Uptake of tracer molecules such as HRP and radioactive amino acids (Avery and Cox, ´77; Chiego et al.,´80; Lasek et al., ´68), although discernible at the electron microscopic level, are not necessarily taken up by all cells at the injection site.They therefore may be incomplete. In addition, both techniques are non-specific (Lasek et al., ´68; Záborszky et al., ´79) and the injection site must be quite large which increases the chance of cell damage and artifacts. Despite the limitations, the autoradiographic method is probably the best long-term technique not requiring the destruction of the sympathetic fibers for identification.

Using electron microscopy, one cannot distinguish non-myelinated sympathetic and sensory axons by straight morphology unless visualization of the adrenergic terminals is made. Although adrenergic dense-cored vesicle preservation can be enhanced with 5-OHDA the intervaricose regions remain unchanged. Since sympathetic terminals are scarce at the apical regions of the tooth (Avery et al., ´80) this technique would be inadequate in identifying pulpal sympathetic axons.

A number of axonal degeneration techniques are available for the study of sympathetic nerve fibers. Injection of neurotoxins such as 6-OHDA or guanethidine results in specific destruction of adrenergic neurons (Heath and Burnstock, ´77; Tranzer and Thoenen, ´68b). However, the extent of destruction with 6-OHDA is dose dependant due to the differential susceptibility of sympathetic neurons (Jonsson and Sachs, ´71; Malmfors and Sachs, ´68). In addition, guanethidine is only effective in rats (Evans et al., ´79).

Immunosympathectomy, the injection of antisera to nerve growth factor, results in almost total destruction of sympathetic ganglia in newborn mammals (Levi-Montalcini and Angeletti, ´66). However, it is incomplete and short-lasting in adults.

Of the degenerative techniques available surgical sympathectomy is the best procedure for inducing degenerative changes in pulpal sympathetic nerve fibers which are discernable at the electron microscopic level. Unilateral surgical sympathectomy (Christensen, ´40; Fehér et al., ´77) results in a complete and irreversible degenerative process (Kirpekar et al., ´62) of the sympathetic neuron derived from that side. Since pulpal sympathetics course through sensory dental nerves (Christensen, ´40; Matthews and Robinson, ´79,´80) surgical removal of the SCG as opposed to nerve sectioning (Arwill et al., ´73; Avery et al., ´71) is the only method guaranteeing destruction of only sympathetic axons. Thus a distinction between non-myelinated sensory and sympathetic axons can be made. An estimation of the proportion of sympathetic axons entering the

pulp can also be obtained since it has been shown that the proportions of non-myelinated axons are similar on right and left sides (Holland and Robinson, ´82,´83) allowing the use of contralateral teeth as controls.

One disadvantage of the technique is that the degeneration of axons and the loss of sympathetic control of the vasculature could alter the morphological detail and subsequent fixation of the dental pulp. It is possible, however, that the vasodilatation produced by sympathectomy (Avery et al., ´71) could be countered by an increase in pulpal tissue pressure (Tønder and Naess, ´78; Weiss et al., ´70) thus affording some vasoregulation. In addition, Fehér et al. (´77) did not detect any significant morphological alterations other than axonal degeneration after pulpal sympathectomy.

Reinnervation of the denervated areas from neighboring intact neurons (Alm and Elmér, ´79; Olson and Malmfors, ´70; Robinson, ´80,´81) is also a potential drawback. However, considering the slow regeneration rates of collateral sprouts (Alm and Elmér, ´79; Robinson, ´80,´81) no regenerating axons should be present if short survival times are used (Fehér et al., ´77; Fried and Hildebrand, ´78).

With the use of this technique two considerations are important: a proper fixative procedure that will preserve both degenerating and intact axons and the morphological features that typify axonal degeneration.

Choice of a Fixative Procedure:

Although a number of fixative procedures are available which adequately preserve adrenergic nerve structure (Hökfelt, '67b; Richards and Tranzer, '70) overall tissue preservation is usually poor. However, the fixative procedure of Machado ('67) and Tranzer and Thoenen ('67) preserves both dense-cored vesicles and general tissue structure. This procedure is the best overall for the study of the morphological relationship of sympathetic and sensory nerve fibers in the dental pulp.

Criteria of Axonal Degeneration:

The onset and rate of degeneration of axons (Thomas and King, '74) and nerve endings (van Orden et al., '67) varies for different organs and fiber types after ganglionectomy or axotomy. Although some authors have claimed that non-myelinated axons persist for long periods (Calabretta et al., '73; Lee, '63) others have reported very short onset and degeneration times (Kirpekar et al., '62; Nathaniel and Pease, '63; Roth and Richardson, '69; Thomas and King, '74). It appears that degenerative changes may advance more rapidly at the periphery as seen in the sympathetic nervous system (Almgren et al., '76; Wakade, '79).

Electron microscopic examination of degenerating pulpal sympathetics has been reported in two studies. Both Fehér et al. ('77) and Fried and Hildebrand ('78) observed degeneration of sympathetic axons shortly after sympathectomy (2 to 7 days) in contrast to myelinated sensory axons which degenerate within two weeks of IAN transection (Fehér et al., '77).

Within 24 hours of surgical (Fehér et al., '77) and chemical sympathectomy (Fried and Hildebrand, '78) subtle alterations are seen in some non-myelinated axons, similar to those seen after surgical transection (Thomas and King, '74). The microtubules become dilated, decrease in number and the endoplasmic reticulum breaks down (Thomas and King, '74). A disruption of the neurofilaments results in an axoplasm filled with granular debris. Some electron dense inclusions may also be seen (Fried and Hildebrand, '78). Although focal swelling similar to that reported by Dyck and Hopkins ('72) was observed it has not been found by others at sites remote from the area of transection (Roth and Richardson, '69; Thomas and King, '74). By 48 hours, marked alterations in the axoplasm were seen including loss of organelles, fragmentation and the appearance of large electron-dense bodies (Fried and Hildebrand, '78) or cytolysosomes (Fehér et al., '77). These bodies may sometimes contain lamellar components (Thomas and King, '74). Filament bundles (Kapellar and Mayor, '69; Matthews, '73) and watery cytoplasm (Dyck and Hopkins, '72) were not observed. The Schwann cells were largely normal and some intact and degenerating axons were found within the same nerve fiber (fig. 5). In addition, small extensions of the Schwann cell were seen protruding into the axons. Fragmentation of the axolemma as described in advanced stages of degeneration (Dyck and Hopkins, '72; Roth and Richardson, '69; Thomas and King, '74) and extrusion of degenerating axons from affected nerve fibers (Calabretta et al., '73) were not described. Axonal debris is thought to be removed by dissolution into the extracellular space or by the perineurium

(Allt, '72), Schwann cells and/or macrophages (Thomas and King, '74). Schwann cells are thought to surround degenerating axonal debris in the latter stages of degeneration (10 to 15 days after transection). They typically form isolated flattened processes often stacked side-by-side in a parallel array (Thomas and King, '74). In addition, dense-bodies derived from the degeneration of axonal organelles has also been noted within Schwann cells (Thomas and King, '74). None of these features were described in the pulpal studies.

No degenerating varicosities were observed by Fehér et al. ('77) or Fried and Hildebrand ('78). This is a reflection of the paucity of adrenergic terminals at the pulpal apical region (Avery et al., '80; Cox and Avery, '78).

Degeneration of axons in normal animals has been described in a number of different areas (Leonhardt, '76; Townes-Anderson and Raviola, '78) including the dental pulp (Fried and Hildebrand, '80a,'81a,b; Holland and Robinson, '82,'83). These pathological alterations appear to be age-related and can be considerable over long periods of time (Fried and Hildebrand, '81a). However, Holland and Robinson ('82,'83) have shown that the amount of degeneration at any one time is small and does not significantly vary from side to side.

Animals:

Unilateral superior cervical sympathectomies were performed on six cats to produce degeneration of sympathetic axons in the tooth pulps on that side.

Cats were chosen as the experimental animal for a variety of reasons. The sympathetic modulation of the excitability of pulpal afferents has only been observed in the dental pulps of cat canines (Edwall and Scott, '71; Matthews, '76). In addition, a recent application of the chemical sympathectomy technique to the cat canine (Fried and Hildebrand, '78) resulted in micrographs illustrating degenerate and intact axons within the same nerve fiber. This suggests that the close approximations of somatic and autonomic axons may account for the sensory modulation. This interaction has not been demonstrated in any other animal.

No control over the age of the animals was possible. Pohto ('72) and Pohto and Antila ('72) have demonstrated a decrease in the amount of sympathetic innervation, which may reflect the retrogressive vascular and neural changes in the pulp, with the progression of age (Bennet et al., '64; Bernick, '67; Fried and Hildebrand, '80a,'81a,b). However, no side-to-side difference in the amount of loss as age increases has been reported. It seems reasonable to expect that an equal loss occurs bilaterally providing no specific injury to a particular side has occurred. In addition, the large inter-animal variation in the number and proportion of axon types does not appear to be related to age, weight or sex (Holland and Robinson, '83).

Anaesthesia:

Animals were anaesthetised using a mixture of alphaxalone-alphadolone (Alfathesin, Glaxo Laboratories) administered according to the following procedure : 12 to 18mg/kg

IM to produce a state of light surgical anaesthesia; 9 to 12mg/kg IV (greater saphenous vein) to produce surgical anaesthesia; and 1ml (12mg) IV when needed to maintain deep anaesthesia.

Surgery:

The SCG was removed unilaterally from 6 adult cats, male and female weighing from 2.3 to 3.5kg. The operation was performed aseptically on the right side in three animals and on the left side in the remaining three: the contralateral side serving as the control.

A midsagittal incision was made in the neck region extending from a point 5cm caudal to the symphysis of the mandible to a point 7 to 8cm caudal to this. The transverse cervical vein was tied off and cut to increase the working area. The vagosympathetic trunk was separated from the common carotid. The vagal and sympathetic components were then separated. Following these structures rostrally, the SCG and the distal ganglion of the vagus were found and separated. Identification of the SCG was based upon two criteria: a) anatomical- the sympathetic trunk is the smaller and more medial of the two; b) physiological- continuous stimulation (5 to 10 volts, 10 to 20 pulses/sec, 10 to 20 msec duration) produced rapid and complete pupillary dilatation of the ipsilateral eye. The SCG, including a few mm of the trunk both caudal and rostral to it, was removed since ectopic postganglionic cell bodies can occasionally be found near the ganglia (Dyck and Hopkins, '72). A topical antibiotic (Neosporin ointment) was applied within the wound site

and the wound sutured. The animals were allowed to recover from the anaesthetic.

Recovery:

A broad-spectrum antibiotic (Ampicillin Sodium, Ayerst) was injected daily (500mg, IM) until perfusion. An external heat source during and following surgery was provided until the animals were fully recovered.

Tests of the Procedure:

The appearance of ipsilateral ptosis and the absence of the pupillary dilatory reflex was tested daily to ensure the efficacy of the procedure. All animals exhibited these conditions.

Survival Times:

The process of degeneration can last a number of days and in order to study the morphological relationship between sensory and sympathetic axons a number of different survival times were chosen. Two animals were sacrificed two days post-surgery in order to detect the early stages of degeneration (Thomas and King, '74). Since surgical sympathectomy of the pulp results in complete degeneration by 7 days (Fehér et al., '77) two animals were sacrificed one week after surgery to detect the maximal loss of axons. Two animals were sacrificed four days after surgery to illustrate intermediate stages of degeneration. Long survival times were not used to exclude the possibility of regeneration from intact axons.

Perfusion: (see Appendix for additional information)

The heads of the animals were perfused via the common carotid arteries using a Cole-Palmer perfusion pump. Initially, a cold (4^{o} C) prewash solution containing 1% procaine hydrochloride and 0.012% heparin in normal saline was administered. This was followed with a cold (4^{o} C) fixative mixture of 3% glutaraldehyde, 0.1% sucrose and 0.5% dextrose in a 0.1M Sorensen's phosphate buffer at pH 7.4. Complete fixation of the cerebral hemispheres indicated good perfusion of the head region.

Decalcification and Post-Fixation:

To prevent damage to the pulpal apices, the mandibular canines and their IANs were carefully removed. The tissues were then immersed in the primary fixative for an additional 2 to 3 hours. Teeth were washed and decalcified in cold EDTA (disodium ethylenediamine tetracetate) according to the technique of Warshawsky and Moore ('67). The teeth were then cut transversely into 1mm sections, washed in 0.1M Sorensen's phosphate buffer overnight and postfixed in cold 2% osmium tetroxide for 2 hours.

Tissue Processing:

The sections were stained "en bloc" with uranyl acetate (UA), dehydrated and embedded in Araldite according to the procedure specified in the Appendix.

Sectioning and Staining:

Each block (from operated and control teeth) was arbitrarily assigned numbers to eliminate bias and the identities were not revealed until all microscopy and data collection had been completed. Blocks 1 to 2 mm from the apex (for each tooth) were sectioned with a Reichert OMU3 ultramicrotome using glass knives. Although sympathetic innervation varies with the level within the tooth no differences are noticable within this small area (Avery et al., '80; Cox and Avery, '78; Pohto, '72). This area was examined to ensure comparison of the same level in all teeth since axons appear to branch as they ascend through the tooth. The pulpal apex appears to be midway between (Holland and Robinson, '83) the extensive cornual innervation (Beasley and Holland, '78) and IAN supply (Holland, '78).

Thick sections, stained with hematoxylin and eosin, were examined by light microscopy to determine the location of the neurovascular bundles. All excess dentine was then trimmed away. Thin sections were cut with glass knives, collected on 0.5% formvar carbon coated slot grids and stained with UA (1 to 11/2 hours) and lead citrate (5 to 10 min).

Microscopy and Data Collection:

Thick sections were examined to determine the entry pattern and location of the pulpal nerves. At higher power, the patency of the pulpal vessels and the cell shrinkage at the pulpal periphery was examined as indicators of good perfusion and fixation.

Thin sections were examined with a Hitachi HU12 electron microscope at X6000. The complete section was scanned in an overlapping fashion. All axons entering the apices (approximately 11,150) were photographed (1630 negatives) at X6000. High power micrographs were taken at X15,600. Prints of all negatives were assembled in montages to avoid repeated counts. The axons were numbered in each apex. Degenerating and intact axons were identified and the presence of dense-cored vesicles and partial ensheathment in nerve fibers were noted. In addition, intimate contact between degenerating and intact axons was quantified. The following observations were made from the micrographs of each apex: a) number of myelinated axons; b) number of intact non-myelinated axons; c) number of degenerating non-myelinated axons as determined by the criteria of Thomas and King ('74); d) number of axons containing dense-cored vesicles; e) number of non-myelinated nerve fibers; f) number of nerve fibers containing degenerating axons only; g) number of nerve fibers containing degenerating and intact axons; h) number of degenerative-intact axon contacts; i) number of intact and degenerating axons exposed (partially or completely) to the extracellular space. Quantitation of these parameters for the operated and control apices was the basis for the comparison of right and left sides.

Pulpal sympathetics make up only a small proportion of the non-myelinated axons in the pulp and a small population should show degenerative features. Thus the proportion of non-myelinated axons within the tooth should decrease as degeneration proceeds. The proportion of loss, however, can be estimated since it has

been shown that the proportion of non-myelinated axons does not vary by much more than 6% between left and right sides of the same animal (Holland and Robinson, ´82,´83). This is in contrast to the high variation seen in the number of axons between sides in the same animal and in the number and proportion of axons between sides from animal to animal. Thus a comparison of the non-myelinated proportions of the operated and the control or unaffected side should give an estimate of the axonal loss as a result of the sympathectomy.

Circumference measurements for myelinated (outer sheath) and non-myelinated axons were made with a Leitz ASL image analysis system. This computer programmed instrument, when calibrated to the micrograph magnification, measures circumference (in µm) after tracing with a pen-sensor over a digitizing tablet. The measurements are printed out on light-sensitive paper. The circumference measurements were then processed by a computer and their frequency distribution analyzed. Control and operated apex counts were then compared using Student´s t and paired t-tests. The circumference measurements of the degenerating axons were analyzed as a population and compared to intact non-myelinated axon distributions.

Results:

Features of Normal Morphology:

Nerves and blood vessels entered the apex of the tooth

through either a single large foramen (fig. 6a) or by several smaller channels (figs. 7,8). The smaller channels contained relatively few nerve fibers and were usually surrounded by cementoblasts and cementum.

The preservation of ultrastructural detail was on the whole, good. The majority of the blood vessels were patent and empty (fig. 6a). Only near the periphery was cell shrinkage evident (fig. 6b).

Nerve fibers and axons were well preserved (figs. 9,10). A distinct basal lamina was found surrounding the nerve fibers (fig. 10). Few myelinated nerve sheaths showed disruption that could be attributed to inadequate fixation (figs. 9,10,11,13). Myelin sheath details (fig. 11) including the inner and outer mesaxons in internodal regions (fig. 12) could be seen. Cross-sections through nodes of Ranvier were occasionally observed (fig. 13).

In most, but not all, nerve fibers the Schwann cell cytoplasm showed greater electron density than the axoplasm (figs. 9,10,14). This aided in axonal identification.

Non-myelinated axons were usually found associated with myelinated nerve fibers and blood vessels (fig. 9). Microtubules, neurofilaments and mitochondria were typically present (fig. 10). Nerve fibers often contained more than one axon (figs. 10,22,23) which were on occasion incompletely ensheathed and partially exposed to the extracellular space (fig. 10). The exposed axons were, however, surrounded by the basal lamina. While the majority

of the axons were separated by Schwann cell cytoplasm some were occasionally found in direct contact with each other (figs. 9,10,15). Clear (fig. 10) and dense-cored vesicles (figs. 9,16) were found within a small number of axons. No adrenergic nerve terminals were observed.

Degenerating Axon Morphology:

The identification of degenerating axons was based upon the criteria of Thomas and King ('74): see Materials and Methods. Degenerating axons were most numerous on the operated sides. No degenerating myelinated axons were observed. A differential degeneration rate was present.

At early stages of degeneration, a few axons were seen containing large numbers of mitochondria and other organelles (figs. 17,18). As the number of organelles increased, identification of the microtubules and neurofilaments became impossible (figs. 19,20). Some of the axons (fig. 20) were significantly larger than normal due to the increased content of organelles and electron dense material.

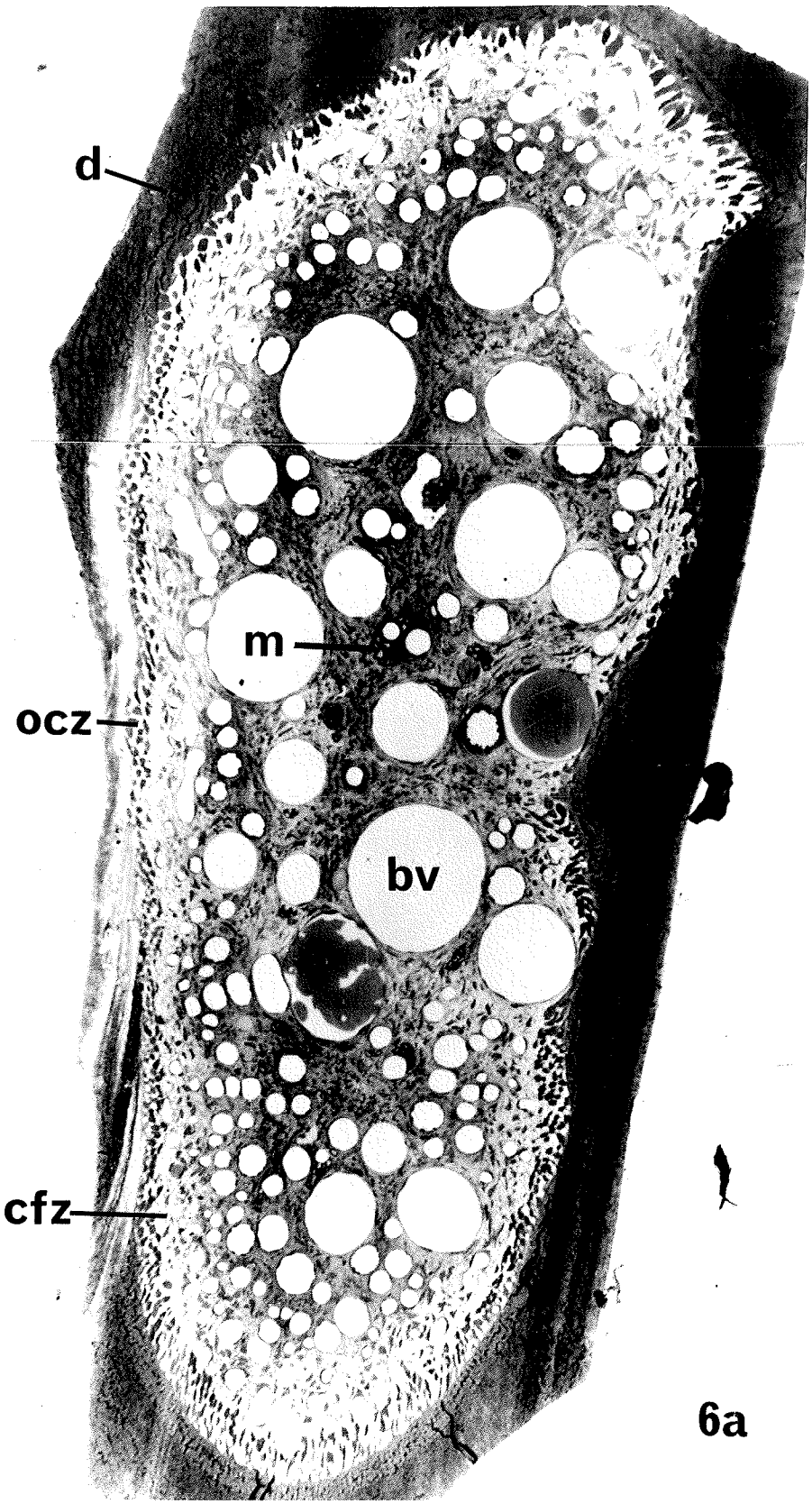
For most axons, the earliest stage of degeneration was characterized by a loss of microtubles and disruption of neurofilaments resulting in a granular and sometimes watery appearance of the axoplasm (figs. 21,22,23). Both intact and degenerating axons were observed within the same Schwann cell (figs. 21,23-27) sometimes in intimate contact with each other (figs. 21,24,26,27). No membrane specializations at contact sites

were seen. Dense-cored vesicles were found in some degenerating axons (figs. 22,24,27).

More advanced stages of degeneration were seen in 4 and 7 day operated sides. The axons contained disrupted cytoplasm with foamy or membrane-like inclusions (figs. 23-27). The axolemma remained intact.

The most advanced stage of degeneration was characterized by a loss of axolemma continuity (figs. 28,29). The axoplasm often contained lamellar membrane-like inclusions (fig. 28). Occasionally, membrane-like bodies were found in a featureless or opaque axoplasm (fig. 29). An indented peripheral cytoplasm with the appearance of empty clefts was occasionally seen possibly due to the loss of degenerating axons (fig. 30).

Figures 6 - 30



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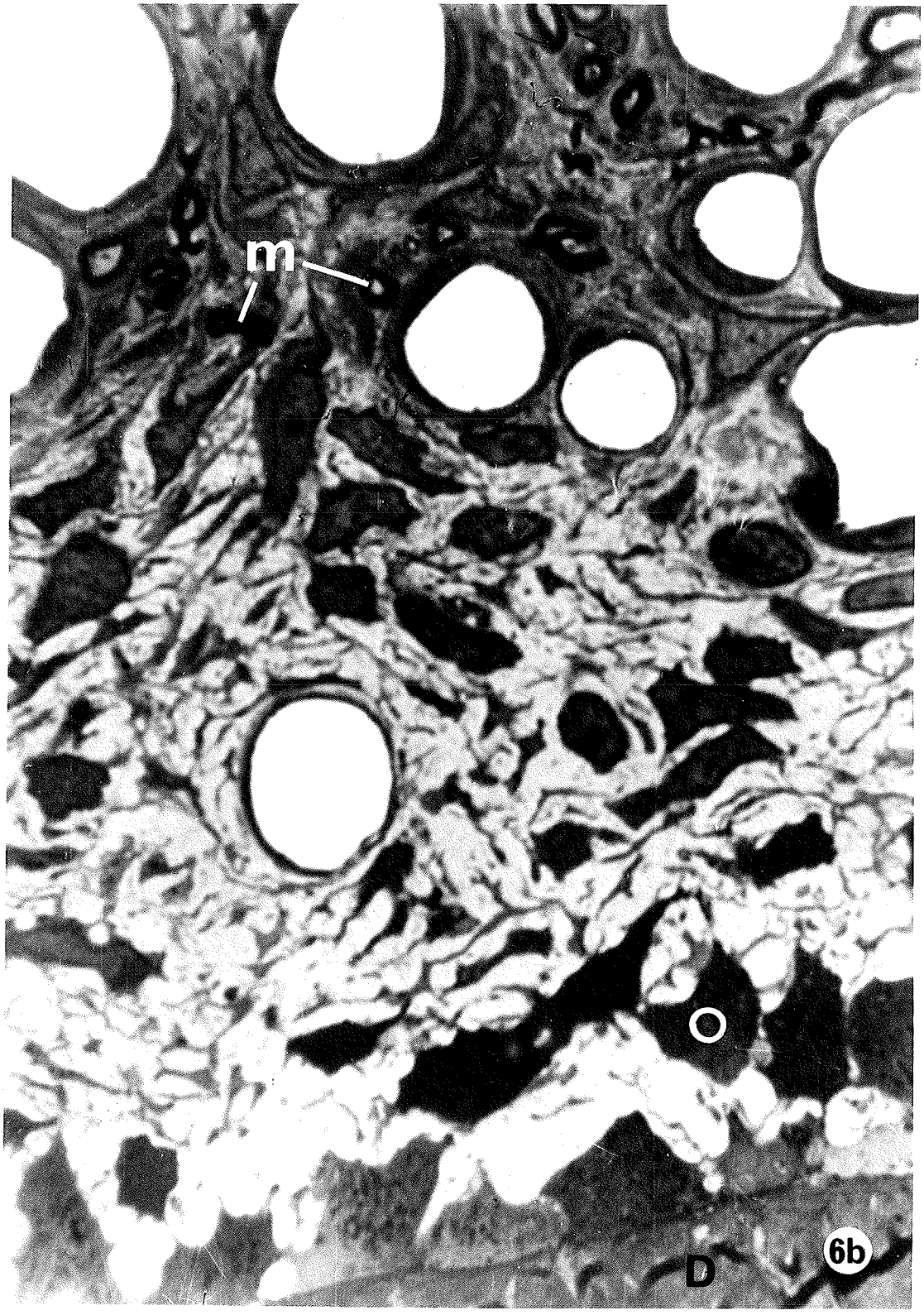
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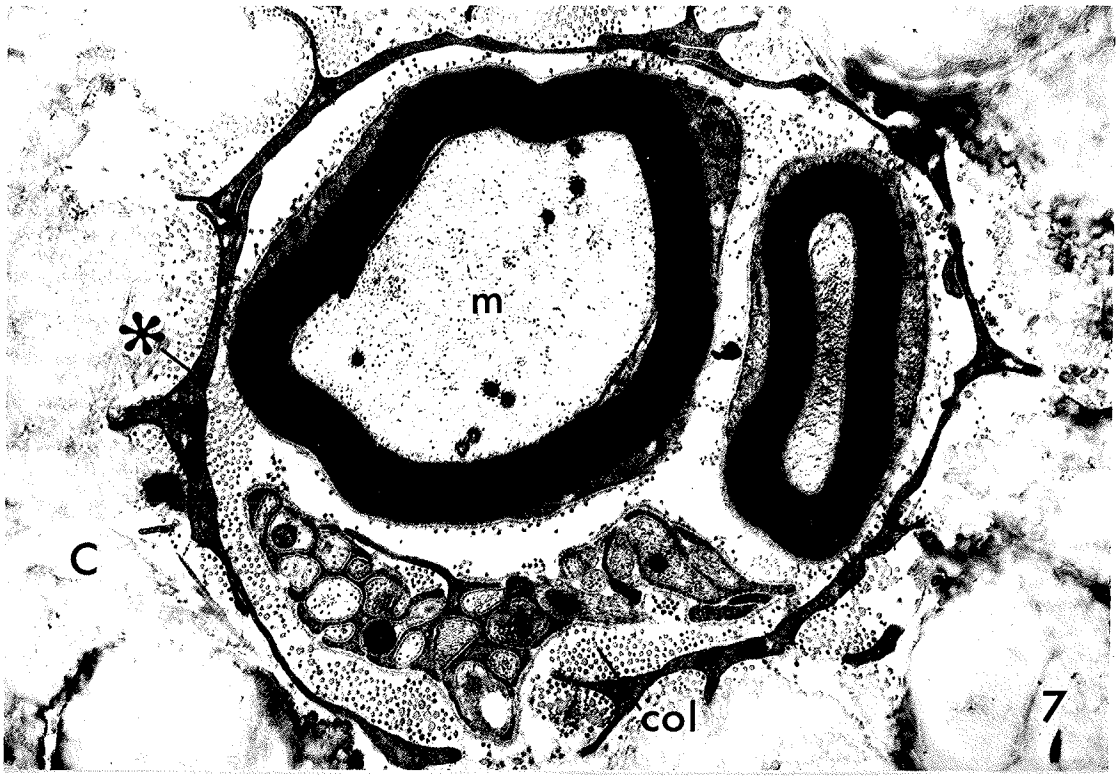
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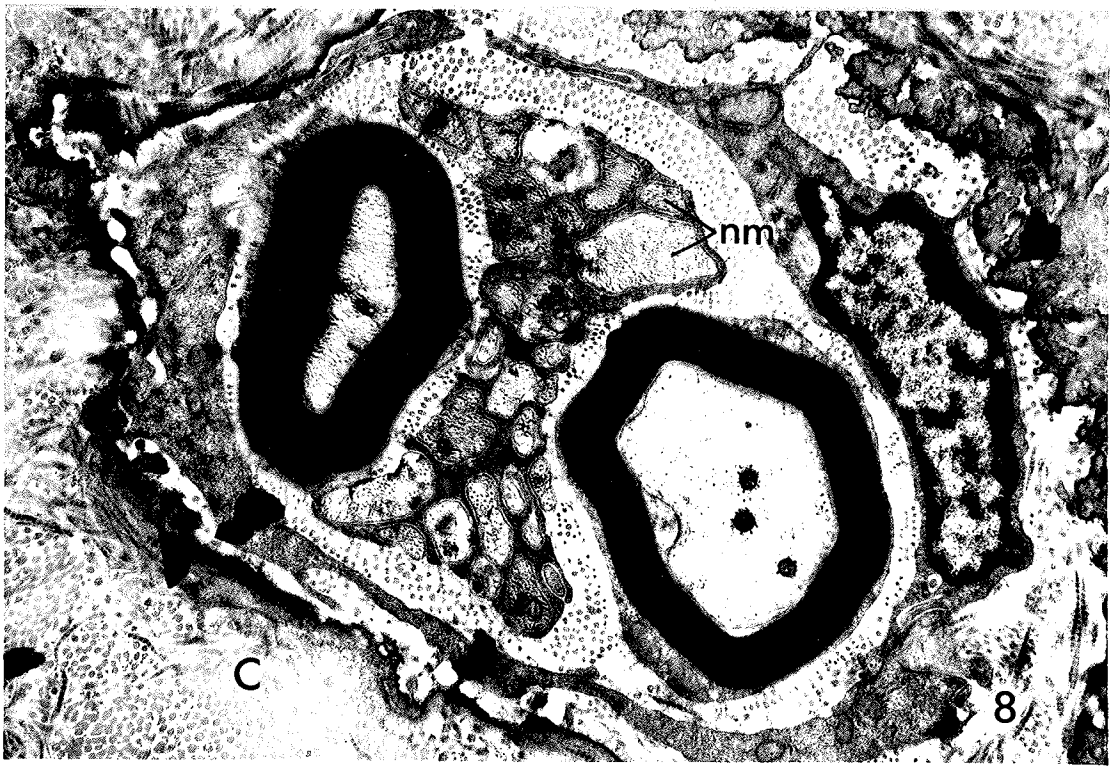
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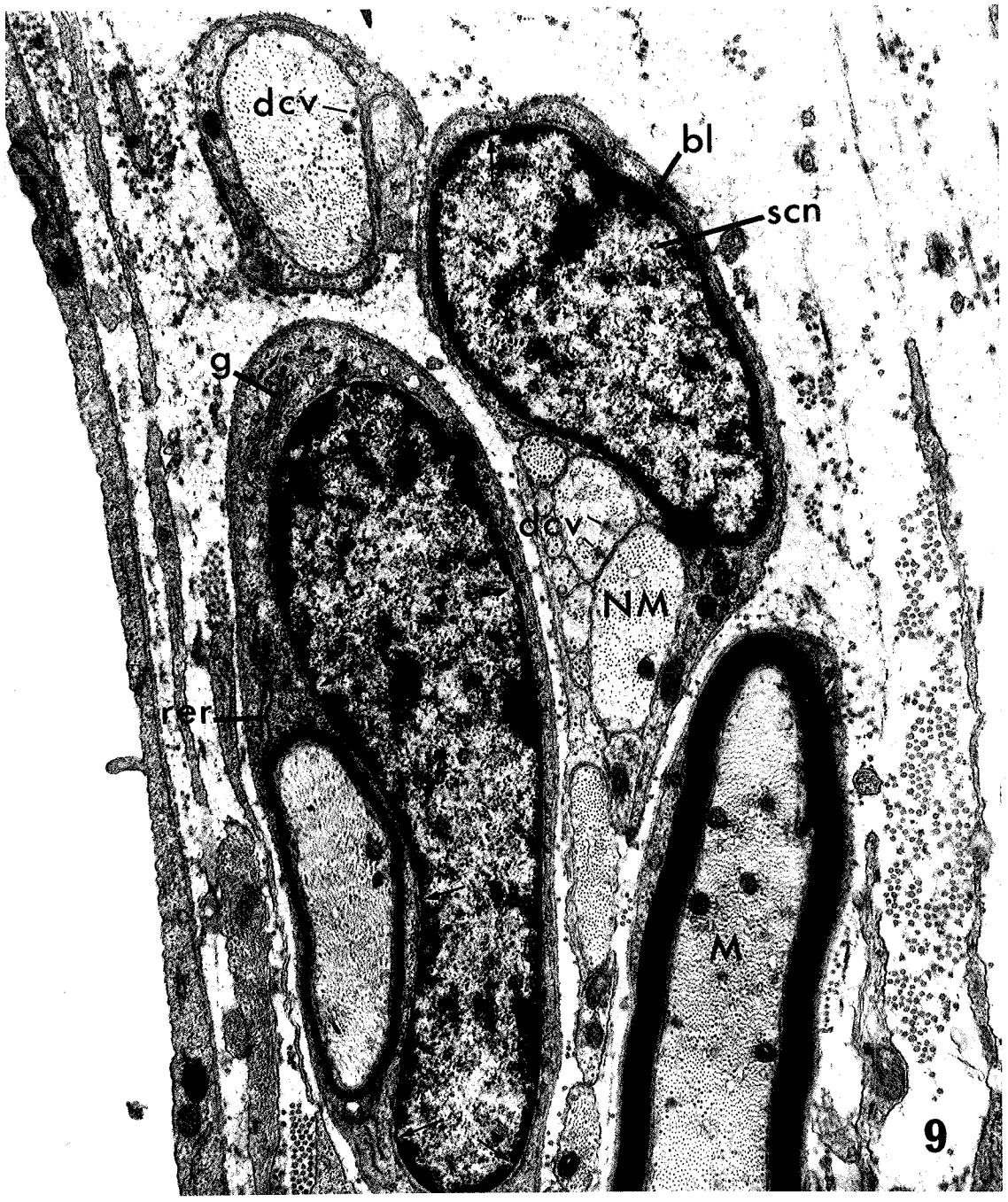
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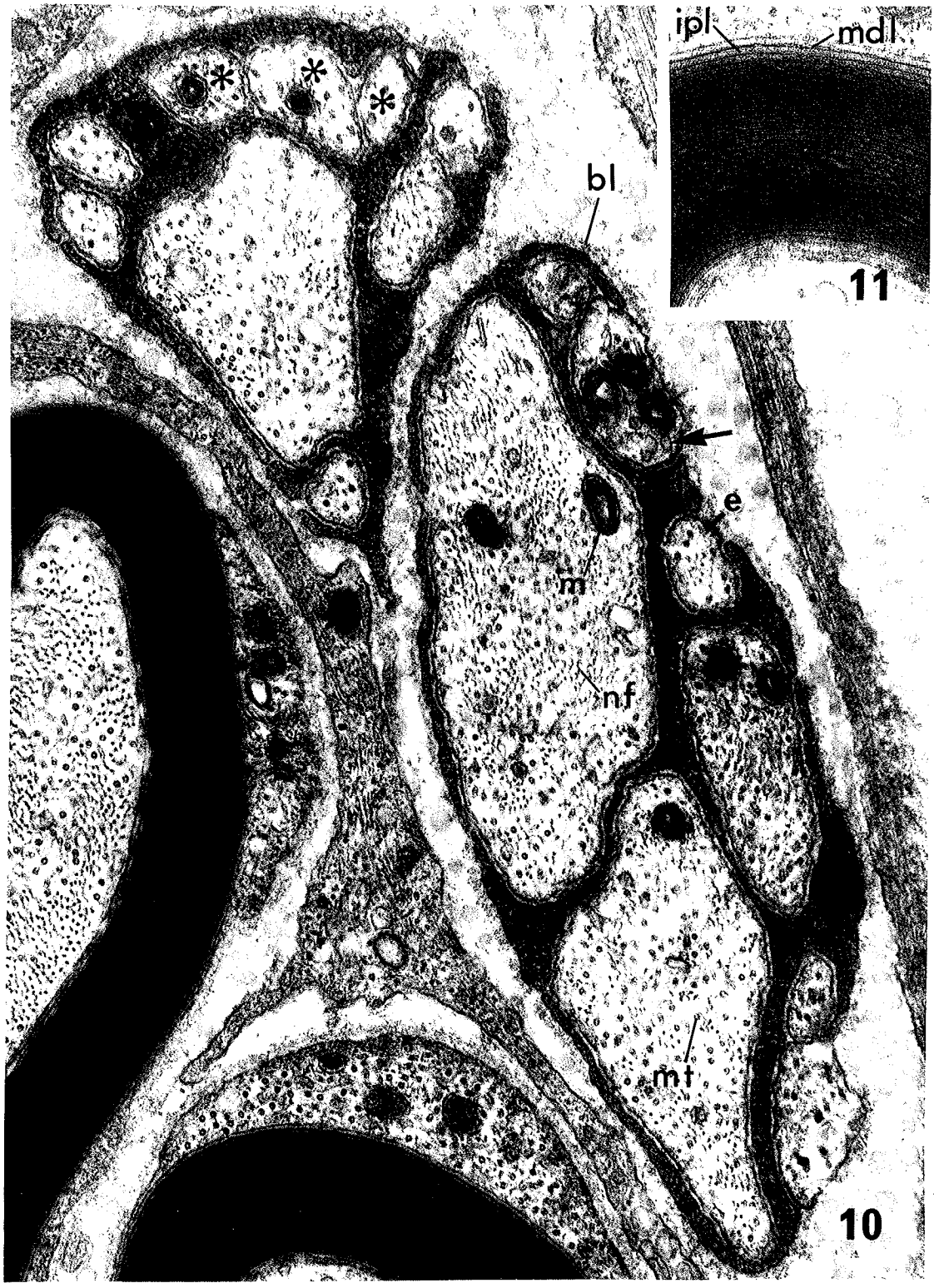
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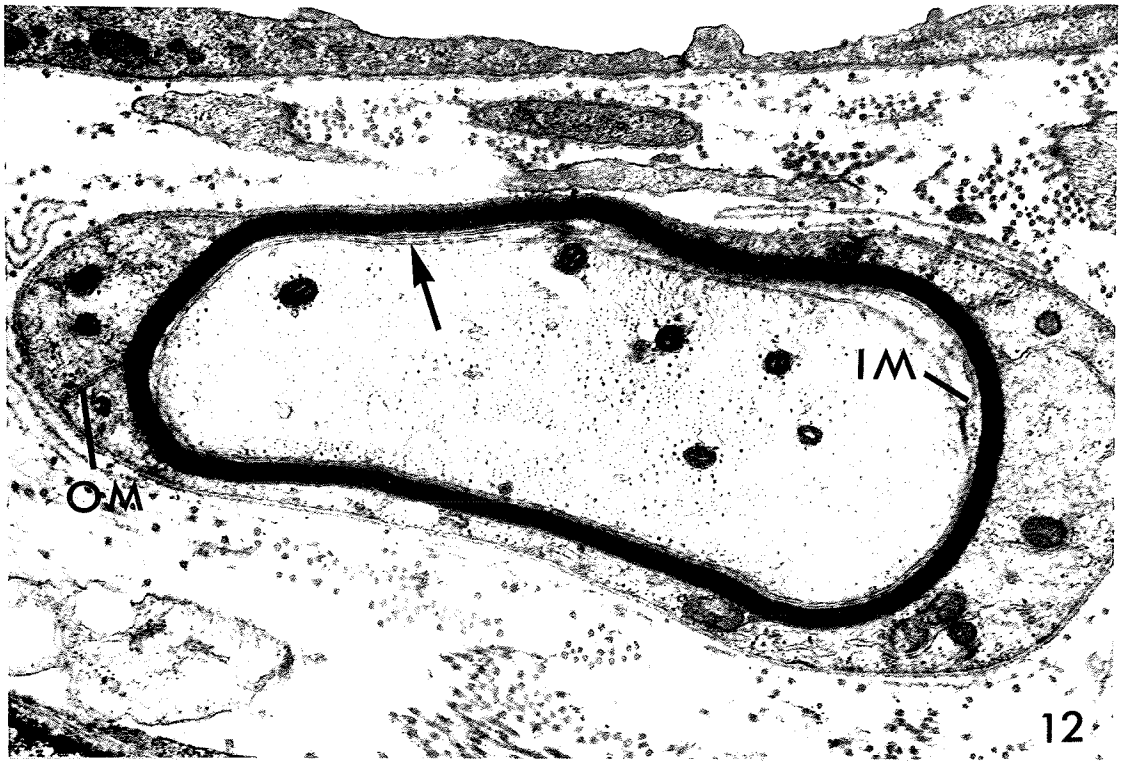

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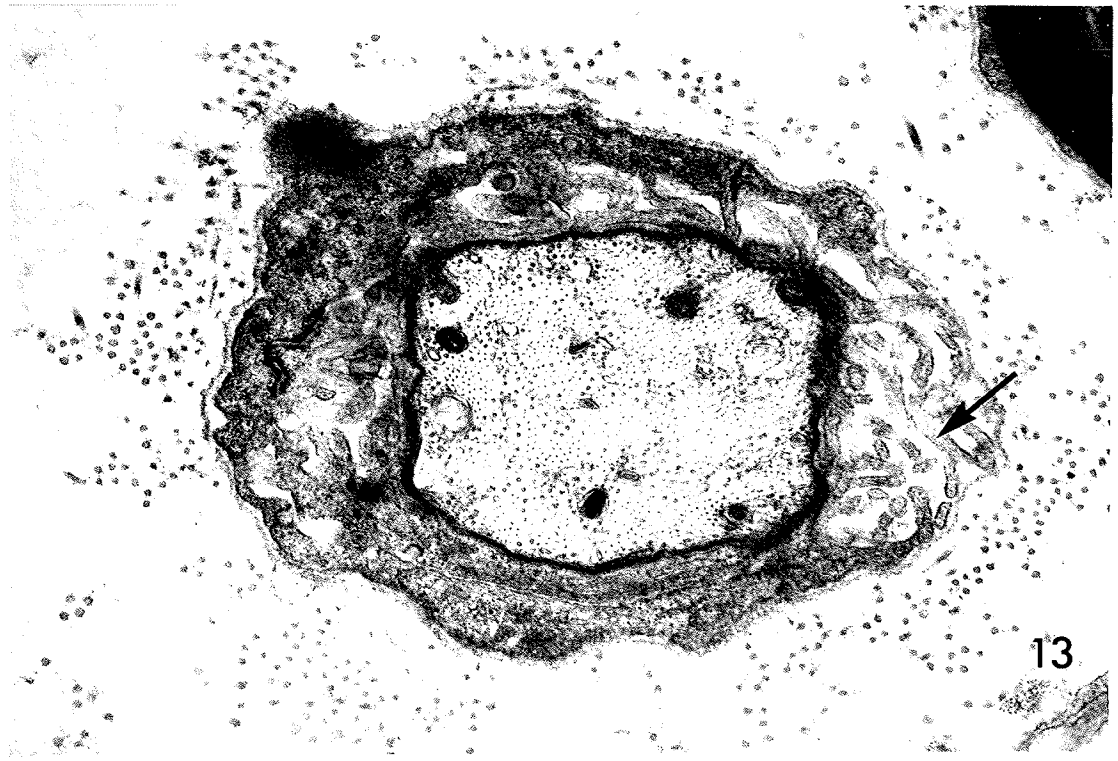

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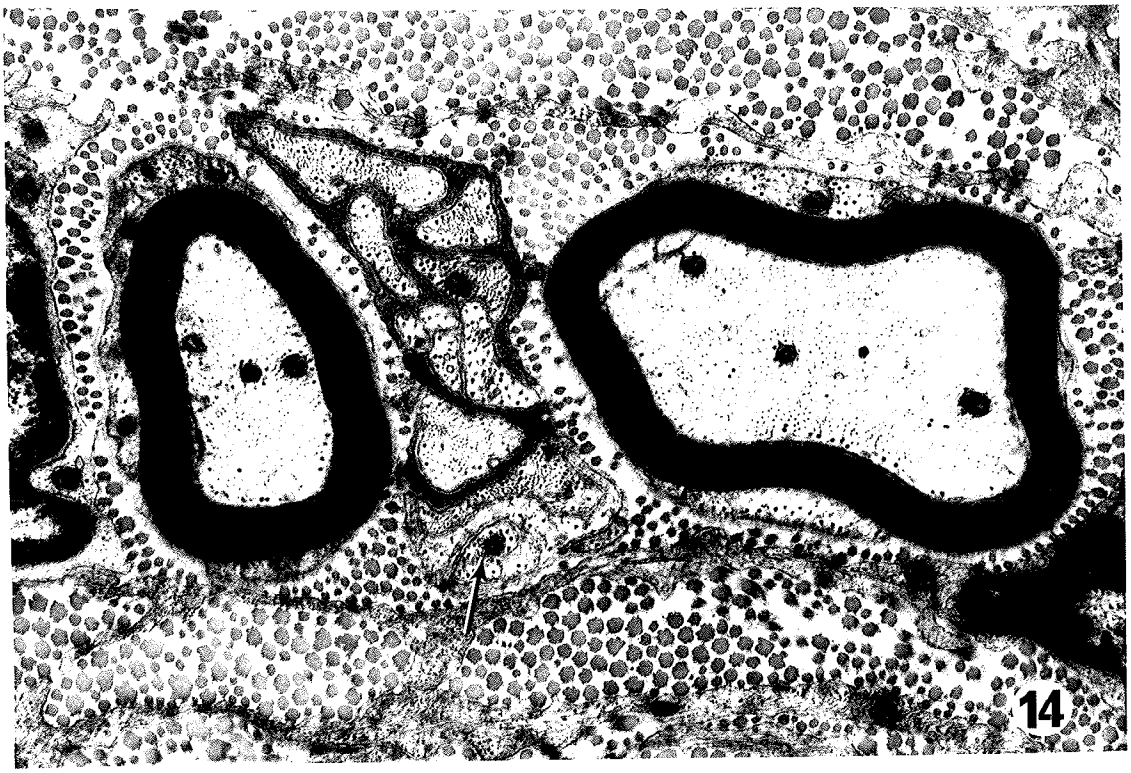
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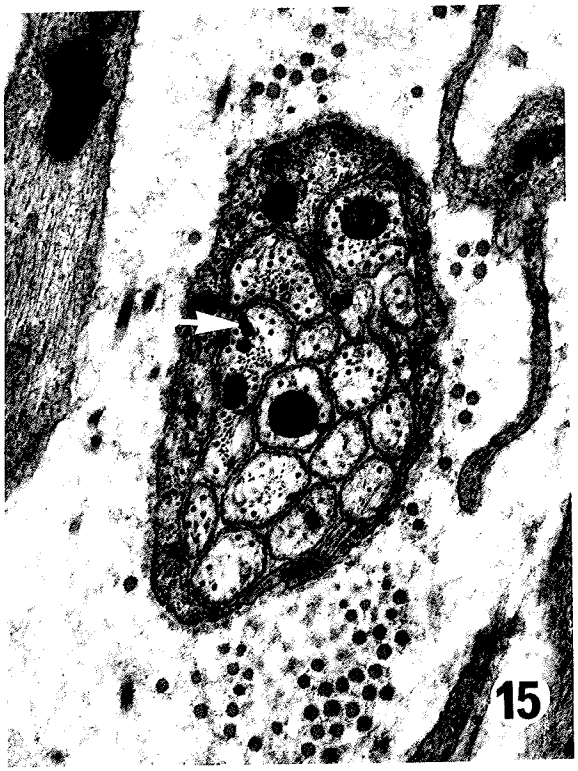
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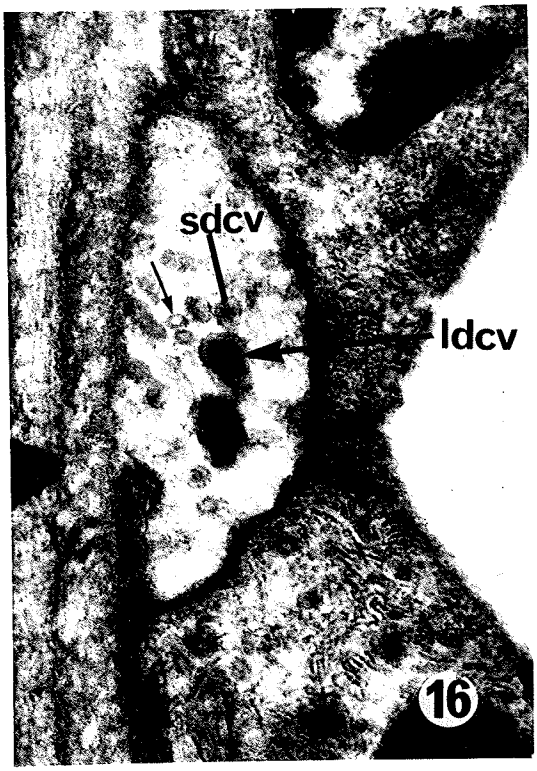
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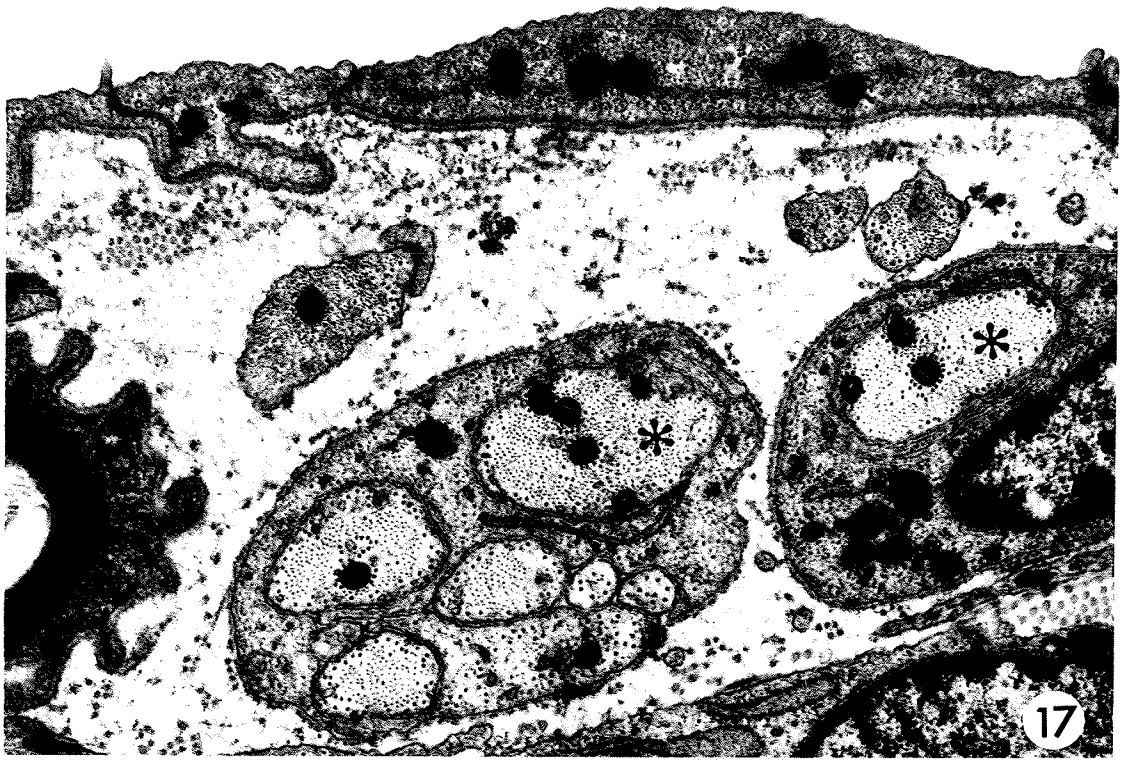
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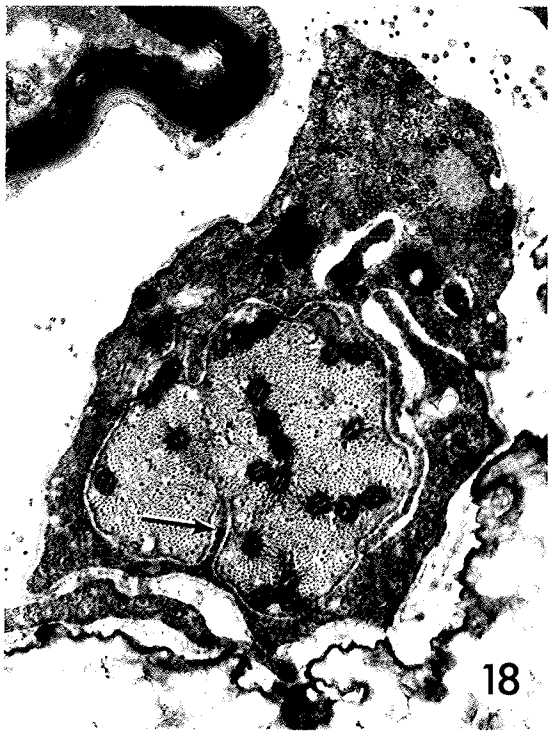
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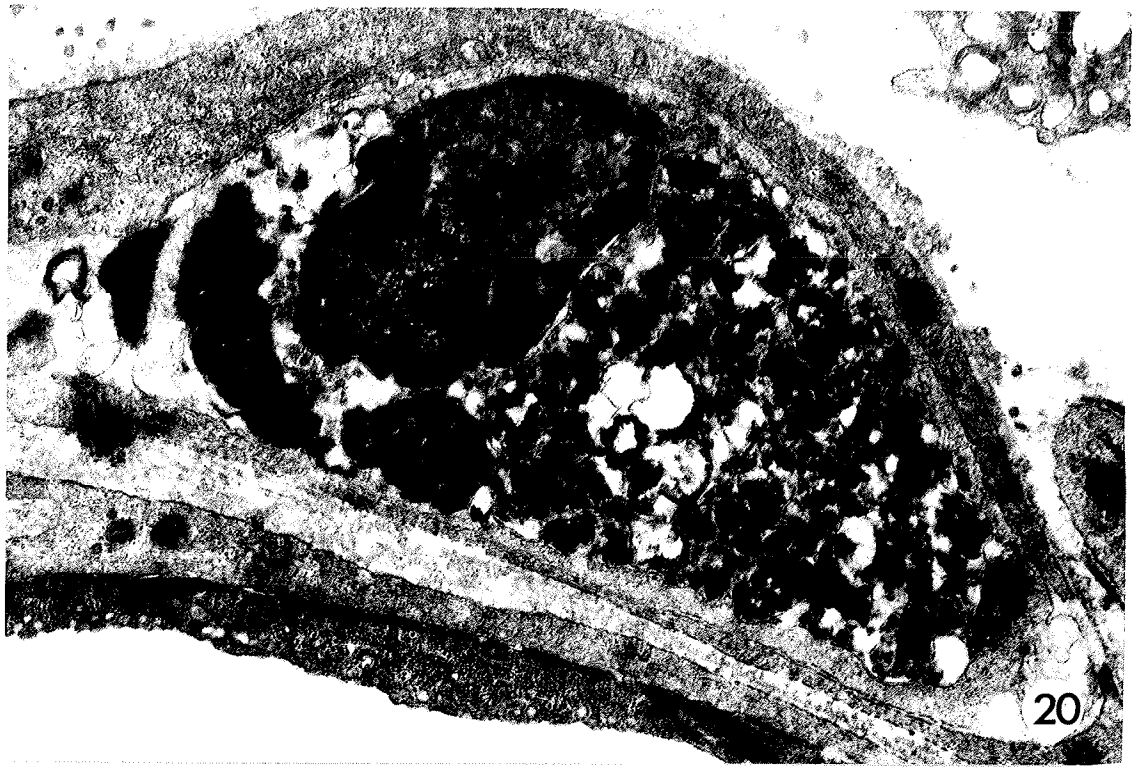
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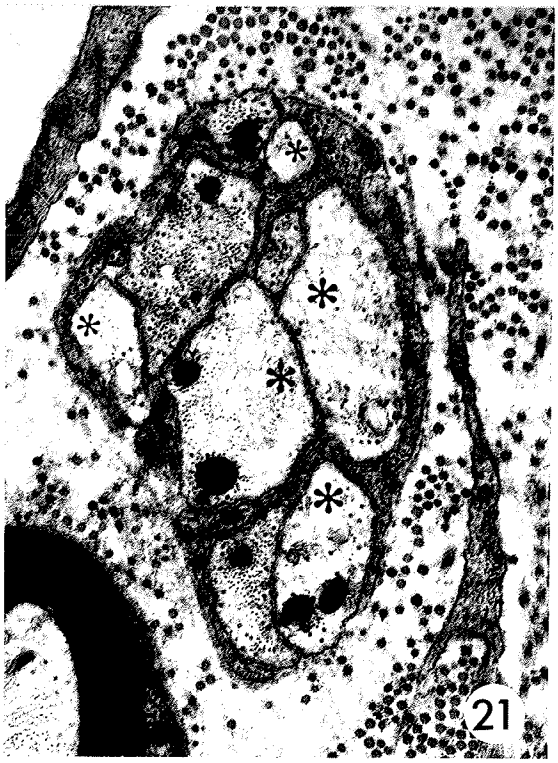
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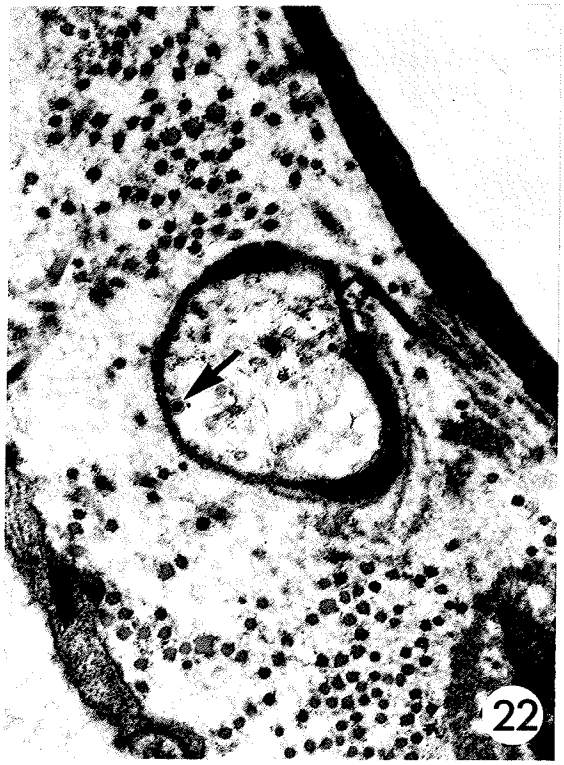
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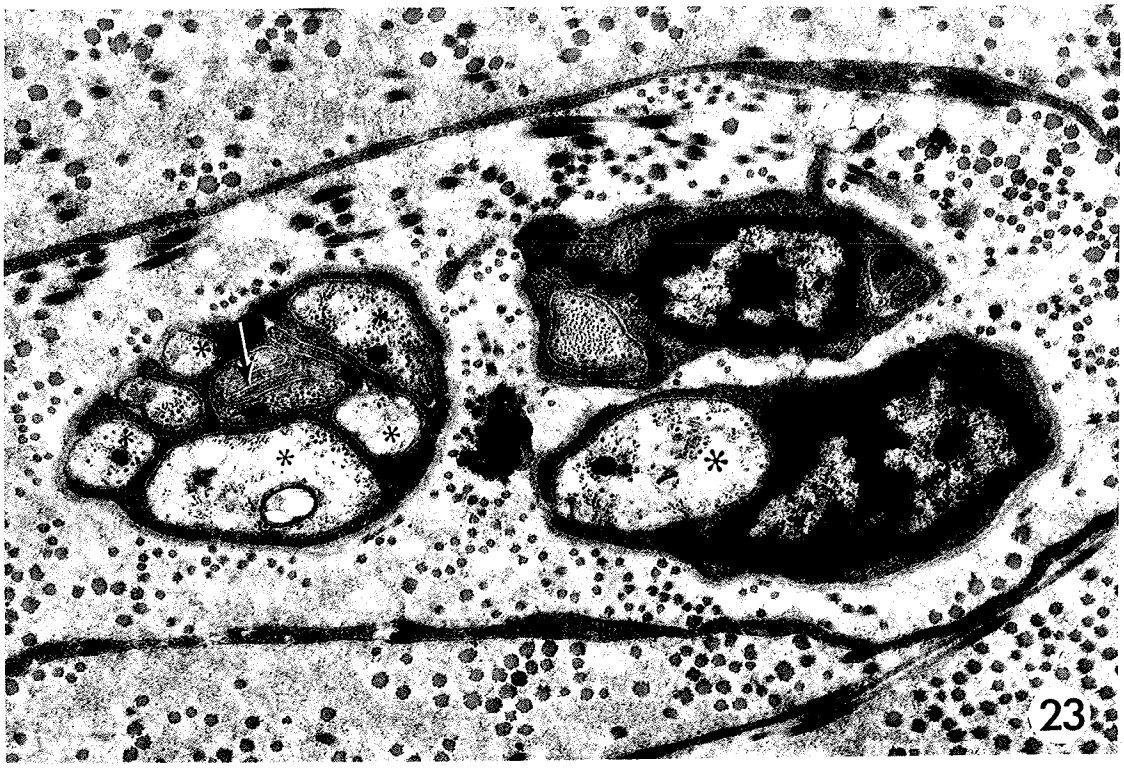

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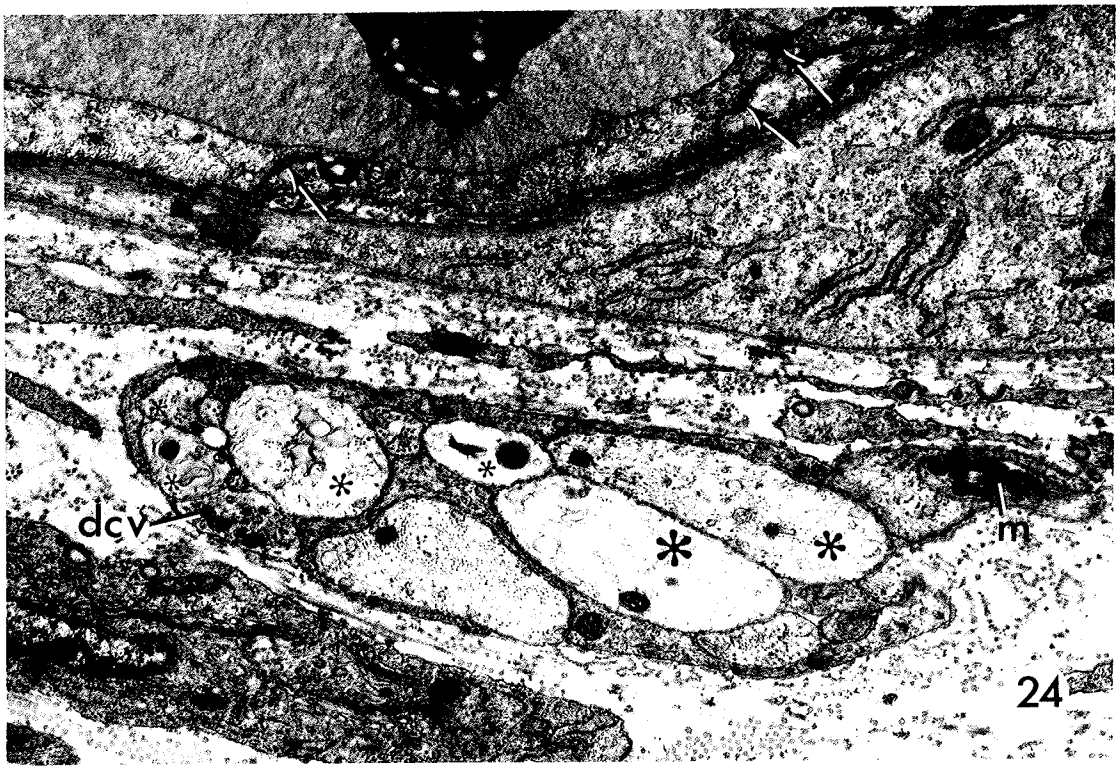

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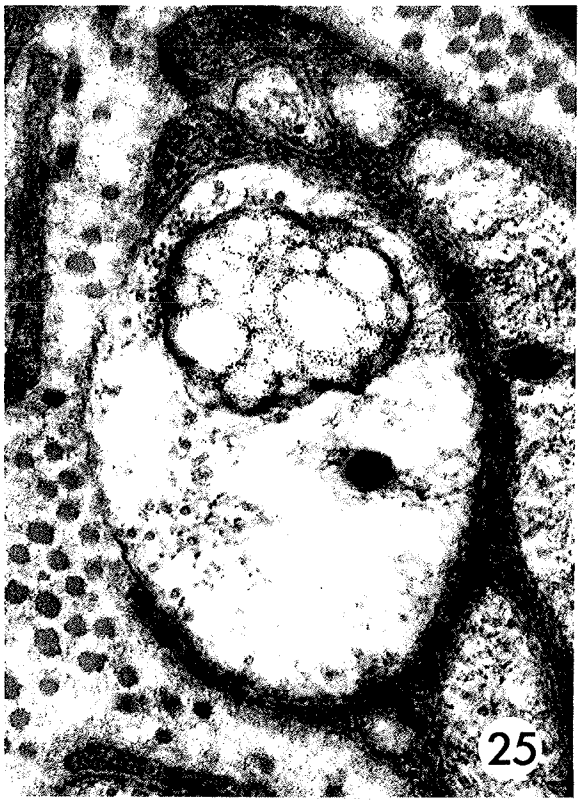

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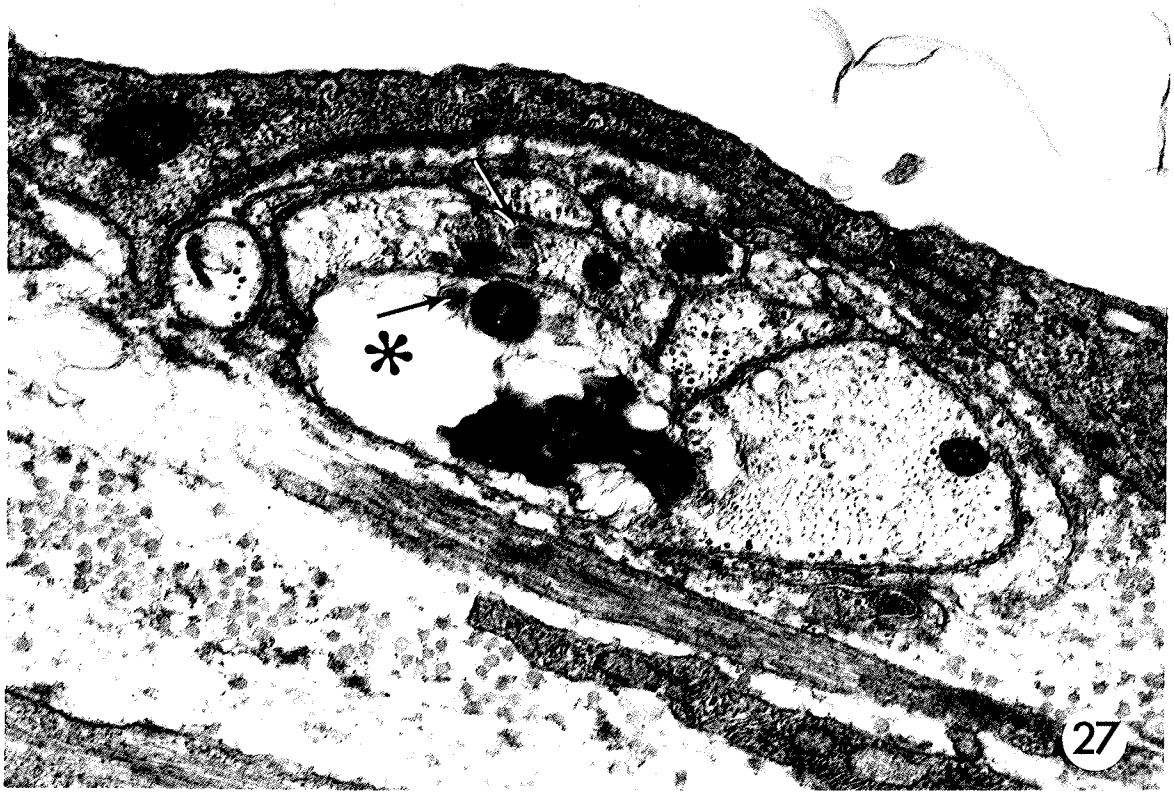

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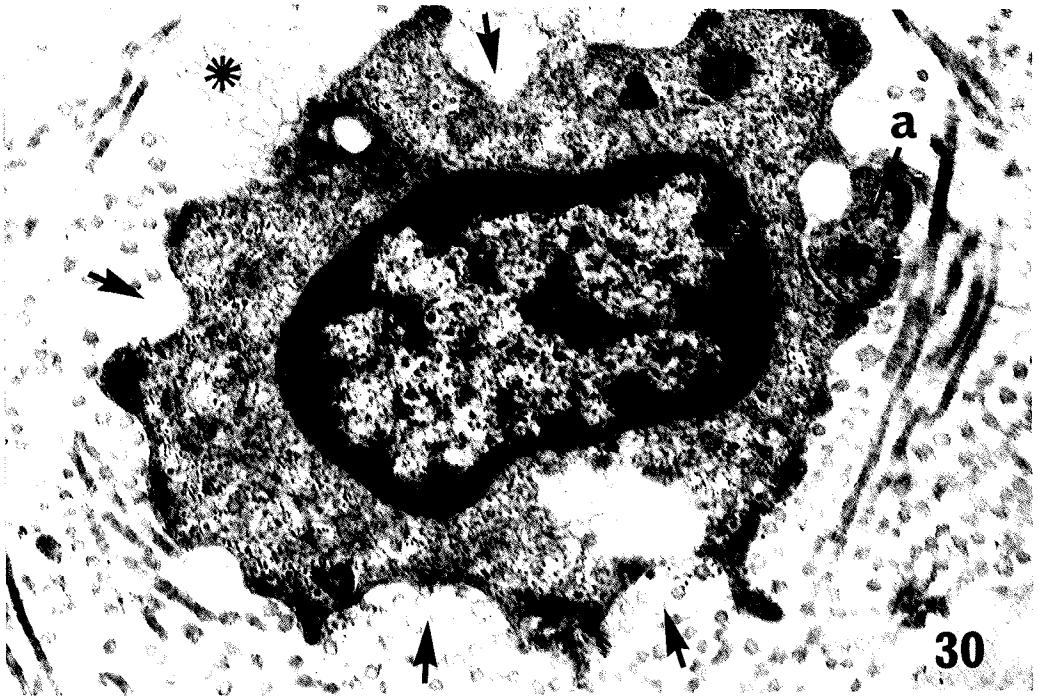
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Quantitative Results:

Comparison of the Number and Proportion of Axon Types of the Right and Left Sides - Table 1:

The total number of axons entering the control apices varied considerably between animals within the range of 556 to 1335. This variability was much less from side-to-side although it could be considerable when non-myelinated axons were compared (from 1.2 to 36.7%). Similarly a high variability in the total number of axons between animals and in the number of non-myelinated axons between sides in control animals (Table 8) was observed by Holland and Robinson (´82,´83).

The proportion of non-myelinated axons within the control apices of different animals varied within the range of 75.9 to 85.6%. Right-to-left variability within the same animal was much less, however (up to 6.7%). Holland and Robinson (´82,´83) have also shown that the proportion of non-myelinated axons can vary considerably between animals but does not vary more than 6.4% between right and left sides of the same animal (Table 8). This variability was not confined to any particular side.

Because of the low variability in the proportion of non-myelinated axons in control animals (Holland and Robinson, ´82,´83) a more accurate estimate of the loss of sympathetic axons can be obtained by comparing the proportion of non-myelinated axons between sides. The operated apices showed a lower % of non-myelinated axons compared to control sides in five of six animals with higher variability in the longer survival

groups. This suggests that the difference may be the result of a reduction in the number of axons due to complete degeneration and loss, rather than just right-left variability.

Comparison of Degeneration of Non-myelinated Axons in Control and Operated Sides - Table 1:

Axonal degeneration, as a result of the sympathectomy, was estimated by taking the difference in the proportion of degeneration between sides.

A small proportion of non-myelinated axons in the control apices (0.8 to 5.6% of the total number of axons) showed features typical of degenerating axons, as described by Thomas and King (´74). A similar but smaller degeneration was seen in control animals (Table 8) with a greater variability occurring between animals than between sides (Holland and Robinson, ´82). In contrast, many more degenerating non-myelinated axons were found in the apices on the operated side (4.8 to 14.9% of the total). In contrast to the low right-to-left variability in control animals (0.0 to 0.8%: Holland and Robinson, ´82,´83) the operated and control side difference ranged from 1.9 to 9.3%. This indicates that the difference in the proportion of degeneration between sides was due to the sympathectomy.

The proportion of degenerating axons in the operated apices decreased as survival time increased. This is seen with examination of the proportion of degeneration with respect to the total number of axons or non-myelinated axons within each apex. Regression-line analysis illustrates a significant decrease in

the % of degeneration in the operated apices as survival time increased (fig. 31).

The Occurence of Dense-Cored Vesicles and Axonal Exposure in Degenerating Axons - Table 2:

Dense-cored vesicles (DCVs) were observed in non-myelinated axons in the control and operated apices. In control apices 2.1 to 7.3% of the total non-myelinated axon population contained DCVs. A similar amount was observed in the operated apices although a more apparent decrease in this proportion was seen as survival time increased.

A small proportion of the axons containing DCVs in the control side also showed features of degeneration. This feature was markedly higher in the operated sides suggesting a sympathetic origin. The fact that some vesiculated axons in the operated apices remained intact seven days after surgery suggests that not all axons containing DCVs are sympathetic in origin.

A high proportion of the degenerating axons in the operated apices contained DCVs, suggesting that they were sympathetic in origin. This proportion was significantly higher ($p<0.01$) than that found in the control apices.

A number of non-myelinated axons were incompletely ensheathed (exposed to the extracellular space) by the Schwann cell cytoplasm. Comparison of the proportion of exposure in the degenerating population to that of the intact population in the

same animals showed no essential differences: i.e., no extrusion of degenerating axons from affected nerve fibers could be detected.

The Number of Non-myelinated Axons per Nerve Fiber - Table 3:

Non-myelinated nerve fibers were distinguished by the presence of one or more non-myelinated axons either completely or partially enveloped by the Schwann cell cytoplasm. A basal lamina was typically found surrounding the nerve fiber.

The number of non-myelinated nerve fibers in the operated and control teeth varied according to the number of non-myelinated axons found at this level. No essential differences were observed between sides.

The mean number of non-myelinated axons per nerve fiber varied from tooth to tooth with no statistically significant difference (paired t-test) between control and operated sides. A range from 1 to 53 axons per nerve fiber was observed.

The Presence of Degenerating Axons and Axonal Contact Within Non-myelinated Nerve Fibers - Table 4:

Detection of the presence of sympathetic nerve fibers in the dental pulp was based upon the comparison of the normal occurence of nerve fibers containing only degenerating axons (in the control side) to the amount found in the operated side after sympathectomy. A small proportion (1.9 to 8.6%) of the nerve fibers in the control apices contained only degenerating axons.

In the operated side this value was significantly ($p<0.01$) higher (7.9 to 14.1%) indicating that a small proportion of nerve fibers are entirely sympathetic in nature.

A higher proportion of the nerve fibers in the control and operated sides contained both degenerating and intact axons. The proportion of mixed nerve fibers was significantly higher ($p<0.01$) in the operated side indicating that the sympathectomy had an influence on the detection of mixed fibers. This proportion decreased somewhat with prolonged survival time but remained greater than that seen in the control, suggesting that the intact axons were not sympathetic in origin.

A large proportion of axons (8.9 to 63.4%) were found together within the same Schwann cell cleft and in contact (membrane-to-membrane apposition between two axons with no intervening cellular process) with one another. No consistent differences in the proportion of non-myelinated axons involved in membrane-to-membrane apposition was seen between sides. However, in the operated apices, a significantly higher ($p<0.05$) proportion of these axons (4.6 to 16.7%) were degenerating. Direct contact between degenerating and intact axons was only common in the 2 day survival groups, where this relationship was 4 times as frequent on the operated side. As survival time increased and the proportion of axonal degeneration decreased, the number of degenerate-intact contacts decreased.

Non-myelinated Axon Circumference - Table 5, Fig. 32:

Since the cross-sectional shape of axons can be frequently irregular (Beasley and Holland, ´78) circumference measurements were made to see whether or not an increase in size of the non-myelinated sympathetics occurred as a result of the sympathectomy. The non-myelinated axon circumferences in each apex were analyzed as a group with respect to their range, mean and mode. The distributions were unimodal with modes of 1.4 or 1.0μm for control and 1.0μm for operated apices. The range varied for the different sides with the operated apices showing slightly larger high-end values, both within the normal range (Holland and Robinson, ´83).

A larger mean size was observed in the operated side in 5 of 6 animals. Using the Student´s t-test it was found that this larger size was significant to the 0.05 level of confidence or higher in 3 of these animals. In one animal, the non-myelinated axons on the control side were significantly larger (p 0.01). In two of the animals which showed a significantly larger mean on the operated side, a significantly larger myelinated circumference ($p<0.01$ and $p<0.05$) was also found (see Table 6). This suggests that the operated apices in these animals were sectioned at a slightly oblique angle.

The degenerating axon population (combined from all operated teeth) was significantly larger ($p<0.01$) compared to the largest non-myelinated axon population observed in all the animals (fig. 32). The degenerating axon population (mean circumference 2.71±0.08μm) had a mode of 1.4μm and a range of 0.6 to 12.1μm.

Myelinated Axon Circumference - Table 6:

Myelinated axon circumference measurements were made to see whether or not the pulpal apices were sectioned obliquely. Since no myelinated axons exhibited degenerative features after unilateral sympathectomy comparison of right and left sides should indicate no significant differences. The myelinated axon circumferences in each apex were analyzed with respect to their range, mean and mode. The circumference distributions were unimodal with modes varying from 9 to 13μm. The circumference ranged from 4 to 35μm, some being outside the A-delta range (larger than 19μm) similar to that seen by Holland and Robinson ('83).

Operated apices showed a similar size distribution to control apices. No statistically significant differences in the mean circumferences between sides in 4 of 6 animals was found. However, in two animals the myelinated axons of the operated sides were significantly larger ($p<0.05$ and $p<0.01$), as were their non-myelinated axons.

Tables 1 - 6

Table 1: Quantitative data comparison of myelinated (M) and non-myelinated (NM) (both intact and degenerating) axons at the apex of control and operated-side teeth

Survival Time (days) and sides (R = right) (L = left)	# M	# NM	Total # axons	Variability in # NM between sides (expressed as a % difference)	% M (of total)	% NM (of total)	Difference in % of NM axons between sdies = % loss	# Deg NM	% Deg of total # of NM	% Deg of total # of axons	Difference in % Deg between sides = % Deg.
2 (R) control	119	706	825	4.3	14.4	85.6	-2.0	46	6.5	5.6	9.3
(L) operated	104	738	842		12.4	87.6		125	16.9	14.9	
2 (L) control	262	825	1087	4.6	24.1	75.9	0.9	52	6.3	4.8	3.9
(R) operated	288	865	1153		25.0	75.0		100	11.6	8.7	
4 (L) control	135	569	704	36.7	19.2	80.8	6.7	20	3.5	2.8	5.8
(R) operated	126	360	486		25.9	74.1		42	11.7	8.6	
4 (R) control	229	1106	1335	28.1	17.2	82.8	4.9	37	3.3	2.8	3.9
(L) operated	225	795	1020		22.1	77.9		68	8.6	6.7	
7 (L) control	130	426	556	1.2	23.4	76.6	4.5	21	4.9	3.8	1.9
(R) operated	167	431	598		27.9	72.1		34	7.9	5.7	
7 (R) control	252	1071	1323	13.1	19.0	81.0	4.6	11	1.0	0.8	4.0
(L) operated	288	931	1219		23.6	76.4		59	6.3	4.8	

Table 2 Quantitative data of the occurence of dense-cored vesicles and axonal exposure in intact and degenerating non-myelinated axons.

Survival times and side	# NM	% of total # of NM axons containing dense-cored vesicles	% of the axons containing DCVs that are degenerate	% of the degenerating axons containing DCV's	% of the degenerating axons that are exposed	% of intact NM axons exposed
2 (R) control	706	5.2	8.1	6.5	34.8	43.9
(L) operated	738	5.8	65.1	22.4	36.8	38.0
2 (L) control	825	7.3	13.3	15.4	26.9	35.2
(R) operated	865	5.8	50.0	25.0	40.0	37.0
4 (L) control	569	2.5	0	0	55.0	41.9
(R) operated	360	2.5	66.7	14.3	50.0	39.9
4 (R) control	1106	4.0	2.3	2.7	35.1	42.5
(L) operated	795	4.0	50.0	23.5	42.6	45.8
7 (L) control	426	2.1	22.2	9.5	23.8	21.0
(R) operated	431	2.3	50.0	14.7	26.5	23.4
7 (R) control	1071	6.2	1.5	9.1	27.3	33.2
(L) operated	931	2.5	34.8	13.6	28.8	28.8

Table 3: Number of non-myelinated axons per nerve fiber

Survival Time and side	#NM Nerve Fibers	Mean # NM axons/nerve fiber ± standard error of the mean	Range
2 (R) control	163	4.3 ± 0.3	1 - 23
(L) operated	161	4.6 ± 0.3	1 - 19
2 (L) control	209	3.9 ± 0.2	1 - 19
(R) operated	201	4.3 ± 0.2	1 - 16
4 (L) control	96	5.9 ± 0.6	1 - 27
(R) operated	71	5.0 ± 0.5	1 - 24
4 (R) control	164	6.8 ± 0.4	1 - 21
(L) operated	157	4.9 ± 0.4	1 - 29
7 (L) control	72	5.9 ± 0.7	1 - 27
(R) operated	93	4.6 ± 0.4	1 - 21
7 (R) control	156	6.9 ± 0.5	1 - 28
(L) operated	151	6.2 ± 0.6	1 - 53

Table 4: The presence of degenerating (D) axons and intact (I) - degenerating axonal contacts within non-myelinated nerve fibers.

Survival Times and sides	% of nerve fibers containing only deg. axons	% nerve fibers with deg. + intact NM axons	# NM axons participating in membrane - to-membrane contacts	% NM axons participating in membrane-to membrane contacts	# axons in contact that are degenerating	% axons in contact that are degenerating	# Deg. axons making D·I contacts	# D·I contacts
2 (R) control	8.6	11.0	402	56.9	23	5.7	17	21
(L) operated	12.4	31.1	458	62.1	56	12.2	48	92
2 (L) control	7.2	12.9	395	47.9	17	4.3	12	16
(R) operated	8.9	23.4	548	63.4	47	8.6	30	52
4 (L) control	4.1	11.5	125	22.0	3	2.4	1	1
(R) operated	14.1	23.9	36	10.0	6	16.7	1	1
4 (R) control	6.1	11.0	582	52.6	5	0.9	5	8
(L) operated	8.9	20.4	327	41.1	17	5.2	11	13
7 (L) control	5.5	13.9	56	13.1	1	1.8	1	1
(R) operated	11.9	16.1	65	15.1	3	4.6	3	3
7 (R) control	1.9	3.8	171	16.0	0	0	0	0
(L) operated	7.9	14.6	83	8.9	5	6.0	0	0

Table 5: Comparison of non-myelinated axon circumferences (µm) in control and operated-side apices.

Survival Times and side	N	Range	Mean	Standard Error of the Mean	Mode	Evaluation of significant size difference (Student's t-test)
2 (R) control	691	0.3-9.8	2.03	± 0.04	1.4	0 > C
(L) operated	698	0.3-11.7	2.19	± 0.06	1.0	(-2.3)
						p <0.05
2 (L) control	811	0.3-10.2	2.02	± 0.04	1.4	C > 0
(R) operated	850	0.3-6.6	1.78	± 0.04	1.0	(-3.7)
						p < 0.01
4 (L) control	556	0.2-7.1	1.76	± 0.05	1.0	0> C
(R) operated	342	0.4-5.9	1.86	± 0.06	1.0	(1.3)
						Not significant
4 (R) control	1090	0.3-5.6	1.42	± 0.03	1.0	0 > C
(L) operated	776	0.3-6.7	1.82	± 0.04	1.0	(-7.4)
						p <0.01
7 (L) control	402	0.2-5.7	1.51	± 0.04	1.0	0 > C
(R) operated	422	0.3-11.9	1.66	± 0.05	1.0	(2.1)
						p <0.05
7 (R) control	1072	0.2-6.8	1.52	± 0.03	1.0	0 > C
(L) operated	914	0.2-12.1	1.54	± 0.03	1.0	(-0.5)
						Not significant
Degenerating (D) axons from operated sides	426	0.6-12.1	2.71	± 0.08	1.4	Compared with 2 day op. side (L) D > NM (5.5) p < 0.01

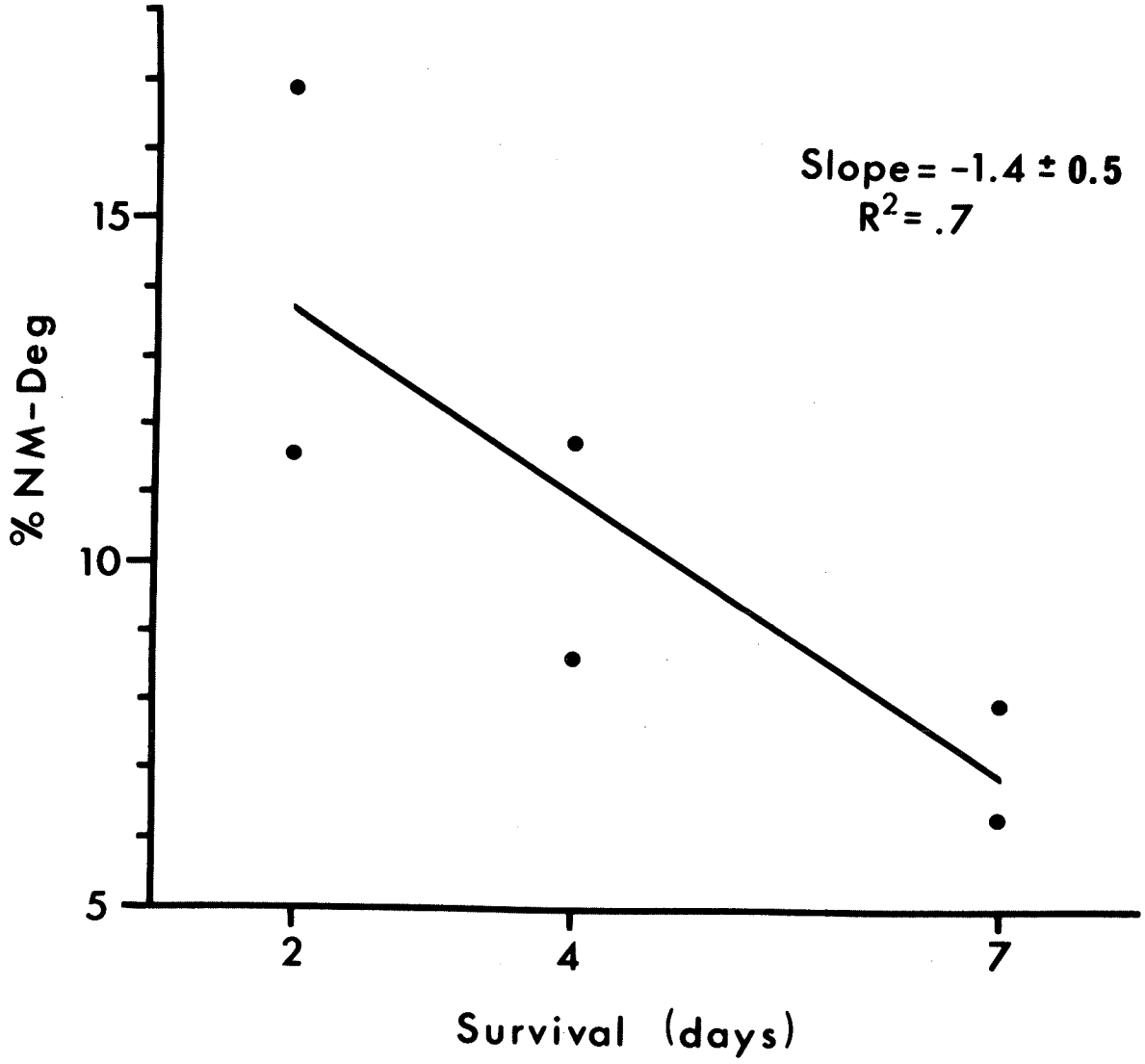
Table 6: Comparison of myelinated axon circumference (μm) in control and operated side apices.

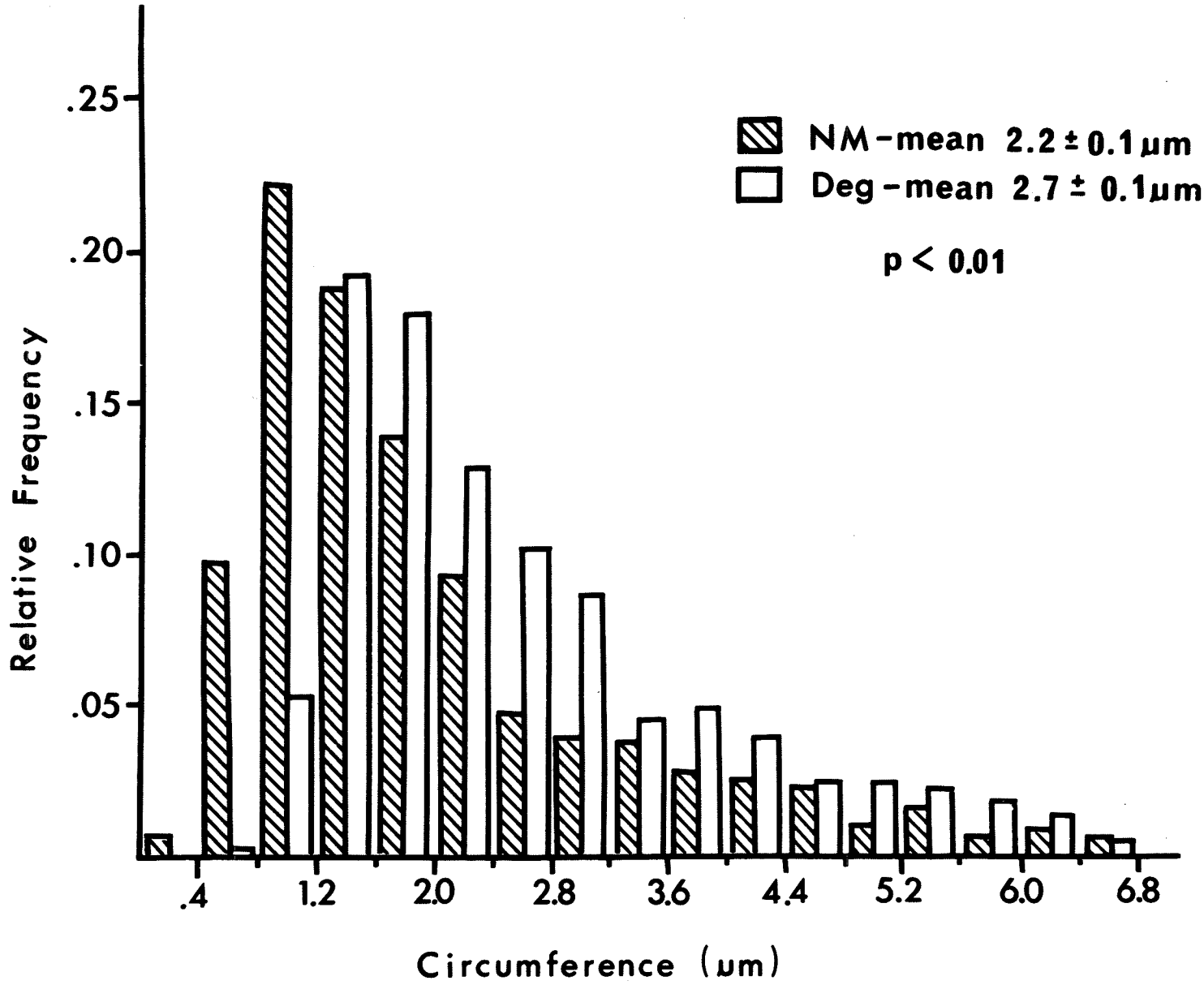
Survival times and side	N	Range	Mean	Standard Error of the Mean	Mode	Evaluation of significant size difference (Student's t-test)
2 (R) control	119	4-23	12.6	± 0.3	11	O>C
(L) operated	96	6-23	13.6	± 0.4	13	(-2.0)
						p <0.05
2 (L) control	242	4-27	14.7	± 0.3	13	C>O
(R) operated	261	4-33	14.2	± 0.3	13	(-0.8)
						Not significant
4 (L) control	121	4-29	13.6	± 0.5	9	C>O
(R) operated	119	4-31	13.2	± 0.5	11	(+0.6)
						Not significant
4 (R) control	216	4-27	11.9	± 0.2	11	O>C
(L) operated	211	6-21	12.9	± 0.2	13	(-2.7)
						p <0.01
7 (L) control	107	4-31	15.3	± 0.6	9	C>O
(R) operated	158	5-29	14.3	± 0.5	13	(-1.2)
						Not significant
7 (R) control	221	4-31	13.6	± 0.4	9	C>O
(L) operated	260	4-35	12.9	± 0.3	11	(1.3)
						Not significant

Figures 31, 32

Fig. 31

Regression-line analysis illustrating the change with time of the % of degenerating non-myelinated axons in operated apices. Analysis of variance showed a significant decrease in the % of degeneration with time ($p<0.01$) with a slope of -1.4 ± 0.5 and intercept at 16.6%.


Slope = -1.4 ± 0.5
R^2 = .7
% NM-Deg
15
10
5
2
4
7
Survival (days)


NM-mean 2.2 ± 0.1 µm
Deg-mean 2.7 ± 0.1 µm
p < 0.01
Relative Frequency
.25
.20
.15
.10
.05
.4
1.2
2.0
2.8
3.6
4.4
5.2
6.0
6.8
Circumference (µm)

Discussion:

The purpose of this experiment was to examine the sensory-sympathetic relationship and the proportion of sympathetic axons found at the apical region of the cat canine. A distinction of the axon types on the basis of a specific destruction of the sympathetic axons was accomplished by the surgical removal of the SCG on one side. Since pulpal sympathetics have not been found to cross the midline (Cox et al., '77a,b; Matthews and Robinson, '80) the contralateral teeth were used as controls. Thus an estimate of the proportion of pulpal sympathetics was obtainable by quantifying the differences in the proportions of non-myelinated axons on both sides following unilateral surgical sympathectomy (see later). This was based upon the finding that the proportion but not the total number of non-myelinated axons on the right and left canines of normal animals are similar (Holland and Robinson, '82,'83). A discussion of the proportion of sympathetics and their relationship to sensory axons follows a consideration of qualitative features revealed by the sympathectomy.

Removal of the SCG Results in a Temporal Degeneration and Loss of Pulpal Axons:

The terminal portions of sympathetic neurons degenerate completely after ganglionectomy or axotomy (van Orden et al., '67). After unilateral sympathectomy pulpal sympathetics can be divided into two groups: those that are in the process of degeneration and those that are lost after complete degeneration

and removal.

Shortly after the sympathectomy, a temporal variation in the amount of axonal degeneration and loss was observed. As would be expected, degenerating axons were detected predominantly in the early stages with complete removal becoming evident later. Regression-line analysis of the proportion of degeneration occurring on the operated side (fig. 31) illustrated a significant decrease over time ($p<0.01$). However, a linear increase in axonal loss with time (calculated from the difference in the % of non-myelinated axons in the control and operated sides: Table 1) was significant only at $p<0.1$. This low probability level was most likely due to the limited number of observations and the wide variation between them. It is, however, indicative of a possible trend of increasing loss with time.

Degenerating Axon Morphology:

Pulpal Sympathetics are Non-myelinated:

Only non-myelinated axons were observed in the process of degeneration after ipsilateral pulpal sympathectomy. Although myelinated postganglionic fibers originating from the SCG have been described (Thompson, '61) no degenerating myelinated axons have been observed in the dental pulp after surgical (Arwill et al., '73; Avery et al., '71; Cox and Avery, '75; Fehér et al., '77) or chemical (Fried and Hildebrand, '78) sympathectomy. In addition, Matthews and Robinson ('80) could only measure non-myelinated conductance velocities from pulpal nerves after

ipsilateral SCG stimulation.

The Morphology and Time Course of Degeneration was Similar to that Found in Other Studies:

The degeneration morphology of pulpal sympathetics was similar to that reported from other pulpal sympathectomy studies (Fehér et al., '77; Fried and Hildebrand, '78). The most common features noted were those seen in portions of nerves distant from the immediate site of injury (Thomas and King, '74). These included the loss of microtubules and granular disruption of filaments, the formation of small osmiophilic bodies and the appearance of a foamy and disrupted, sometimes watery (Dyck and Hopkins, '72), axoplasm. Finally, axonal debris was noted in the interstitial spaces.

Localized accumulations of organelles within greatly enlarged axons were occasionally found as reported in other studies (Kapeller and Mayor, '69; Matthews, '73; Thomas and King, '74; Roth and Richardson, '69). Few axons of this type were seen since features of this type are found mainly in the vicinity of transection.

No extrusion (unensheathment) of degenerating axons from the Schwann cell cytoplasm (Calabretta et al., '73) could be quantitatively demonstrated with the comparison of normal and degenerating axons. It is possible that the degenerating axonal population was too small to illustrate a significant extrusion. This, however, seems unlikely as Thomas and King ('74) failed to find any extrusion and noted instead that either Schwann cells or

macrophages disposed of the axonal debris. Although the presence of macrophages was not investigated, protrusions of Schwann cell cytoplasm into degenerating axons were noted. However, the morphology typical of Schwann cell envelopment of degenerating axons (Thomas and King, ´74) was not observed. This suggests that only a slight reactive condition existed within the Schwann cells as was reported to occur in the tooth pulp after chemical sympathectomy (Fried and Hildebrand, ´78).

Contrary to that found elsewhere (Calabretta et al., ´73; Lee, ´63) the onset and rate of degeneration induced by pulpal sympathectomy was quite fast. Similar results were obtained for pulpal sympathetics (Fehér et al., ´77; Fried and Hildebrand, ´78) and other fiber systems (Kirpekar et al., ´62; Nathaniel and Pease, ´63; Roth and Richardson, ´69; Thomas and King, ´74).

Degenerating Sympathetic Axons Were Found Associated with Blood Vesels and Nerve Bundles:

Degenerating sympathetic axons were distributed throughout the pulp within nerve fibers usually associated with blood vessels and nerve fibers as described elsewhere (Avery et al., ´80; Fehér et al., ´77; Pohto, ´72; Pohto and Antila, ´68a,b,´72). However, sympathetic nerve fibers were not found in the odontogenic zone (Avery et al., ´80) and were rare in vascular-independant areas (Avery et al., ´80; Pohto and Antila, ´68a,´72).

No Sympathetic Nerve Terminals were Observed in the Apical

Cross-sections:

No sympathetic terminals or varicosities with the characteristics described elsewhere (Hamberger et al., '63; Norberg, '67) were found in the apical sections examined. Although dense-cored vesicles of two sizes were observed, serial sections were not examined to determine whether the small DCVs were actually large vesicles cut in a tangential manner. Fehér et al. ('77) also did not find any specialized nerve terminals at apical levels after SCG transection. It seems that a paucity of sympathetic varicosities exists in the root pulps, as described by Avery et al. ('80). They found 5.6±0.9 terminals per mouse molar root pulp using serial section light microscopy. Thus the chances of sectioning through a terminal at this level are quite small.

The Degenerating Axon Population was Significantly Larger in Size in Comparison to the Normal Axon Population:

Circumference measurements of myelinated and non-myelinated axons in the control teeth showed a bimodal size distribution similar to that described previously (Bueltman et al., '72; Holland and Robinson, '83; Johnsen and Johns, '78; Johnsen and Karlsson, '74). When compared to the apical regions, the greatest size differences in the circumference distributions from other studies were found in the cornual region of the cat canine (Beasley and Holland, '78). The smaller size distributions in their study suggests that the axons progressively decrease in size as they ascend through the pulp, probably due to progressive

branching and unsheathing (Engström and Ohman, ´60; Holland and Robinson, ´83). However, the proportion of unsheathing of myelinated axons within the tooth (Harris and Griffin, ´68) is unknown.

The mean non-myelinated axon size was greater in the operated side in most animals, possibly due to swelling of the degenerating sympathetic population. This phenomenon has been reported elsewhere (Dyck and Hopkins, ´72) including the dental pulp after SCG transection (Fehér et al., ´77) and chemical sympathectomy (Fried and Hildebrand, ´78). However, quantitative data was not provided. As the proportion of sympathetic axons in the operated apices was small, this effect was not always statistically significant. Furthermore, the operated side apices of two animals contained significantly larger myelinated axons as well. Since pulpal sympathetics are entirely non-myelinated it appears that in these two animals, the operated apices were obliquely sectioned. Thus detection of swelling with such a small degenerating population cannot be achieved by comparing operated and control circumference measurements.

Comparisons of the degenerating axon population from all operated apices to the largest non-myelinated axon population (Table 5, Fig. 32) illustrated a significantly larger size of the degenerating axons. This difference may either be due to axonal swelling or to an inherently larger size of the sympathetics within the tooth.

The Presence of Degenerating Axons in Control Apices and DCVs in

Normal Axons in Operated Apices is Not Due to a Contralateral Sympathetic Innervation:

The major component of axonal degeneration was found in the side ipsilateral to SCG removal and was thus due to the sympathectomy (see later). However, a small number of degenerating axons were also found in the control teeth. In addition, a number of intact axons containing DCVs were observed in teeth ipsilateral to the SCG removal. These findings suggest that a contralateral sympathetic innervation of the cat canine may be present. However, other explanations are also possible and appear more reasonable.

In comparison to control animals (Table 8; Holland and Robinson, ´82,´83) a higher than normal level of degeneration was seen in the control teeth of the present study. This may be due to differences between animals and not necessarily to a contralateral extension. It is also possible that the degenerating axons were a result of poor fixation although nearby axons appeared well preserved. If fixation was a major factor it could be assumed that the same proportion of degeneration would occur in the operated apices as is seen in the small right-to-left variation of control animals (Holland and Robinson, ´82,´83). If this were the case, the difference between control and operated sides (in the proportion of degenerating non-myelinated axons) would be a true reflection of the sympathetic nerve supply.

The degenerating axons on the control side may be a part of a normal ageing process since both retrogressive vascular and

neuronal changes have been observed in the dental pulp with the progression of age (Bennet et al., '64; Fried and Hildebrand, '80a,'81a,b). It appears that the presence of degenerating axons is a normal occur nce in a variety of tissues (Leonhardt, '76; Townes-Anderson and Raviola, '78) with the dental pulp not being an exception.

The presence of normal axons containing DCVs in operated apices is also suggestive of a contralateral sympathetic innervation. However, available data suggests otherwise. Horseradish peroxidase tracer studies (Avery and Cox, '77; Chiego et al., '80; Cox et al., '77) failed to demonstrate a sympathetic crossover. Stimulation of the contralateral SCG produced no change in pulpal blood flow (Ogilvie, '67,'69) nor did it produce recordable neural activity from the pulp (Matthews and Robinson, '80). The resolution of these techniques may have been inadequate, however. For example, responses could be recorded from sympathetic fibers in only 6 of 27 canines on the side ipsilateral to SCG stimulation (Matthews and Robinson, '80) and a smaller contralateral extension may have gone undetected.

The inability to detect a sympathetic crossover as far as the canine does not preclude the possibility that one is present in more anterior teeth (incisors). For example, this difficulty may be similar to that seen for establishing a sensory crossover (Arvidsson, '75; Byers and Kish, '76; Byers and Matthews, '81; Fuller et al., '79; Furstman et al., '75; Lisney, '78; Matthews and Lisney, '78; Nord and Rolice, '80; Pimendis and Hinds, '77b; Robinson, '80,'81; Wilson et al., '80).

A number of other explanations for the presence of DCVs in normal axons of operated side apices are available. They may be ipsilateral sympathetics which have not yet entered early degenerative stages. This seems unlikely since the DCVs were found in 7 day pulps when all sympathetics should have been completely degenerated (Fehér et al., '77). Another possibility is that the sympathectomies were incomplete. This seems unlikely as part of the sympathetic trunk rostral and caudal to the ganglion was removed and ipsilateral ptosis together with loss of the pupillary dilatory reflex, persisted in all animals. In addition, only small numbers of ganglion cell bodies have been found immediately caudal to the SCG (Dyck and Hopkins, '72) and Christensen ('40) could find no evidence of projections to the dental pulp from cells more caudal than the SCG.

The vesiculated axons may represent sympathetic reinnervation from the contralateral side. Although intact sympathetic neurons can grow into adrenergically denervated tissues (Olson and Malmfors, '70) and regenerating neurons can cross the midline of the body, this appears to be a very slow process (Alm and Elmér, '79; Robinson, '80,'81). For example, regenerating axons (Fehér et al., '77) and their neural activity (Robinson, '80) are absent from the dental pulp for 6 to 9 weeks after total denervation. In addition, none of the axons examined in the operated sides had the characteristics of regenerating axons (Dyck and Hopkins, '72). Thus the vesiculated axons are not likely due to a regenerating population.

The vesiculated axons are most likely normal cholinergic

axons. This explanation seems reasonable since acetylcholinesterase has been demonstrated in the pulpal nerves of many species (Avery et al., '71; Kukletová et al., '68; Pohto and Antila, '68b,'72; Sticht, '67; TenCate and Shelton, '66) and large DCVs are known to occur in normal cholinergic axons (Burnstock and Robinson, '67; Grillo, '66; Tranzer et al., '69).

The Proportion of Sympathetic Axons in the Apical Dental Pulp:

As described previously, a major objective of this study was to establish the proportion of sympathetic axons at the pulpal apex (expressed as a % of the total number of axons entering the tooth). A comparison of the proportions of non-myelinated and myelinated axons in the control teeth to that found in other studies is followed by a discussion of the sympathetic contribution to pulpal innervation.

Control Data:

A comparison of the data from the control teeth (Table 1) with that obtained in other studies illustrates similarities in the total number and proportions of axon types (see Table 7). A highly variable number of axons is found at the apical level (Bueltman et al., '72; Johnsen and Johns, '78; Johnsen and Karlsson, '74). This variability is best seen in the studies by Fried and Hildebrand ('81a) and Holland and Robinson ('82,'83). In addition, a high proportion of non-myelinated axons was observed in the control teeth, similar to that seen by others (Table 7).

Table 7: Quantitative data summary of pulpal axons at various levels from a number of studies

Author	Source	Tooth	Level	Total # Axons	% NM
Bueltman et al., '72	Marmoset	Canines Incisors	Apex	3136	66.4
Johnsen and Johns, '78	Human	Canines Incisors	Apex	2601 1950	86.1 86.1
Johnsen and Karlsson, '74	Cat	Incisor	Apex	558	77.4
Mumford and Bowsher, '76	Cat	Incisor	Apex	-	72.2
Fried and Hildebrand, '81a	Cat	Incisor	Apex	20 - 448	81 - 90
Beasley and Holland, '78	Cat	Canine	Cornua	3,470	81
Holland and Robinson, '82, '83	Cat	Canine	Apex	670 - 1903	56.0 - 79.6
Present Study	Cat	Canine	Apex	556 - 1335	75.9 - 85.6

The cornual region of the cat canine (Beasley and Holland, '78) contained a much larger number of axons than the apical control teeth. The majority of these (81%) were non-myelinated. The large number at this higher level suggests that branching of the axons occurs as they ascend within the pulp. Since it is not known to what extent the myelinated axons lose their sheath in the periphery, one cannot comment on the relative contribution of the myelinated and non-myelinated axons to the branching.

The Proportion of Sympathetic Axons is Calculated on the Basis of the Loss and Degeneration Observed After Sympathectomy:

Pulpal sympathectomy results in degeneration and loss of non-myelinated axons. Since a temporal variation in the proportion of degeneration and possibly loss is observed any accurate estimate of pulpal sympathetics requires the combination of these two values. In order to determine whether the degeneration and loss was the result of the sympathectomy a comparison to the normal degeneration and side-to-side variation in control animals is important. The loss and degeneration is expressed as a proportion of the total number of axons in order to obtain an estimate of the sympathetic contribution to the total innervation of the dental pulp.

The Difference in the Total Proportion of Non-myelinated Axons Between Right and Left Sides is Similar to the Side-to-Side Variation in Unoperated Animals:

Animal-to-animal variation in both the total number of axons and in the % of non-myelinated axons (Table 1) is much greater than that found between sides in the same animal. This is similar to that seen by Holland and Robinson (´82,´83) in their examination of the apical region of normal cat canines (Table 8). They also observed a larger variation in the total number of non-myelinated axons (from 20.7 to 27.5% difference) than the % of non-myelinated axons between sides in the same animal (from 3.4 to 6.4%). Therefore, the proportion of axonal loss due to the sympathectomy was estimated by taking the difference in the proportion of non-myelinated axons in the control and operated sides.

Comparison of axonal loss to normal side-to-side variation in unoperated control animals (Holland and Robinson, ´82,´83) indicates that the loss in the sympathectomized animals (Table 1) was within the value of maximum right-to-left variation (±6.4%). However, the loss detected in the longer survival groups always occurred on the operated side suggesting that the sympathectomy was the determining factor in the side-to-side variation.

Considerably More Degenerate Axons are Detectable in the Operated Sides of the Experimental Animals than are Found in the Control Animals:

The proportion of degeneration of pulpal axons due to unilateral sympathectomy was obtained by taking the difference in the proportion of degenerating axons from the control and operated sides (Table 1). This method of calculation was based

Table 8: Left and right side comparison of axon numbers at the apical region of cat canines in three control animals (after Holland and Robinson, '82, '83).

	A		B		C	
	Left	Right	Left	Right	Left	Right
Total # axons	796	670	1550	1903	996	771
# NM	473	375	1021	1376	793	575
Variability in # of NM between L+R (expressed as a % difference)	20.7		25.8		27.5	
% NM	59.4	56.0	65.9	72.3	79.6	74.6
Difference in % NM between sides	3.4		6.4		5.0	
# Deg. NM	19	11	11	13	11	9
% Deg. NM	2.4	1.6	0.7	0.7	1.1	1.2
Difference in % Deg. between sides	0.8		0		0.1	
Total Difference between sides	4.2%		6.4%		5.1%	

upon the assumption that the small amount of axonal degeneration found on the control side is a normal phenomenon either due to ageing (Bennet et al., '64; Bernick, '67; Fried and Hildebrand, '80a,'81a,b) or fixation and that a similar proportion exists on the operated sides. Thus the amount of degeneration on both sides would tend to cancel each other out and the remaining difference would be due to the sympathectomy.

Comparison of the degeneration in operated and unoperated animals (Holland and Robinson, '82,'83) indicates that a large proportion of degeneraton occurred as a result of the sympathectomy. Operated apices contained up to 14.9% degeneration whereas the control apices never exceeded 5.6% (Table 1). The proportion of degeneration due to the sympathectomy (calculated as the difference between control and operated sides) showed a range of 1.9 to 9.3%. In contrast, normal degeneration within operated apices (Table 8) was much lower (0.7 to 2.4%) and the right and left variation never exceeded 0.8%. That the major component of degeneration was the result of the sympathectomy is also indicated by the significantly larger ($p<0.01$) component of vesiculated degenerating axons in the operated apices.

As a Result of the Small Changes and Normal Side-to-Side Variation an Accurate Estimate of the Sympathetic Contribution to Pulpal Innervation is Difficult to Determine:

To obtain a meaningful quantitative estimate of the sympathetic component of pulpal innervation it is important to

know the relative contribution of the pulpal sympathectomy to the observed degeneration and loss in comparison to that found in normal animals. It is questionable whether or not the calculated axonal loss in the experimental animals is the result of the sympathectomy since the normal side-to-side variation in control animals is similar. That the loss is the result of the sympathectomy is suggested by the fact that the loss detected in the later survival groups always occurred on the operated side. In contrast, comparison of axonal degeneration in experimental and control animals indicates that the degeneration observed was the result of the sympathectomy.

Ideally, the proportion of sympathetic axons entering the pulpal apices would be the combined values of percentage loss and degeneration. Considering the normal side-to-side variation (Holland and Robinson, ´82,´83) an accurate estimate is difficult to determine.

Combination of the loss and degeneration present at the apical level of the cat canine (Table 9) indicates that the sympathetic component contributes 8.1±4.4% of the total number of axons entering the teeth.

For a mandibular canine containing, for example, 1000 axons approximately 80 would be of sympathetic origin (if calculated mean is used). Since each sympathetic axon can possess a large number of terminals (Pohto and Antila, ´68a,b) more sympathetic varicosities should be found in the cat canine than that described in the mouse mandibular molars (Avery et al., ´80). This is to be expected since higher numbers of sympathetic fibers

Table 9

Survival Time (days)	% Loss	% Deg.	Total (% sympathetic)
2	-2.0	9.3	7.3
	0.9	3.9	4.8
4	6.7	5.8	12.5
	4.9	3.9	8.8
7	4.5	1.9	6.4
	4.6	4.0	8.6
			Mean 8.1 ± 4.4%

are found in more anterior teeth (Pohto, ´72; Pohto and Antila, ´72).

This study confirms quantitatively what has been suggested by previous qualitative studies (Anneroth and Norberg, ´68; Arwill et al., ´73; Avery et al., ´71; Christensen, ´40; Fehér et al., ´77; Fried and Hildebrand, ´78; Kukletová et al., ´68; Larsson and Linde, ´71; Pohto, ´72; Pohto and Antila, ´68a,b,´72; Waterson, ´67,´69) that a relatively small proportion of non-myelinated axons entering the tooth are sympathetic. Perhaps more importantly it establishes that the large majority of C-fibers entering the pulpal apex are sensory.

Degenerate Axons are Commonly Found in Contact with Intact Axons on the Experimental Side Suggesting that Sympathetic-Sensory Apposition is Common in the Dental Pulp:

Although a small proportion of nerve fibers in the dental pulp are entirely sympathetic in nature (Table 4) a large proportion of nerve fibers containing both degenerating and intact axons were observed. In some cases the two types of axons were found within the same Schwann cell cleft where membrane-to-membrane axonal contacts were seen. These contacts were more numerous in the operated apices. No specialized membranes or junctions were found.

The possibility that the mixed fibers were due to a reactive change of the Schwann cell as a result of the presence of nearby degenerating axons is unlikely. Axonal debris is usually removed by dissolution into the extracellular space or perineurium (Allt,

´72) or uptake by Schwann cells and/or macrophages (Thomas and King, ´74). Schwann cell reactivity of this type is usually seen in later stages of degeneration where they typically form isolated flattened processes, often stacked side-by-side in a parallel array, around the degenerating axonal debris (Thomas and King, ´74). These complexes are usually not found together with other axons in the same cleft. Features with this morphology were not observed in the operated apices. Only a slight reactivity (indicated by the occasional protrusion of Schwann cell cytoplasm into the degenerating axon) was observed.

The majority of the degenerating axons involved in the contacts were sympathetic in origin since sympathectomy results in a significant increase in degeneration and contact over that found in normal or unoperated sides. That the intact axons were non-sympathetic or sensory was based on the fact that sympathectomy results in the complete degeneration of the sympathetic neuron (Kirpekar et al., ´62) and sympathetic axons should have showed features of degeneration by two days (Fehér et al., ´77; Fried and Hildebrand, ´78). The sympathectomies were most likely complete since part of the rostral and caudal extensions of the SCG were also removed. In addition, the existence of a sympathetic crossover is doubtful (Avery and Cox, ´77; Chiego et al., ´80; Cox et al., ´77; Christensen, ´40; Matthews and Robinson, ´80; Ogilvie, ´67,´69). There is, however, no way of proving that the intact axons observed in this study are not sympathetics which have not yet entered the early degeneration stages. Concomitant injection of HRP or a radioactive tracer into the trigeminal ganglion could solve this

point.

The presence of degenerate-intact axonal contacts could provide a morphological substrate for the modulation of sensory output concomitant with chemical stimulation of dentine (Edwall and Scott, '71; Matthews, '76). The presence of a direct mechanism is suggested from the presence of the membrane appositions. Fried and Hildebrand ('78) have also published micrographs illustrating degenerate axons within nerve fibers occupied by other intact axons after 6-OHDA treatment (fig. 5). However, it is possible that these normal axons are unaffected sympathetics since the extent of destruction of adrenergic fibers with this method is dose dependant (Jonsson and Sachs, '71). Irregardless, the cumulated evidence suggests that the apposition is between sensory and sympathetic neurons.

The existence of two axon types within the same nerve fiber is not a novel finding. Adrenergic and cholinergic axons have also been observed within the same fibers in intramuscular nerve bundles (Ehinger et al., '70; Gabella, '76).

No membrane specializations or gap junctions were observed between the degenerate and intact axon membranes. It is not known whether an electrotonic spread of potential between these axons could occur at these sites since gap junctions and/or the extracellular space are usually considered to be a low resistance shunt pathway (Pappas et al., '71; Ishii et al., '71). In addition, if Gasser's ('55) computations are correct, a high degree of membrane-to-membrane apposition would be required to allow cross-excitation, this being more than that observed in

Raschkow's plexus (Holland, '80a). Cross-excitation could occur however, if high degrees of contact were present or if the apposed membranes were of low resistivity (Bennet, '77).

The fact that no membrane specializations were observed does not exclude the possibility of gap junctions and/or terminals between the sympathetic and sensory axons at higher pulpal levels. In fact, most adrenergic terminals are located in the distal pulp (Avery et al., '80). In addition, Holland ('80a) has shown that axons in Raschkow's plexus may be in contact for at least 7 μm and it is possible that some of the contacts which include gap junctions (Holland, '75b,'76a,'80a) may have been of sympathetic and sensory origin. The existence of this type of interaction may be present at higher levels considering the existence of sensory axon coupling within the odontoblast layer (Matthews, '77a,b; Matthews and Holland, '75).

The presence of normal and degenerate axons in the same nerve fiber does not rule out the possibility of indirect mechanisms (Matthews, '76) affecting the sensory receptors. The presence of sympathetic axons near pulpal blood vessels observed in this and other studies (Anneroth and Norberg, '68; Avery et al., '80; Cox and Avery, '75; Kukletová et al., '68; Larsson and Linde, '71; Pohto, '72; Pohto and Antila, '68a,b,'72; Scheinin and Light, '69; Waterson, '67,'69) indicates that sympathetic control of blood flow and tissue pressure may play a role in modulating the sensory response to the chemical stimulation of dentine. In addition, the odontoblast which could be acting as a sensory receptor (Anderson et al., '70; Matthews, '72; Matthews

and Holland, '75) may also be controlled by pulpal sympathetics since sympathetic fibers have been described in the odontoblast zone (Avery and Cox, '77; Avery et al., '80; Cox and Avery, '75,'78). Further studies are clearly needed to establish the mechanism(s) of sympathetic-sensory interaction.

Suggestions for Further Studies:

This study raises questions concerning pulpal sympathetics:

1. What is the quantitative and qualitative distribution of sympathetic axons at different pulpal levels? This could be answered with examination of midcrown and cornual regions.

2. Are sensory axons unequivocally present within the same nerve fiber as sympathetic axons and to what extent are the axons in contact? This problem could be answered by surgical sympathectomy concomitant with HRP or H^3-amino acid labelling of the trigeminal ganglion. The presence of labelled and degenerating axons within the same nerve fiber as examined by electron microscopy would confirm the relationship. In addition, reconstruction of the axonal contact areas by examination of serial sections could shed light on their extent.

3. Can sympathetic reinnervation from the contralateral side occur after unilateral sympathectomy and if so, how long would it take? This experiment could be done with light microscopy. Identification of the regenerating adrenergic nerve fibers would be possible using fluorescence techniques

(Falck, ´62) since adrenergic neurons contain adequate concentrations of NA during ontogenesis in growth cones and axonal sprouts (Olson and Malmfors, ´70).

4. Is there a differential sympathetic innervation of anterior or posterior teeth? Examination of different teeth utilizing a surgical sympathectomy or histochemical fluorescence technique could answer this question.

Conclusions:

Unilateral surgical sympathectomy resulted in the degeneration and loss of approximately 8.1±4.4% of the total number of axons (or 16.9% of the total number of non-myelinated axons) in the apical region of the cat´s mandibular canine. All degenerating sympathetic axons were non-myelinated. By exclusion, this study supports the idea that the large majority of C-fibers entering the pulpal apex are sensory and that most, if not all, of the myelinated axons are afferent in nature. The degenerating axons, a few of which were still present 7 days post-operatively, presented the same morphological appearance as described by Thomas and King (´74). These axons were found in most areas of the apex usually associated with blood vessels and/or nerve bundles. The degenerate axons were found to be of significantly larger size than the normal non-myelinated axons. No extrusion of axons from Schwann cells due to degeneration was found. No evidence for a sympathetic crossover was found. Degenerate sympathetic and intact, possibly sensory, axons were observed together sometimes in contact (membrane apposition without any

gap junctions) with each other within the same Schwann cells. The possibility that this provides a morphological substrate for the direct modulation of sensory activity concomitant with chemical stimulation of dentine (Matthews, '76) was discussed.

Appendix

Sorensen's Phosphate Buffer:

To make 1 litre of stock solution (0.5M) combine 800 ml of 0.5M Na_2HPO_4 (Sodium Phosphate Dibasic) and 200 ml 0.5M KH_2PO_4 (Potassium Phosphate Monobasic). Add slowly until correct pH 7.2 to 7.4 is obtained.

Fixative:

To make 3% Glutaraldehyde in 0.1M Sorensen's Phosphate buffer combine 180 ml of 25% Glutaraldehyde (TAAB Laboratories), 300 ml of 0.5M Sorensen's, 1.5 grams of sucrose (0.1%) and 7.5 grams of dextrose (0.5%). Make up to 1300 ml with double distilled H_2O, adjust pH to 7.2 to 7.4 and bring final volume to 1500 ml. Place in ice-bath before use.

Heparinized Saline Prewash:

Add 1 gram procaine hydrochloride and 12 mg heparin to 100 ml 0.9% NaCl.

EDTA Decalcifying Solution:

Dissolve 82.6 grams EDTA (disodium ethylenediamine tetraacetate) and 8.2 grams NaOH pellets in 1500 ml double distilled H_2O. Adjust pH to 7.2 to 7.4 and bring final volume to 2000 ml.

Uranyl Acetate:

To make a saturated solution of uranyl acetate (UA) dissolve 5 to 7 grams UA in 100 ml double distilled H_2O. Stir for 2 hours, filter and centrifuge for 1 hour. Staining time around 1 hour.

Lead Citrate:

Dissolve 1 pellet (0.1 grams) of NaOH in 12 ml double distilled H_2O and add 70 mg lead citrate. Dissolve and bring volume to 25 ml. Centrifuge fresh solution for 10 min before use. Stain grids for 10 to 15 min in staining chamber and rinse with 0.01M NaOH.

Tissue Processing:

The sections were stained "en bloc" with uranyl acetate (UA), dehydrated and embedded in Araldite according to the following:

a) 2% osmium tetroxide	2 hrs
b) double distilled H_2O	2 10min changes
c) 8% UA (aqueous)	30 min
d) 8% UA (50% alcohol)	30 min
e) 8% UA (75% alcohol)	15 min
f) 100% alcohol	15 min
g) 100% absolute alcohol	15 min
h) propylene oxide (PO)	15 min
i) PO	15 min
j) PO/Araldite (50/50)	2 hrs-overnight
k) fresh Araldite	24 hrs

l) fresh Araldite		
	35-40° oven	overnight
m) 60° oven		overnight

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