I N N E R V A T I O N

of the

T E E T H

Henry W. Noble, L.D.S.,H.D.D.

January, 1962.

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Summary.

The combination of methods described in the thesis to demonstrate the nerves of the dental pulp gave rapid and consistent results which were superior to those obtained by previously described processes. In the author's experience, the great stumbling-block to the successful demonstration of pulpal nerves was the harmful effect of the acid used to decalcify the dentine. The ability of chelating agents to perform at neutral or alkaline pH suggested that the tooth might be decalcified without harm to the staining affinities of the pulpal nerves. The selection of an alkaline pH actually improved the selectivity of the silver method employed and was later discovered to be based upon an accepted method of improving silver impregnation.

The success of the methods adopted permitted the observation of the pattern of distribution of the nerve fibres in a large number of permanent teeth from patients of all age groups.

The/

The author was able to demonstrate that nerve fibres were present in the odontoblast layer and in the predentine zone of uncalcified dentine matrix. The affinity of the inner border of the calcified dentine for the silver stain prevented the fine terminal nerve fibres from being easily followed beyond this layer. There was, however, ample evidence in support of the belief that nerve fibres did extend into the layers of calcified dentine. It is believed that the majority were calcified within the intertubular matrix. An occasional nerve fibre may have been enclosed beside the dentinal process of an odontoblast and, by virtue of its intratubular position, may have escaped the wave of calcification which passes through the intertubular matrix.

The attempt which was made to demonstrate the presence of nerve fibres in the dentine matrix by means of the electron microscope was unsuccessful. Nevertheless the author still believes that it will be possible to demonstrate silver impregnated nerve fibres within the predentine zone and so prove conclusively the relationship between the nerve fibres and the collagen fibres of the dentine matrix. He also believes that the question of

the/

the presence of fine intratubular nerve fibres (described as being 0.2 μ in diameter) within the calcified dentine matrix will only be satisfactorily proved or disproved by evidence from the electron microscope. In the numerous electron micrographs of the dentinal tubules in cross-section from areas where fine intratubular fibres have been described, the author was unable to observe any appearance which might confirm their existence.

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Contents.

continued.

Acknowledgment.

I wish to thank Professor James Aitchison, Director of Dental Education at Glasgow University, for help and encouragement. Mr. John Houston of the Orthodontic Department, Glasgow Dental Hospital, rendered great assistance in maintaining a constant supply of specimens.

Acknowledgment is made in the text of the extensive use made of the silver staining method developed by Dr. H. S. D. Garven and Mr. F. W. Gairns. I am also indebted to Mr. Gairns for the friendly interest which he has taken in the investigation.

Electron microscope facilities in the Anatomy Department were placed at my disposal by Professor G. M. Wyburn. I am particularly grateful to Dr. A. D. Hally for assistance with technical difficulties associated with the preparation of specimens for the electron microscope. The electron micrographs were taken by Mr. H. Johnstone whose patience with many of my earlier attempts at specimen preparation was considerable.

To these my thanks.

Introduction.

The explanation of the sensitivity of human dentine remains an unsolved and controversial problem.

Brashear (1937) reviewed the extensive literature upon the subject and concluded:-

> "In spite of the earnest labours of over one half century, it is amazing that while so much is known about the nervous system of the dental pulp, much is still unknown - the actual methods of the peripheral distribution of the nerves being buried in obscurity."

Twenty years later, we find that the present uncertainty is commented upon by Fearnhead (1957) who states that:-

> "None of the workers who have attempted to demonstrate nerves in this tissue (dentine) can be said to have done so unequivocally. The literature .....remains inconclusive because most workers have apparently disregarded the possibility of mistakes in their interpretation.
>
> Only a few observers who claim to have demonstrated nerves in the dentine proper have supported their observations with photomicrographs."

It is the purpose of this investigation to attempt, by modern histological techniques, to demonstrate the peripheral distribution of the pulpal nerves by an examination of the relationship between these nerves and the dentine.

Historical Review.

The discussion of pain of dental origin may well have preceded the written word, and allusions to the sensitivity of dental tissues appear in many early medical treatises. The earlier references concern the explanation of the painful sensations caused by pathological conditions in and around the teeth.

In a work attributed to the Emperor Hoang-Ti, founder of Chinese medicine, who is believed to have lived around 2637 B.C., a chapter is devoted to toothache. (Weinberger, 1926)

Hippocrates of Cos (460 - 377 B.C.) believed that the pain of toothache was caused by "pituita insinuating itself under the roots of the teeth".

Archigenes (48 - 117 A.D.) notices, for the first time, that in certain cases of toothache the interior of the tooth was affected, and concluded that this "inflammation of the pulp" caused the pain. (Weinberger, 1926)

A more fundamental and systematic approach to the subject/

subject of dental pain was inspired by the work of Galen (130 - 200 A.D.) who declared that teeth were bones, supplied with sensory nerves by the third pair of cranial nerves, of which he classified seven pairs. (Lufkin, 1948) He recorded two sites of pain, one in the tooth and the other in the surrounding tissues. (Latham, 1901)

Aetius of Amida (502 - 575 A.D.) taught that the teeth receive tiny ramifications of sensitive nerves through a small hole at the end of every root. (Weinberger, 1926)

The first treatise upon dental anatomy and histology, "Libellus de Dentibus" (1563), was produced by Bartholomaeus Eustachius (1520 - 1574). He examined the structure of the teeth with great care and described the dental follicles, their blood vessels and nerves.

The development of the compound microscope by Leeuwenhoeck in the mid-seventeenth century, enabled great advances to be made in every aspect of histology. Leeuwenhoeck (1678) also gave the first description of the microscopical appearance of dentine from observations made upon one of his own teeth.

"Having some time since applied a glass, esteemed/

> esteemed a good one, to observe the structure of the teeth .... it has plainly appeared that the whole tooth was made up of small, straight and transparent pipes. Six or 700 of these pipes put together exceed not the thickness of one hair of a man's beard."

A misconception held by many of the earlier writers was expressed by Hunter (1778), who stated that dentine was not a sensitive tissue. He had, presumably, been misled by the insensitivity of the exposed surface of dentine when a tooth is worn by mastication or attacked by caries. He believed that the "exquisite sensibility" of the nerves within the pulp cavity was partly due to the speed with which the calcified tissues conducted thermal changes.

Duval (1833) declared that calcified dentine was a sensitive tissue and added that sensitivity was most marked immediately within the amelo-dentinal junction.

The distribution of the nerves within the pulp of a tooth was studied by Raschow (1835) who declared that the pulp had one large and a few smaller nerves; the latter breaking up near the surface of the pulp into an exceedingly fine plexus now called the Plexus of Raschow. He also observed that, embryologically, true nervous filaments/

filaments could not be distinguished in the pulp until its vascularity had been established. The importance of this observation was not appreciated until it was confirmed, over one hundred years later, by workers who realised that some of the very sensitive outermost layers of dentine were formed before the pulp became innervated.

Tomes (1856) noted that the sensitivity of the dentine is dependent upon its connection with the pulp of the tooth, and that it has no inherent sensibility in its own hard tissue. He drew attention, for the first time, to the fact that each dentinal tubule is permanently tenanted by a soft fibril which, after passing from the pulp into the tubule, follows its ramifications. He was unable to determine how these dentinal fibrils terminated in the pulp; whether they arose from cells or were in any way connected with the nerves of the pulp. He was in no doubt that dentine owed its sensation to the presence of dentinal fibrils and did not believe that it was necessary to assume the fibrils to be actual nerves before allowing them the power of communicating sensation. On the contrary he suggested that the greater degree of sensitivity in the dentine adjacent to the enamel may be fully accounted for on the supposition that the fibrils, in/

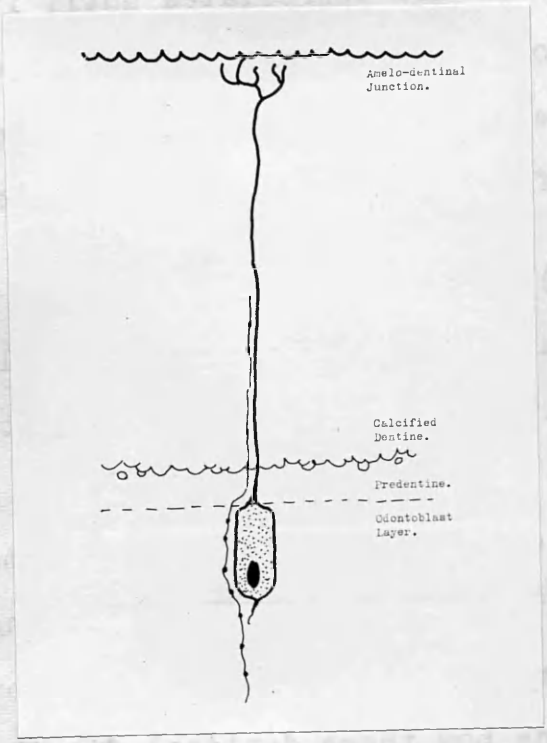
Amelo-dentinal
Junction.
Calcified
Dentine.
Predentine.
Odontoblast
Layer.

Fig. 1.

in a manner similar to nerves, exhibit greatest sensibility at the point of their ultimate distribution.

Boll (1868) gave the first detailed microscopic description of the innervation of the dental pulp. He demonstrated an abundance of fine non-medullated nerve fibres which he traced from the larger medullated fibres to the odontoblast layer. His observations were made upon sections of pulp tissue removed from rodent incisors, which he treated with a 1/32% solution of chromic acid. His attempts to decalcify the teeth and prepare sections of the pulp tissue 'in situ' resulted in the destruction of the fine nerve fibrils. He expressed the opinion, however, that the nerve fibres which he observed did not end in the odontoblast layer but continued, to follow an intratubular course within the dentine. (Fig. 1.)

Pulps from the teeth of dog, cat and calf were examined by Legros and Magitot (1879). They described terminal nerve filaments arising from cells below the odontoblast layer. These cells were interpolated between the pulpal nerve fibres and the odontoblast cells. (Fig. 2). Legros and Magitot believed that the fibrils which occupy the dentinal tubules do not transmit sensation but are themselves "les points d'impression directe".

"Bödecker/

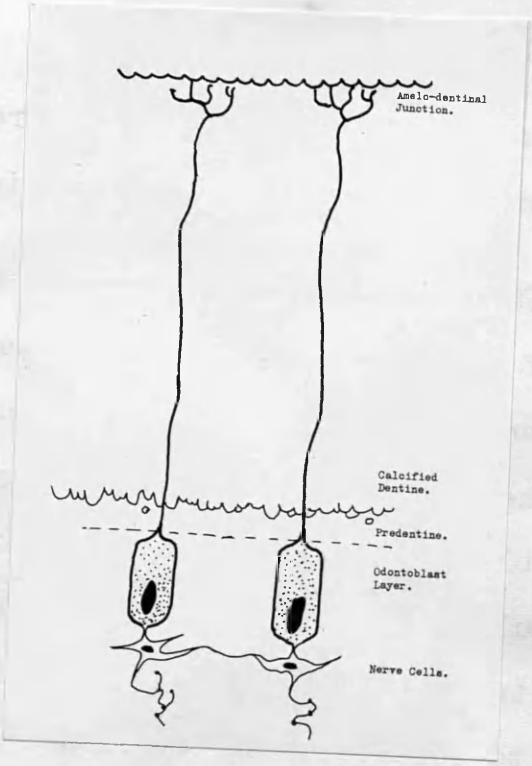
Amelo-dentinal Junction.
Calcified Dentine.
Predentine.
Odontoblast Layer.
Nerve Cells.

Fig. 2.

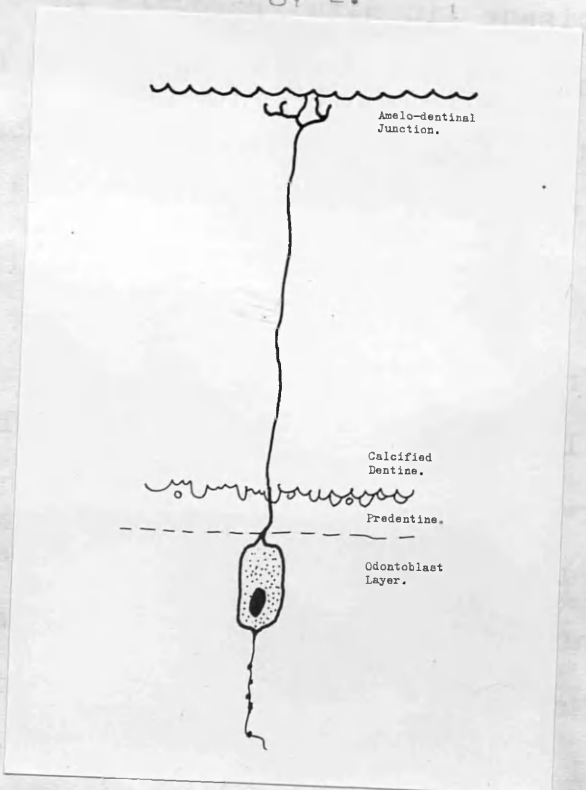
Amelo-dentinal Junction.
Calcified Dentine.
Predentine.
Odontoblast Layer.

Fig. 3.

"Bodecker (1882) treated sections of a nine-months foetal pulp with ½% solution of gold chloride followed by reduction in daylight. He observed that the medullated nerve fibres, upon approaching the periphery of the pulp, are destitute of their myelin sheath and split up into numerous extremely delicate beaded fibrillae. He traced the latter between the odontoblast cells, to which they appeared to be connected by means of delicate conical offshoots.

The conception of the odontoblast cell as a terminal nervous element was carried a stage further by Aitchison Robertson (1891), who asserted that the pulpal processes of the odontoblast cells were continuous with nerve fibres. (Fig. 3.) He therefore considered the odontoblast and its dentinal process to be the terminal organ of the nerve fibre.

In a series of articles, Retzius (1892, 1893 and 1894) examined the course of the pulpal nerves in different classes of vertebrates. In fish, he described non-medullated fibres which arose from a dense plexus in the soft tissues in which the teeth were situated. These/

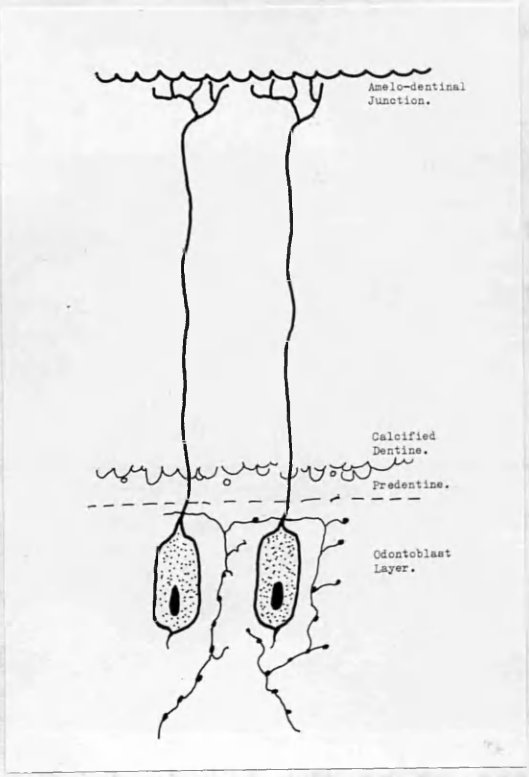
Amelo-dentinal
Junction.
Calcified
Dentine.
Predentine.
Odontoblast
Layer.

Fig. 4.

These fibres spread out thickly, to form free endings terminating within the pulp tissue. He traced the nerves in reptilian teeth from the middle of the pulp to the odontoblast layer, which they penetrated. They then terminated on the outer surface of the pulp as free extremities directly below the dentine. In young mice, he described nerve fibres everywhere in the pulp and found that they again terminated between the odontoblast layer and the inner surface of the dentine. (Fig. 4.) The Golgi method was used to stain these nerves. Retzius did not find nerves within the dentine.

Without committing himself as to the existence of nerve fibrils in dentine, Charles Tomes (1894) drew attention to the difficulties surrounding this problem.

> "..... in those tissues which are naturally so thin as to present great facilities for examination, nerves of a degree of fineness unknown elsewhere have been demonstrated; in other words, the easier the tissue is to investigate, the finer the nerves which have been seen in it, while dentine is among the most difficult substances conceivable for the demonstration of fine nerve fibrils, if such exist in it."

An unspecified methylene blue method was used by Morgenstern (1897) who observed many nerve fibres with free/

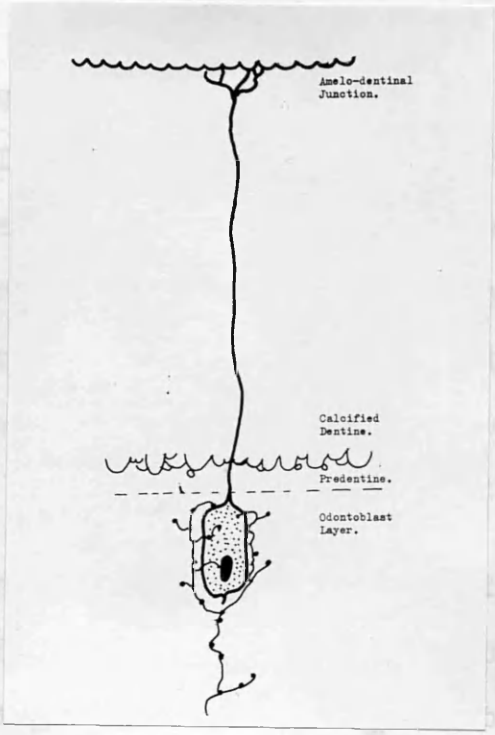
Amelo-dentinal
Junction.
Calcified
Dentine.
Predentine.
Odontoblast
Layer.

Fig. 5.

free terminations within the pulp. Others showed bead-like enlargements, beyond which a short supplementary fibre extended and sometimes terminated in peculiar cell-like discs which he believed were nerve endings and which occurred frequently in the odontoblastic zone.

Those authors who claimed they had observed nerve fibres within the dentine were severely criticised by Hopewell-Smith (1903). He expressed the strong conviction that the pulpal nerves terminated in a basket-work of varicose fibres which embraced and were often closely attached to the cell walls of the individual odontoblasts. He traced the "sensory current", in this way, from the amelo-dentinal junction through the dentinal processes, odontoblast cells and arborisations of non-medullated nerves to the medullated fibres of the pulp. (Fig. 5.)

The distribution of the nerves of the dental pulp and their relationship with dentine were demonstrated by Mummery (1912). He used several methods but preferred Beckwith's modification of Freud's gold chloride method. He found that in young growing teeth, nerve fibres were very adundant at the cornua, where they passed in wavy bundles/

bundles between the odontoblasts and entered the dentinal tubules before further division of these bundles took place. At the lateral portions of the pulp, neurofibrils passing from the main nerve trunks formed an intricate plexus beneath the odontoblasts and were again collected into large strands of neurofibrils, which passed directly into dentinal tubules. He described large fibrils with bead-like enlargements at intervals and other fine fibrils with a minutely dotted appearance. He was convinced that he was not mistaking connective tissue fibres for nerves.

Support for Mummery's views soon came from Dependorf (1913), who demonstrated nerve fibres from the pulp entering the dentinal tubules. Dependorf also found other nerve fibres which formed a wide-meshed network within the dentine matrix.

In an article dealing with physiological and pathological aspects of oral and dental pain, Hopewell-Smith (1915) reaffirmed his belief that the processes of the odontoblasts in the dentinal tubules possessed the properties of sensory nerves. He believed that the ultimate ramifications of the non-medullated nerve fibrillae/

fibrillae of the pulp arborised about the odontoblasts which, in turn, acted as sensation transmitters, although they were non-neutral in origin.

In a later publication, Mummery (1916) confirmed his previous findings and criticised the views expressed by Hopewell-Smith. A controversy regarding the problem of the innervation of the teeth ensured. Hopewell-Smith (1916) refuted Mummery's criticisms and stated that the nerve fibres demonstrated by Mummery were connective tissue fibres. In reply, Mummery (1916a) reported that he had submitted his specimens to Sir E. A. Schäfer, Professor E. H. Starling, Mr. Charles S. Tomes and Professor Arthur S. Underwood and produced letters from these eminent authorities confirming his belief that the fibres which he had demonstrated were, in fact, nerve fibres.

As a result of further investigations, Mummery (1920) described a system of non-medullated fibres, which arose from the medullated nerves of the pulp and formed the nerve plexus of Raschow. Immediately below the odontoblasts he found a series of nerve cells which, at their central ends, were connected by synapses with fibres of the plexus of Raschow. Their peripheral ends were drawn out into long/

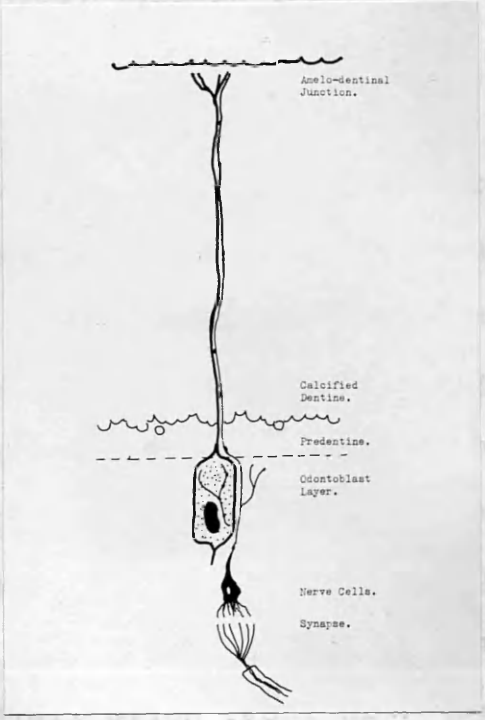
Amelo-dentinal Junction.
Calcified Dentine.
Predentine.
Odontoblast Layer.
Nerve Cells.
Synapse.

Fig. 6.

long fibres which passed through the odontoblast layer to enter the dentinal tubules and end near the amelo-dentinal junction. (Fig. 6.)

Montfort (1923) claimed she had demonstrated neuroblasts and embryonic nerve cells related to the odontoblast layer, in the tooth-germs of newly born cats and where dentine formation was just about to begin. She believed that these cells left fine nerve fibrils within the dentinal tubules, as dentine formation proceeded, and that they later transmitted stimuli through the dentine to the pulp. Her observations were therefore similar to those made by Mummery.

Mummery's experiments were repeated by Stewart (1927) in an attempt either to confirm or refute the conception of dentine innervation expressed by Mummery. Stewart successfully reproduced the fibres described by Mummery and agreed that they closely resembled nerve fibres. He was unable to demonstrate nerve cells in the pulp and when Mummery supplied sections from which he had made this observation, Stewart could not agree with Mummery's interpretation of the appearances. Stewart then discovered that/

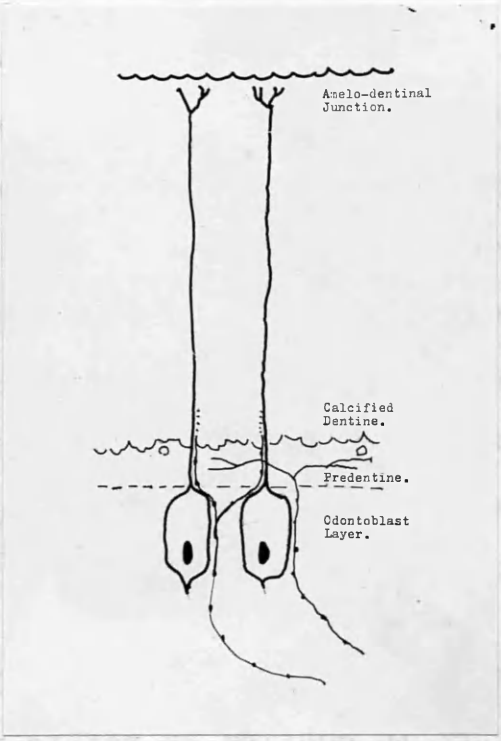
Amelo-dentinal
Junction.
Calcified
Dentine.
Predentine.
Odontoblast
Layer.

Fig. 7.

that when the sensory nerve supplying the tooth in a cat was served, the "fibres of Mummery" were still present when all the sensory nerve fibres in the pulp had degenerated. In a later experiment, Stewart (1928) showed that these fibres were still present after the sympathetic plexus, which supplied the pulp, had also been severed. He thus came to the conclusion that the fibres observed by Mummery were not nervous in origin.

Lewinsky and Stewart (1936) confirmed the work of Sealey (1923) and Tojado (1927) demonstrating intratubular and intertubular nerve fibres in the predentine. They described two types of fibres. Very fine fibres, continuous with the nerves in the pulp, entered the odontoblast layer, branched, and could occasionally be seen entering the dentinal tubules. Coarser fibres ran through the odontoblast layer without branching and entered predentine where they ran at right angles to the tubules and branched. (Fig. 7.) They could not trace either type of fibre into the fully calcified dentine and put forward the suggestion that the terminations were destroyed by the complete calcification of the dentine.

Van der Sprenkel/

Van der Sprenkel (1936) claimed that all myelinated pulp nerves terminated as intradentinal nerve endings and that all non-myelinated fibres terminated within the walls of blood vessels. He believed that almost every Tomes' fibre was provided with an intracytoplasmic nerve fibre which would even accompany the Tomes' fibre into the enamel in the case of an enamel spindle. These extravagant opinions were by no means supported by the observations which he described, nor by the illustrations in his publications, most of which were retouched photomicrographs.

An earlier article by Tiegs (1932) met with a certain amount of criticism. He had published only drawings of his preparations and had confused his account by the introduction of a new term, the subdentinal zone. In a further article (Tiegs, 1938) he included photomicrographs and explained that his 'subdentinal zone' was synonymous with the odontogenic zone or predentine. His findings remained unaltered and included a description of numerous fine nerves, arising as branches from coarser nerves, which passed through the odontoblast layer to the predentine where they turned and, with much branching, ran for considerable distances below the calcified dentine before/

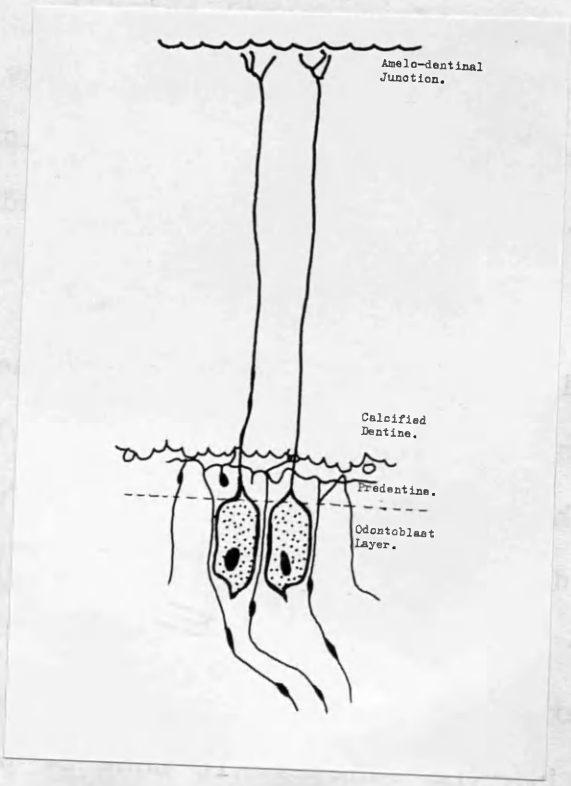
Amelo-dentinal
Junction.
Calcified
Dentine.
Predentine.
Odontoblast
Layer.

Fig. 8.

before they ended in association with a Tomes' fibre. Sometimes a nerve fibre, after penetrating the odontoblast layer, accompanied a Tomes' fibre as far as the calcified dentine where it disappeared in the densely stained inner border of the calcified dentine. He was never able to observe nerve fibres within the calcified dentine. He believed that fine nerve fibres terminated with minute end-organs on the surface of the odontoblast cell or its processes. (N.B. The present author's findings do not confirm the constant association of nerve fibre with Tomes' process although he is in agreement with Tiegs so far as the extension of nerve fibres into predentine is concerned. No end-organs have however been observed in the authors sections as claimed by Tiegs.)

Bradlaw (1939) described simple and complex looping of nerve fibres within the zone of predentine. He also observed nerve fibres which ran tangentially between the odontoblast layer and the dentine and claimed that they formed a definite nerve plexus in this position. Numerous round or pear-shaped bodies attached to these nerve fibres were, he thought, either nerve cells or end-organs. He traced definite nerve fibres into the calcified dentine. (Fig. 8.) (N.B. Similar round or pear-shaped/

pear-shaped bodies attached to nerve fibres within the predentine are described by the present author but a different interpretation of their origin and significance will be advanced.)

Wasserman (1939) employed the Bodian silver impregnation method in his study of the innervation of rat teeth. He discussed the development of the innervation of all teeth and observed that nerves did not begin to enter the dentine papilla until some time between the eighth and fifteenth days of post-natal life. In the early stages there were only a few nerve fibres to be found in connection with the blood vessels and no peripheral plexus had developed. While a more complicated innervation developed in the molar teeth at a later stage, the persistently growing incisors retained this primitive innervation and were merely permanent tooth-germs as regards their innervation. In the molar teeth Wasserman noted fine nerve fibres passing from the peripheral plexus in the pulp to the odontoblast layer where they terminated in minute beads attached to the odontoblast cell. In certain cases he noted an indentation of the nuclear membrane of the odontoblast cell, which led him to believe that the endings might have been intracytoplasmic. He also believed/

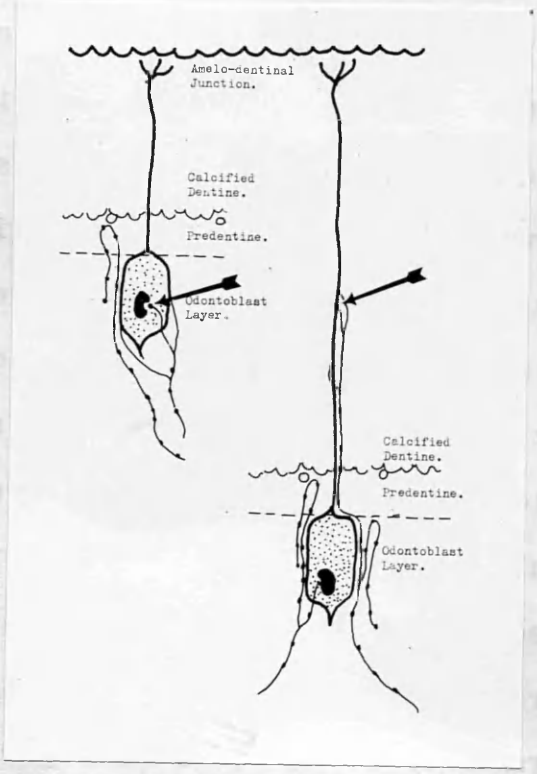
Amelo-dentinal Junction.
Calcified Dentine.
Predentine.
Odontoblast Layer.
Calcified Dentine.
Predentine.
Odontoblast Layer.

Fig. 9.

believed that this attachment to, or penetration of, the odontoblast cell membrane at an early stage in dentine formation may have led to the presence of intratubular nerves attached to the dentinal processes of the odontoblasts in the fully formed teeth. Many fine fibres were also seen to loop into the odontoblast layer and, occasionally, into the predentine. (Fig. 9.) Wasserman considered the whole tooth to be an innervated dermal papilla and was in no doubt that the ability of the dentine to receive stimuli was due to the nerves within the dentinal tubules. (N.B. In the present investigation minute beads were frequently observed on the fine nerve fibres in the odontoblast layer. When well stained, the nerves became so fine that the point at which they disappeared from sight was not usually regarded as the termination of the nerve fibre. This was confirmed by the electron microscope which showed that the finest nerve fibres were beyond the resolving power of the light microscope. Consequently, the author is very doubtful if the beads described by Wasserman really indicated the terminations of the nerve fibres which he observed. Indentation of the nucleus of the odontoblast cell has been frequently observed in electron micrographs but/

but no associated nerve fibre has been observed.)

The silver nitrate techniques of Romanes (1950) and Ungewitter (1951) were used by Powers (1952) to demonstrate the nerve fibres in the pulp and dentine of human and rat teeth. She came to the conclusion that there were nerve fibres in the calcified dentine and that, in their course through this hard tissue, they presented many varicosities and terminated in so-called nerve endings. Although the methods used by Powers do not produce such intense staining of the inner border of calcified dentine, they require the use of thin paraffin sections which are not suitable for tracing fine, tortuous nerve fibres from their origin to their terminal distribution.

Convincing evidence in favour of the innervation of the calcified dentine was presented by Cocker and Hatton (1955). They found that in many sections where the predentine contained numerous fine fibres; these could sometimes be traced into the calcified dentine where they were very soon lost. In a few cases, long nerve fibres could be traced from the pulp through the predentine and into the calcified dentine, reaching as far as half-way through the thickness of the calcified dentine. These long fibres ran/

ran parallel to the direction of the tubules but it was not possible to determine whether their position was intertubular or intratubular. Two untouched photomicrographs were presented showing the course of these fibres within the predentine and calcified dentine but their origin in an undisputed pulpal nerve fibre was not demonstrated.

A protargol method was used by Philipp (1955) to examine the peripheral distribution of the nerves of the dental pulp. He came to the conclusion that the nerve plexus of Raschow did not exist. Nerve fibres were traced from a pulpal nerve bundle directly to the odontoblast layer and the predentine. By the use of sections through the predentine and parallel to the surface of the pulp, he discovered nerve fibres following both an intratubular and an intertubular course, changing from one position to the other. He believed that the nerve fibres were restricted to the predentine and did not invade the fully calcified dentine and concluded that the agent by which sensory stimuli were received was the Tomes' fibre which transmitted the stimulation to the predentinal nerve fibres. (N.B. It is difficult to understand how Philipp can believe that nerve fibres are restricted to the predentine. Even though the production of/

of physiological secondary dentine is a slow process in elderly patients, predentine is always only a stage in the development of fully calcified dentine and any nerve fibres in an intertubular position in the predentine must eventually lie within the fully calcified dentine. It is, of course, extremely unlikely that any nerve fibres exist in the calcified dentine in a vital state or that they retain the same silver staining properties as they possessed before calcification.)

Using the Bielschowsky-Gros Method, Falin (1956) reported that medullated nerve fibres passed through the odontoblast layer into the predentine. Here the non-medullated nerve endings turned in a direction parallel to the inner surface of the dentine and divided into two parts. One branch curved back into the odontoblast layer and another ended in the predentine. From the predentine some nerve fibres entered dentinal tubules but it was not possible to trace where they ended and how deeply they penetrated the calcified dentine. Fewer nerve endings were found in the radicular pulp canals and the plexus of Raschow, usually well developed in the pulp of the crown was absent. No nerve fibres were observed in the predentine of the root canal walls.

(N.B./

(N.B. In the present study, nerve fibres were frequently observed in the predentine of the root canal walls.)

The innervation of the dental germs in foetal sheep of various body lengths was studied by Maccaferri (1957). He confirmed the findings of earlier workers concerning the absence of nerve fibres from the dental papilla until the calcification of dentine had commenced. The tooth germs were progressively innervated, commencing with the deciduous central incisors by a few nerve fibres which proceeded along the longitudinal axis of the dental papilla and were visible only in central sections. Later, dense nerve fasciculi followed the course of the vessels. From these small nerve bundles, fine non-medullated fibres branched out to innervate the peripheral portion of the dentine papilla where their course followed that of the vessels.

Tissues which are especially rich in collagenous and precollagenous fibres, such as the pulp, periodontal membrane and gingival tissues present a problem when silver impregnation methods are used for nerve study. Bernick (1955) described a method of pretreatment of tissues with a proteolytic enzyme such as pepsin or papain which/

which removed the collagenous elements and suppressed their argyrophilic response. Applying this method to the study of the innervation of the dental tissues, Bernick (1957) described the course of the pulpal nerves in an extensive interlacing plexus at the pulpo-odontoblastic border. Both medullated and non-medullated nerve fibres pass from this plexus to the odontoblast layer where they terminated as naked fibres among the odontoblasts. A few non-medullated fibres continued into the predentine but, for the most part, they formed loops and returned to the odontoblast layer where they terminated among the odontoblasts. He described exteroceptive nerve fibres, independent of blood vessels, in the pulps of the developing permanent teeth in a four year old boy. (N.B. Pretreatment of the tissue sections such as that recommended by Bernick (1955) illustrates the difficulty which many authors experience in obtaining adequately selective impregnation of the nerve fibres. The present author does not agree that Bernick's method is helpful when one of the points to be determined is the exact relationship of the nerve fibres to the collagenous elements which are being digested and removed.)

Many of the findings noted by recent investigators were/

were confirmed by Rapp, Avery and Rector (1957). They described a network of fibres beneath the zone of Weil from which many non-medullated nerve fibrils passed to terminate among the odontoblasts. No neutral endings were noted on the odontoblasts or on their protoplasmic processes. A few fibres entered the predentine where some followed an intratubular course while others appeared to be positioned in the matrix of the predentine between the tubules. They compared sections of teeth from patients aged ten years and sixty years and found that more nerve fibres were present in the predentine of the older teeth. The explanation given was that the nerve fibrils which formerly existed among the odontoblasts, were included in the predentine as it was laid down in a pulpal direction. They also suggested that the dentine contained more nerve fibres than were observed.

Continuing his investigation of intratubular nerve fibres in the calcified dentine, Fearnhead (1958) reported that these fibres had an approximate diameter of 0.2 μ and were not present until an advanced stage of tooth development had been reached. He noted that the dentine papilla did not appear to receive its first nerve fibres until after vascularisation, when pioneer nerve fibres accompanied/

accompanied the developing vessels. He believed that the intratubular nerves grew into preformed dentinal tubules rather than passively becoming enclosed in the tubules as the dentine was formed. He suggested that the complex arrangement of nerve fibres in teeth could be explained by an exhuberant growth of nerves in a confined space which became progressively smaller with age. (N.B. Fearnhead is now the principal exponent of a dentine actively innervated by presumably exteroceptive nerve fibres. His explanation of the intratubular nerve fibres cannot, of course, apply to the intertubular fibres within the developing dentine matrix. The author agrees with Fearnhead, however, in the importance of demonstrating by means of electron micrographs the existence and location of the fine terminal nerve fibres which are almost beyond the resolving power of the light microscope.)

The electron microscope was used by Uchizona and Homma (1959) in their study of human dental pulp nerves. They confirmed the presence of medullated and non-medullated fibres and calculated their diameters. They found that medullated fibres ranged from 1 to 6 μ with the majority between 2 and 3 μ. Most non-medullated fibres had a diameter/

diameter of between 1 and 2 μ. They found that the myelin sheath was usually between 0.5 and 1.0 μ and that its thickness was apparently independent of the fibre diameter. (N.B. The nerves studied by these authors were centrally positioned in a pulp horn. The location and relationships of the fine terminal fibres within the odontoblast layer and in the developing dentine matrix remain undemonstrated.)

Bibliography.

Bernick, S. 1955. Silver impregnation of nerves after enzymatic removal of collagenous elements. Stain Technol. 30 : 253.

Bernick, S. 1957. Innervation of the teeth and periodontium after enzymatic removal ofcollagenous elements. Oral Surg., Oral Med. & Oral Path. 10 : 323.

Bödecker, C. F. W. 1882. The minute anatomy, physiology, pathology and therapeutics of the dental pulp. Dent. Cosmos. 24 : 292.

Boll, F. 1868. Untersuchung über die Zahnpulpa. Archiv. für Micr. Anat. 4 : 73.

Bradlaw, R. V. 1939. The histology and histopathology of the dental innervation. Proc. roy. Soc. Med., Sect. Odont. 32 : 1040.

Brashear, A. D. 1937. The controversy concerning the innervation of teeth. J. dent. Res. 16 : 5.

Cocker, R. and Hatton, J. M. 1955. A note on the innervation of human dentine. J. Anat., Lond. 89 : 189.

Dependorf. 1913. Beitrage zur Kenntnis der Innervierung der menschlichen Zahnpulpa und des Dentins. Deutsche Monatschr. für Zahnheilk. 31 : 689.

Duval, J. R. 1833. Mem. de L'Acad. roy. de Med. ii : 197.

Falin/

Falin, L. I. 1956. Morfologia receptorov zuba. Stomat., Moscow. 6 : 7. See also:-

Falin, L. I. 1957. The morphology of tooth receptors. Dent. Abstr. 2 : 495.

Fearnhead, R. W. 1957. Histological evidence for the innervation of human dentine. J. Anat., Lond. 91 : 267.

Fearnhead, R. W. 1958. Neurohistology of dentine. Internat. dent. J. 8 : 4.

Hopewell-Smith, A. 1903. The histology and pathohistology of the teeth. The Dental Manufacturing Co., London.

Hopewell-Smith, A. 1915. Pain and other oral sensations. Dent. Cosmos. 57 : 494.

Hopewell-Smith, A. 1916. The so-called innervation of the dentine: an epicriticism. Dent. Cosmos. 58 : 421.

Hunter, J. 1778. Natural history of the teeth. 2nd ed. J. Johnson, London,

Latham, V. A. 1901. The literature of the pulp. J. Amer. Med. Ass. 37 : 86.

Leeuwenhoeck, A. 1678. Microscopical observations of the structure of teeth and other bones. Phil. Trans. roy. Soc. No. 140 Page 1002.

Legros, Ch. and Magitot, E. 1879. Morphologie du follicule dentaire chez les mammiferes. J. de l'Anat et de la Phys. 25 : 248.

Lewinsky/

Lewinsky, W. and Stewart, D. 1936. The innervation of the dentine. J. Anat., Lond. 70 : 349.

Lufkin, A. W. 1948. A history of dentistry. 2nd ed. Kimpton, London.

Maccaferri, A. 1957. L'innervazione del germe dentale. Riv. ital. Stomat. 12 : 446. See also:-

Maccaferri, A. 1958. The Innervation of the tooth germ. Dent. Abstr. 3 : 301.

Montfort, J. C. 1923. Researches on dental innervation. Dent. Cosmos. 65 : 917.

Morgenstern, M. 1897. Investigations of the nerves of the pulp by the methylene blue method. Internat. dent. J. 28 : 255.

Mummery, J. H. 1912. On the distribution of the nerves of the dental pulp. Phil. Trans. roy. Soc. Ser. B 202 : 337.

Mummery, J. H. 1916. The innervation of dentine. Dent. Cosmos. 58 : 803.

Mummery, J. H. 1920. On the nerve-end cells of the dental pulp. Phil. Trans. roy. Soc. Ser. B 336 : 321.

Philipp, J. J. 1955. Extent of distribution of nerve fibres in the human tooth. J. Amer. dent. Ass. 50 : 22.

Powers, M. M. 1952. The staining of nerve fibres in teeth. J. dent. Res. 31 : 383.

Rapp/

Rapp, R., Avery, K. J.and Rector,R. A. 1957. A study of the distribution of nerves in human teeth. J. Canad. dent. Ass. 23 : 447.

Raschow, J. 1835. Die Gesundheitslehre des Mundes. Ludwigsburg.

Retzius, G. 1892. Zur Kenntnisse der Nervenendigungen in den Zähnen. Biol. Untersuch., Neue Folge. 4 : 65.

Retzius, G. 1893. Ueber die Nervenendigungen in den Zähnen bei Amphibien. Biol. Untersuch., Neue Folge. 5 : 40.

Retzius, G. 1894. Zur Kenntniss der Endigungsweise der Nerven in den Zähnen. Biol. Untersuch., Neue Folge. 6 : 64.

Robertson, W. G. A. 1891. On the relations of nerves to odontoblasts and on the growth of dentine. Trans. roy. Soc. Edin. 36 : 323.

Romanes, G. J. 1950. The staining of nerve fibres in paraffin sections with silver. J. Anat., Lond. 84 : 104.

Sealey, V. T. 1932. Austral. J. Dent. 36 : 1.

Stewart, D. 1927. The problem of the innervation of the dentine. J. Anat., Lond. 61 : 439.

Stewart, D. 1928. Further observations on the innervation of the teeth and the fibres of Mummery. J. Anat., Lond. 63 : 1.

Tiegs, O. W. 1932. Nerve endings in human teeth. J. Anat., Lond. 66 : 622.

Tiegs/

Tiegs, O. W. 1938. Further remarks on the terminations of the nerve fibres in human teeth. J. Anat., Lond. 72 : 234.

Tojoda, M. 1927. Nerves in the dentine. Brit. dent. J. 48 : 1325.

Tomes, C. S. 1894. Dental anatomy. 4th ed. page 45. J. & A. Churchill, London.

Tomes, J. 1856. On the presence of fibrils of soft tissue in the dentinal tubules. Phil. Trans. roy. Soc. 146 : 515.

Uchizono, K. and Homma, K. 1959. Electron microscopy of nerves of the human tooth pulp. J. dent. Res. 38 : 1133.

Ungewitter, L. H. 1951. Urea silver nitrate method for nerve fibres and nerve endings. Stain Technol. 26 : 73.

van der Sprenkel, H. B. 1936. Microscopical investigation of the innervation of the tooth and its surroundings. J. Anat., Lond. 70 : 233.

Wasserman, F. 1939. The innervation of the teeth. J. Amer. dent. Ass. 26 : 1097.

Weinberger, B. W. 1926. Orthodontics: an historical review of its origin and evolution. Vol. 1. C. V. Mosby Co., St. Louis.

Aims of the Investigation.

The lack of unanimity among authors, even during the last decade, emphasises the fact that this subject still poses many unresolved problems. Technical difficulties associated with the demonstration of nerve fibres in dental tissues are largely responsible for the divergent opinions expressed. Importance has therefore been attached in this investigation to techniques which can illustrate the details of the innervation with greater clarity and less ambiguity.

The aspects of the problem which were selected for this investigation may be summarised as follows:

(1) Can the nerves in sections of teeth stained by silver methods be more clearly demonstrated after demineralisation by sodium ethylenediaminetetraacetate at an alkaline pH than after demineralisation by a stronger inorganic or organic acid?

(2) What is the distribution pattern of nerve fibres in the pulp of the erupted tooth?

(3) Are nerve fibres present in the odontoblast layer, predentine and dentine?

(4) What additional information concerning the structure and distribution of the pulpal nerve fibres can be obtained by use of the electron microscope?

(5)/

(5) Can the electron microscope demonstrate accurately the position occupied by the nerve fibres as they pass through the odontoblast layer and enter the dentine matrix?

Material.

The investigation was limited to the examination of non-carious human teeth of the permanent dentition. In each case a record was kept of the nomenclature of the tooth and the age of the patient from which it was extracted. The reason for extraction varied; most teeth were removed for orthodontic reasons, some were unerupted or impacted and a small number were extracted after accidents. Most of the teeth therefore came from fairly young persons although no sound tooth with a vital pulp from an older person was ever refused. When the processing of a tooth could be commenced immediately after its removal from the patient, portions of the pulp were prepared for examination by the electron microscope. A total of 157 teeth from 123 patients were used in the investigation. The type of tooth used is shown in Table I and the age of patient varied as in Table II.

Table I.

Teeth used in the Investigation.

Upper Teeth		Lower Teeth	
Tooth	Number Examined	Tooth	Number Examined
1^1	6	1_1	7
1^2	17	1_2	9
C	4	C	4
Pm^1	49	Pm_1	12
Pm^2	18	Pm_2	9
M^1	7	M_1	4
M^2	1	M_2	1
M^3	3	M_3	6
Total Upper	105	Total Lower	52
		" Upper	105
		Total Teeth	157

Table II.

Age of Patient from which Teeth were extracted.

	Number of Patients.
- 10 years	19
11 - 20 years	89
21 - 30 years	6
31 - 40 years	5
41 - 50 years	3
51 - 60 years	1
Total Patients	123

Methods.

Similar principles govern the preparation of sections for the optical microscope and the preparation of sections for the electron microscope. There are, however, considerable differences in the reagents employed, in the materials used and in the techniques employed in handling the smaller specimens and the thinner sections obtained for use in the electron microscope. It will therefore be more convenient to consider the methods separately and according to the manner in which the sections will subsequently be examined.

Fig. 10.

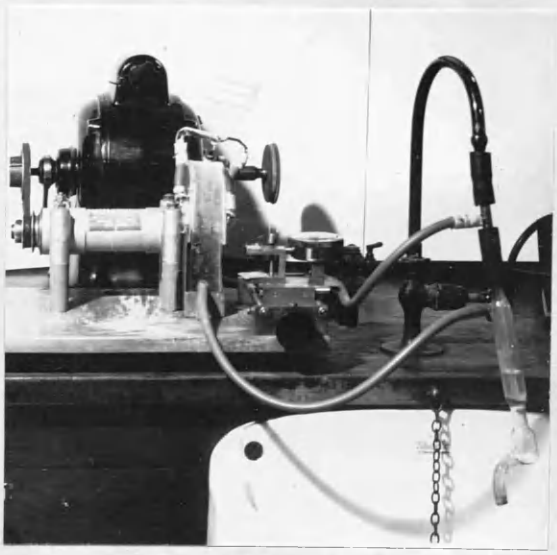

Fig. 11.

Methods employed for Optical Microscopy.

Fixing and Rough Grinding.

Extracted teeth were immediately placed in a 12 per cent neutral formalin solution to which had been added 15 per cent sucrose.

As is well known, a stock solution of formalin becomes acid upon standing. The addition of sufficient magnesium carbonate to the stock solution of formalin counteracts this tendency and ensures that neutral formalin may be filtered off as required. The addition of 15 per cent sucrose to the 12 per cent neutral formalin solution is in accordance with the recommendation by Barnett and Palade (1958) concerning the balancing of the osmolarity of all solutions to 0.44M with sucrose.

Within 24 hours of extraction a hole 1.5 mm. in diameter was drilled through the root as close to the apex as possible and perpendicular to the plane in which the tooth was later to be sectioned. (Fig. 10.) In a multi-rooted tooth the larger root was always selected. A machine for grinding sections (Fig. 11.) was then used to prepare a slice 1.5 mm. thick and containing the tissue from which the sections were to be prepared. A thin carborundum disc 3 inches in diameter and 0.005 inches thick (Fig. 12.) and which revolved at a speed

of/

Fig. 12.

Fig. 13.

of 6,000 r.p.m. under a constant stream of cold water was used to cut the desired slice. (Fig. 13.)

Demineralisation.

Demineralisation was accomplished by means of the chelating action of a 0.5 M solution of sodium ethylene-diaminetetraacetate at a pH of 7.4 This solution was prepared by adding approximately 70 ml. of 20% solium hydroxide solution to a mixture of 36.5 g. ethylenediamine-tetraacetic acid and 100 ml. distilled water until the acid dissolved. A careful reading of the pH was then taken, using an electric pH meter, and further sodium hydroxide solution was added to bring the pH to 7.4 Distilled water was then added to bring the total volume to 250 ml. (The further addition of 15% sucrose to this solution did not impede the process of demineralisation nor the subsequent staining of the sections and did impart excellent cutting qualities to the specimen when trans-ferred directly to the stage of the freezing microtome following demineralisation.)

The tooth slab was suspended from a glass hook in a test tube containing 20 ml. of chelating solution (Fig. 14.)/

Fig. 14.

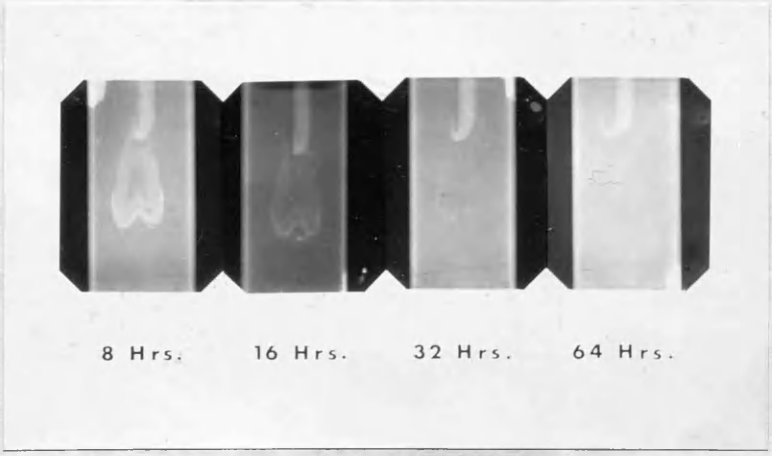

Fig. 15.

(Fig. 14.) and placed in an incubator at 60° C for 72 hours. At the end of this period radiographical examination of the specimen in the test tube confirmed that satisfactory decalcification had been achieved. (Fig. 15.)

Preparation of Sections and Staining.

The demineralised specimen was removed from the chelating solution, rinsed in distilled water and placed upon the stage of a freezing microtome. Sections 10 - 15 μ thick were prepared and collected in a bath of distilled water. Selected sections were transferred to a further bath of distilled water in preparation for staining by a modification of the Bielschowsky method described by Garven and Gairns (1952). The sections were now taken, one or two at a time, and immersed in approximately 10 ml. of a 20% solution of silver nitrate for a period which varied from 5 to 30 minutes. A box covered this vessel in order to exclude daylight. A section was then transferred by means of a clean glass rod to a bath containing 20% formalin in distilled water. The section was not allowed to rest in this bath but was quickly moved around and transferred to a second similar bath after/

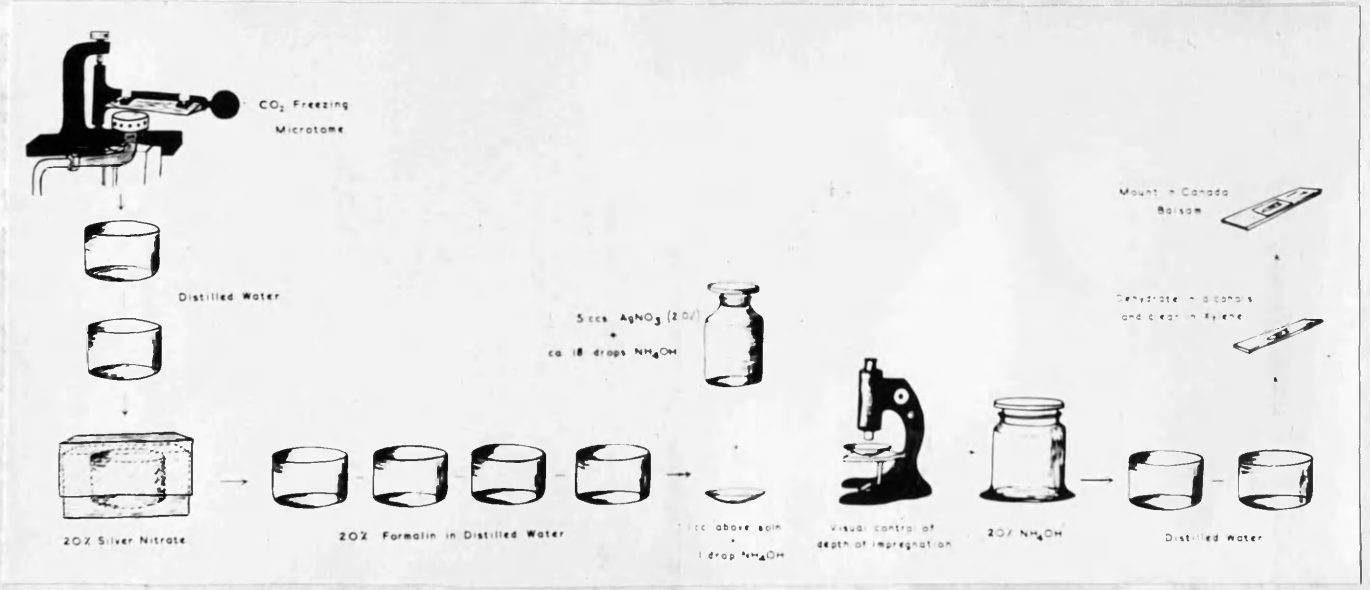
CO_2 Freezing
Microtome
Distilled Water
20% Silver Nitrate
20% Formalin in Distilled Water
5 ccs $AgNO_3$ (2.0%)
+
ca. 18 drops NH_4OH
1 cc. above soln
+
1 drop NH_4OH
Visual control of
depth of impregnation
20% NH_4OH
Distilled Water
Dehydrate in alcohols
and clear in Xylene
Mount in Canada
Balsam

Fig. 16.

after an interval of approximately 10 to 15 seconds. The section was then taken through three further baths of 20% formalin in distilled water. The section was removed from the last formalin bath upon a narrow strip of filter paper, blotted gently by contact with another piece of filter paper, and rapidly deposited in about 1 ml. of ammonical silver nitrate solution in a watch glass. The watch glass was placed upon the stage of a microscope where the blackening of the nerve fibres could be observed. A satisfactory degree of impregnation was normally achieved within 1 to 5 minutes, whereupon the section was rapidly transferred to a 12.5% solution of 25 vols. per cent ammonia in distilled water. The section remained in this bath for at least 10 minutes but not longer than 1 hour and was then washed in distilled water, counterstained with haemalum if desired, and mounted after dehydration and clearing in Canada Blasam dissolved in xylene. The various stages in the above process are represented in diagrammatic form in Fig. 16. The degree and nature of the impregnation was found to vary according to the total time spent in the four baths of 20% formalin in distilled water and according to the manner in which the ammoniacal silver nitrate/

nitrate bath was prepared. With practice, however, satisfactorily consistent results could be achieved, giving a precise differentiation of the nerve fibres in the pulp and predentine.

Discussion of Methods.

The rapid preparation of a thickly ground slice containing the pulp was desirable for several important reasons.

1) The fixative penetrated more rapidly.
2) Demineralisation was more rapid since so much unnecessary tooth substance had been removed.
3) The process of orientation of the specimen upon the stage of the freezing microtome was greatly facilitated. Slight discrepancies in the plane of the coarsely ground slice were easily rectified since the outline and position of the pulp could usually be clearly seen.

No harmful effects resulted from the exposure of the pulp tissue at this stage but careful orientation of the tooth before grinding could ensure that a thin layer of dentine was left covering the pulp tissue in all teeth except molars. The hole could be drilled and the tooth ground/

ground within 25 minutes; the tooth slice was then immediately returned to the neutral formalin fixative. Prolonged immersion in the fixative did not impair the staining affinities and a minimum period of four days was regarded as adequate.

Rapid and thorough fixation is particularly important in a histological investigation of nerve detail. Specimen bottles containing the fixative were supplied to the clinical departments in which the teeth were removed in order that they might be immediately transferred to the fixative.

Young (1945) postulated a continual flow of material from the nerve cell body down the nerve fibre and a leak over its surface. He believes that this pressure maintains the organisation of the substance of the axoplasm and that if it is removed (as by cutting the fibre) the axon is severely disturbed.

Arwill (1958) noted the problem of fixing the specimen rapidly enough to prevent the onset of post-mortem changes. He suggested that nerve tissues were particularly susceptible to such changes and that the semi-fluid axoplasm readily formed droplets which were visualised/

visualised as characteristic swellings or 'beads' along the length of the fibre. He believed that if the fixing was rapid and effective no beads formed.

The selection of ethylenediaminetetracetic acid (hereinafter referred to as EDTA) as a demineralsising agent was not fortuitous but proved to have been even more advantageous and important than had at first been realised. The ability of EDTA to demineralise at a neutral or slightly alkaline pH without disturbing the soft tissues of the pulp by gas formation or other chemical side-effects was the main reason for its selection in the first instance. It was soon discovered that the process of demineralisation was reasonably rapid when a slice was prepared as described and that a further increase in speed could be obtained by carrying out the process in an incubator at 60°C without sacrificing the quality of the staining in any way. It was later realised that the 72 hour period of exposure to the buffered EDTA solution at a temperature of 60°C was in fact similar to the process of immersion in a heated buffer which precedes silver impregnation in so many of the other methods. (e.g. Rowles and Brain 1960, Fearnhead and Linder 1956, etc.)/

1956, etc.)

Such exposure to a buffer in an incubator and over a fairly long period of time appears to be desirable in order to condition the cells and tissues in preparation for the silver impregnating solution. The process of silver impregnation is known to be a complicated physico-chemical process in which such factors as pH, temperature, time, concentration of the silver nitrate solution and many other factors are inter-related to encourage the specific affinity of the nerve tissue for the silver nitrate.

The specimen may be removed from the chelating solution, given a brief rinse in distilled water and immediately placed on the stage of the freezing microtome. No elaborate process of neutralisation is necessary as in the case of tissues demineralised by a strong acid. The buffered EDTA chelating solution is harmless to the metal edge of the microtome knife and does not appear to interfere with the action of the silver nitrate solution even if a trace were to be inadvertently carried across within the tissue section.

Several/

Several important advantages resulted from the use of the particular silver method selected. Many of the following points were mentioned by Garven and Gairns in their account of this staining method. (Garven and Gairns, 1952) They are, however, enumerated in recognition of the important part which this method played in the success of the investigation.

1) The method was rapid.
2) The quality of the impregnation was easily controlled.
3) The method was capable of deep, selective impregnation of nerve fibres alone or a more generalised and less specific impregnation of other argyrophilic structures.
4) The few solutions required were simple in composition and were easily prepared.
5) The resulting impregnation was stable and did not readily alter or disappear.
6) The method was suitable for any desired thickness of section up to 25 μ. Sections of this thickness permitted the distribution of a nerve to be followed over large areas of the section.

Control/

Control over the final result could be most easily exerted during the preparation of the ammoniacal silver nitrate bath. Slight variations in the composition of this bath produced marked differences in the selectivity of the impregnation and in the speed with which it proceded. If the ammonia was slightly in excess in this solution, nothing was stained. As the proportion of ammonia decreased, firstly the nerve elements alone stained intensely and then, with lesser proportions of ammonia, the other argyrophilic elements in the section began to be impregnated more strongly. The speed with which the elements of the tissue became impregnated was in inverse proportion to the quantity of ammonia added to this bath.

The control provided by the composition of the ammoniacal silver nitrate bath permitted variations of the impregnation over fairly wide limits. These limits were set by the earlier treatment of the specimen such as the nature of the fixative and the length of time during which the specimen was immersed in it, the pH of the buffered EDTA solution and the length of time that the specimen remained in it for the purpose of demineralisation.

The classical type of impregnation, with the nerve fibres stained intensely black and the surrounding tissue unstained, was not the ideal result in an investigation concerning the location and distribution of nerve fibres. A slight degree of impregnation in the neighbouring cells and tissues was not considered an undesirable feature. In sections where the background staining was insufficient, the section could be counterstained in haemalum with no effect upon the intensity of the silver impregnation of the nerve fibres. Any acid stain such as eosin decolourised the impregnated fibres.

If it was desired to increase the intensity of the impregnation, the sections were toned in a bath of gold chloride. Before toning, the nerve fibres stained brown to black were surrounded by the other tissue stained in various shades of light brown. After toning, the nerves and the various impregnated tissue elements were all stained blue-black to varying degrees.

Methods employed for Electron Microscopy.

The investigation of the terminal distribution of the pulpal nerves in the odontoblast layer and in the predentine involved the detection of nerves so fine that they approached the limits of resolution of the light microscope. It was not possible to stain these fine nerve fibres to a degree which made them stand out clearly from the background and at the same time allow the background to stain sufficiently for a fine nerve fibre to be accurately located. The superior powers of resolution, at very high magnifications, of the electron microscope were enlisted in the solution of this problem. It was believed that the main problem would be the satisfactory identification of a nerve fibre among the other fibres of the odontoblast layer and dentine matrix. A method was therefore devised which used the silver staining method to locate a suitable area of the ondotoblast layer or predentine matrix for more detailed examination in the electron microscope. It was expected that the silver impregnation would also satisfactorily distinguish the nerve fibres from others in the electron micrographs.

The/

The silver staining method had not previously been used for this purpose. It was later discovered that the idea was in line with several other methods utilising the staining effect of a solution of a heavy metal salt to accentuate the electron density of some particular tissue component. (Watson, 1958a & b and Churg, Mautner & Grishman, 1958)

The assistance of the electron microscope has been enlisted in several investigations involving the submicroscopic organisation and distribution of vertebrate nerve fibres. Fernandez-Moran (1950, 1952, 1957a, 1957b, & 1958) has elucidated the appearances seen in fine nerve fibres at the high magnifications available. The successful application of the resolving power of the electron microscope enabled Geren (1954) to explain the origin of myelin by the infolding of the Schwann cell membrane. Detailed investigations of the fine structure of a nerve fibre have also been carried out by Palay and Palade (1955) and numerous other workers.

A preliminary examination of the dental pulp as a whole was carried out in order to gain familiarity with/

with the appearance of the various structural elements at the high magnifications obtainable.

Isolated Pulp Tissue.

The pulp of an extracted tooth was immediately exposed by splitting the tooth with a blow from a hammer. The exposed surface of the pulp was at once flooded with a cold 1% solution of veronal acetate buffered osmium tetroxide. (Palade, 1952) A suitable portion of the pulp was then carefully dissected from the cavity within the dentine and placed in a larger volume of fixative for 1 to 4 hours at room temperature. The specimen was then taken up through the alcohols to absolute alcohol before being transferred to a 4:1 mixture of n-butyl methacrylate and methyl methacrylate. Embedding was carried out in a Park Davis No.1 gelatin capsule containing the 4:1 mixture of methacrylate monomers to which 1 per cent Perkadox PDB 50 was added as a catalyst; the capsule then being suspended in an oven at 60 deg. C for at least 12 hours to effect polymerisation.

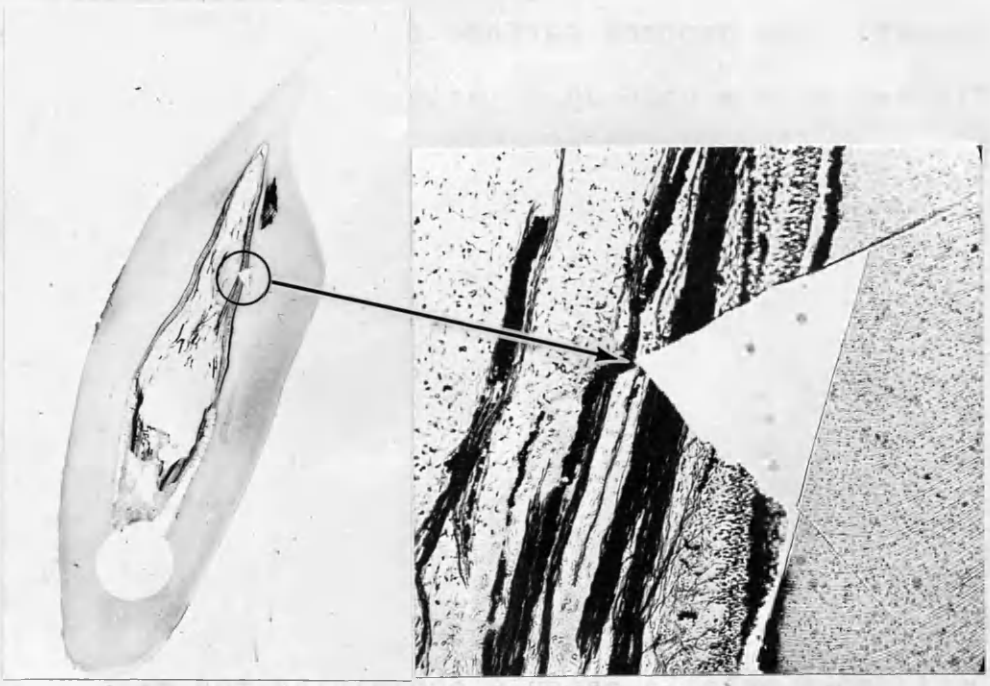

Fig. 17. Shows silvered section and enlargement of area from which portion was removed for further sectioning.

Demineralised, Frozen, Silver Impregnated Sections.

A freshly extracted tooth was ground to expose the pulp on each side of the plane from which sections were desired. The roughly prepared slice containing the pulp tissue was fixed by immersion for at least four days in 12% formalin neutralised by the addition of excess magnesium carbonate. Demineralisation was carried out by suspension in a solution of EDTA buffered to pH 7.4 for 60 hours at a temperature of 60 deg. C. Frozen sections of the pulp tissue and the surrounding dentine were then prepared. The sections were transferred to distilled water and then impregnated by the silver diamine ion modification of the Bielschowsky-Gros method described by Garven and Gairns (1952). The stained section was then placed on a microscope slide which had previously been covered by a layer of dental wax, and was kept moist by distilled water. A suitably impregnated area was selected under the microscope and the section was cut by a minute piece of razor blade held in a dental broach holder to dissect out the small area from which ultr-thin sections were to be prepared. (Fig. 17.) This small area/

area of the original frozen section was then taken up to absolute alcohol while still on the wax coated slide before being rinsed with several changes of the 4:1 mixture of n-butyl and methyl methacrylate monomers and finally transferred by a pipette to a gelatin capsule containing the methacrylate mixture and catalyst. Polymerisation was then carried out at 60 deg. C for at least 12 hours.

Demineralised Dentine and Pulp.

The freshly extracted tooth was immediately mounted upon the specimen holder of the section grinding machine and a slice approximately 1 mm. in thickness was removed and placed in 1% buffered osmium tetroxide solution (Zetterqvist, 1956) within six minutes of its removal from the patient. After 2 to 4 hours in the fixative, the specimen was rinsed briefly and placed in a 0.5 M solution of EDTA buffered to a pH of 7.4 (Nikiforuk and Sreebny, 1953) and with 15% sucrose added (Barnett and Palade, 1958). The specimen remained at the top of a column of this solution in a test tube overnight at room temperature.

On/

On the following morning, blocks of tissue not more than 2 mm. in length were cut with a sharp razor and passed through an ascending series of alcohols to absolute alcohol. They were then transferred to a 4:1 mixture of n-butyl methacrylate and methyl methacrylate. 0.2% of 2,4-dichlorobenzoyl peroxide was added to the final resin mixture and the specimen was polymerised in a gelatin capsule at 60 deg. C for at least 12 hours.

Ultra-thin sections of the specimens prepared by each of the above methods were cut upon a Cooke and Perkins ultramicrotome and examined in a Phillips EM 75 B electron microscope with stigmator.

Discussion of Methods.

As with the preparation of specimens for light microscopy, certain factors have to be carefully controlled if a satisfactory result is to be obtained. Some difficulties are inherent in the techniques and methods employed and would be present in an examination of any tissue or structure at such high magnifications. Other/

Other difficulties result from the selection of the particular tissue to which the methods are applied. The factors which require special attention may be summarised under the following headings.

1) Prompt Fixation. Electron microscopy demands much more exacting standards of prompt and adequate fixation than does light microscopy. It is accepted that post-mortem changes commence as a cell or tissue is removed from the living body. Such changes are not normally appreciable by light microscopy unless a delay of from several minutes to several hours (depending upon the tissue) occurs between death and fixation. Under the very high magnifications of the electron microscope, this degree of latitude is severely curtailed and fixation should commence at least as soon as death. Under ideal circumstances, in experimental animals, 'in vivo' perfusion of the fixative may ensure that fixation causes the death of a cell or tissue and so fixation and death occur simultaneously.

The dental pulp, immured within the relatively impermeable calcified dental tissues, is not amenable to rapid/

to rapid fixation by the swift penetration of the fixing fluid. The pulp of a freshly extracted tooth must therefore be exposed or laid bare over a large part of its surface if rapid fixation is to occur. This may be accomplished by either of two methods.

The tooth may be cracked open by a blow from a hammer or by pressure in a vise. Fixative is immediately applied to the exposed surface of the pulp which may then be carefully dissected from its cavity within the dentine. It has been demonstrated that this method does not satisfactorily preserve the delicate structure of the pulp matrix which may be severely disrupted by the compression of the elastic dentine just before it fractures.

Alternatively, the freshly extracted tooth may be sectioned by a carborundum slitting disc revolving at high speed under water jets and the calcified tissue on each side of the pulp removed. The resulting slice of calcified tissue containing the exposed pulp may then be rapidly immersed in a fixative which is enabled to penetrate the pulp tissue from each side. It has been observed that this method produced remarkably little distortion/

distortion of the pulp matrix.

2) Demineralisation. Prolonged immersion of the pulp tissue in a decalcifying fluid, even if previously fixed, results in an appreciable loss and distortion of the constituents of the cells and tissues when observed at high magnifications. This harmful effect may be minimised by the use of the chelating agent, EDTA, buffered to a pH of 7.4 This is preferable to the use of any strong acid. Demineralisation is, however, only necessary when sections are to be prepared from the periphery of the pulp where dentine is likely to be included. It can usually be avoided if more central portions of the pulp are to be examined.

3) Orientation. Primary orientation is possible by careful placing of the specimen in the gelatin embedding capsule. This may be assisted by the use of partially polymerised monomer of a syrupy consistency. When the methacrylate block containing the specimen is trimmed, a field containing nerve trunks is selected under a binocular stereoscopic microscope. This is facilitated by the fact that nerve trunks containing myelinated fibres are more intensely stained by the osmium/

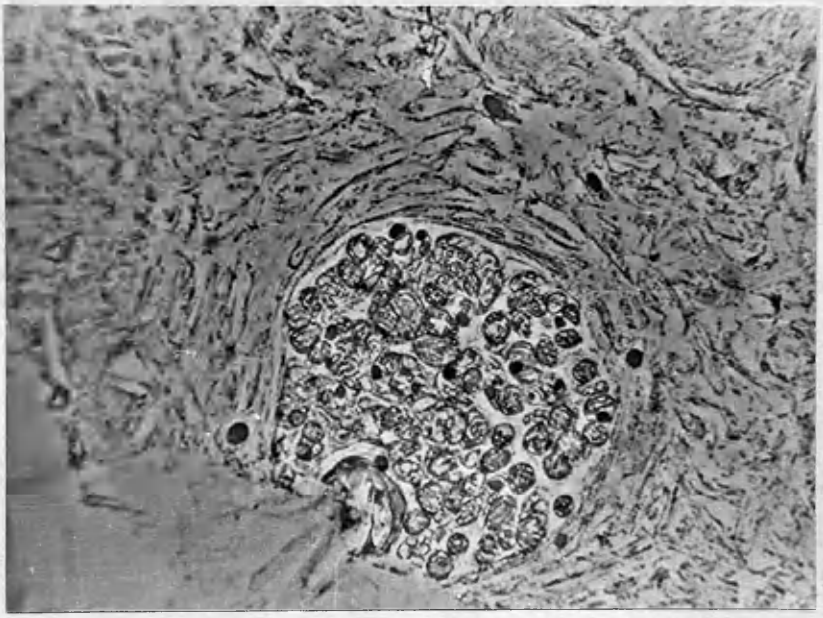

Fig. 18.

osmium fixative. A final check that the sections do indeed show the desired structures may be rapidly made by examining a slightly thicker section under the phase contrast microscope after the methacrylate has been removed. (Fig. 18.)

Bibliography.

Arwill, T. 1958. Innervation of the teeth. Trans. Royal Schools of Dentistry, Stockholm and Umeå. Series 2 : 3.

Barrnett, R. J. and Palade, G. E. 1958. Applications of histochemistry to electron microscopy. J. Histochem. Cytochem. 6 : 1.

Churg, J., Mautner, W. and Grishman, E. 1958. Silver impregnation for electron microscopy. J. Biophys. and Biochem. Cytol. 4 : 841.

Fearnhead, R. W. and Linder, J. E. 1956. Observations on the silver impregnation of nerve fibres in teeth. J. Anat., Lond. 90 : 228.

Fernandez-Moran, H. 1950. Sheath and axon structures in the internode portion of vertebrate myelinated nerve fibres. Exptl. Cell Res. 1 : 309.

Fernandez-Moran, H. 1952. The submicroscopic organisation of vertebrate nerve fibres. Exptl. Cell Res. 3 : 282.

Fernandez-Moran, H. and Finean, J. B. 1957. Electron microscope and low angle x-ray diffraction studies of the nerve myelin sheath. J. Biophysic. and Biochem. Cytol. 3 : 725.

Fernandez-Moran, H. 1958. Fine structure of the light receptors in the compound eyes of insects. Exptl. Cell Res. Suppl. 5 : 586.

Garven/

Garven, H. S. D. and Gairns, F. W. 1952. The silver diamine ion staining of peripheral nerve elements and the interpretation of the results: with a modification of the Bielschowsky-Gros method for frozen sections. Quart. J. exp. Physiol. 37 : 131.

Geren, B. B. 1954. The formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. Exptl. Cell Res. 7 : 558.

Nikiforuk, G. and Streebny, L. M. 1953. Demineralisation of hard tissues by chelating agents at neutral pH. J. dent. Res. 32 : 859.

Palade, G. E. 1952. A study of fixation for electron microscopy. J. exp. Med. 95 : 285.

Palay, S. L. and Palade, G. E. 1955. The fine structure of neurons. J. Biophys. and Biochem. Cytol. 1 : 69.

Rowles, S. L. and Brain, E. B. 1960. An improved silver method for staining nerve fibres in decalcified sections of teeth. Arch. oral Biol. 2 : 64.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophysic. and Biochem. Cytol. 4 : 475 & 727.

Young, J. Z. 1945. The history of the shape of a nerve fibre. from Essays on growth and form. ed. Le Gros Clark and Medawar. Clarendon Press, Oxford.

Zetterqvist, H. 1956. The ultrastructure of the columnar absorbing cells of the mouse jejunum. Stockholm.

Observations by Optical Microscopy.

The Identification of Nerve Fibres.

In any method which demonstrates the innervation of a tissue by silver impregnation, the recognition of a nerve fibre must not be permitted to rest upon the fact that the fibre is impregnated. Many fibres, other than nerve fibres, may be impregnated by silver nitrate and the identification of a nerve fibre should also depend upon morphological considerations. The nerve fibre is therefore recognised by its origin, configuration and position and not simply by the fact that the fibre is silver impregnated. Methods vary, however, in the degree to which this difficulty of interpretation is present.

The method described by Garven and Gairns (1952) which was modified to demonstrate nerve fibres in demineralised frozen sections was found to be capable of such a high degree of selective impregnation of the nerve fibres that no difficulty of interpretation arose. It was possible, in most cases, to manipulate the method/

the method in such a way that the nerve fibres appear dense black upon an absolutely unstained background. However, such a high degree of specificity only proved to be an embarrassment in the examination of the sections, since it left some doubt as to the precise location of the nerve fibres and their relationship to the other elements of the pulp and dentine. Consequently an attempt was usually made to permit a slight degree of impregnation of certain other elements of the pulp and dentine. It was noted that silver staining of the other elements of the pulp and dentine took place in strict order as follows:-

Red Blood Corpuscles,
Nucleoli,
Inner border of calcified Dentine,
Nuclei of Blood Vessel walls,
Pulp cells and Odontoblasts,
Rest of calcified Dentine,
Reticular fibres of Pulp and von Korff's fibres,
Predentine.

It is to be noted that the von Korff's fibres, which/

which require careful differentiation from the nerve fibres in most silver staining methods, only appear in the most heavily stained sections in this method. The early staining of the inner calcified border of the dentine made it impossible to trace any of the nerve fibres into the dentine beyond the zone of predentine.

Many previous investigators using less specific staining methods were faced by the difficulty of differentiating satisfactorily between nerve fibres and argyrophilic connective tissue fibres. In order to clarify the interpretation of their results, control sections or specimens were treated by some other method more specific for the confusing connective tissue elements. Confusion did not arise in the present investigation and the control experiments performed did not play an important part in the recognition and identification of the nerve fibres.

The Distribution of Nerves in the Human Dental Pulp.

Large nerve bundles, from three to eight in number and varying considerably in size, arrived in the pulp of the tooth through the apical foramen. During their course along the pulp canal or canals of the tooth the bundles of medullated nerve fibres followed a very straight course within the central area of the pulp tissue in the root canal and were usually seen to invest or be in close association with an artery. Branching was infrequent and when it did occur the divisions diverged only slightly before continuing in the same direction. Occasionally a few fibres would leave one of the larger trunks and follow a more tortuous course laterally towards the wall of the pulp canal where they would ramify below the odontoblast layer or pass between the odontoblasts to reach the predentine. The surface of the pulp tissue within the root canal was, however, relatively lacking in nerve fibres in comparison with the profusion of branching fibres present on the surface of the pulp in the coronal pulp chamber.

As the pulp canal widened into the pulp chamber of the/

REFERENCE FIG. 19 etc.

All photomicrographs are now specially mounted and are placed at the end of the thesis. This is to prevent cracking as they are all large photomicrographs.

of the tooth the bundles of nerve fibres diverged towards the lateral walls of the pulp chamber. (Fig. 19.) Branching became much more frequent and the course of those bundles which accompanied arterioles became more tortuous. Nerve fibres were frequently to be seen wrapped around branching arterioles and capillaries in a spiral manner. Occasionally it would appear that a nerve fibre which looped over the bifurcation of a vessel had been dragged in an occlusal direction. The successive divisions of the larger nerve trunks resulted in the structure of the fibres which comprised them being more clearly visible. (Fig. 22.) They were seen to be composed of beaded and unbeaded fibres of varying dimensions. A final branching took place in the cell-rich zone towards the surface of the dental pulp where the many finer nerve fibres formed a very dense interlacing parietal plexus. (Fig. 27.) From this plexus a large number of exceedingly fine nerve fibres were seen to pass across the cell-free zone and between the cells of the odontoblast layer (Fig. 28.) to the zone of predentine (Fig. 29.) where they could frequently be seen following an intertubular course within the matrix at right angles to the dentinal tubules/

tubules and parallel to the inner border of the calcified dentine. (Fig. 30.) Although there was occasionally strong evidence for the belief that a nerve fibre might enter the calcified dentine this could never be satisfactorily proved. In all well impregnated sections the inner border of the calcified dentine was found to be intensely argyrophilic and the method was therefore unsuited to the demonstration of nerve fibres within the calcified dentine.

The Nerve Plexus of Raschow.

Raschow (1835) described the manner in which the smaller nerves towards the periphery of the pulp break up into an exceedingly fine plexus. The term 'plexus' is capable of two interpretations. In a capillary plexus or network there is a pronounced degree of anastomosis between the various capillary looks which form the network. In the terminal plexus of lightly myelinated nerve fibres in the dental pulp, there is no evidence that anastomosis takes place and the apparent network results from the interweaving course of the successively finer subdivisions of the nerve branches. (Fig. 21.)

Several authors have drawn attention to the fact that nerve fibres were not present in the dentine papilla before the formation of the dentine commenced. The nerve plexus did not make its appearance until the formation of the pulp chamber in the crown of the tooth had been completed by the deposition of the primary dentine. Thereafter, a gradual increase was seen in the number of fibres lying a short distance from the surface of the pulp and parallel to the inner surface of the/

of the dentine. (Fig. 25.) To begine with, this plexus increased in prominence as a result of the growth of the pulpal nerve fibres as the full innervation of the pulp gradually developed. Another factor which increased the prominence of this plexus was the formation, in later years, of physiological secondary dentine which gradually diminished the size of the pulp chamber. It was believed that pulp cells and connective tissue elements atrophied or contributed to the formation of the matrix of the physiological secondary dentine. For the majority of the nerve fibres, however, there was no alternative but to be crowded into the peripheral plexus.

The dense peripheral plexus in the dental pulp of an adult did not appear to serve any sensory function which could not have been carried out by a more direct and less profuse innervation. It is therefore suggested that the plexus arises as a result of topographical considerations concerned with the growth of nerve fibres within a confined space and, later, the shrinkage of this space.

The distribution of the nerve plexus of Raschow within/

within the pulp of a tooth was of interest in that it appeared to be related to those surfaces of the pulp chamber at which external stimuli most frequently arrived. While it was therefore a constant feature of the coronal pulp chamber it was not observed in the root canal. In premolar and molar teeth, the plexus was prominent on the upper surface of the pulp adjacent to the roof of the pulp chamber and it was normally absent from the floor of the pulp chamber. Although present on the buccal and lingual sides of the pulp chamber, it was slightly more prominent on the buccal side than on the lingual side of the pulp.

Beads, Varicosities or Fusiform Enlargements.

The beaded appearance of some pulp nerve fibres was noted by many of the earlier investigators (e.g. Bödecker, 1882; Hopewell-Smith, 1903; Mummery, 1912) and was used as a feature which distinguished nerve fibres from connective tissue fibres. A similar appearance was described by Mohuidin (1950) in nerve fibres in deciduous teeth but was interpreted by him as evidence of degeneration. Arwill (1958) suggested that the characteristic beads or swellings along a nerve fibre were fixation artefacts resulting from droplet formation of the semi-fluid axoplasm. Meyer (1954) has however observed these ovoid or fusiform enlargements on nerve fibres growing 'in vitro'. Fischer (1946) also noted, in connection with tissue culture experiments, that varicosities formed and disappeared and that sometimes a peristaltic movement in a distal direction could be detected.

It was noted that, in those methods where the selective intensity of impregnation did not or could not differentiate between axoplasm and cell nuclei, the swellings of the nerve axons were sometimes/

sometimes mistakenly regarded as Schwann cell nuclei. Arwill (1958) therefore talked about ".... the Schwann cells that accompany the fibres and form characteristic spindle-shaped swellings."

In the present investigation the following observations were made upon the occurrence and appearance of these beads or enlargements. Firstly, they were always present in the pulps of teeth from all age groups and the speed of fixation within practical limits did not appear to affect their number size or appearance. Secondly, it was discovered that, in order that they should be satisfactorily demonstrated, a slightly heavier degree of impregnation was required than was necessary to demonstrate the nerve fibre. Thus in some lightly impregnated sections gaps would be seen in nerve fibres which, under higher magnification, could be seen to be occupied by a very faintly impregnated varicosity or enlargement. It thus appeared that the slight degree of resistance to staining offered by these beads or varicosities was indicative of some minor chemical or physical variation in their composition relative to the main portion of the fibre. The third striking/

striking feature concerning these enlargements was their ubiquity. When sections through a main perivascular nerve trunk were thin enough to show the internal arrangement of the fibres, many varicosities were observed. (Fig. 41.) Occasionally, however, very fine filaments could be seen which apparently did not possess them. The varicosities were also to be observed on most of the nerve fibres present in the parietal plexus. (Fig. 26.) They were also to be seen on most of the fine filaments which coursed from the peripheral plexus across the Basal Layer of Weil and between the odontoblast layer of cells. (Fig. 28.) Occasionally a swelling was to be seen on that part of a terminal nerve fibre which actually penetrated the predentine and it is believed that the 'pear drop' formation described in these fibres by Bradlaw (1936) results from the distortion of a varicosity included in the developing dentine matrix. (Fig. 37.) The size of the varicosity was observed to bear a relationship to the thickness of the fibre on which it was located. Thus large ovoid swellings normally found on the thicker fibres present in the larger nerve bundles were usually present near the centre of the pulp. (Fig. 43.) Smaller swellings/

swellings were to be seen on the finer fibres in the peripheral plexus (Fig. 39.) and minute beads were located upon the extremely fine fibres which penetrated the odontoblast layer. (Fig. 36.) Difficulty was experienced in examining the structure of these swellings under light microscopy since a degree of magnification was required which approached the limits of resolution of the microscope. In some of the larger beads, however, it was possible to observe that the swelling consisted of a loose arrangement of lengthwise orientated fibres or filaments which were gathered together at each pole of the fusiform enlargement to continue as the more dense and compact nerve fibre. (Figs. 43 & 44.) The filaments disclosed within the bead are known as neurofibrils. Maximov and Bloom (1950) note that they are distributed as a complicated network throughout the nerve cell body and spread into all processes including the finest terminal ramifications. The swelling therefore appeared to be due to a localised accumulation of interfibrillar substance or axoplasm. Young (1945) suggested that there was a continual flow of material from the nerve cell body down the nerve fibre and a leak over its surface. He believed that this pressure maintained the organ-

isation/

organisation of the substance of the axoplasm and that if it was removed, as by cutting the fibre, the normal structure of the axon became disorganised.

When a tooth was extracted, the nerves were severed at a point relatively close to the terminal distribution of the fibres. The collapse of the axons in the pulp following the sudden loss of pressure was thus more certain and more severe than in other tissues where a similar drop in pressure did not occur because the nerve cell itself was removed with the specimen or a greater length of axon reduced the effect.

Branching of Nerve Trunks and Fibres.

As mentioned above, branchingof nerve trunks was infrequent during the course of the nerve bundle along the root canal but became more frequent as the pulp chamber was reached. (Fig. 19.) The branching sometimes accompanied division of the blood vessel to which the nerve trunk was closely related. In the pulp chamber the nerves branched as they passed towards the surface of the pulp. This type of division involved a straightforward redistribution of existing nerve fibres into the two branches. Towards the surface of the pulp and particularly in the region of the peripheral plexus, branching of single nerve fibres took place in which there was an actual dichotomy of the nerve fibre. It was this type of branching which was largely responsible for the profusion of fine nerve fibres within the peripheral plexus. Since this type of branching limited the power of the brain to localise the source of any stimulus it only occurred at a late stage in the distribution of a nerve fibre. A swelling was often present at the site ofsuch a division in a nerve fibre. When suitably stained, the neurofibrillae in the undivided nerve fibre were seen to be distributed into the branches within the web-like triangular swelling.

The Relationship between Nerve Fibres and Blood Vessels.

As they entered the apical foramen, the largest bundles of nerve fibres were usually closely related to the arterioles. The larger vessels retained the sheath of nerve fibres as they passed through the pulp canal to reach the pulp chamber. When the blood vessel branched, each division was usually accompanied by some of the nerve fibres. Most of the nerve fibres, however, appeared to sever their connection with the blood vessels before reaching the subodontoblastic layer. Small capillaries often passed through this layer to loop between the cells of the odontoblast layer but in no case were they accompanied by nerve fibres at this stage.

In many cases the nerve fibres followed a spiral course around the blood vessel (Fig. 24.), occasionally in both directions at the same time. Where a blood vessel branched, nerve fibres would frequently be seen looped over the bifurcation and intertwined with the divisions in a most intricate manner. It has been asserted by many authors that two types of nerve fibres were present within the pulp tissue; myelinated sensory fibres and fine/

fine non-myelinated fibres of the autonomic nervous system. The staining method employed did not differentiate between myelinated and non-myelinated nerve fibres and, while it was noted that numerous fine nerve fibres were present in the bundles of coarser fibres passing along the pulp canal, it was impossible to confirm this finding.

Careful examination of the walls of blood vessels accompanied by nerve fibres failed to disclose any organised nerve ending or end-plate in contact with the vessel wall.

The Relationship between Nerve Fibres and Secondary Dentine.

The gradual deposition of physiological secondary dentine upon the walls of the pulp chamber had an effect upon the pattern of distribution of the nerve fibres within the pulp. In the younger pulp, the terminal nerve fibres which passed among the odontoblast cells were fine and not so numerous as in the older pulp. The plexus of Raschow was more prominent in the older pulp where a gradual decrease in the size of the pulp cavity had occurred. The peripheral pulpal nerves which branched so frequently in the subodontoblastic zone were heaped up, as it were, by the retreating odontoblast layer. This concentration was particularly noticeable in the pulp horn, where the greatest reduction in space took place. (Fig. 20.)

It appeared probable that nerve fibres might have become entangled in the fibrous elements from the pulp which were incorporated in the matrix of subsequent layers of secondary dentine. It was believed that this was the correct explanation of the course taken by the nerve fibre which passed from the pulp tissue between the odontoblast cells to follow an intertubular course within/

within the dentine matrix. The expectation that this should have occurred more frequently in an older tooth was in accordance with the observations made during this investigation.

Such an explanation of the relationship between the nerve fibres of the pulp and the developing dentine explained many of the different forms which this relationship took. Thus, when a nerve fibre looped into the predentine and returned to the pulp, it did so, not because it had grown along this path, but because a penultimate part of the nerve fibre was included in the developing dentine. (Fig. 29.) Fibres which branched within the predentine layer of matrix did so because the division was included in the developing matrix following the entanglement of one or more of the fine terminal branches. (Figs. 30 & 31.) If only one branch of a dividing nerve fibre was previously caught in the developing matrix, the other branch looped back into the pulp tissue. This configuration was given special mention by Falin (1956). A nerve fibre enclosed within the developing matrix might follow the course of a tubule and be situated within the lumen or calcified within/

within the wall. On the other hand, the nerve fibre might follow the horizontal stratification of the intertubular fibrillar matrix at right angles to the direction of the tubules. (Fig. 34.) As more and more of a nerve fibre became engulfed within the developing matrix, the nerve fibre seen passing between the odontoblast cells to enter the predentine ceased to be a fine terminal filament and, particularly in older teeth, was seen to be a thicker fibre such as is normally seen in a more central position in the pulp. (Fig. 30.)

The Relationship between Nerve Fibres and Pulp Stones.

Severe dental pain in an otherwise sound tooth has occasionally been attributed to pulp stones. The manner in which the growth of the pulp stone caused the painful sensation was usually vaguely attributed to pressure. A more detailed explanation of the relationship between the growth of the mass of calcified tissue within the pulp and the nerves was suggested following careful examination of silver impregnated sections in a few cases where pulp stones were present.

The common explanation given; that the pressure of the growing calcified mass on the nerve fibre produced the pain; was not acceptable. The growth of the calcified mass was slow and gradual and could therefore bear little relationship to the sudden, severe spasms of pain which were experienced. It was observed, however, that a blood vessel surrounded by nerve fibres might, by its mere presence, cause an enlarging mass of calcified tissue to grow around it rather than push it to one side. The blood vessel and its surrounding nerve fibres now lay in a deep groove which extended along one side of the pulp stone. It was believed/

believed that the painful sensation was caused by occasional, transient variations in the dilation of the blood vessel which would be of no account in the normal pulp but which pressed the nerve tissue firmly against the hard unyielding sheath of calcified tissue in the affected pulp.

Observations by Electron Microscopy.

The Identification of Nerve Fibres.

When sections 0.01 μ in thickness are prepared from an area of pulp tissue not greater than 0.5 mm. by 0.25 mm., it is not surprising to find that many sections can be prepared in which no nerve fibres are present. In many other cases the lack of thickness and the small area covered by the section can render a nerve fibre quite unrecognisable. Certain other factors, however, favour easy recognition. The myelin sheath, if present, is normally heavily stained by the osmium fixative. The uninterrupted length of the nerve fibre is not usually a factor of much assistance in this connection because the sections are so thin that the slightest undulation on the part of the nerve fibre carries it immediately outwith the plane of section. When a nerve trunk is cut in cross-section, however, the repetitive pattern of round figures, all nearly equal in diameter, is quite distinctive. (Fig. 45.) The fine nerve fibres among the cells and tissues of the pulp, without a definite myelin sheath, are all too/

too easily overlooked at magnifications where cell boundaries and other landmarks of light histology are absent. It was for this reason that experiments were undertaken to see if the method of identification by silver impregnation could be employed in the electron microscope. Silver possesses a sufficiently high atomic number (47) for any precipitate to be readily discernible under the electron microscope. As was previously observed in connection with the identification of nerve fibres under the light microscope, the precipitation of silver in the tissues could be controlled in such a way that the nerve axons were specifically stained. It was therefore expected that the precise and exclusive localisation of the electron-dense silver particles within the axon would constitute a reliable and accurate method of identifying the nerve fibre in the extremely thin sections and at the high magnifications used in the electron microscope. This proved to be an entirely reliable method of identifying nerve fibres with or without a myelin sheath but the damage sustained by the tissue elements during the chemical staining process was a considerable disadvantage. (Fig. 46.)

The Schwann Cell - Axon Relationship.

The interdependent relationship between an axon and its accompanying Schwann cell has been studied extensively. Much confusion has surrounded the question of the origin of the myelin sheath and the precise meaning of the term 'non-myelinated'. In recent years the structure and formation of the myelin sheath of peripheral nerves have been closely investigated with the electron microscope following the discovery by Geren (1954) that the myelin sheath was produced by a spiralling of the mesaxon of the Schwann cell around the axon. This idea became known as the 'jelly-roll' theory. It was also discovered that many fine nerve fibres which appeared naked and were described as non-myelinated, do in fact possess traces of myelin and are in almost every case invested by Schwann cell cytoplasm. The degree to which Schwann cell cytoplasm is wrapped around the axon now appears to be the feature which distinguishes the myelinated from the non-myelinated nerve fibre. The belief that the Schwann cell completely enclosed an axon within its cytoplasm was proved incorrect in the case of non-myelinated/

non-myelinated fibres was proved incorrect by the investigations of Gasser (1952 & 1955). He showed that the Schwann cell was simply folded around each axon and that the continuity of the Schwann cell membrane was not interrupted. The thin membrane formed as the enveloping lips of the Schwann cell cytoplasm came together around the axon was called by Gasser a 'mesaxon'. (Fig. 47.) In 1954, during a study of chick embryonic nerve fibres, Geren postulated a similar relationship between the axons and their related Schwann cells in the case of myelinated fibres. She found that the mesaxon was greatly elongated in a spiral around the axon and suggested that adult compact myelin consisted of closely packed Schwann cell membranes. (Fig.48.) The lamellated structure of myelin was first visualised by electron microscopy by Fernandez-Moran in 1950. The lamellae were found to repeat radially at a period of about 120 Å. The importance of the myelin sheath in providing insulation of the axon cylinder against the loss of nervous current traversing it during its activity was confirmed by Peters and Muir (1959). They found that the nerves of developing rats were,at an early stage,/

stage, composed of small closely packed naked axons, isolated from the surrounding tissues by a peripheral layer of Schwann cell cytoplasm. When this nerve was stimulated, it was only possible to produce generalised movements from a rat foetus (Angulo, 1932; Baron, 1941) as would be expected when mutual interference was possible between axons. Later in development, the Schwann cells invaded the nerve, producing partitions which separated the axons into smaller bundles. This process continued until each axon possessed a number of Schwann cells aligned along its length. Specific movements now resulted from stimulation in place of the earlier generalised response.

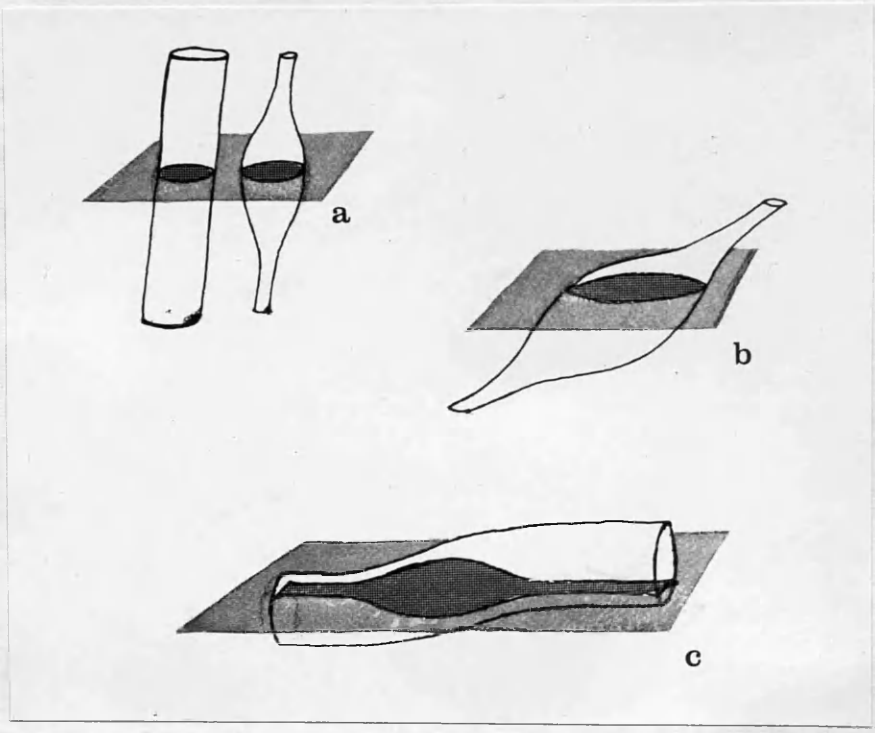

Fig. 49.

The Structure of the Axon.

Sections of large bundles of nerve fibres were prepared from freshly isolated pulp tissue and examined in the electron microscope. The axons were observed to consist of an outer limiting membrane surrounding a relatively structureless axoplasm. Mitochondria were present at irregular intervals and appeared as darker ovoid masses, their longer axes in line with the length of the fibre.

The smallest nerve fibre observed was approximately 0.05 μ or 500 Å in diameter. The average thickness of the sections was approximately 100 Å. Thus it was never possible to observe a nerve fibre lying entirely within the thickness of the section and the beads or swellings which were so typical of nerve fibres in the much thicker sections used for optical microscopy could not be identified. In a true cross-section of a nerve fibre, a swelling on a small nerve fibre was always observed as a normal cross-section of a thicker fibre. (Fig. 49a) In an oblique section through a thin fibre with a swelling, the appearance in such thin sections was always consistent with that/

that of a section through a thicker fibre. (Fig. 49b) When sectioned in a plane parallel to the long axis of the nerve fibre, any swelling observed could be due to a twist in the nerve fibre so that for a short distance it showed its true thickness. (Fig. 49c) When it was observed in silver impregnated sections under the light microscope, that the diameter of a bead or swelling was often as much as ten times the diameter of the axon upon which it was situated; it was realised that any estimation of the thickness of an individual nerve fibre or any attempt to build up a spectrum of nerve calibers would be very misleading if based upon electron microscope appearances.

In the ultra-thin sections prepared from selected areas of thick, frozen, decalcified sections which had been silver impregnated, the nerve fibres presented an interesting appearance. The metallic silver particles were seen to be confined to the axons; the myelin sheaths being unstained. (Fig. 50.) The silver particles were not distributed at random within the axon but were obviously arranged along lengthwise orientated fibrils which were themselves invisible. (Fig. 51.) It was thus confirmed that, in a nerve fibre/

fibre impregnated with silver by this method (Garven and Gairns, 1952), the blackness of the nerve fibre was due to the combined effect of the impregnated neurofibrils within each axon.

The Odontoblast Layer.

An attempt was first made to trace the fine nerve fibres within the odontoblast layer in ultra-thin sections of silver impregnated material. By means of the initial silver impregnation it was established that many suitable nerve fibres within the odontoblast layer were present in the minute block of tissue selected for ultra-thin sectioning. The knowledge that the silver particles were situated exclusively within the axon of the nerve fibre assisted in the location of the nerve fibre for photography in the electron microscope. The results were, however, very disappointing because of the severe damage suffered by the odontoblast layer of cells. Thus, although the nerve fibre could be distinguished it was quite impossible to confirm that the surrounding cells were odontoblasts.

Ultra-thin sections were then prepared of material which had not been stained by the silver method. In such sections the odontoblast layer of cells showed a greatly improved degree of preservation. A surprising feature of these electron micrographs was the lack of definition/

definition of the odontoblast cell membrane. (Fig. 52.) The membrane which, in light microscopy is so definite and straight, proved most difficult to follow around the boundary of the cell and occasionally appeared to interdigitate in a most complex manner with the membrane of neighbouring cells. It had also been expected that the only intercellular elements in the odontoblast layer would be nerve fibres or von Korff fibres and it was hoped that the cross-banded appearance of the latter would enable a distinction to be made. In fact, a large variety of vesicles, globules and fibres was present (Fig. 52.) and the identification of nerve fibres by a process of elimination proved impossible.

The line dividing an odontoblast cell from the developing dentine matrix was also surprisingly difficult to follow. (Fig. 53.) The pulpo-dentinal junction of light microscopy could not be detected and there were many vesicles and globules present between the odontoblast cell and the developing matrix which were obviously not nerve fibres.

The Dentine.

In spite of numerous attempts by different methods, it was not possible to detect nerve fibres within the dentine either in an intertubular position within the predentine layer or in an intratubular position. Even when it was established by preliminary silver staining that nerve fibres were present within the predentine, it was still not possible to locate the nerve fibre among the dense layers of interweaving collagen fibres.

Many sections were prepared from the coronal dentine close to the pulp horns and roof of the pulp chamber where some authors have described intratubular nerve fibres. Although satisfactory preservation of the dentinal process of the odontoblast was achieved, no evidence of the presence of nerve fibres within the dentinal tubules was obtained. (Figs. 54 & 55.)

Bibliography.

Angulo. 1932. See Peters, A. 1961. Electron Microscope in Anatomy, Proceedings of a Symposium on the Ultrastructure of Cells, Anatomical Society of Great Britain and Ireland.

Baron. 1941. As above.

Arwill, T. 1958. Innervation of the teeth. Trans. Royal Schools of Dentistry, Stockholm and Umeå. Series 2 : 3.

Bödecker, C. F. W. 1882. The minute anatomy, physiology, pathology and therapeutics of the dental pulp. Dent. Cosmos. 24 : 292.

Falin, L. I. 1956. The morphology of tooth receptors. Dent. Abstr. 2: 495.

Fernandez-Moran, H. 1950. Sheath and axon structures in the internode portion of vertebrate myelinated nerve fibres. Exptl. Cell Res. 1: 309.

Fischer, A. 1946. Biology of Tissue Cells. Copenhagen.

Garven, H. S. D. and Gairns, F. W. 1952. The silver diamine ion staining of peripheral nerve elements and the interpretation of the results, with a modification of the Bielschowsky-Gros method for frozen sections. Quart. J. exp. Physiol. 37 : 131.

Gasser, H. S. 1952. Cold Spring Harbour Symposia Quant. Biol. 17 : 32.

Gasser/

Gasser, H. S. 1955. Properties of dorsal root unmedullated fibres on the two sides of the ganglion. J. Gen. Physiol. 38 : 709.

Geren, B. B. 1954. The formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. Exptl. Cell Res. 7 : 558.

Hopewell-Smith, A. 1903. The histology and pathohistology of the teeth. The Dental Manufacturing Co., London.

Maximov, A. A. and Bloom, W. 1948. Text-book of Histology. 5th ed. W. B. Saunders & Co., Philadelphia.

Meyer, H. 1954. Electron microscope study of nerve fibres grown 'in vitro'. Exptl. Cell Res. 7 : 15.

Mohiuddin, A. 1950. The fate of the nerves of the deciduous teeth. J. Anat., Lond. 84 : 319.

Mummery, J. H. 1912. On the distribution of the nerves of the dental pulp. Phil. Trans. roy. Soc. Series B 202 : 337.

Peters, A. and Muir, A. R. 1959. The relationship between axons and Schwann cells during the development of peripheral nerves in the rat. Quart. J. exp. Physiol. 44 : 117.

Raschow, J. 1835. Die Gesundheitslehre des Mundes. Ludwigsburg.

Young, J. Z. 1945. The history of the shape of a nerve fibre. from Essays on growth and form. ed. Le Gros Clark and Medawar. Clarendon Press, Oxford.

Discussion.

The introductory survey of literature upon the innervation of dentine shows the lack of unanimity which exists even among recent authors. Agreement is only possible concerning two basic facts:

a) Nerves are present in the pulp of a tooth,

b) When exposed, the surface of dentine is sensitive to a variety of stimuli; tactile, thermal and chemical.

The structure and distribution of the nerves and the method of transmission of sensation through dentine are among the most highly debatable points.

The present study cannot claim to have advanced our knowledge of the structure and extent of the nerve supply but it does clarify our conception of many aspects of the histology of the innervation.

Any remarks concerning the sensitivity of the dentine or the transmission of stimuli to the pulp are very speculative when made by a histologist from a morphological point of view. Nevertheless such observations/

observations must fit into the wider picture of the physiology of the transmission of sensation from tooth tissues.

The following conclusions have been reached in the light of the results of the present investigation and of past experience.

1) That no special sensory nerve endings or end-organs are present in the pulp or dentine.
2) That nowhere else on the surface of the body does such a profuse innervation relay so little sensory information.
3) That the transmission of sensation in dentine is non-neural and is a by-product of the basic property of irritability on the part of the odontoblast cell, whereby the cell is stimulated to assist in the formation of additional protective calcified matrix on the pulpal surface if the peripheral portion of the cell process receives mildly harmful stimuli.

4)/

4) That, although odontoblasts are not nerve cells, they were unique in their resemblance to nerve cells in that they support long extensions of their cytoplasm hundreds of times longer than the length of the cell itself. This continuity of cytoplasm over long distances is one of the essential prerequisites for the transmission of stimuli, neural or otherwise.

5) That, in the healthy intact tooth, the nerves of the pulp do not assist in the provision of any sensory information from the oral cavity which it not more readily perceived by the nerves of the lips, tongue, gingivae and periodontal membranes..

6) That the transmission of stimuli from the pulp is usually dependent upon the previous carious destruction or other loss of tooth tissue.

7) The author accepts the view that the dental pulp is merely a specialised dermal papilla and believes that the profuse innervation is an expression of the desire of the nervous tissue to experience external stimuli.

8)/

8) That the pulp behaves similarly to a visceral organ in giving rise to referred pain.

9) That factors other than the abundance of nerve fibres are responsible for the intensity of the pain experienced when the pulp is inflamed. It may be said that the pain in any inflamed tissue is inversely proportional to the ease with which it is permitted to swell. Swelling and congestion of the nasal mucosa is relatively painless in comparison with an inflammation below the unyielding skin of a finger tip. No swelling is possible in the case of the dental pulp and the rise of pressure which results from any dilation or increased permeability of the pulpal capillaries is rapid and severe. This, rather than the profusion of nerve fibres present, is responsible for the severity of the pain.

10) That in their efforts to reach a position where they could pick up external stimuli the nerves branch most abundantly below and between the odontoblasts on the surface of the pulp.

11) That from their position around and between the odontoblast/

odontoblast cells, it is only to be expected that many nerve fibres should be passively incorporated in the developing dentine; either in an intratubular position where they may remain vital or in an intratubular position in the matrix where they are liable to become engulfed in the advancing front of calcification.

12) That there is a great similarity between this occurrence and the development of intra-epithelial nerve fibres in the skin and mucosae where fine nerves fibres are occasionally observed to be caught in the stream of epithelial cells and carried so far as the granular or keratinised layers.

13) That, asmight be reasonably expected, the degree to which nerve fibres become involved in the developing matrix is greater in old age and at any localised area of secondary dentine formation. If, as is believed, nerve fibres are unintentionally included in the developing dentine matrix, then the likelihood of such an occurrence increases the longer a peripheral nerve fibre lies exposed to this possibility near the surface of the pulp. A localised/

A localised increase in the rate of deposition of secondary dentine has the same effect.

14) That the inclusion of a nerve fibre within the calcifying dentine matrix is a very common occurrence and is obviously not a painful one. This lends indirect support to the alternative explanation given of the pain associated with pulp stones.

In conclusion; it is believed that the intensity of pain which can come from a damaged pulp and the super-abundance of nerve fibres present have led histologists to search for some highly organised receptor mechanism.

The isolation of the dental pulp from external stimuli is so complete, however, that there is every reason to suppose that the morphological arrangements for the reception of stimuli should be poorly developed and unspecialised.

It should be recalled that there is frequently a relationship between excessive proliferation and absence or lack of specialisation in animal tissues.

If the/

If the dental pulp has evolved from a dermal papilla, the changes on its surface have been such as to eliminate the possibility of an accompanying specialisation of any sensory receptor mechanisms.

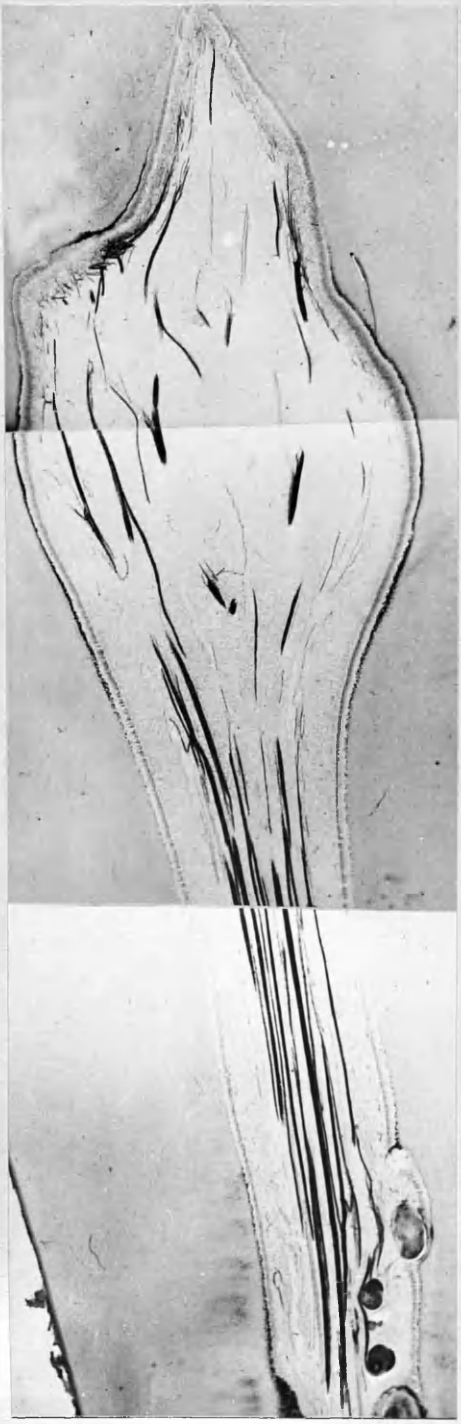

Fig. 19. General distribution of nerves within the dental pulp. The progressive subdivision of the nerve trunks entering the pulp canals results in a rich supply of terminal filaments to the surface layers of the coronal part of the pulp.

X 10

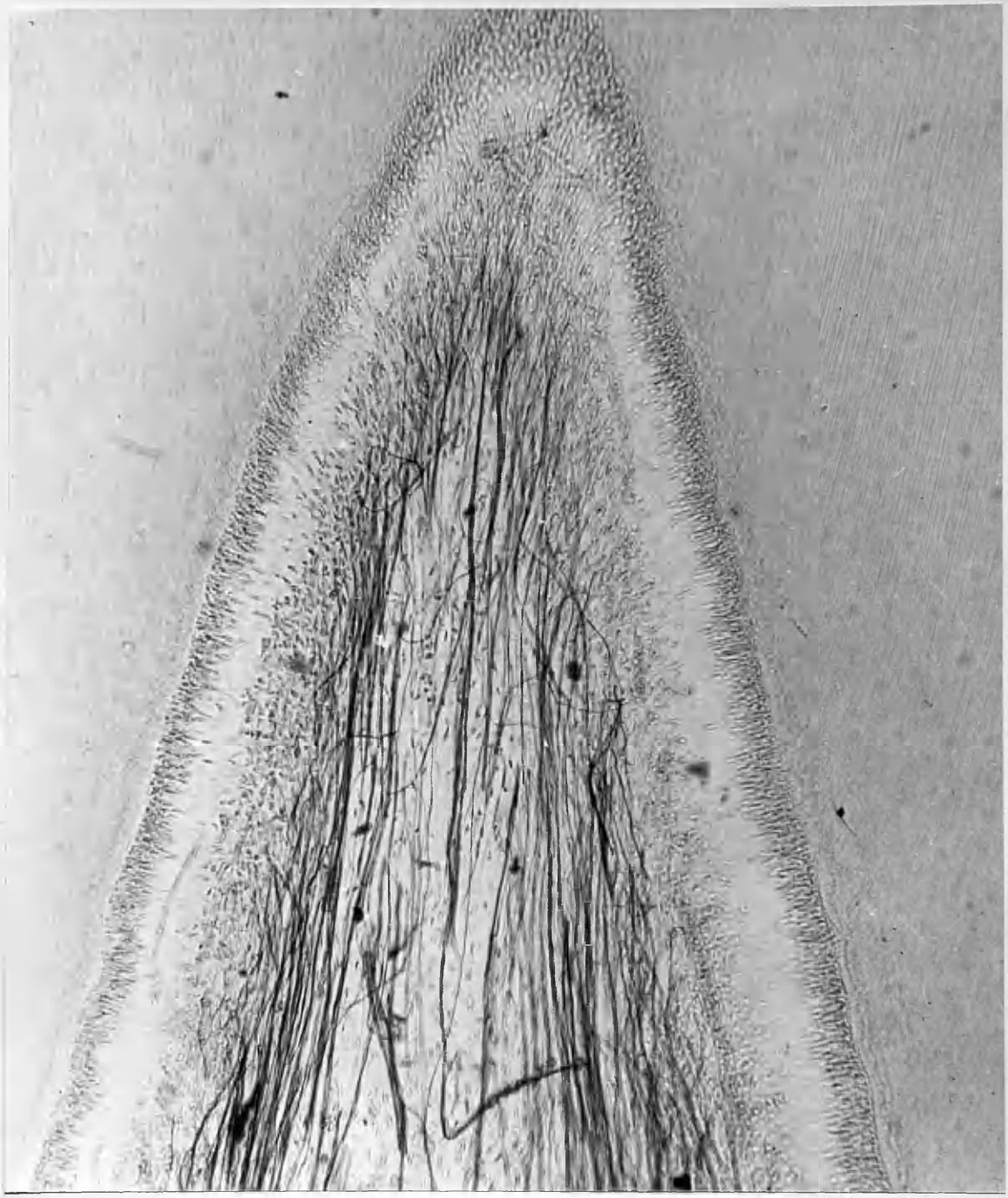

Fig. 20. The pulp horns are always well supplied with nerve fibres. Note the position of the peripheral plexus on the inner surface of the cell-rich zone.

X 182

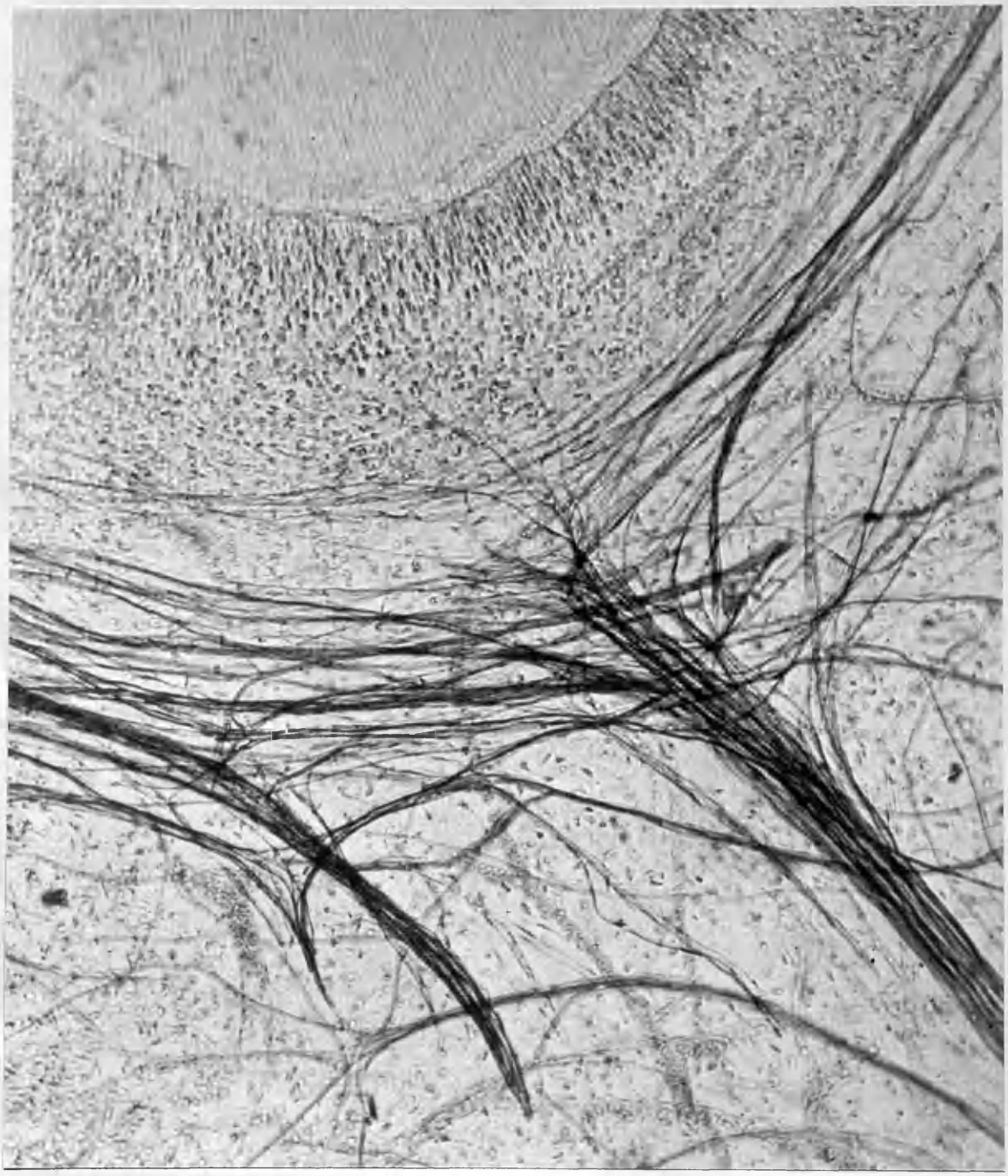

Fig. 21. A large nerve trunk follows a straight course to the roof of the pulp chamber where it divides and supplies many branches to the sub-odontoblastic plexus.

X 322

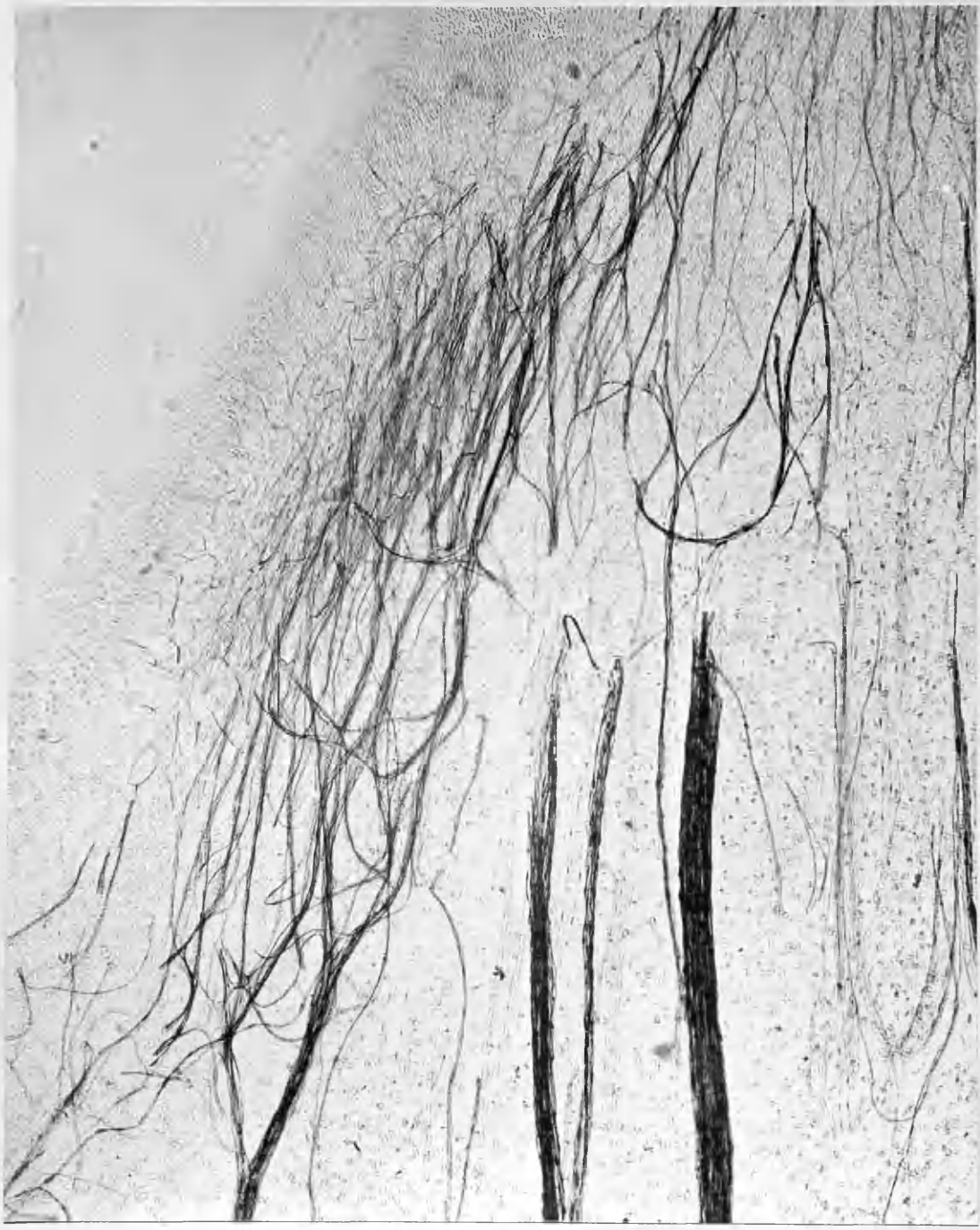

Fig. 22. The irregular course followed by many of the branching nerve fibres in the sub-odontoblastic zone is very difficult to follow and results in the matted 'plexus' of nerves.

X 50

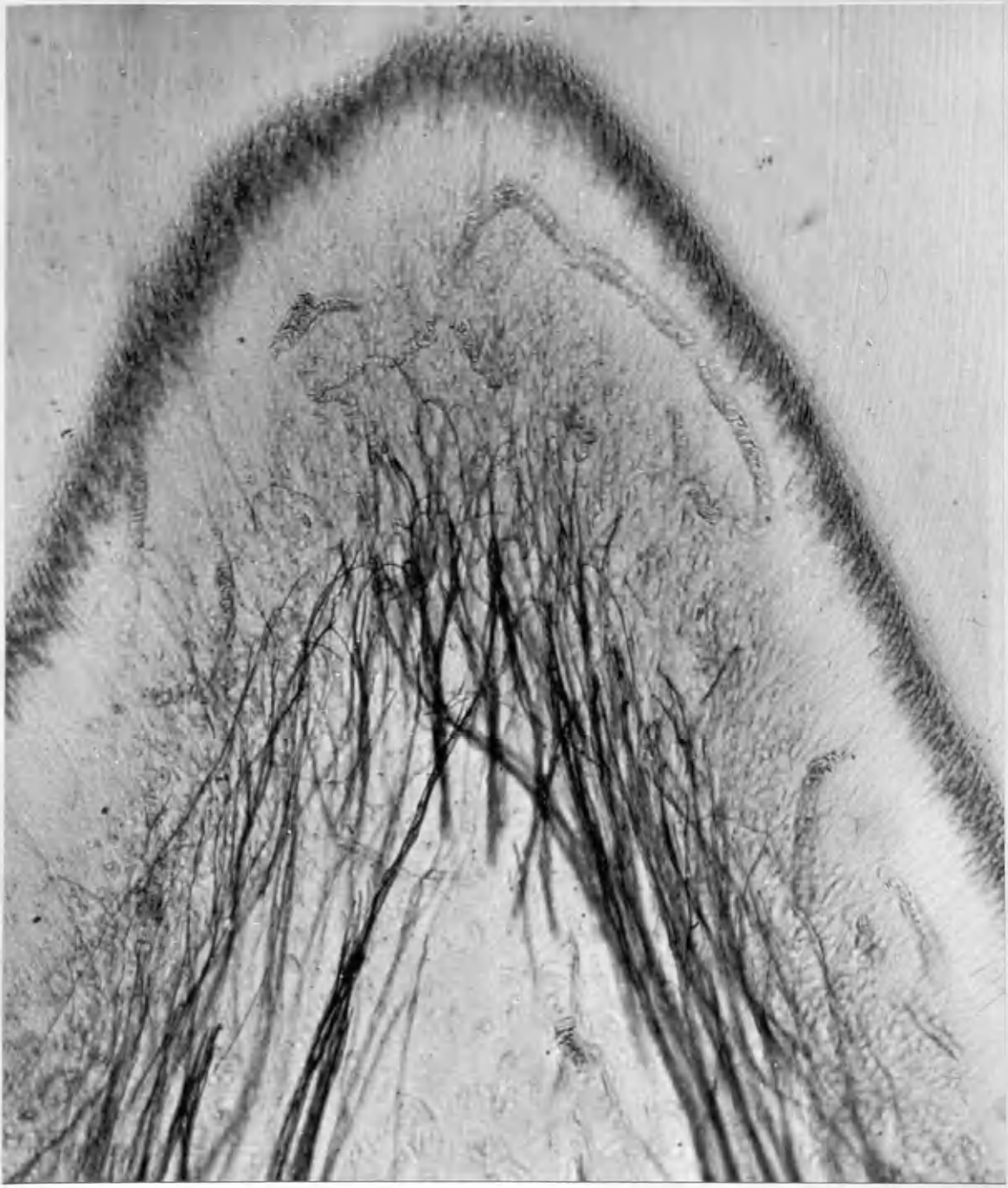

Fig. 23. Many nerve fibres loop below the cell-rich zone and return in an apical direction. That the odontoblast layer is well supplied by capillary blood vessels is also clearly shown.

X 292

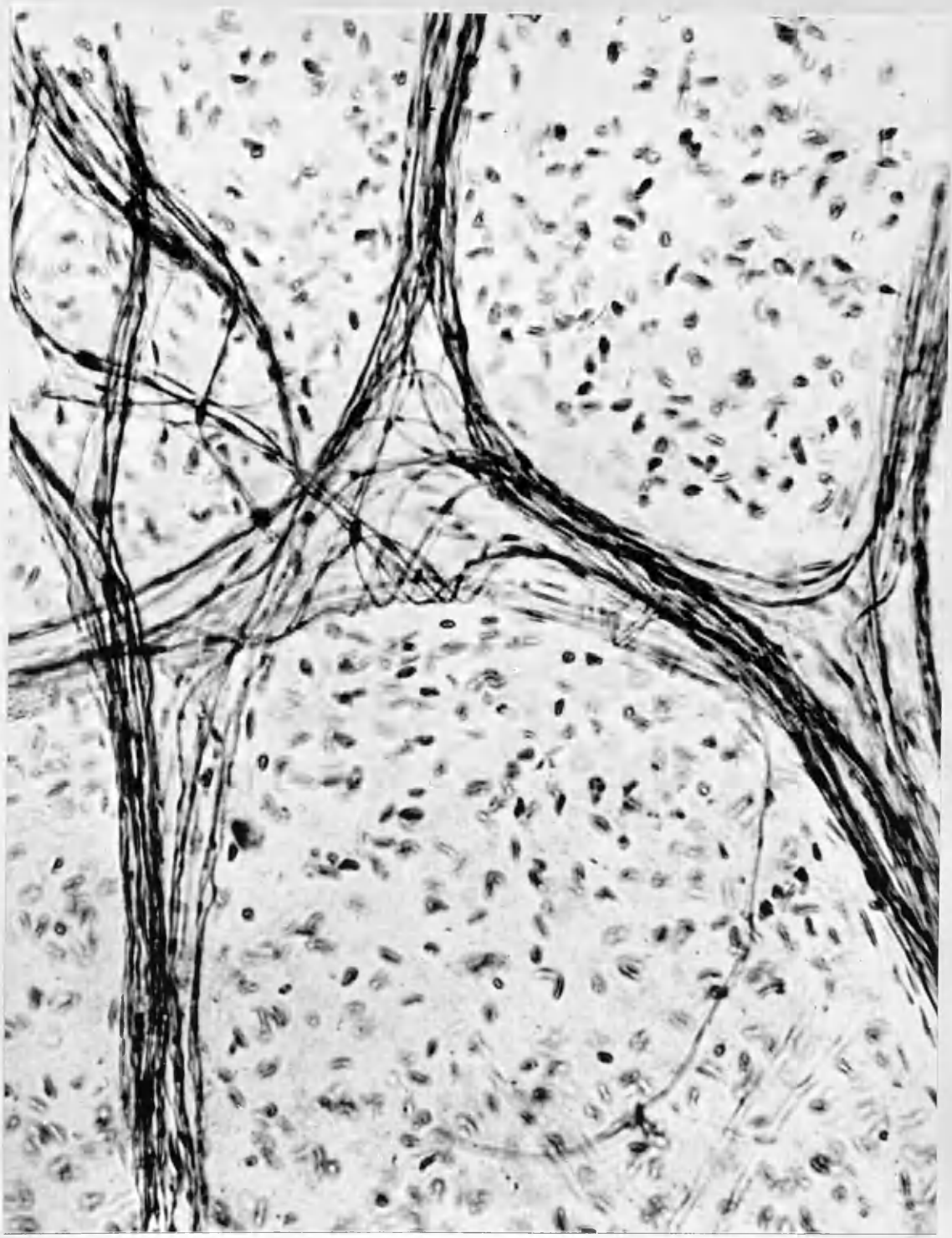

Fig. 24. Beaded nerve fibres loop round a branching arteriole.

X 270

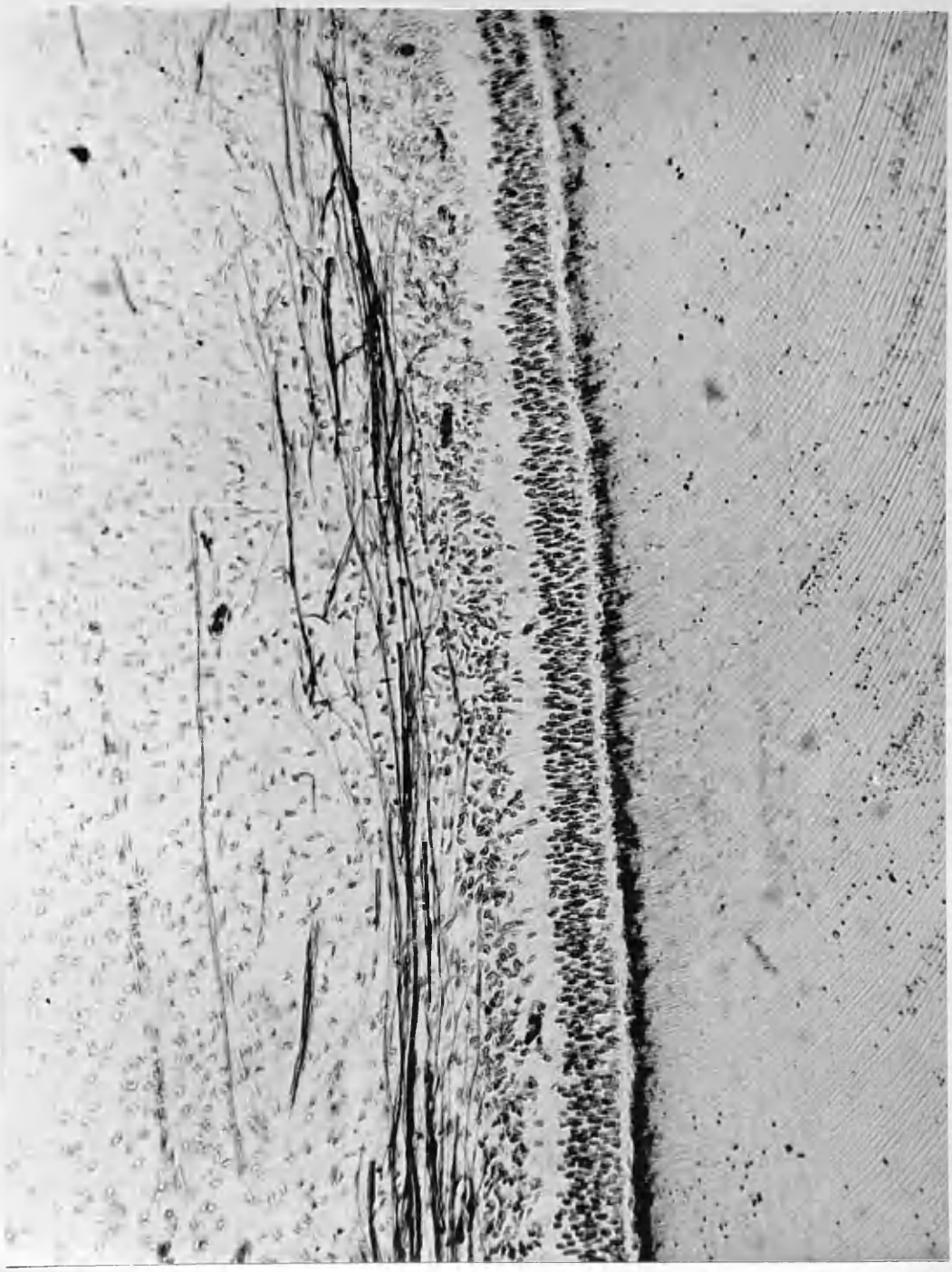

Fig. 25. The thicker fibres of the plexus are located within the pulp below the so-called cell-rich zone.

X 280

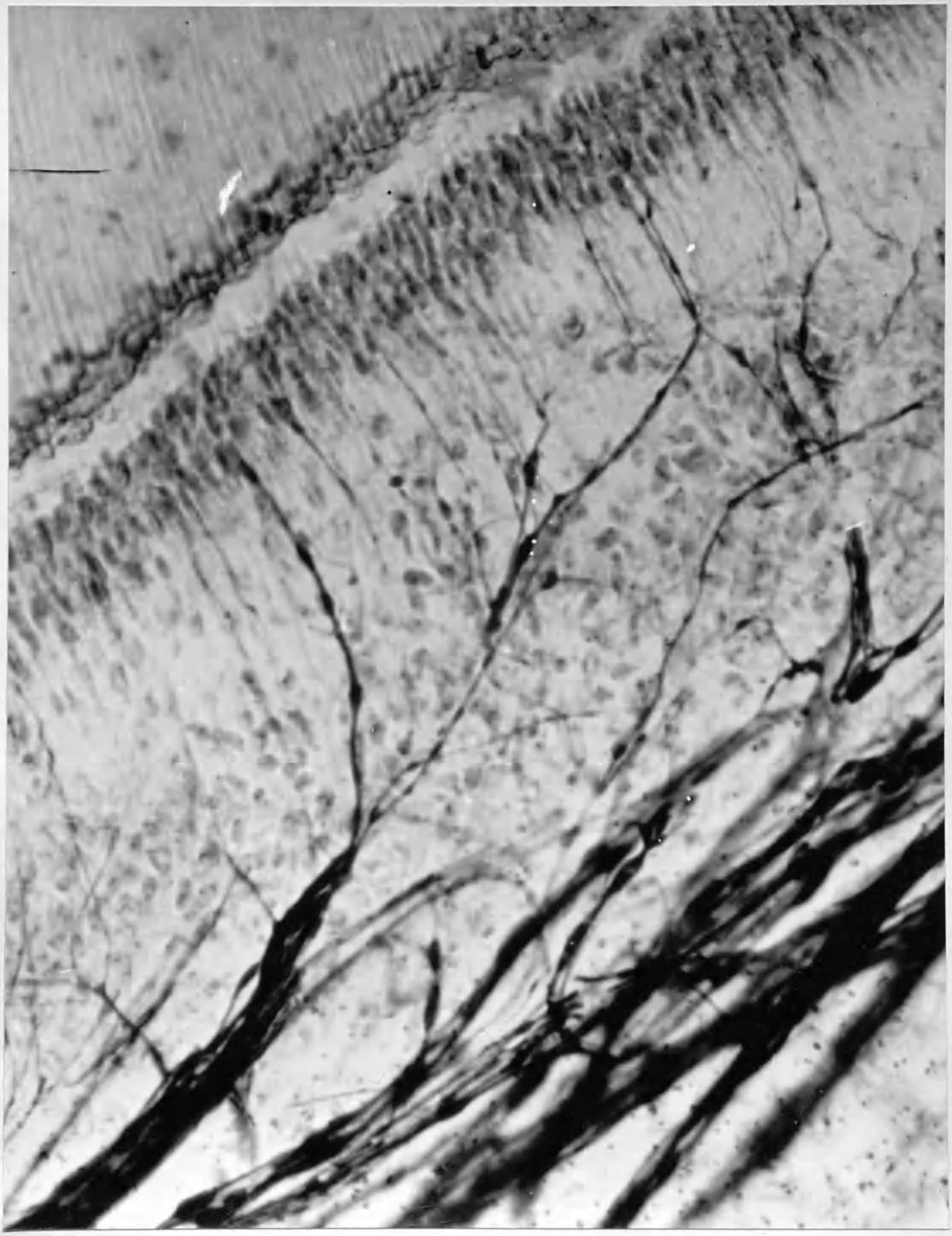

Fig. 26. The beads or fusiform enlargements are seen to occur on large and fine fibres alike in the plexus and as they cross the cell-free zone to enter the odontoblast layer.

X 560

Fig. 27. Below the cell-rich zone most of the thicker fibres run in a direction parallel to the surface of the pulp; whereas in the cell-free zone the general direction of the fibres which run to the odontoblast layer is perpendicular to the pulp surface.

X 400

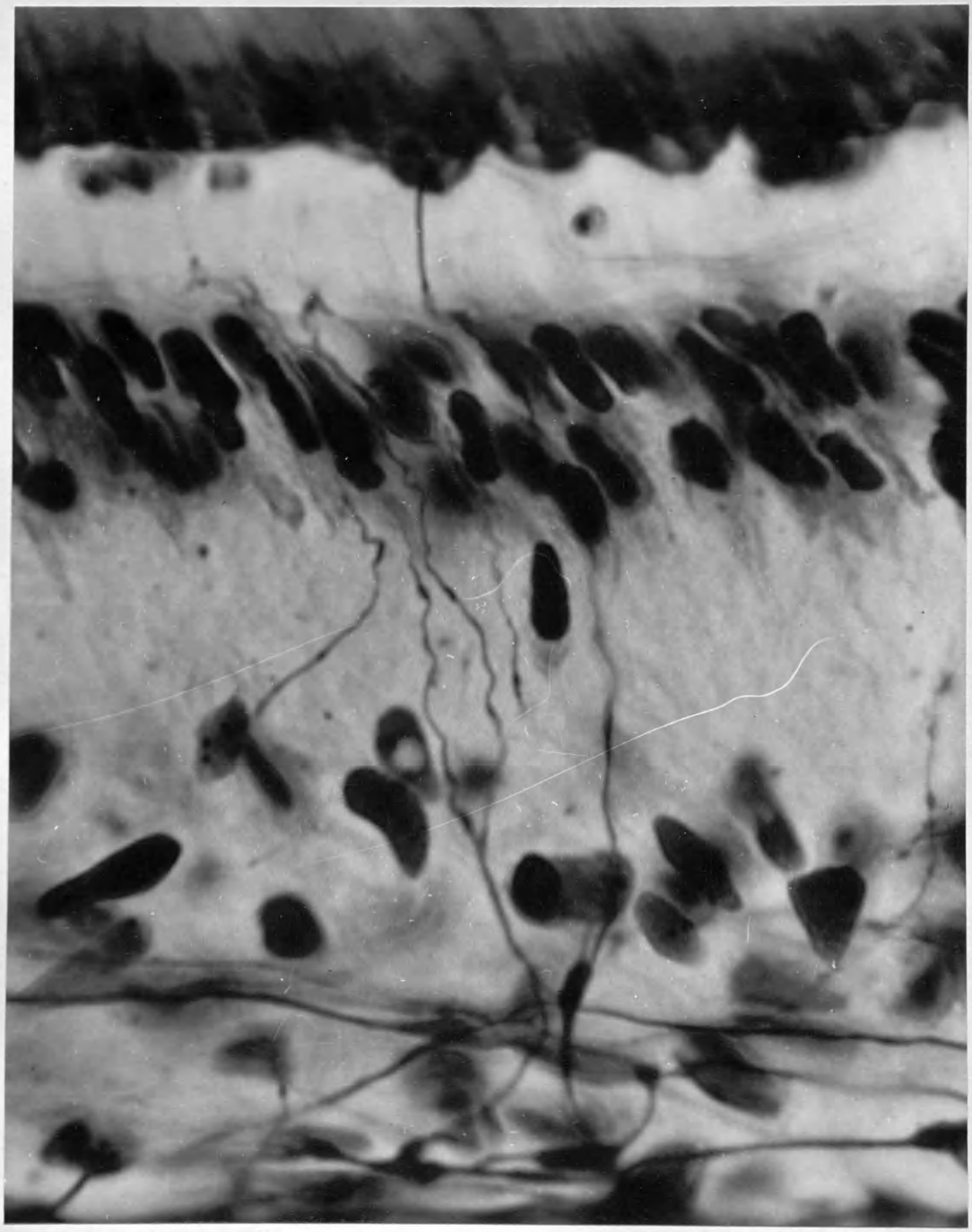

Fig. 28. Shows the course of several fibres through the odontoblast layer to the pulpo-dentinal junction and the predentine.

X 1,800

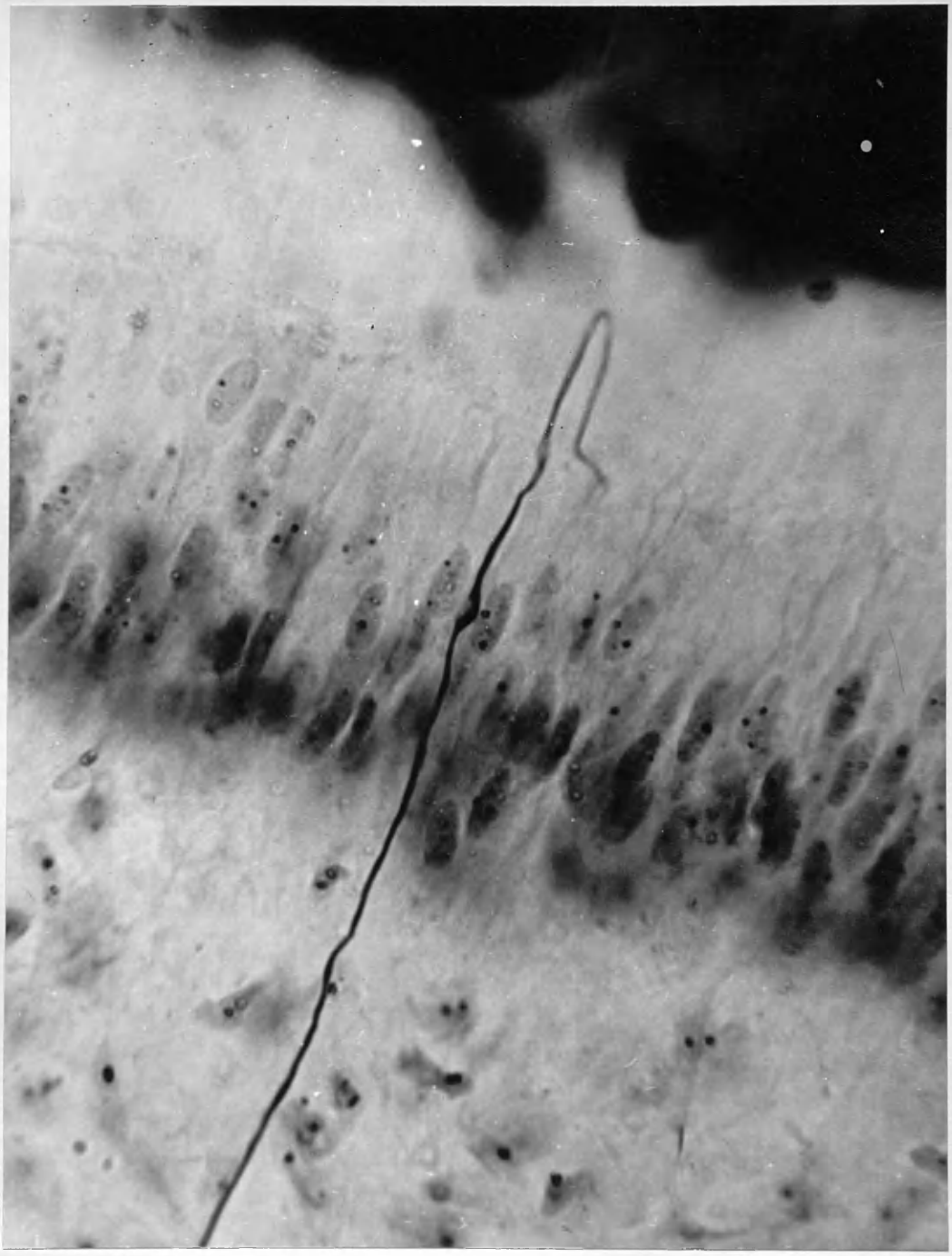

Fig. 29. The nerve fibre passes through the odontoblast layer, loops in the predentine and returns to the odontoblast layer. This is a common configuration.

X 1,400

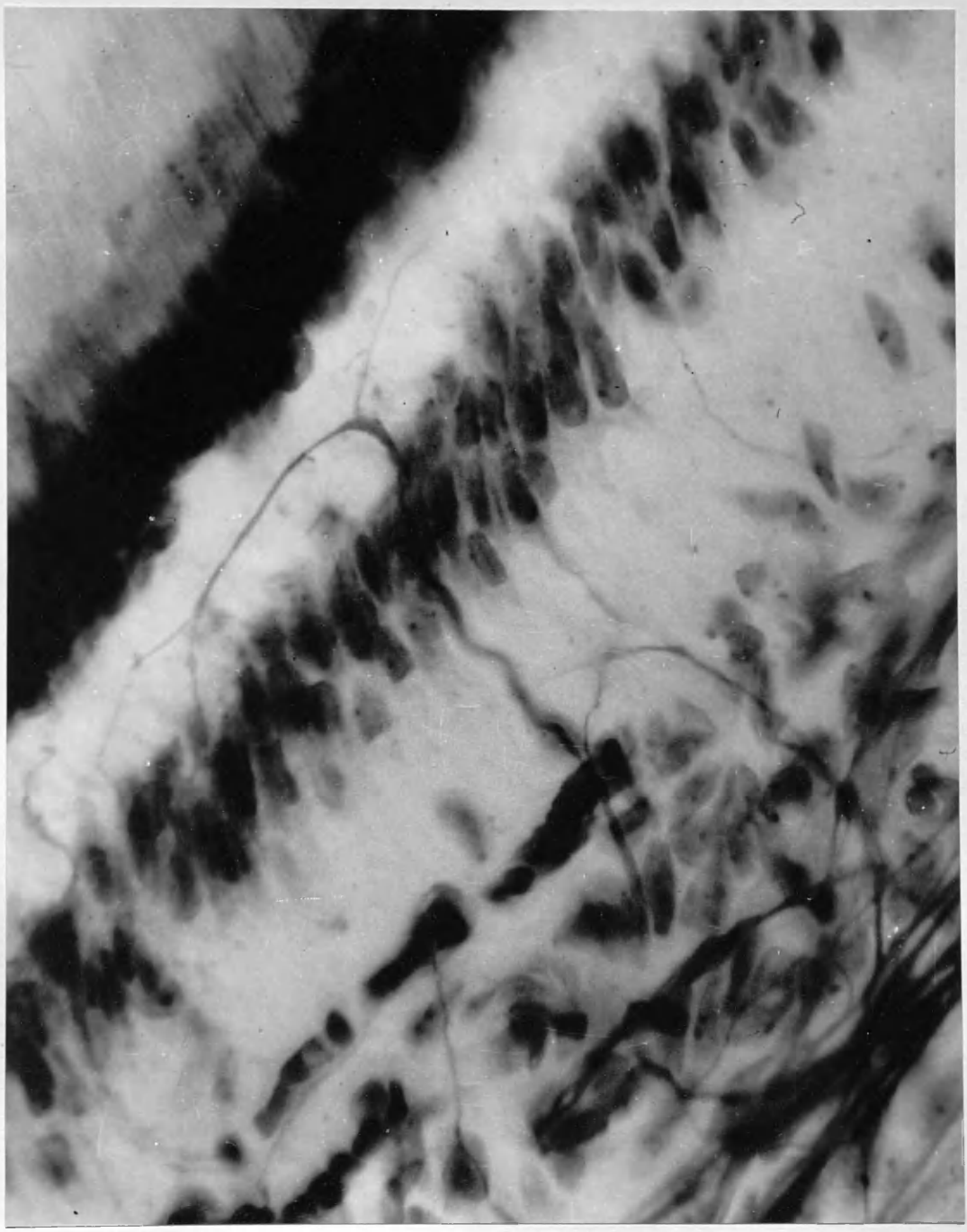

Fig. 30. A T-shaped branching of a larger nerve fibre at the pulpo-dentinal junction is quite common. It is believed to result from the inclusion of one or more of the finer terminal filaments in earlier layers of formed dentine matrix.

X 1,400

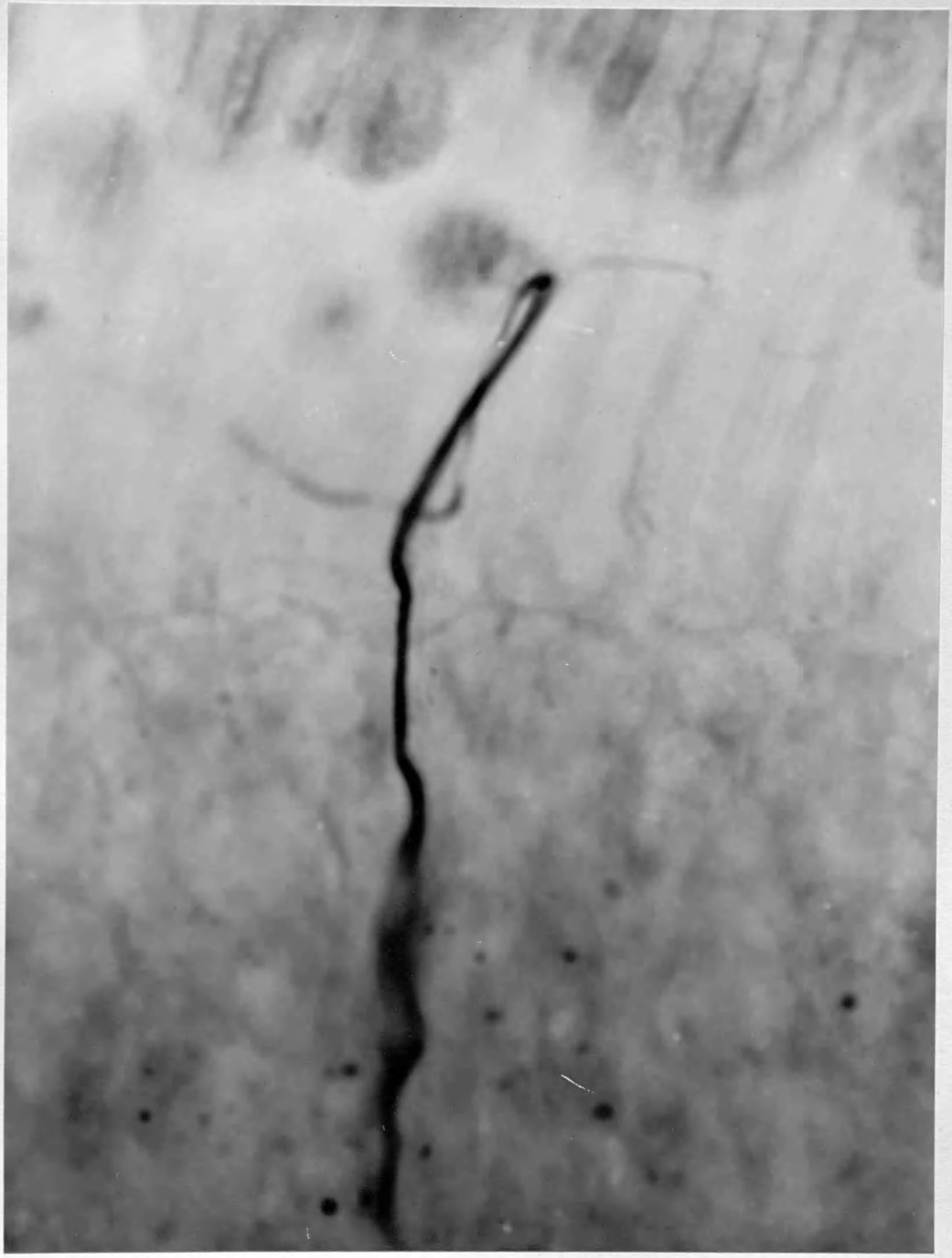

Fig. 31. The two terminal filaments of this dividing nerve fibre are included in different layers of the dentine matrix.

X 2,350

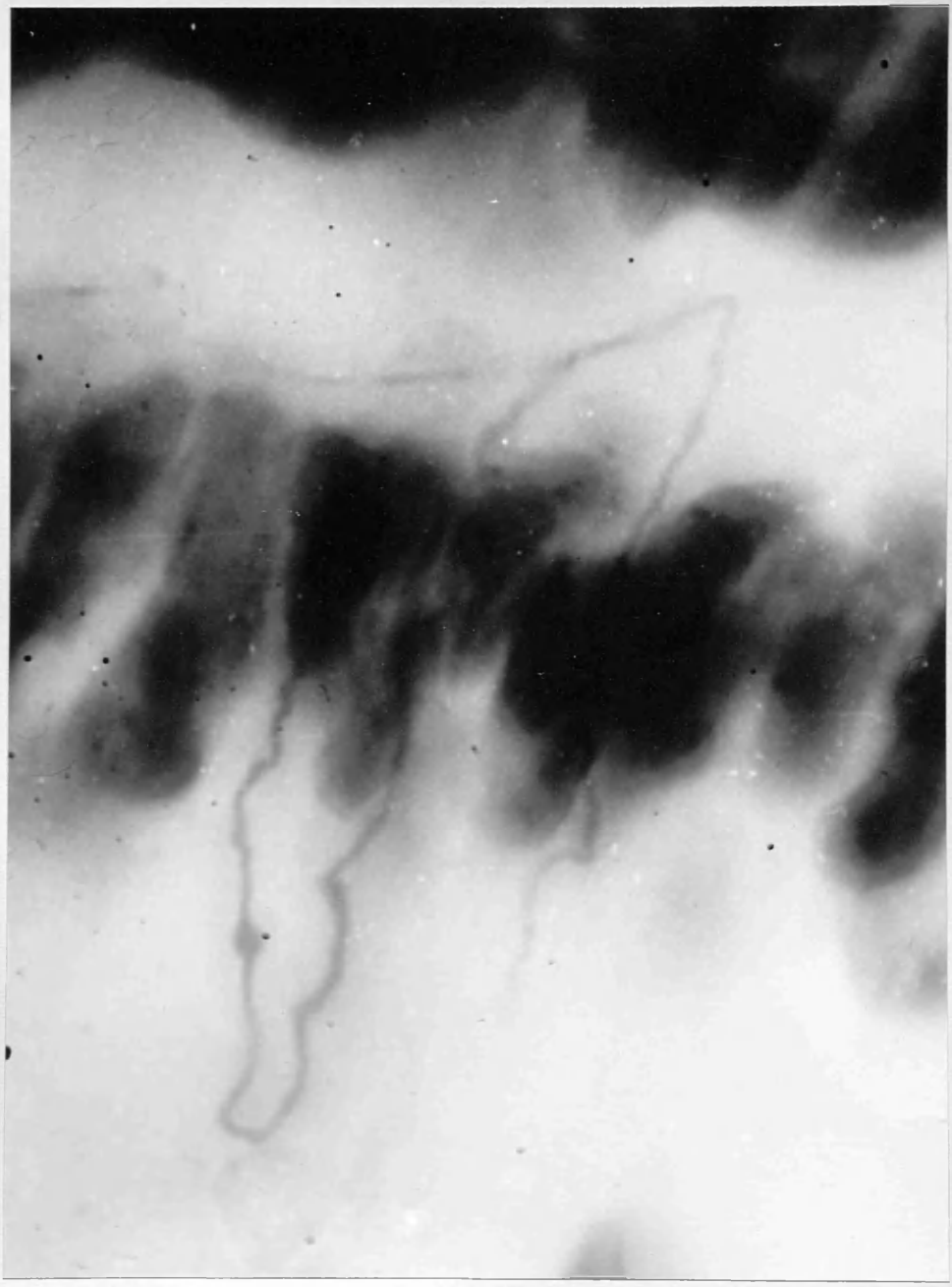

Fig. 32. As a result of a complicated entanglement in the forming predentine, this nerve fibre appears to loop into the pulp from the dentine side of the odontoblast layer.

X 3,100

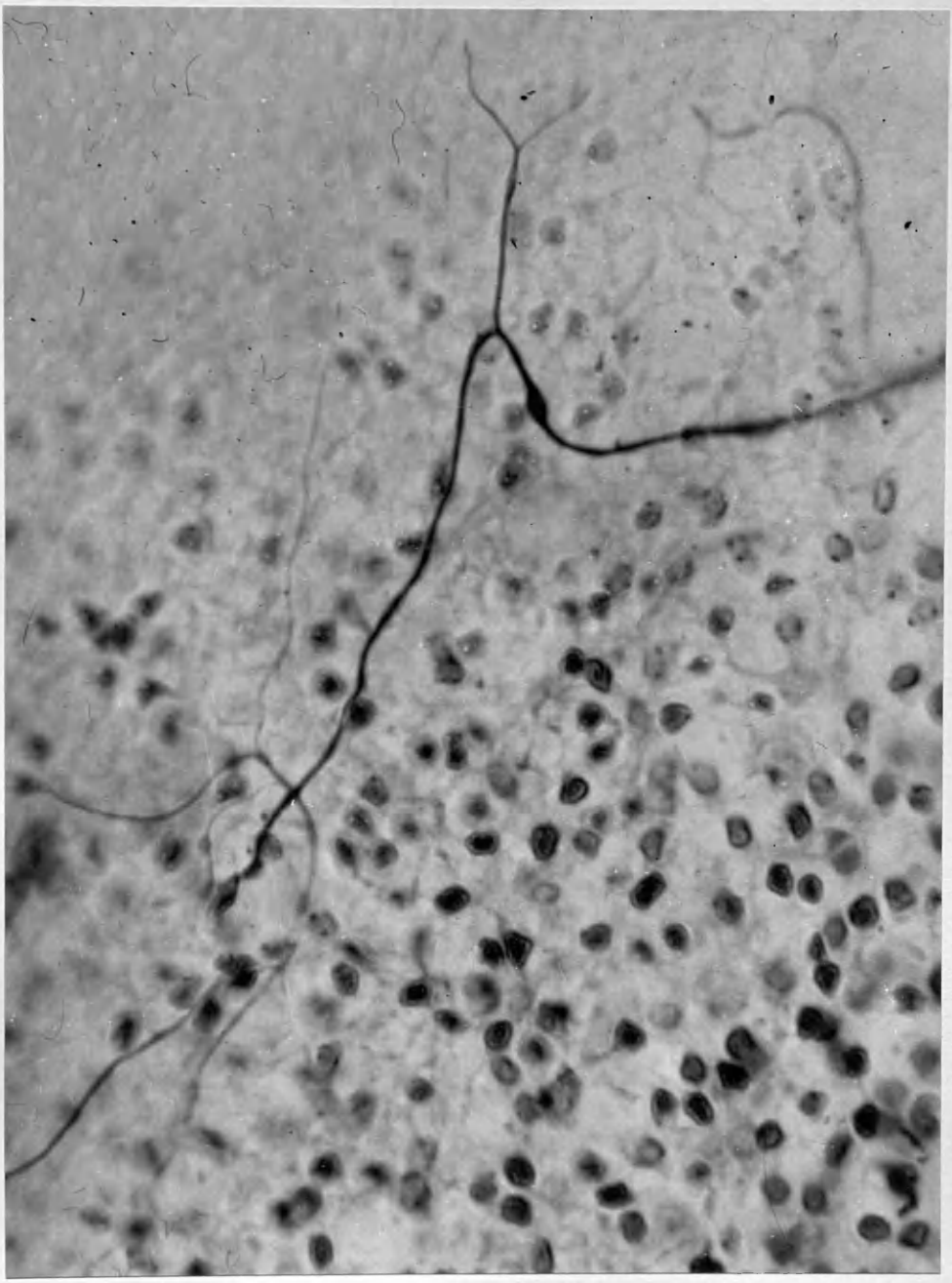

Fig. 33. An oblique section through the pulpo-dentinal junction shows the branching network of fine nerve fibres which may exist in this position.
X 1,030

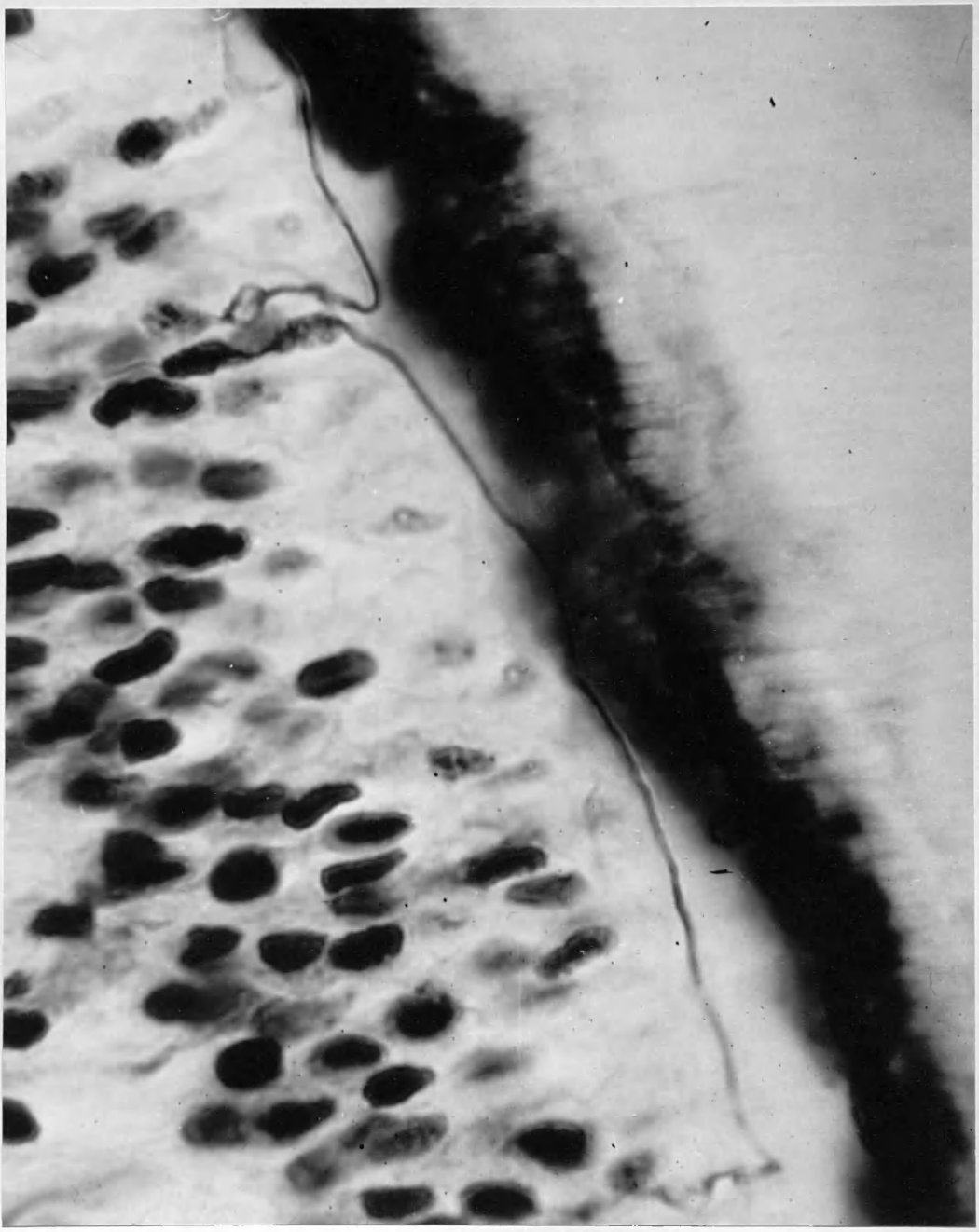

Fig. 34. Nerve fibres trapped in the predentine layer await the advancing front of mineralisation of the dentine matrix.

X 1,160

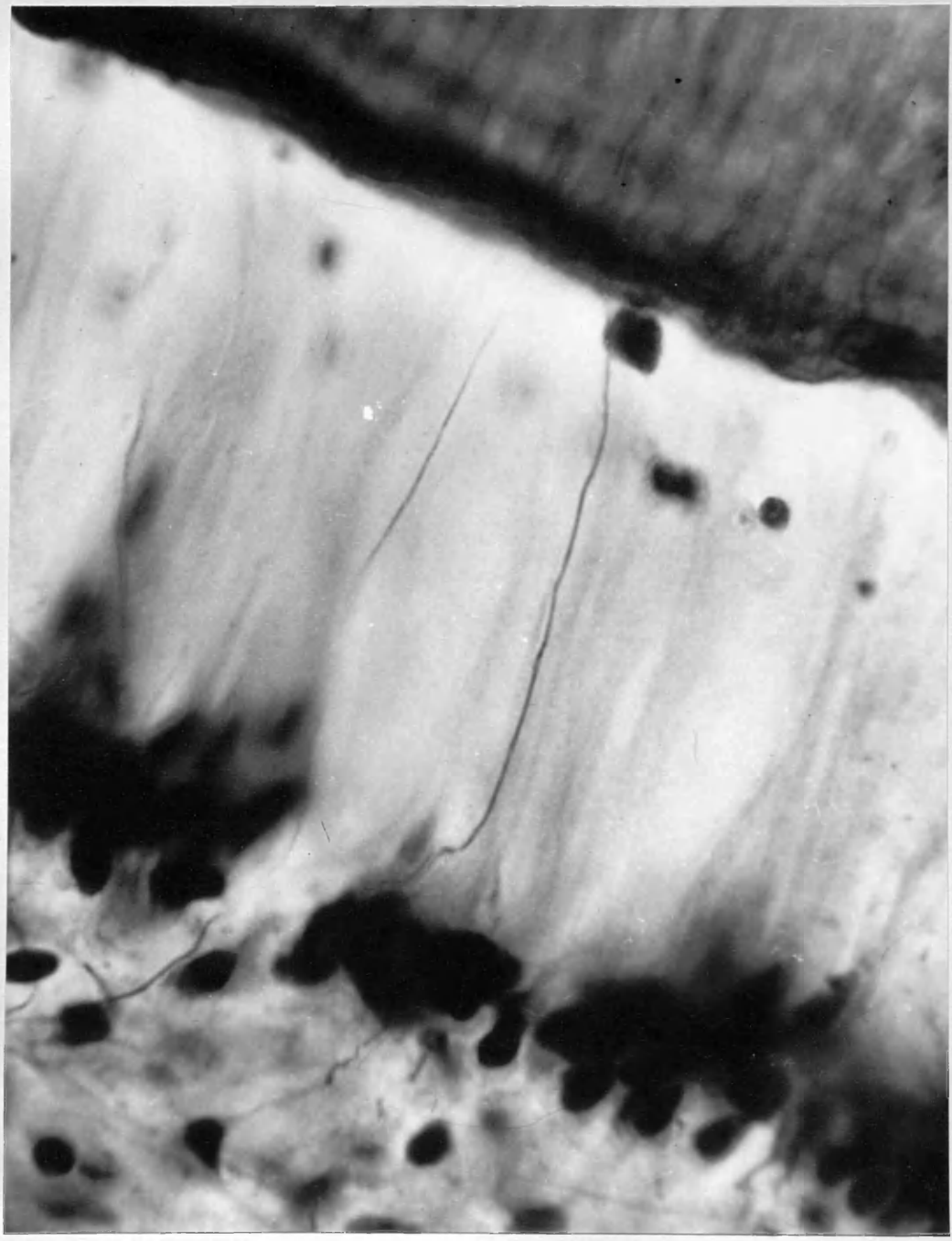

Fig. 35. During the formation of adventitial secondary dentine, the predentine zone widens. The length of nerve fibre embedded in the predentine is thus increased; the nerve does not move with the rapidly advancing front of predentine formation.

X 1,400

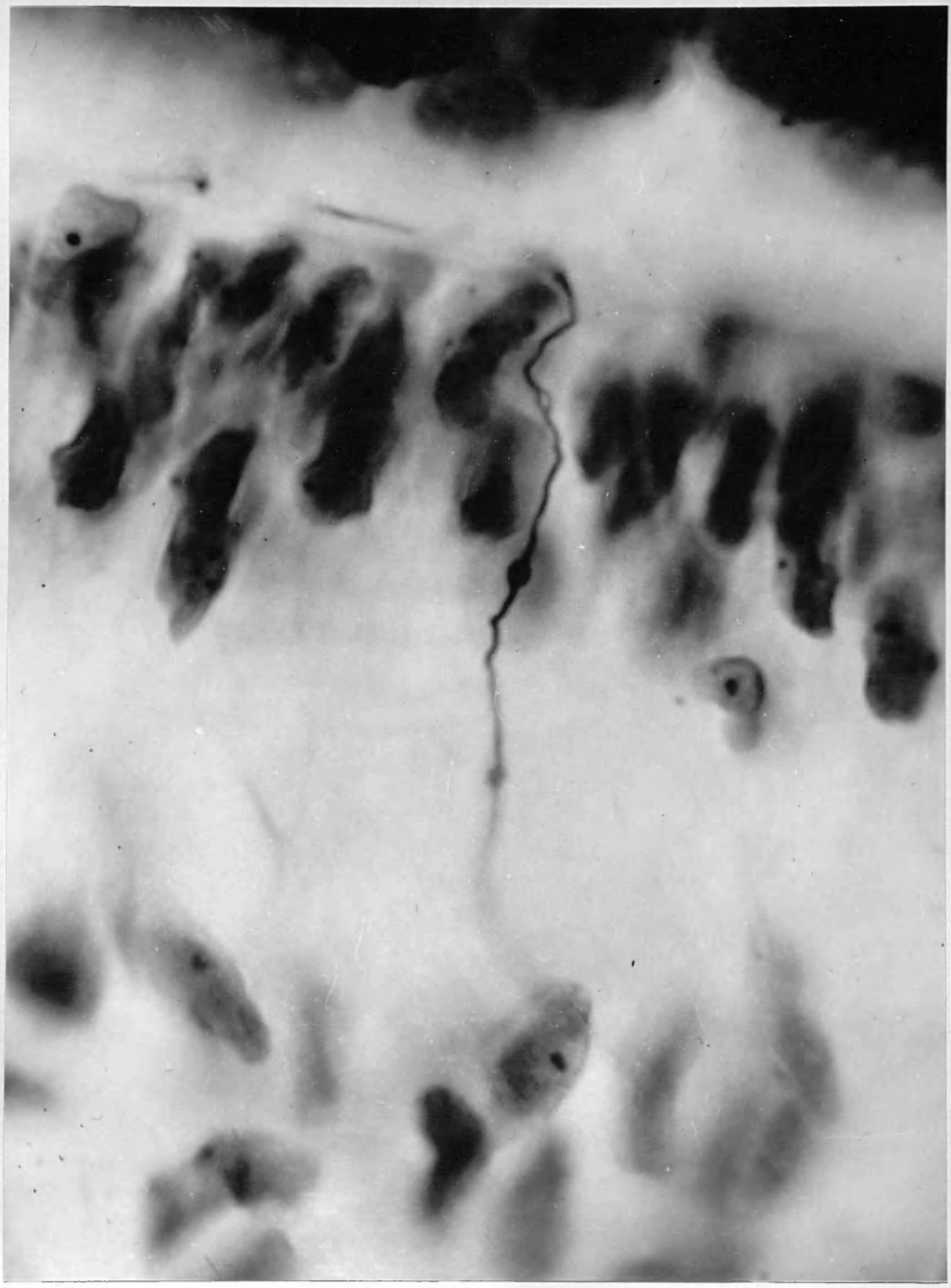

Fig. 36. Some beads on the fine terminal nerve fibres stain intensely black throughout while others have a clear unstained central area.

X 3,300

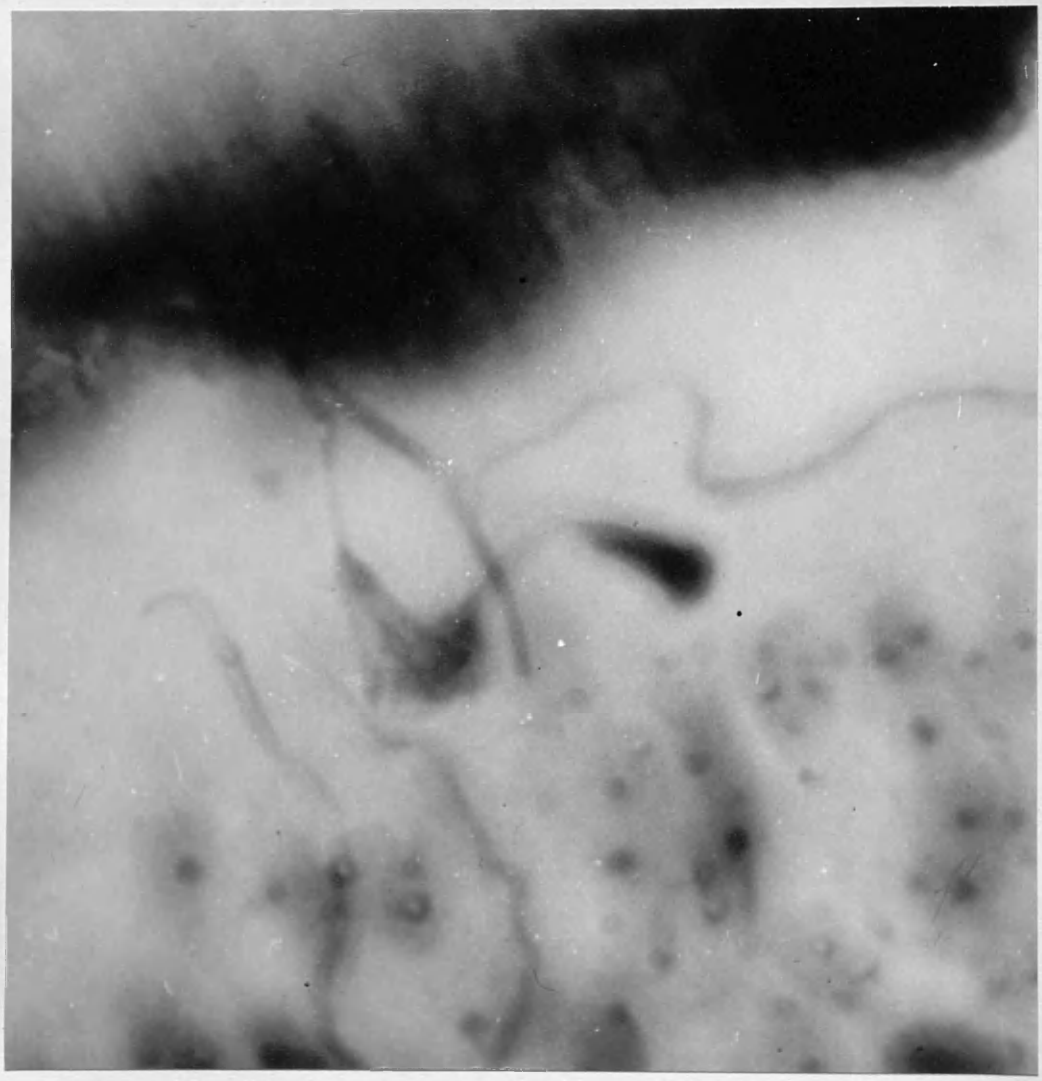

Fig. 37. The nerve fibre usually pursues a tortuous, three-dimensional course through the predentine and is difficult to follow. A large pear-shaped mass can be seen.

X 3,845

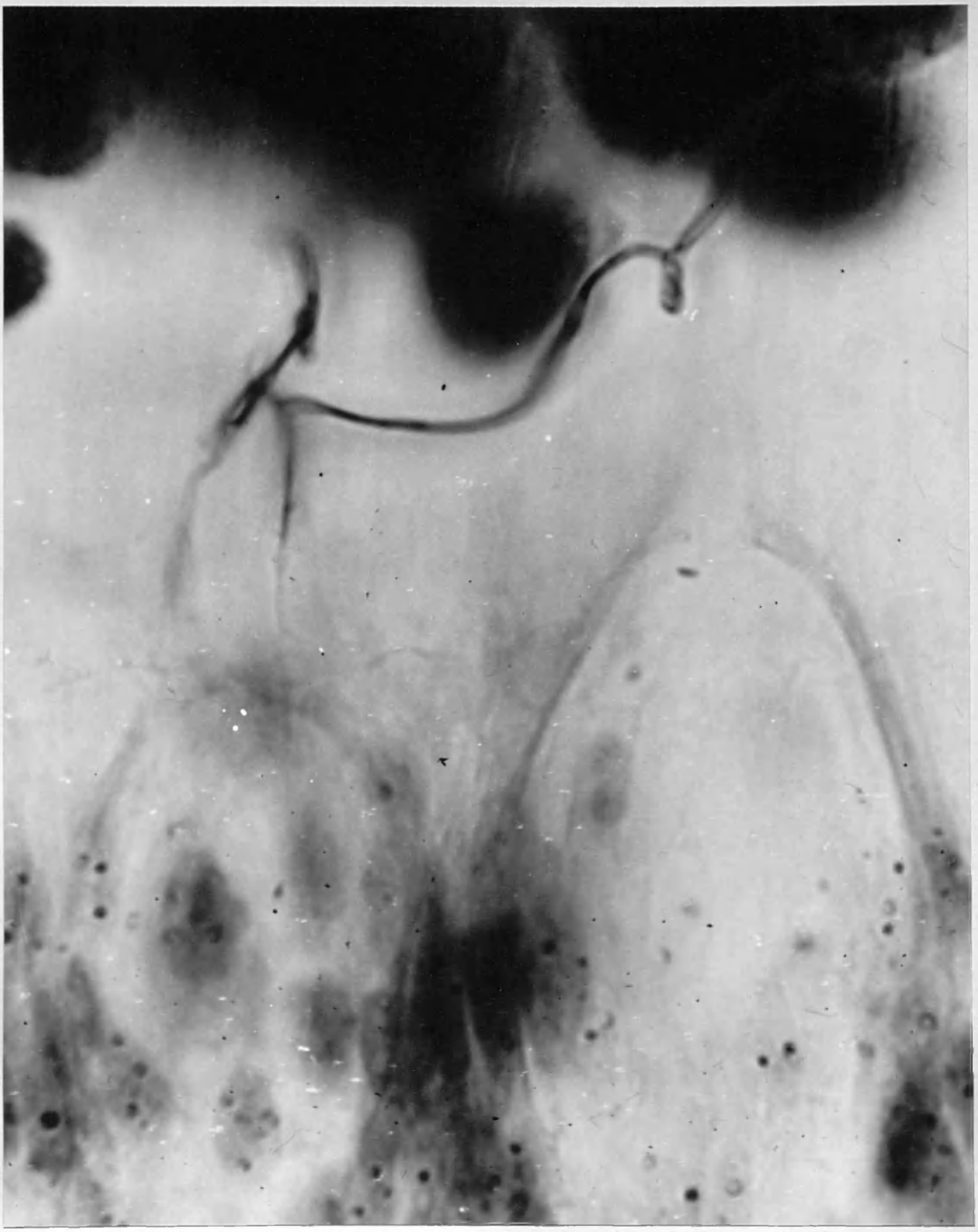

Fig. 38. Occasionally, small pear-shaped masses are observed hanging from nerve fibres which pass transversely through the dentine matrix. They are believed to result from deformation of beads or varicosities during matrix formation.

X 1,840

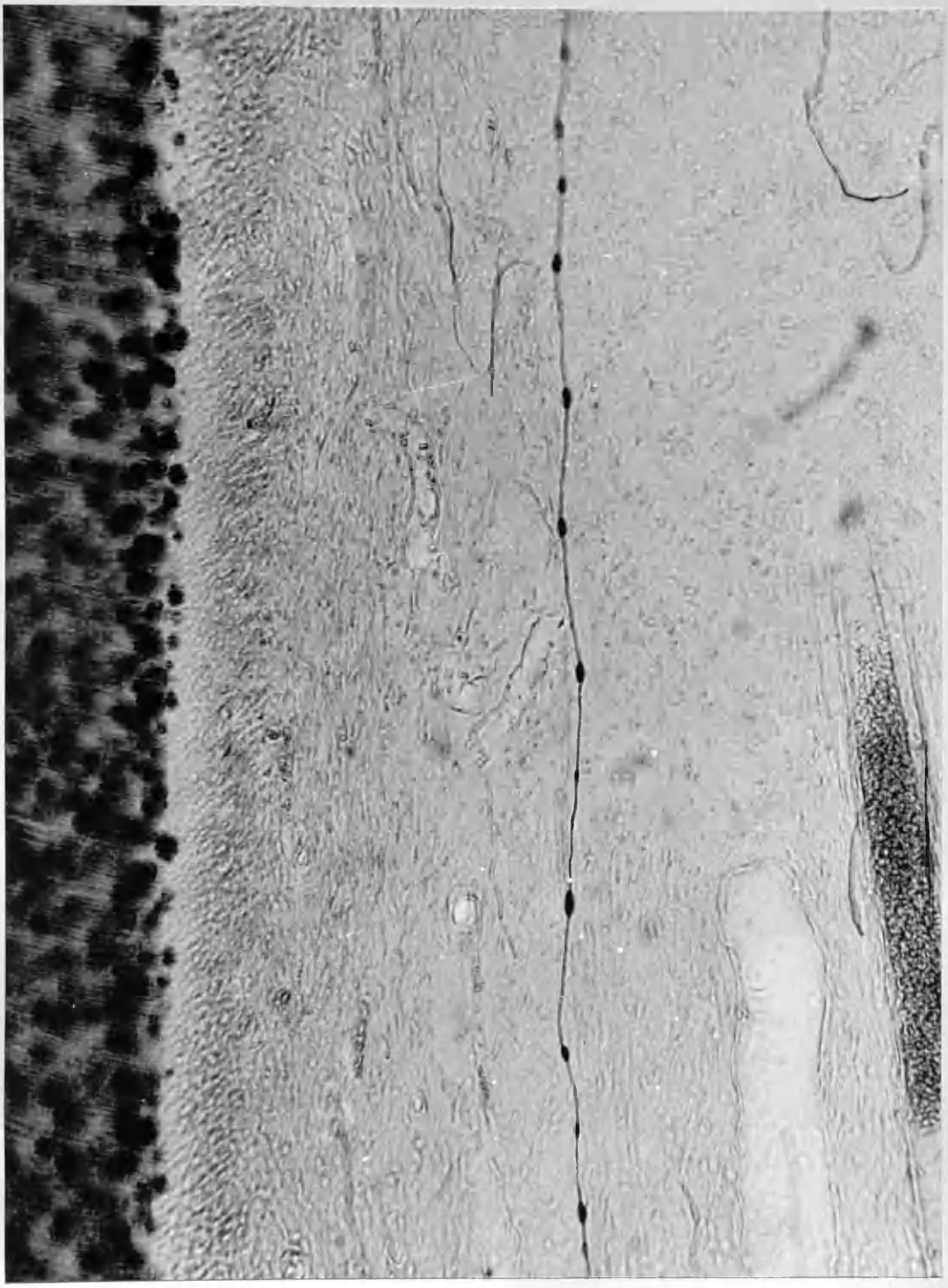

Fig. 39. The beads or swellings do not appear to be spaced at regular intervals nor is their size constant. There is only a rough correlation between the size of the beads and the thickness of the nerve fibre.

X 256

Fig. 40. It is important that beads or swellings should not be confused with stained nuclei of Schwann cells which lie in close proximity to the nerve fibre.

X 2,150

Fig. 41. The beads are present at all stages in the distribution of nerve fibres. They are shown among the fibres in a large nerve trunk in the pulp.

X 1,600

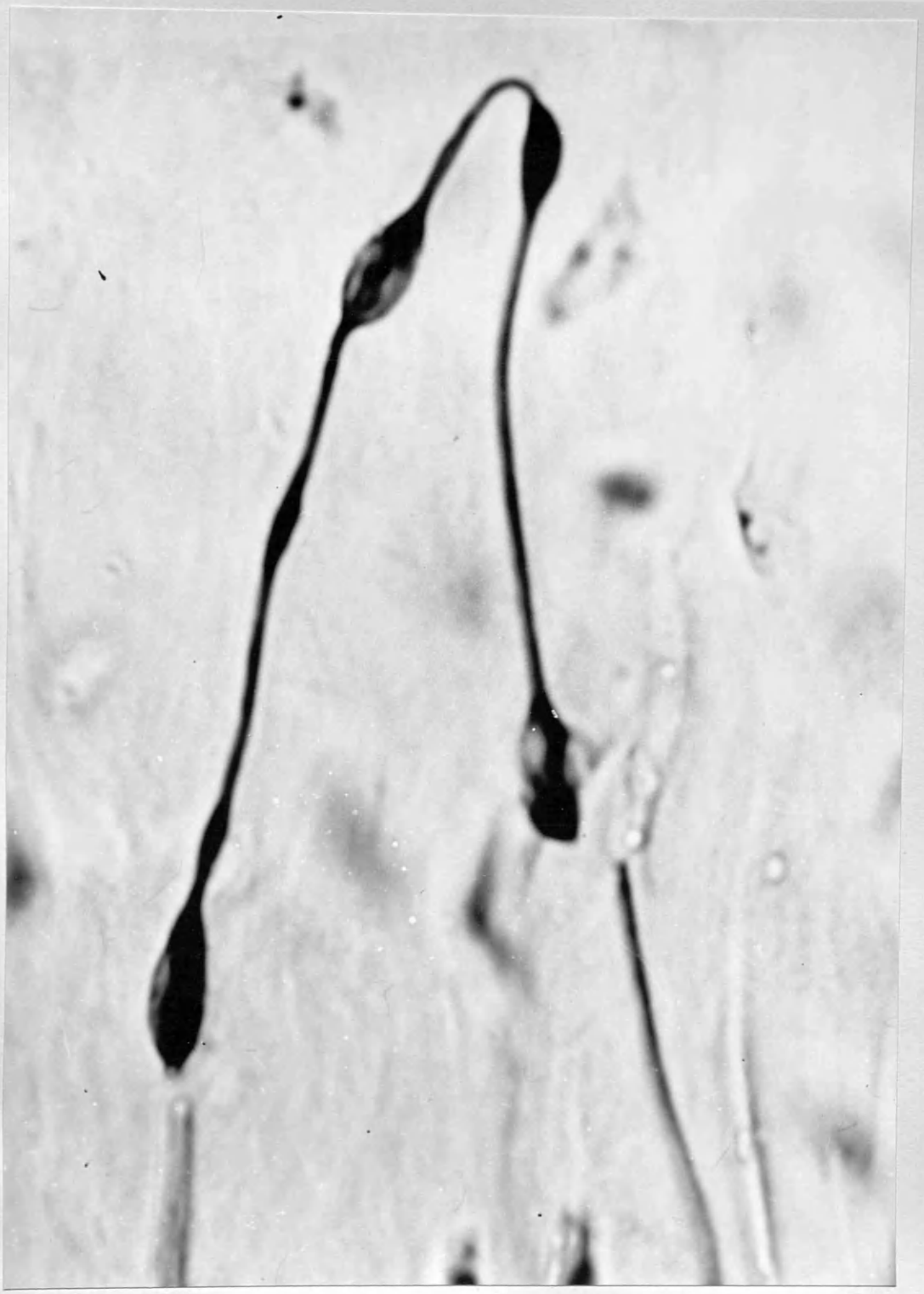

Fig. 42. It was observed that the junction between a nerve fibre and a bead was often a point of weakness in a nerve.

X 2,600

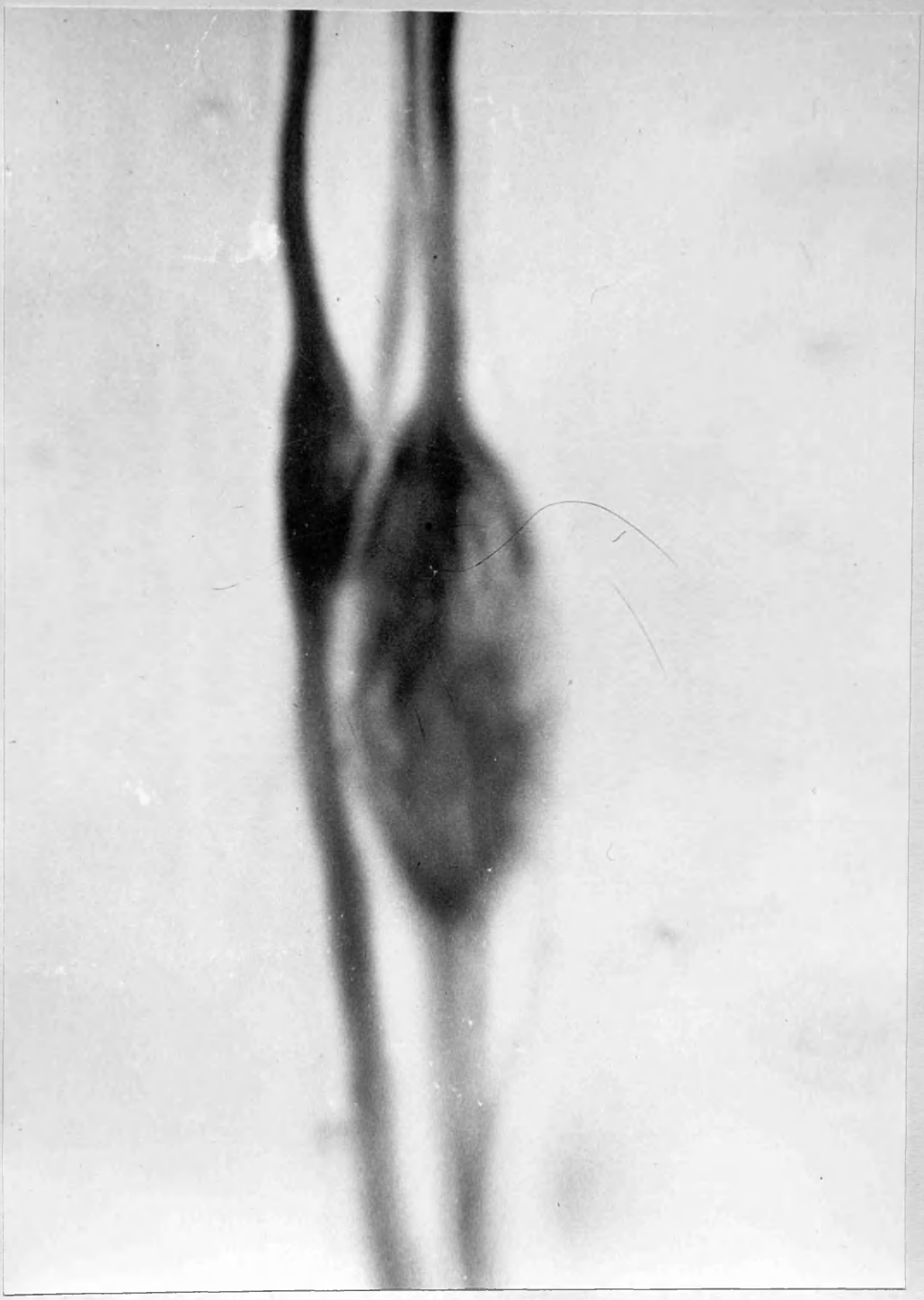

Fig. 43. The fact that the bead may also possess a myelin sheath is supported by this illustration where two neighbouring fibres are obviously separated by the unstained myelin layers.

X 4,100

Fig. 44. The internal structure cannot be visualised with accuracy by the light microscope but there is a suggestion that the bead contains lengthwise orientated fibrils which unravel at each pole.

X 14,600

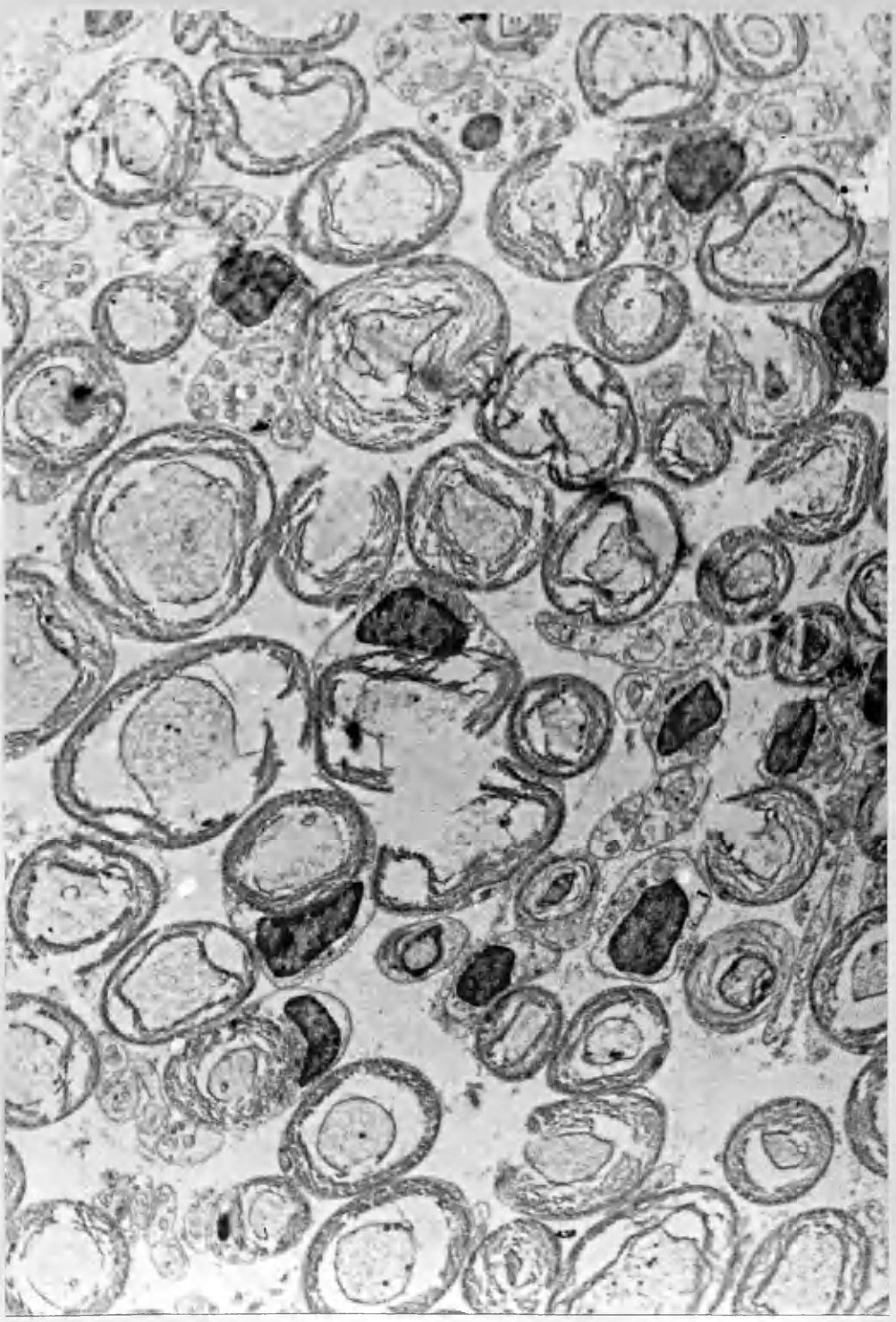

Fig. 45. Electron micrograph of nerve bundle consisting of myelinated and non-myelinated nerve fibres. The arrangement of the myelin has been greatly disturbed during processing.

X 4,200

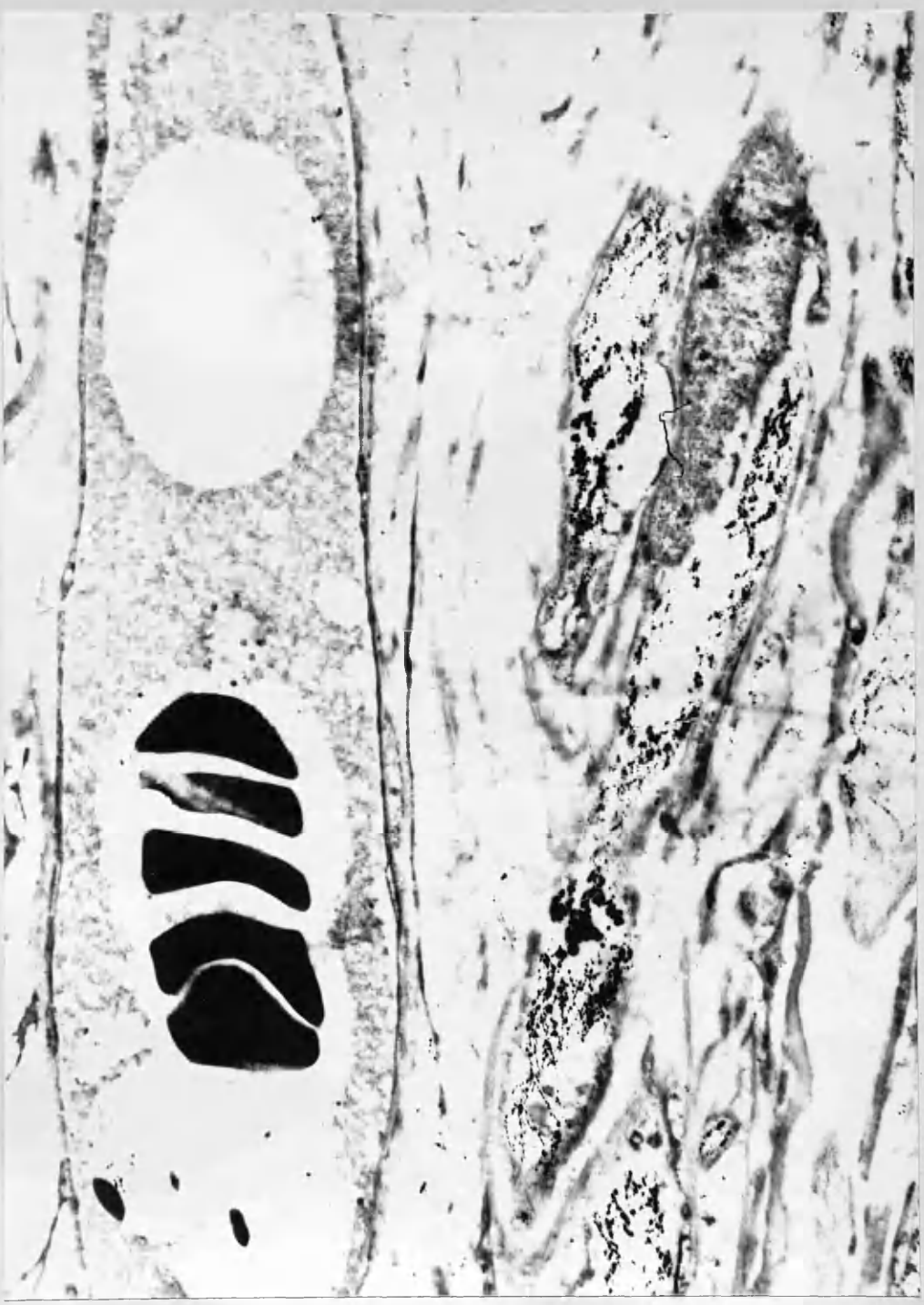

Fig. 46. Electron micrograph of a capillary with red blood corpuscles. Several longitudinally sectioned silver impregnated nerve fibres can be identified in the tissues beside the capillary.

X 4,200

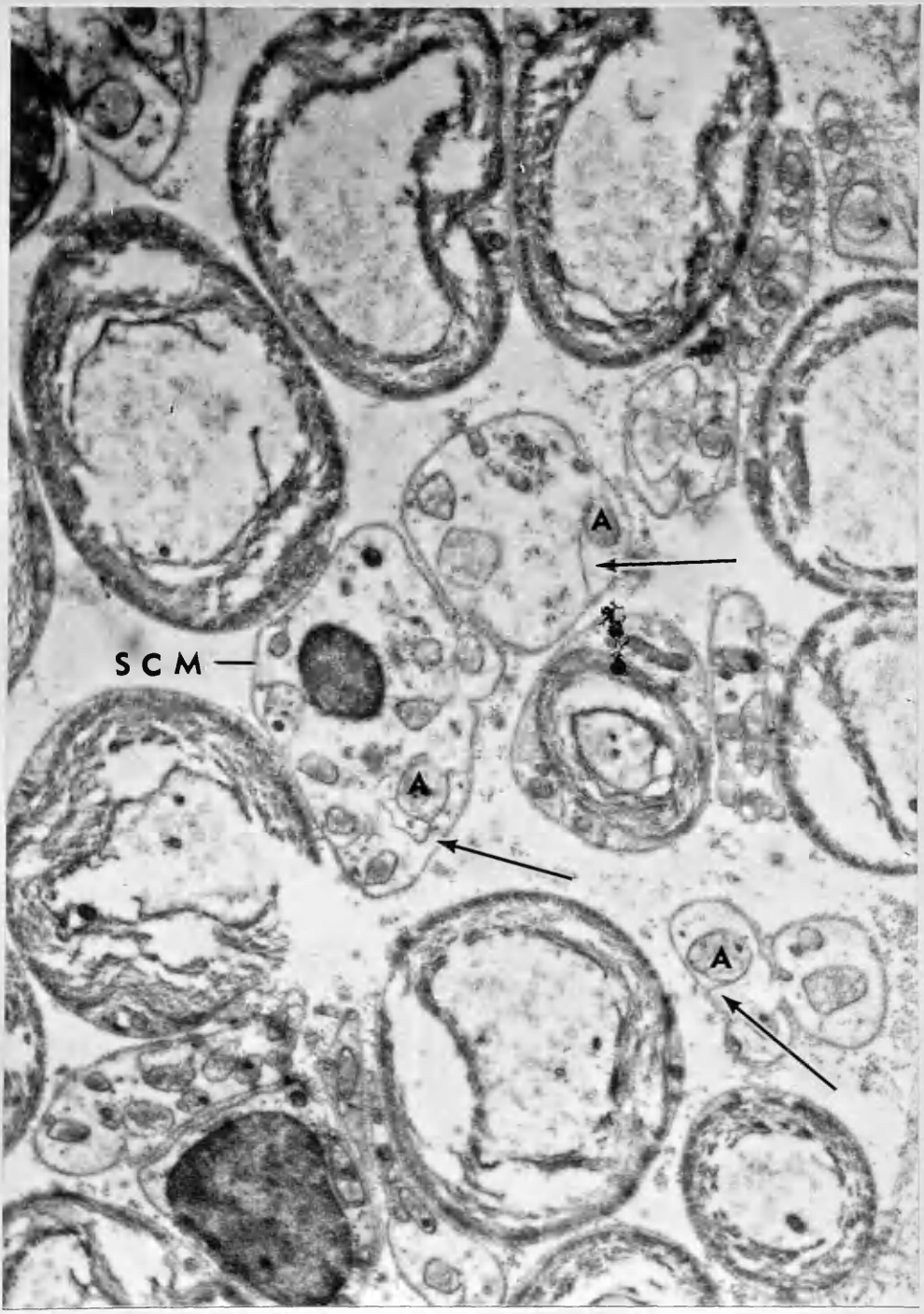

Fig. 47. The relationship between the Schwann cell membrane and numerous non-myelinated axons are shown. (SCM) and (A). Three mesaxons are indicated by arrows.

X 11,600

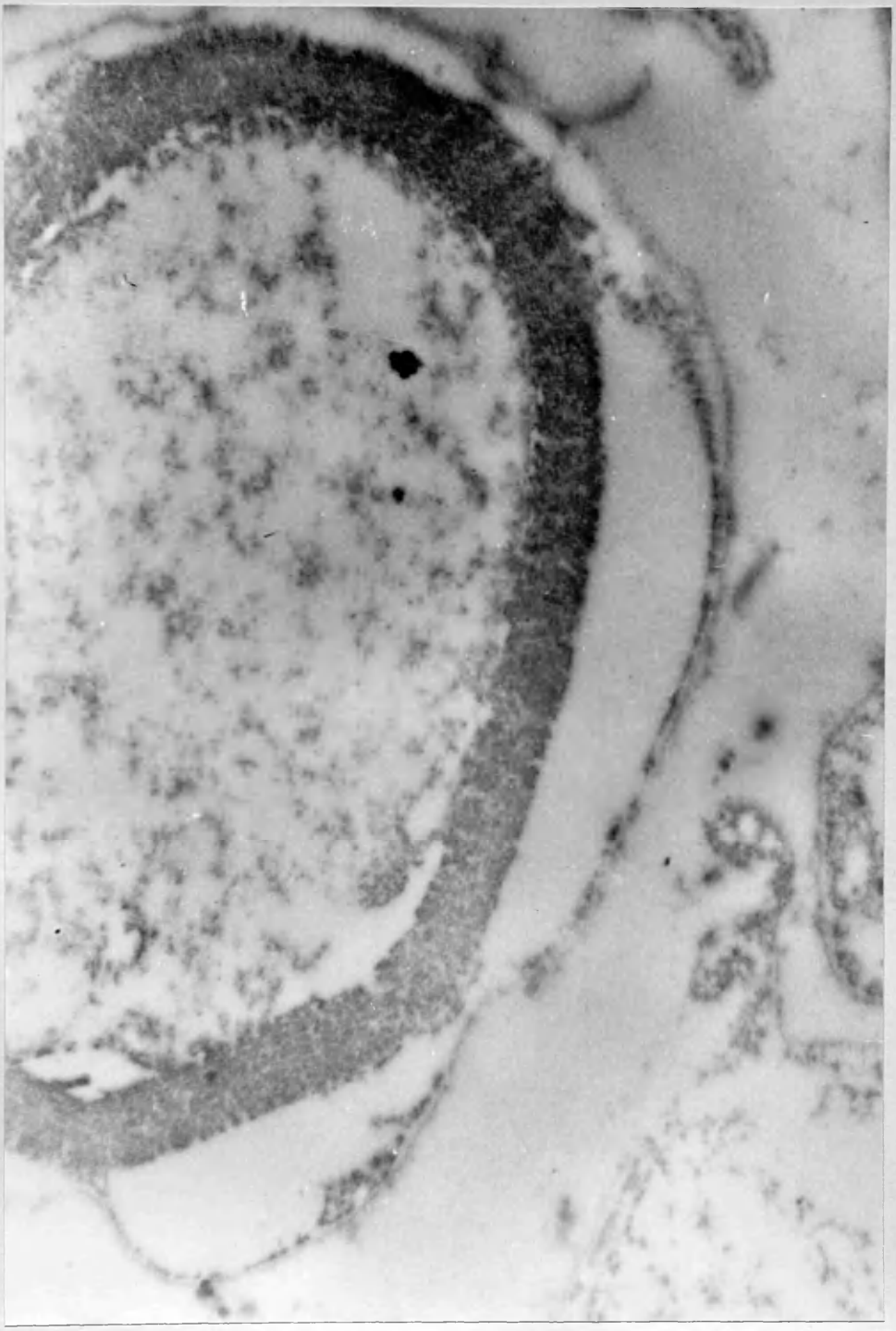

Fig. 48. Although poorly preserved, the laminated nature of the myelin sheath can be seen in this electron micrograph of a myelinated fibre.

X 34,000

Fig. 50. The silver particles are confined to the axon while the myelin sheath and outer connective tissue fibres are not impregnated.

X 22,000

Fig. 51. The arrangement of the silver particles in this axon suggests that the longitudinally orientated neurofibrils are impregnated.

X 11,600

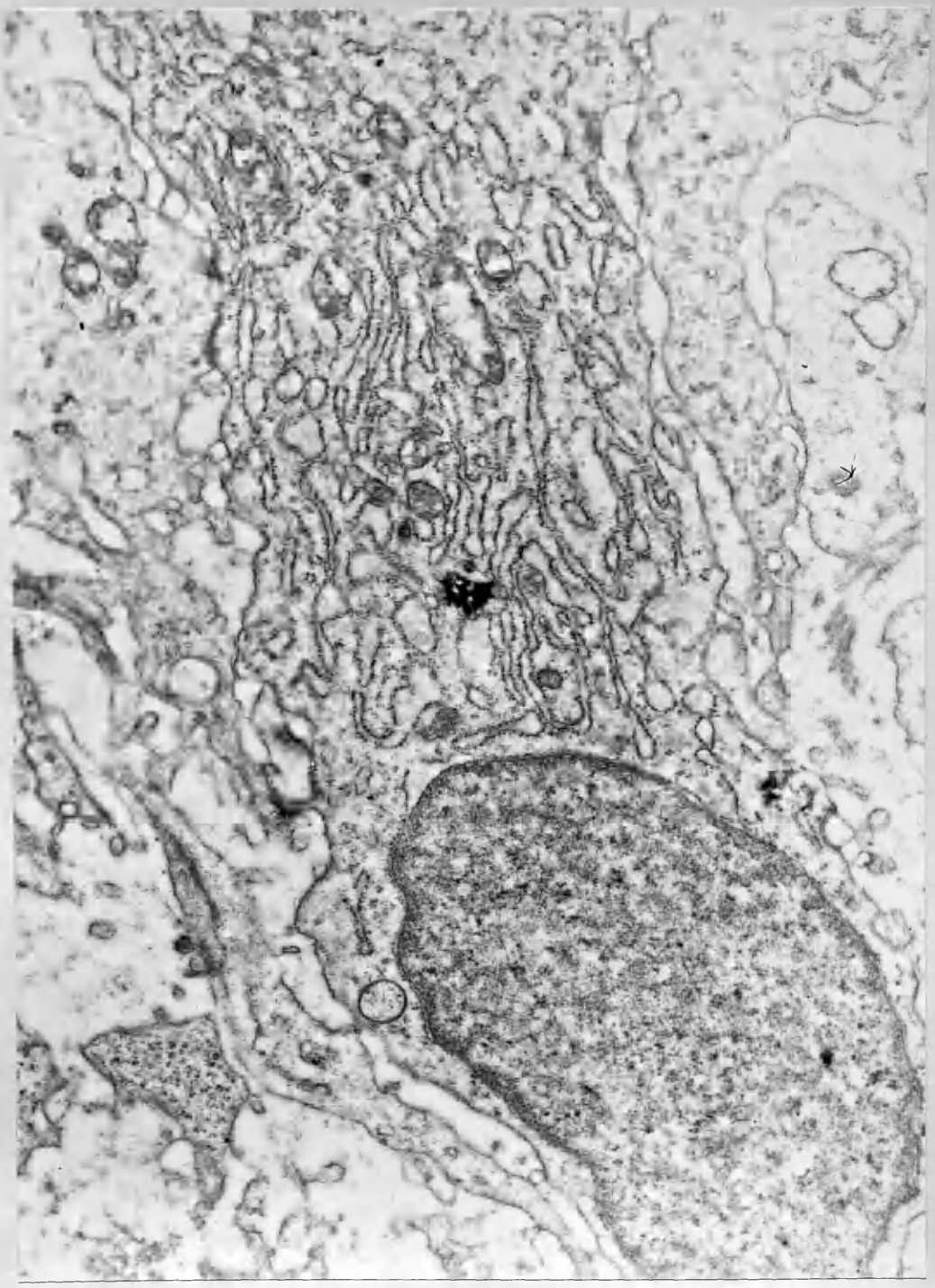

Fig. 52. The large nucleus belongs to an odontoblast cell whose column of cytoplasm extends towards the top of the illustration. The confusion between intra-cellular and extra-cellular structures can be seen.

X 11,600

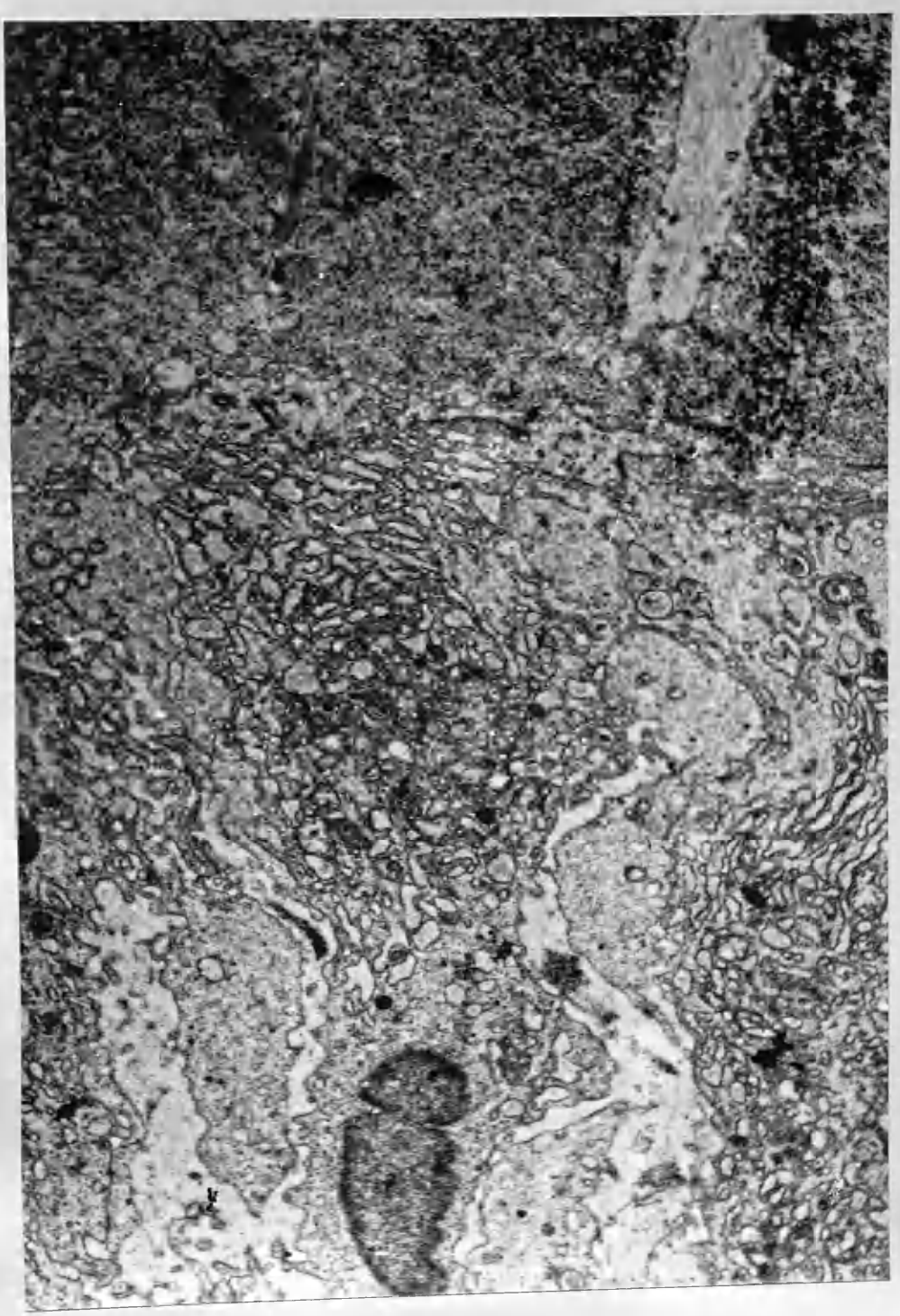

Fig. 53. The junction between the odontoblast layer of cells and the dentine matrix is poorly defined in electron micrographs.

X 5,000

Fig. 54. Dentine cut parallel to the direction of the tubules. The preservation of theodontoblast process suggested that the fine nerve fibres, if present, should also be able to withstand the processing procedures.

X 7,600

Fig. 55. Dentine sectioned across the tubules. No intra-tubular nerve fibres could be recognised in the many electron micrographs of this type which were examined.

X 11,600