Section of Odontology

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Paper

The Neurohistology of Human Dentine

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John Hunter (1770), writing about the sensitivity of the teeth, observed that this sensitivity is due to the nerve in the cavity of a tooth and his account reads: 'We do not observe the same violent effect from any other nerve in the body being exposed either by wound or sore, as we do from the exposure of the nerve of a tooth. Perhaps the reason for the intenseness, as well as the quickness of the sense of heat and cold in the teeth, may be owing to their communicating these to the nerve sooner than any other part of the body.'

It is not surprising, therefore, that the point of greatest controversy since Hunter's comments has been whether sensory nerves end at the periphery of the pulp or whether they end in the calcified part of the dentine.

None of the research workers who have since attempted to demonstrate nerves in this tissue can be said to have done so unequivocally. The literature, which has been reviewed by Brashear (1937), Bernick (1948) and Arwill (1958), remains inconclusive.

One of the most important objectives in the interpretation of histological data is to attempt to relate histological appearances with the living state. Almost invariably the early research which has stood the test of time is that of such workers as Retzius, Schwann, Tomes, Boll and many others who, in spite of great technical difficulties and, by modern standards, crude instruments, gave us vivid and accurate factual descriptions of the tissues which they studied, although their interpretation was often in error. It is significant that these workers examined freshly obtained tissues which were often unfixed and they applied only the simplest of chemical and physical tests to them. It is the evolution of the complicated series of manipulations now commonly employed in modern histology and which purport to show structures more clearly, that has given rise to complex artifacts which are extremely difficult to interpret and which tend to obscure an understanding of cytology as a vital process. In fact there are cases where failure to appreciate the distinction between the living state and the appearance of fixed sections has led to a retardation rather than an advance of knowledge. The innervation of human dentine is a problem which should be viewed in this light.

My approach to the problem of the neurohistology of dentine was, therefore, to study first the nerve fibres in vertebrate skin in the fresh unfixed and unstained state and then after staining with methylene blue, using the techniques of Schabadasch (1935). The results were then compared with those obtained from sectioned material after various fixative, embedding, decalcification and metallic impregnation procedures. The morphology of the nerves demonstrated by these techniques was compared carefully with that shown by other workers, namely Weddell & Zander (1950), Harrison (1907, 1910), Speidel (1932, 1933, 1935, 1942) and Hughes (1953). Many of the simpler experiments of these workers were repeated in order to gain experience of the appearance of the morphology of nerve fibre terminations under various conditions. For example, growing nerves were watched for short periods in the tails of living amphibian larvæ and post-mortem changes were followed in methylene-blue preparations of terminal nerve fibres in mammalian skin.

In the naked terminal portion of nerves, marked changes in the outline of the axon are frequently present in the form of minute bead-like swellings. Fusiform and vesicular enlargements also occur in this part of the nerve and these are often associated with changes in the staining density of the axoplasm. The full significance of these changes in the outline form and the changes in staining density of the axon is by no means established. It is quite possible that beading, swelling and vesiculation of axoplasm are post-mortem changes or are produced by treatment with fixatives. This is

not a completely satisfactory explanation, however, since according to Speidel (1935, 1942) many of these structural features can be observed in living nerves. The small spherical, densely staining beads are often, but not invariably, arranged regularly along the axon near its termination. The diameter of axons possessing such beading is usually below 1 μ and there is no supporting Schwann cell. Less frequently, small spherical enlargements with unstained centres containing vacuoles can be found in the axon; these I call vesicular swellings to distinguish them from the spindle-shaped or fusiform swellings with unstained centres that also occur. Fusiform bodies with unstained centres are frequently formed at the junction of two or more branches. Occasionally, at what appears to be the growing tip of an axon, flattened film-like areas of lightly stained axoplasm, often containing droplets of stained material and vacuoles, may be found. These strongly resemble the growth cones of growing nerves. The bead-like, vesicular and fusiform structures, therefore, provide a valuable guide for the identification of small nerve fibres. These structural characteristics of small terminal axons are, furthermore, related to the state of the axoplasm at the time of fixation (Fig 3).

My own study of the neurohistology of human dentine has been made mostly on silver impregnated material. It is appropriate at this juncture, therefore, to outline the morphological characteristics of silver impregnated fibres which are regarded as nerve fibres in this study. Silver impregnated fibres not associated with Schwann cells were regarded as nerve fibres only if they satisfied the following six criteria:

(1) The diameter of the fibres should fall within the size range of terminal nerves, i.e. 1·0 μ and less.

(2) The silver deposited in or on the fibres should possess a fine grain size resulting in an apparently smooth impregnation in obvious contrast to the coarse grained deposit of silver more commonly found in non-neural connective tissue fibres. This criterion is only valid for the silver technique finally adopted for this study, since the quality of the impregnation depends entirely on the care with which the various stages in the impregnation are controlled (Fearnhead & Linder 1956).

(3) The presence of densely impregnated beads, or lightly impregnated fusiform or vesicular swellings, situated periodically along the fibres, was regarded as an important morphological characteristic indicative of small terminal naked axons.

(4) The presence of branching, in fibres, which also satisfied conditions (1) to (3) was regarded as an additional feature consistent with the morphology of terminal nerve fibres.

(5) The criteria (1) to (4) were only regarded as valid if, in the same section, fibres of similar size and morphology could be found in continuity with larger nerves.

(6) That the fibres in the dentine and pulps of teeth which satisfy all the previous criteria, should disappear following nerve resection experiments.

Material and Methods

Both pre-natal and post-natal human teeth were studied. The pre-natal material consisted of the complete or partial dentitions of 13 fœtuses ranging from approximately 6 weeks to 7 months menstrual age. I am indebted to Professor J D Boyd for the loan of 5 of these specimens. These were the only preparations impregnated with silver by the De Castro method. The post-natal material consisted of 18 deciduous teeth with an age range of 3 days to 9 years, 29 permanent teeth obtained from patients between the ages of 10 years and 53 years, and 25 permanent teeth of unknown age. Some of the teeth studied were carious; some, extracted for orthodontic purposes, were apparently caries free. With the exception of the De Castro impregnated sections the fixatives used were either 10% neutral formol saline, or Bouin's fixative. Decalcification was achieved with formic acid/sodium formate solution according to the method of Kristensen (1948). The silver impregnation method used was that described by myself and Linder in 1956. A monkey was used for the experimental section of the nerve supply to the teeth.

Observations

Pioneer nerve fibres arrive very early in the embryonic tissues of the jaws and quite large nerve trunks are present and well established in the tissue in which the tooth germs develop and grow. According to Hogg (1941) intra-epithelial sensory nerves can be demonstrated and correlated with functional capability in human fœtuses of 8–14 weeks menstrual age, when the fœtus is approximately 30 mm crown-rump length.

The dentine papilla is small in this early stage of tooth development and nerve fibres can be demonstrated in close relationship to the tooth germ. In spite of their close proximity to the base of the dentine papilla, the nerve fibres are orientated tangentially to the tooth germ and do not enter the substance of the dentine papilla (Fig 1A). The bending of these fibres suggests that they had either deviated in their course to avoid the growing tooth germ or that the tooth germ had pushed its way between groups of fibres already present. In the light of current views each of these possibilities is reasonable since not only may the path of the growing nerve be deflected by the orientation

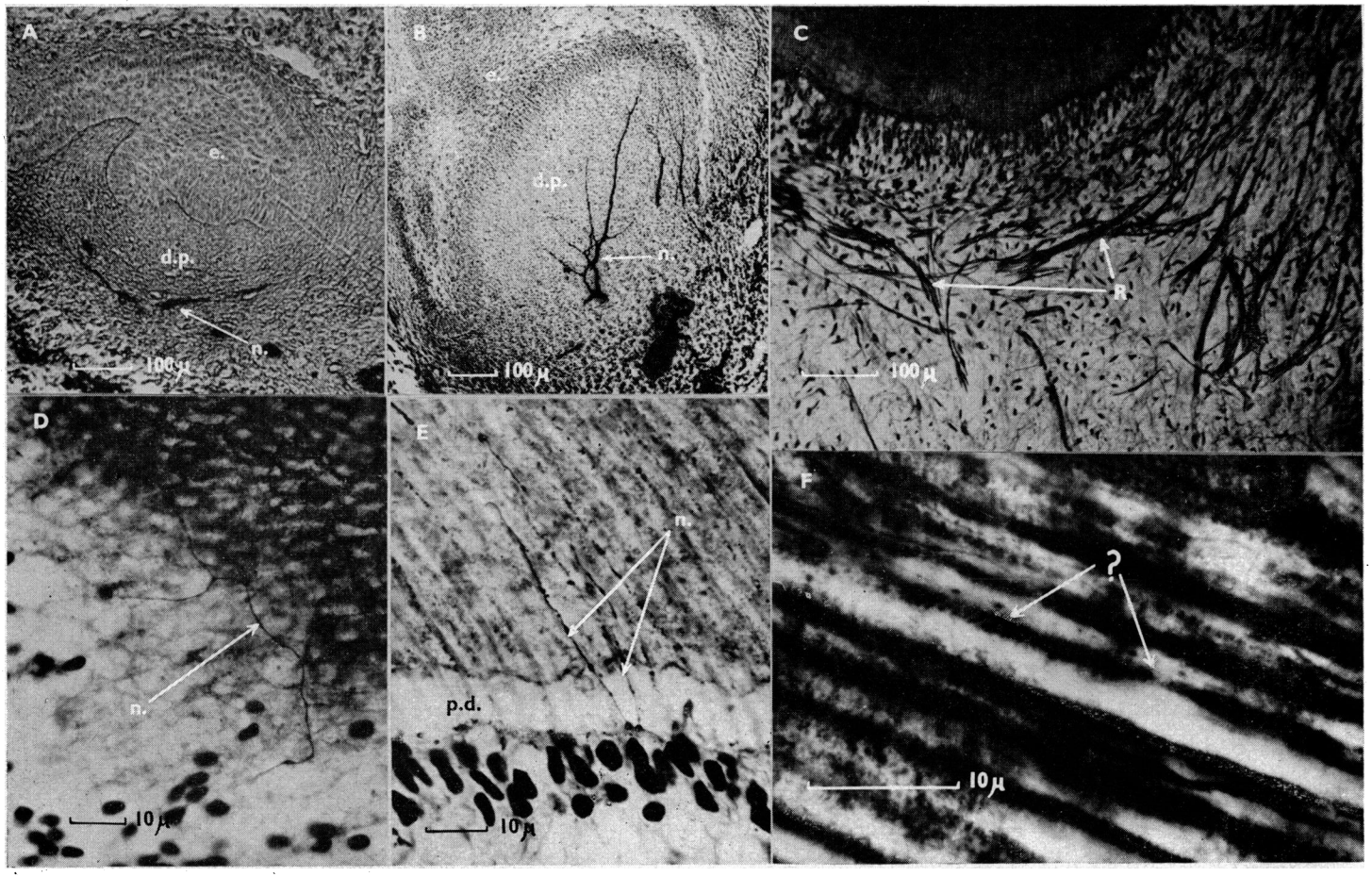
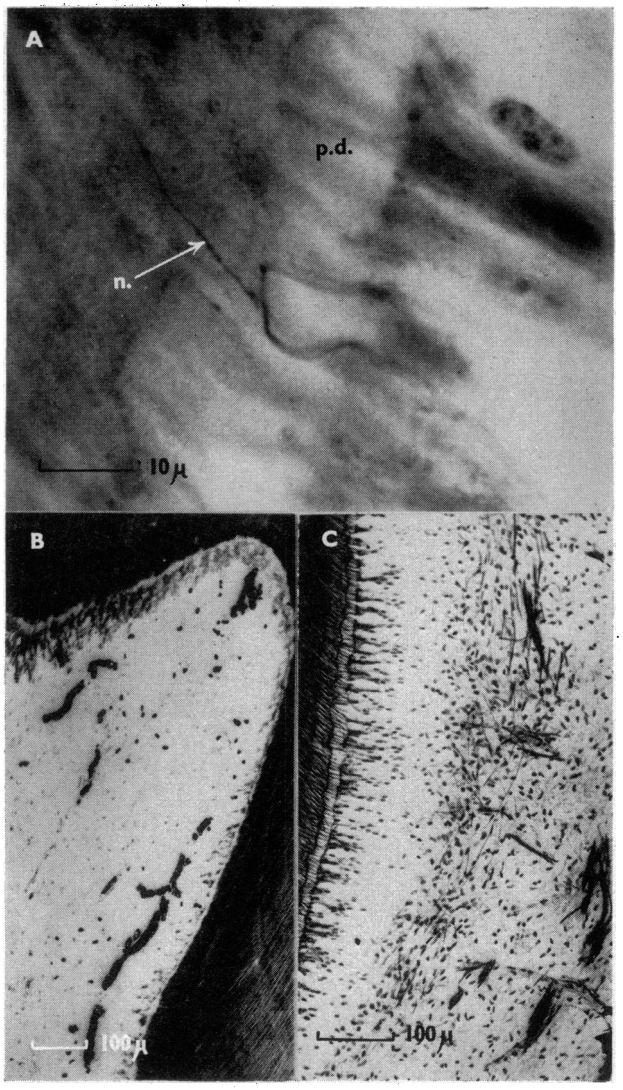


Fig 1A, *Longitudinal section through an incisor tooth germ of a male human fœtus 48 mm C.R. length. De Castro silver impregnation method.* **B**, *Longitudinal section through a canine tooth germ in the 'Bell stage' of tooth development in female human fœtus 68 mm C.R. length. De Castro silver impregnation method.* **C**, *Longitudinal section of a human lower molar tooth.* **D**, *Branching nerve fibres of the marginal plexus. Tangential section through the pulp-dentine junction of a human pre-molar tooth.* **E**, *Longitudinal section through crown of a human molar tooth showing beaded intratubular nerve fibres arising from a nerve fibre of the marginal plexus.* **F**, *Silver impregnated beaded fibre of uncertain identity in the outer third of the dentine.* n = *nerve fibres*, e = *enamel organ*, d.p. = *dentine papilla*, d.t. = *dentinal tubule*, p.d. = *predentine*, R. = '*plexus of Raschkow*'. *Unless otherwise stated all sections were impregnated with silver by the method described by Fearnhead & Linder (1956)*



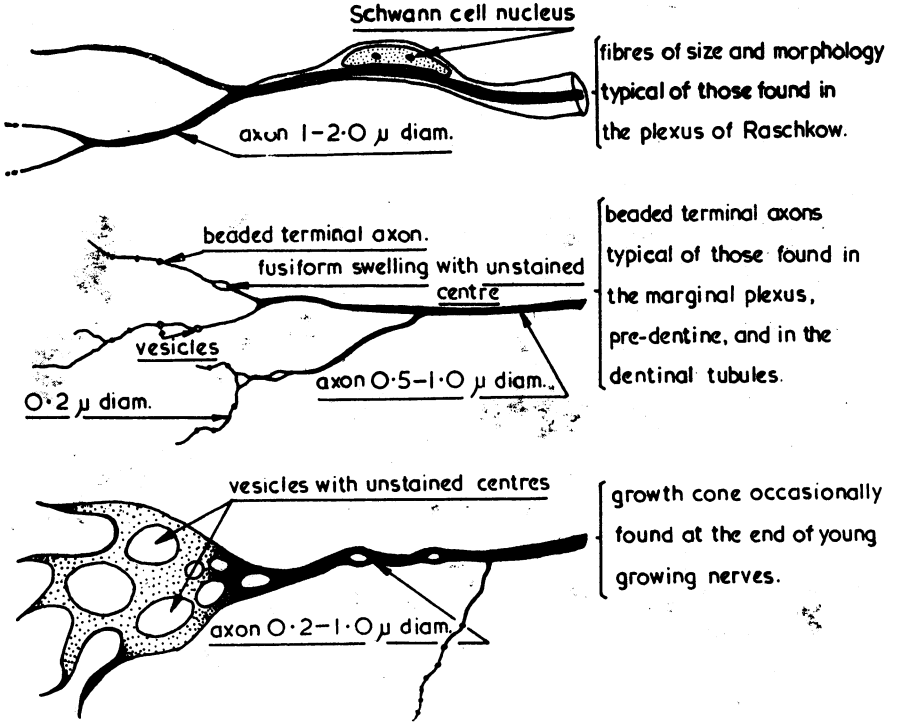


Fig 3 *Diagram illustrating some of the characteristic features of nerve morphology commonly observed in this study*

←Fig 2 A, *An intratubular nerve fibre in the dentine of a lower molar tooth of a monkey* (Cercocebus fuliginosus). B, *Appearance of a longitudinal section of the pulp-dentine junction of the crown of a molar tooth of a monkey* (Cercopithecus sabæus) *one month after resection of the inferior dental nerve.* C, *A similar region showing the typical innervation of the normal pulp, for comparison with* B. n=*nerve fibres,* p.d.=*predentine.*

of the cell interfaces at the base of the developing tooth germ but angulation of the nerve fibres can be caused by growing organs pushing against them (Weiss 1955).

The first pioneer nerve fibres arrive in the dentine papilla during the late bell stage of tooth germ development and usually follow the course of the vessels (Fig 1B). By the time dentinogenesis commences most tooth germs possess one or more nerve fibres. These observations have also been recorded by Maccaferri (1956) and Madajan (1958). These pioneer nerve fibres have a diameter of approximately 1·0–2·0μ and less. During the period of development commencing with the onset of dentinogenesis and ending with the completion of the root, there is a marked increase in the number of nerve fibres of the pulp in both deciduous and permanent teeth, the diameter of the fibres remaining relatively small. In the permanent teeth with root formation nearing completion, the plexus of Raschkow was present in most cases, whereas this plexiform arrangement of nerves was not apparent in the deciduous teeth at a similar stage of development.

In deciduous teeth approximately twelve to sixteen months elapses from the commencement of dentinogenesis, at which time it may be assumed that the first pioneer nerve fibres are present, to the time when the root is completed. The corresponding period of development for permanent teeth is, however, much longer, namely six to nine years. This great difference in the time available for development has a particularly important bearing on the pattern of the innervation. It is quite clear that it is impossible to compare the pattern and density of the innervation of the deciduous and permanent teeth at morphologically similar developmental stages without taking this discrepancy in developmental timing into consideration.

In the young fully-formed tooth, varying numbers of nerve bundles, some containing few fibres and some containing many fibres of different sizes, enter the pulp canal through the apical foraminæ and the lateral canals. These nerves usually, but not invariably, accompany blood vessels; the majority are myelinated, a few only being nonmyelinated. Some of the nonmyelinated nerves can be followed for considerable distances and are often in very close proximity to the walls of vessels. In the pulp canal, most of the nerves remain associated with the vessels, although one or two fibres may leave the main bundle to continue on an independent course. Very infrequently single fibres approach the odontoblasts in the root region and occasionally small branches arise from these fibres, some of them passing between the odontoblasts towards the predentine.

The majority of the pulpal nerves pass uninterrupted, up the root canal towards the pulp chamber. Within the root canal the fibres occasionally divide, the branches continuing in the same direction as the parent fibre. Once the pulp chamber is reached – and this is especially evident in teeth with large pulp chambers such as molars – single fibres and small groups of fibres leave the main nerve trunks, spreading out towards the roof and walls of the pulp chamber (Fig 1C).

In incisor and canine pulp chambers the nerve fibres contained within the main nerve trunks hold to a fairly direct course towards the pulp cornu; those situated directly under the pulp cornu may pass without branching between the odontoblasts towards the dentine and appear to enter the dentinal tubules without changing direction. The very small conical areas of the pulp cornua seem to be the only situation on the crown where this happens to such a marked degree.

On approaching the 'cell-rich' zone adjacent to the 'cell-free' zone in the other regions of the pulp chamber, namely the walls and the roof of the pulp chamber between cusps, the majority of the nerves alter their course abruptly, often changing their direction several times. The angulation of the fibres in this situation seems to be random, the fibres turning off in all directions. In addition to changing their direction, many of the fibres divide, some quite frequently. This results in a complex interlacing 'basket-work' of fibres under the roof and walls of the pulp chamber, the plexus of Raschkow (Fig 1C). The fibres arising from the plexus of Raschkow cross the cell-free zone of Weil obliquely to reach the odontoblasts and then turn either abruptly between the odontoblasts towards the dentine or back towards the pulp.

From examination of the methylene blue and silver preparations, it is evident that the branching nerve fibres in the plexus of Raschkow have become reduced in diameter as a result of losing most of their myelin sheaths, although many of them still possess a Schwann cell covering. The variation of fibre sizes in the plexus of Raschkow ranges from 0·5μ to 2·0–3·0μ.

Most of the nerve fibres which pass between the odontoblasts change direction once more on reaching the predentine surface. Here there is a marked tendency for the fibres to divide into numerous branches. This plexiform arrangement

of nerves on the surface of the predentine is the marginal plexus referred to by Bradlaw (1936, 1939). Most of the marginal plexus is formed by naked axons which have a diameter of approximately 0·1μ–0·5μ. In the silver preparations these fibres have the distinctive morphology of small terminal nerve fibres. Some of the branches from the marginal plexus follow the odontoblasts into the dentinal tubules in the predentine; others become embedded in the substances of the predentine. Branches from these latter fibres may enter a dentinal tubule from the predentine and, changing direction, pass either towards the calcified dentine or back towards the pulp. Occasionally, among the fibres of the marginal plexus or those embedded in the predentine, a nerve fibre of larger dimensions with attendant Schwann cells may be found. These larger fibres nearly always loop back towards the pulp, although small branches may arise from them to enter the tubules (Fig 1 D, E).

In the dentinal tubules in calcified dentine of many specimens, small beaded fibres were found lying close to the process of the odontoblast, between it and the wall of the tubule. These fibres have a diameter of 0·2μ and less and the beads on them vary in size from 0·4μ–0·8μ. All these fibres are smoothly impregnated with silver and many can be followed in continuity with fibres of the marginal plexus. These fibres possess all the distinctive morphological characteristics of small terminal nerve fibres.

The validity of assuming that these silver impregnated beaded fibres are nerve fibres from a purely histological viewpoint has been fully dealt with in an earlier publication (Fearnhead 1957).

Although the intratubular nerve fibres often appear to be very closely applied to the surfaces of the odontoblast process, no synaptic or special form of connexion with the odontoblast process could be demonstrated. In fact, in sections where some shrinkage had occurred, nerve and odontoblast had become separated without apparent damage to the cell interfaces. This suggests that although there is an intimate contact between axon and odontoblast, there is no fixed organized connexion between them. It also refutes the idea that the intratubular nerve fibres penetrate the cell wall of the odontoblast to end intracellularly as suggested by Robertson (1891), Bernick (1948).

The intratubular fibrils that could be traced in continuity with nerves in the predentine or pulp were counted. There are many more nerves entering the dentinal tubules in the crown than in the region of the cervix. In the root, intratubular nerve fibrils were only occasionally seen. In all the fully formed teeth studied the general pattern of distribution was the same, although individual regional counts varied from tooth to tooth (Fearnhead 1957).

Although 312 teeth of various ages were studied this was not considered to be a large enough group from which quantitative conclusions could be drawn about differences in the peripheral density of the innervation between deciduous and permanent teeth. The number of nerves in the pulp is known to differ from tooth to tooth (Graf & Bjorlin 1951) and it is also quite possible that an increase in the density of the innervation of teeth may be related to age, disease, physical trauma or chemical treatment.

Although the junction between the enamel and dentine is especially sensitive, nerve fibres have been demonstrated within dentinal tubules for only a short part of their length. The question whether the peripheral portion of the dentine is innervated must, therefore, be regarded as still unsettled. Fernández-Morán (1952) and others have shown that submicroscopic nerve fibrils exist in the central nervous system and De Robertis & Sotelo (1952) have shown that the pseudopodal processes of neurites grown in tissue culture may possess submicroscopic extensions of their cytoplasm. It is quite possible, therefore, that terminal nerve fibrils too small to be resolved by optical microscopy may extend as far as the enamel-dentine or cementum-dentine junction as continuations of the intratubular fibres described in this work. It was hoped that silver deposited on the surfaces of such fibrils might bring them within the range of the optical microscope since silver deposited on nerve fibres during physical development may result in an increase in their diameter. In many of the silver preparations some of the dentinal tubules contained extremely thin fibrils very near the dentine-enamel junction (Fig 1F). These fibrils could not be identified as nerves with the light microscope, but it is possible that the greater resolving power of the electron microscope may help to elucidate their true character, when a reliable method for the fixation of dentine for electron microscopy becomes available.

Stewart (1927) cut the inferior dental nerve on one side of the jaw in cats in order to test the identity of the fibres demonstrated by the method used by Mummery (1916, 1924). Because unilateral resection of the nerve supply did not remove the fibres stained by Mummery's technique, he was able to show conclusively that the fibres described by Mummery were not nerve fibres. This type of experiment has

its greatest value when the fibres under consideration are not nerve fibres since, as they are not removed by the experimental procedure, they can be positively identified in both control and experimental sections. The reverse, that is a negative result, has not the same value, however, for although structures showing in the control sections may not be present in the section after the experiment, it is not always possible to say with certainty that they were present in the material on the experimental side before the experiment began. This has a special importance in the present case, since all the observations made on the innervation of dentine in this study point to the fact that the degree of innervation varies widely from tooth to tooth, even in different regions in the same tooth. It was, therefore, important to choose an experimental animal most likely to possess an innervation similar to that of man. Teeth from most of the common laboratory animals were examined histologically but monkeys were found to possess an innervation most closely comparable with that of man. All the features of innervation of the teeth of monkeys closely paralleled that of human teeth including the presence of intratubular dentinal nerve fibres (Fig 2A).

The right inferior dental nerve of a green monkey (*Cercopithecus sabæus*) was cut in its bony canal and a short length removed without damaging the accompanying inferior dental artery. The animal was sacrificed one month later, by which time it was hoped that complete degeneration of the resected nerves would have occurred. This, in fact, was the case, only a few isolated nerve fibres remaining in the pulps of the teeth on the operated side (Fig 2B, C). The plexus of Raschkow had disappeared and nowhere could beaded terminal nerve fibres be found in the position of the marginal plexus, in the predentine or in the tubules, whereas in the control material intratubular nerve fibres could be demonstrated. The fibres which remained after resection were of small diameter, were confined to the middle zone of the pulp tissue and were closely applied to the vessels; it was assumed that these were of autonomic origin which had escaped resection. It was considered, therefore, that the experimental section of the sensory nerve supply proved the validity of the histological criteria adopted for the identification of nerve fibre terminations in this study.

One of the most striking impressions gained from an examination of the human specimens obtained from patients in the older age group is the remarkable persistence of the nerve fibres of quite normal morphology which were frequently found enclosed in newly forming irregular secondary dentine. It is impossible to say how long these nerves, or those that often become embedded in predentine, survive in this situation.

Mohiuddin (1950) studied the degenerative changes in the nerves of deciduous teeth, which were about to be shed. He was able to demonstrate degenerative changes in the pulps of teeth in the process of being shed and suggested that these changes were confined to axons 1 μ or more in diameter. Smaller fibres, according to this worker, apparently disappeared without any characteristic changes demonstrable with the silver method he used. He concluded that the onset of degeneration precedes root resorption and that this degeneration was confined to axons in the pulp and did not spread centrally to involve the axons in the main dental nerve trunks.

In the present study, some deciduous teeth were selected which, on the appearance of hæmatoxylin and eosin stained sections, were judged to have been well fixed. The roots of these teeth were fully formed and in some resorption had not begun; in others resorption had just commenced. Silver preparations of these teeth were examined to see whether there was any evidence that might support the hypothesis that 'spontaneous' degeneration occurs in human deciduous teeth just prior to resorption. Some nerve fibres were found in the resorbing teeth and the morphology of these fibres was similar to the morphological appearance described as a degenerative change by Mohiuddin, although many were apparently normal. However, some fibres possessing a similar morphology were also found in the teeth of the permanent dentition in which there was no evidence of resorption. It is considered that no special significance can be ascribed to these observations even if it could be proved that the changes were truly degenerative and not a result of fixation, since Weddell (1942) has shown that degenerating nerve fibres can nearly always be found in any group of otherwise normal preterminal nerves.

It seems, therefore, that there is little evidence to support Mohiuddin's claim that resorption is under nervous control, at any rate, in human teeth.

I wish in conclusion to make quite clear that this work only attempts to establish the presence of nerves in some of the tubules of calcified human dentine. It does not pretend to explain the mechanism involved in the transduction of a stimulus applied to the dentine, into an impulse carried by the nerve. Whether this occurs in the tubule or at the surface of the pulp or in both

places cannot be solved by a morphological study. One is, however, tempted to speculate a little and this I will do along a different line of thought.

It is impossible to examine the quite large amount of material studied in this work without gaining an impression of vital processes at work. Recently Hattyasy (1961) has come to the same view. Smoothness, beading, vesiculation, and growth cones have been observed in the tails of amphibian larvæ, by Speidel (1942), in different parts of the same nerve. It seems very likely, therefore, that these morphological changes in the axons are reversible ones which may be taking place all the time in all nerves, even in adults, and may represent axonal changes perhaps associated with changes in local environmental conditions.

Nerves that have entered the dental pulp have little chance of finding their way out again. The dental pulp, therefore, provides a very suitable region for the histological study of factors which might influence the peripheral density of nerve terminations. The nerve fibres, having once innervated the pulp, appear to continue their growth long after the tooth reaches its final form and to continue to grow after reaching what one would assume to be an adequate saturation of the tissue by sensory nerves. The view expressed by Weiss (1934, 1955) that the peripheral saturation of a tissue by terminal nerve fibres is under the control of some 'ordering factor' does not therefore seem to apply to tooth pulps, at least in man and many other mammals. The apparent 'overgrowth' of nerves in tooth pulps, giving rise to the plexus of Raschkow, might result from either the expression of an excessive growth potential, the absence of an inhibitory mechanism. Alternatively it may be that these nerves have the same growth span as other nerves, but since they are contained within a bony case they are not subjected to the same wear and tear processes which otherwise might normally cause a balance to struck between tissue loss and replacement.

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