HISTOLOGICAL EVIDENCE FOR THE INNERVATION OF HUMAN DENTINE

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The sensitivity of human dentine strongly suggests that it possesses a rich innervation. None of the workers who have attempted to demonstrate nerves in this tissue can be said to have done so unequivocally. The literature, which has been reviewed by Brashear (1937) and Bernick (1948), remains inconclusive because most workers have apparently disregarded the possibility of mistakes in their interpretation. Many histologists agree that nerves can be demonstrated in the predentine, and Bradlaw (1939) refers to intra-tubular fibrils in this region. Only a few observers who claim to have demonstrated nerves in dentine proper have supported their observations with photomicrographs. Powers (1952), who figured a retouched photomicrograph, believed that the nerves she demonstrated were situated in the dentine matrix between the tubules. Cocker & Hatten (1955), who used the same silver technique as Powers, were not prepared to say whether the silver impregnated fibres in their sections were situated in the dentinal tubules or the dentine matrix. Tojoda (1934*a*, *b*) described in some detail many types of silver impregnated fibrils within the dentinal tubules and, apparently relying on the specificity of silver for nerve tissue, seemed to regard all of them as special types of nerve endings.

It is the purpose of this paper to show that small intra-tubular nerve fibrils do extend into the dentine in close relationship to the odontoblast process (Pl. 1, fig. 8*a*, *b*), and to attempt by conventional histological techniques to eliminate the possibility of misinterpretation.

MATERIALS AND METHODS

Non-carious and slightly carious human premolars and molars from subjects between the ages 10–14 and 18–24 years were obtained immediately after extraction, which was performed under general or local anaesthesia. The approximal surfaces of some of the teeth were ground down nearly to the pulp in chilled (4° C.) normal saline. In others the roots were either cut off or large holes were drilled into the dentine in parts not required for study. The teeth were then placed in 10% formol saline for not less than 7 days. Formic acid/sodium formate solution (Kristensen, 1948) was used for decalcification, and the majority of the teeth were double embedded in celloidin paraffin. Other embedding materials used were paraffin wax, celloidin, Nonex (Miles & Linder, 1952), or Ester Wax (Steedman, 1947). Celloidin sections were cut at a thickness of 15 μ, paraffin sections at 10 μ and Nonex and Ester Wax sections at 7 μ. The sections mounted in series were then impregnated with silver by a modified Holmes technique (Fearnhead & Linder, 1956). The quality of the

fixation was judged from the appearance of haematoxylin and eosin stained sections and the appearance of unstained sections examined by phase-contrast microscopy. The matrix of the 'translucent area' around the dentinal tubules and the cytoplasm of the odontoblast processes was demonstrated by Held's molybdic acid haematoxylin stain (Pl. 1, fig. 6). Sections of undecalcified dentine fixed in buffered pH 7·2 osmium tetroxide (Palade, 1952) and embedded in methylmethacrylate, were cut with a diamond-knife similar to that described by Fernández-Morán (1953).

Silver impregnated intra-tubular nerve fibrils were counted over measured lengths of the pulp-dentine junction in three regions of four teeth, the crown, the cervix and the root, see Appendix, Text-fig. 3, and Table 1 *a–c*.

RESULTS

Fixation

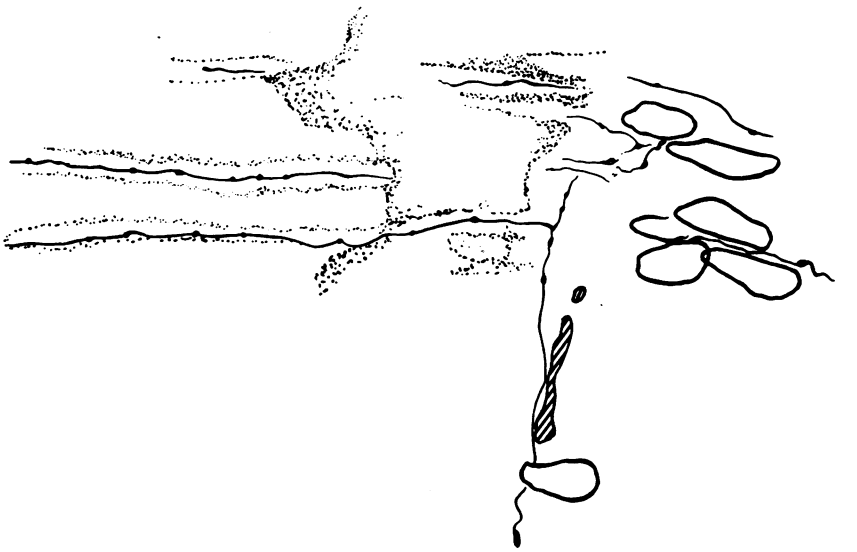
In this study a considerable amount of care was taken to select only those teeth in which fixation of the contents of the dentinal tubules was thought to be good. Unstained sections were examined with the phase-contrast microscope, and other sections were stained with haematoxylin and eosin in order to assess the quality of the fixation before impregnating the series with silver. The specimens were discarded when the pulp and odontoblasts showed vacuolation and other changes attributable to faulty fixation. In the majority of the remainder the continuous unbroken outline of the odontoblast process could be seen within many of the tubules. This was found more frequently in teeth which had been cut in half before being placed in the fixative. These specimens were regarded as being well fixed and were therefore used for the silver impregnation. In many specimens where exposure of large areas of dentine had not been obtained before fixation, the contents of the tubules appeared to be broken up into droplets, although the fixation of the pulp was often quite good. Such sections were regarded as being poorly fixed and discarded (Pl. 1, fig. 7).

Silver impregnation

In silver-treated sections of well-fixed dentine numerous small beaded fibrils could be detected lying in close relationship to the process of the odontoblast, between it and the wall of the tubule. Although in places the intra-tubular nerve fibrils were in very close association with the odontoblast process, they did not appear to be embedded in its protoplasm nor did they possess small collateral fibrils embracing it as described by Tojoda (1934*a*, *b*). Measurement of the beaded fibrils showed them to have a diameter of 0·2 μ or less, and the beads varied in size from 0·4 to 0·8 μ. These fibrils therefore have the size and distinctive moniliform morphology of small terminal nerve fibrils (Weddell & Glees, 1941). All were heavily impregnated with silver and many of them could be traced in continuity with similar fibrils in the predentine which in turn could be traced to nerve fibrils situated on the surface of the predentine (Pl. 1, figs. 3, 10). The lowest beaded intra-tubular fibril illustrated in Text-fig. 1 and Pl. 1, fig. 1, was traced as far as 0·4 mm. into the dentine from the point at which it left a branch in the predentine. This is the greatest distance that any single fibril was traced when in continuity with a nerve in the pulp or predentine. Fibrils having a similar size and morphology were noted, however, much

further (1·5 mm.) in the dentine (Pl. 1, fig. 9). It was impossible to establish the continuity between these fibrils and nerves in the pulp because of the many small curvatures in the dentinal tubules and the wavy or spiral course of the beaded fibrils within the tubules (Pl. 1, fig. 8*a*, *b*).

Counts were made of intra-tubular fibrils that could be traced in continuity with nerves in the predentine or pulp. The number of nerves entering the dentinal tubules in the crown was much higher than those entering tubules in the region of the cervix. In the root, intra-tubular fibrils were only occasionally seen. The



Text-fig. 1. Camera lucida drawing of the field illustrated in Pl. 1.

statistical significance of the differences found between the numbers of fibrils in each of these three regions on ten serial sections from four teeth is dealt with in the Appendix. In all the teeth studied so far the general pattern of distribution was the same, although individual regional counts varied from tooth to tooth.

DISCUSSION

As a result of recent work it has become necessary to reconsider the classical concept of the structure of dentine. Bradford (1950, 1951, 1955) has shown that the dentinal tubule is surrounded by an area of dentine which appears translucent when ground sections are viewed by transmitted light and for which he coined the term 'translucent area'. This term is not altogether satisfactory since it refers to only one particular aspect of the physical properties of the peritubular region. Takuma, Kurahashi, Yoshioka & Yamaguchi (1956) use the term 'peritubular matrix' or 'secondary matrix' because they found that the matrix of the tubule walls appeared different in character from the rest of the dentine matrix when studied with the electron microscope. Shroff, Williamson, Bertrand & Hall (1956) prefer to use the term calcified canicular sheath to describe the walls of the dentinal tubules. Each

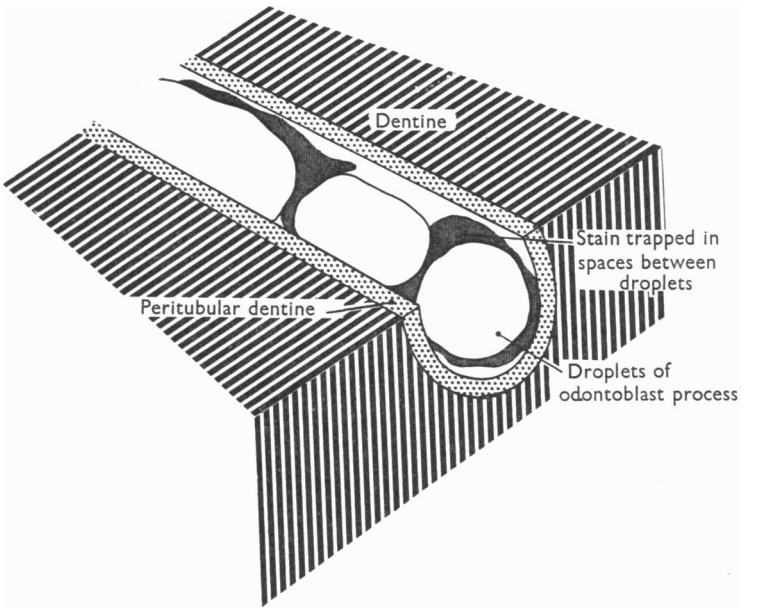
of these names is equally unsatisfactory since they refer to only one component of the structure of this region. Since little is known about the mode of formation, structure, function, or ageing processes of the dentine immediately surrounding the tubules, it might be better to employ the term 'peritubular dentine' for this region. This at least has the merit of recording where this special region may be found without placing the emphasis on any one particular feature of its structure. This term is therefore used in the remainder of this study.

The peritubular dentine consists of a delicate matrix which takes up stains more readily at its inner and outer border and is thought to be highly calcified. When dentine is decalcified for the preparation of histological sections, the delicate matrix of the peritubular dentine may be lost, disrupted, or it may remain as a sheath which can be seen to surround the odontoblast process. Sometimes the matrix appears as an empty tube when for some reason, such as poor fixation, the contents of the tubule are lost. When ultra-thin sections, cut from human dentine without previous decalcification, were examined with the electron microscope, the peritubular dentine was revealed as an electron dense region which appeared to consist of fibres orientated differently from the inter-tubular dentine matrix, (Pl. 1, fig. 4). Removal of the methacrylate, and brief (30 sec.) decalcification in sodium formate/formic acid, removed the substance responsible for the electron density and left behind what appeared to be the matrix of the peritubular dentine (Pl. 1, fig. 5). In addition, in the sections decalcified on the electron microscope grids, the junction between the peritubular dentine and the adjacent inter-tubular dentine was particularly well marked. This junction represents the position of the 'Neumanns sheath' referred to by earlier workers. These observations are obviously of considerable importance in the interpretation of the contents of dentinal tubules, since the tubules, which were originally thought to have a diameter of 3–5μ, in reality vary in size from 1·0 to 1·5μ at the periphery of the dentine, to 2·5–3·5μ at the pulpal end. This of course limits the size of nerve fibrils and odontoblast processes which could be accommodated within them.

It might be argued that the silver impregnated beaded filaments were not nerve fibrils at all, but merely represented the matrix of the peritubular dentine impregnated with silver. If the matrix of the peritubular dentine were impregnated, it might be expected to envelop the odontoblast process in the form of a reticulated sheath. The fibrils shown in Pl. 1, figs. 1, 3 and 8*a*, *b*, however, have a diameter of 0·2μ and less, and quickly go out of focus at different levels within the same tubule. Furthermore, the matrix of the peritubular dentine can be stained by Held's molybdic acid haematoxylin method, when it can be seen to surround the odontoblast process and to have a morphology quite different from silver impregnated nerve fibrils. Some places could be found in the sections where the plane of the microtome knife had divided the dentinal tubule and its contents longitudinally. In such regions much more precise resolution of the structure of the dentinal tubules is possible. Both in sections stained with Held's molybdic acid haematoxylin and unstained sections viewed with the phase-contrast microscope, five linear structures were identified representing the inner and outer margins of the peritubular dentine, either side of the tubule, and a thicker central odontoblast process surrounded by a small space (Pl. 1, fig. 6).

In addition to these structures, in some tubules, very small spherical droplets (diameter approx. 0·5 μ) were seen closely applied to the surface of the odontoblast processes (Pl. 1, fig. 6). The spherical droplets did not stain with Held's molybdic acid stain, and appeared refractile in phase-contrast preparations. It is impossible to say whether these droplets are inclusions within the odontoblast process or whether they are lying on the surface of it. In the latter case they might represent unstained beads of an intra-tubular nerve fibril.

It was mentioned earlier that delay in fixation can cause the dentinal process of the odontoblast to break up into droplets. It was thought therefore that reduced silver in the spaces between the tubule wall and the droplets might resemble small nerve fibres (Text-fig. 2). Sections from teeth in which droplet formation was known to



Text-fig. 2. Diagram to show how silver might simulate nerve fibres by becoming reduced in the spaces between droplets formed from the odontoblast processes.

have occurred were impregnated with silver. It was hoped that silver penetrating the tubules might become trapped between droplets and tubule walls and later become reduced in this situation. This was achieved in some sections by omitting the washing and sulphite stage of the impregnation. The result is shown in Pl. 1, fig. 7. The distribution of the silver is quite distinctive in such preparations, and once seen is not likely to be confused with the appearance of silver impregnated nerve fibrils.

Many of Tojoda's illustrations show small intra-tubular beaded fibrils which have a morphology similar to the fibrils regarded as nerves in the present study. Unfortunately he did not record precisely how far into the dentine he was able to trace these fibrils. Tojoda (1934*a*, *b*), who ground away considerable amounts of enamel and dentine in order to obtain good fixation, describes and figures intra-tubular nerves having very small collateral 'sprouts' (Sprossen) clasping the odontoblast processes. This appearance can be attributed to an increase in argyro-

philia of the matrix of the peritubular dentine or of the odontoblast process, which might be due to over-heating, since it has been shown that in the region of a dental bur cutting into dentine without lubrication, temperatures as high as 368° F. can occur (Henschel, 1944). In the present study, in sections of teeth in which a small hole had been drilled prior to fixation, a zone of altered silver affinity could be clearly detected in the dentine around the hole and in the pulp cells immediately beneath it (Pl. 1, fig. 2), despite the fact that the bur had been irrigated with cold saline during cutting. The contents of the tubules in this region had a foam-like appearance almost certainly due to heating. Examined at high magnification, the contents of the tubules in this altered zone possessed a striking resemblance to the collateral sprouts figured by Tojoda. It is not meant to imply by this that grinding before fixation is valueless, but merely to indicate that care must be taken in the interpretation of the histology of dentinal tubules in close relationship to surfaces exposed in this way. Ambrose (1943) criticized the histological evidence for the innervation of dentine on the basis that the odontoblast processes themselves were being mistaken for nerve fibrils. This may well be the case in some instances. In sections prepared by the method used in the present study, however, the odontoblast process is often coloured a delicate pink; its outline is smooth and not beaded and it has a diameter of about 1 μ. The diameter of the odontoblast process is therefore approximately five times greater than the beaded nerve fibrils described in this paper. Furthermore, Pl. 1, figs. 8*a*, *b*, are photomicrographs of a section taken at different focus levels which show a small nerve fibril and a lightly coloured odontoblast process together in the same tubule. Although the silver technique used in this study gives consistent results it is impossible to say whether every nerve fibre in every section is impregnated, and this criticism is of course equally valid for any silver method. It seems certain from the results of the counts, however, that by no means every dentinal tubule contains a nerve fibril. The variation in the number of intra-tubular nerve fibrils counted in the three regions of the teeth studied has some significance in establishing the identity of these fibrils, since it is unlikely that artefacts would be distributed in such a constant pattern.

Although the junction between the enamel and dentine is especially sensitive, nerve fibrils have been demonstrated within dentinal tubules for only 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 intra-tubular nerve fibrils 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, according to Romanes (1950), silver deposited on nerve fibrils during physical development results in an increase in their diameter. It is of interest in this respect that in many of the silver preparations some of the dentinal tubules contained extremely thin fibrils very near the dentine-enamel junction (Pl. 1, fig. 9). These fibres could not be identified as nerve fibres

with the light microscope, but it is possible that the greater resolving power of the electron microscope may help to elucidate their true character. It is also worthy of note that occasionally dentinal tubules containing more than one fibril have been discovered by electron microscopical methods (Scott, 1955). Consideration of this problem would be incomplete without mention of the recent observations of Shroff *et al.* (1956). These workers examined shadowed replicas of freshly fractured dentine, polished surfaces of dentine and ultra-thin sections of decalcified dentine with the electron microscope. In their preparations they were able to identify several distinct structural layers in the odontoblast process. They describe these layers as a central core of a labile protein nature surrounded by a thin organic sheath which in turn is surrounded by a thick material possessing some of the properties of myelin. Around this 'myelin-like' layer is a thin outer sheath composed of fibrils which appear to be collagen. The myelin-like sheath is osmiophilic, soluble in hot alcohol, and stains blue with acidified methylene-blue, the latter apparently being regarded as a specific stain for myelin. On the basis of these observations they conclude that there is a close similarity between this layered submicroscopic structure of the odontoblast and the submicroscopic structure of vertebrate nerve fibrils as described by Fernández Morán (1950*a*, *b*). Shroff *et al.* (1956) suggest therefore that if their observations are correct the odontoblasts may function as some form of receptor cell, a suggestion which revives the hypothesis put forward by Hopewell-Smith (1893) and supported more recently by Philipp (1955). The evidence used by Shroff and his co-workers is open to criticism, however; for example, it is very doubtful whether osmiophilia, solubility in hot alcohol, or staining with acidified methylene blue can be regarded as properties possessed by myelin alone. It is also difficult to see how the suggestion put forward by these workers can be reconciled with the observations of Geren (1954) who has demonstrated the formation of myelin sheaths from Schwann cell membranes.

Finally, the replica method does not always permit an accurate interpretation of soft tissue structure. It is important, therefore, to develop techniques for preparing ultra-thin sections of dentine suitable for electron microscopy in which the contents of the tubules are undisturbed. Fixation of the dentine is obviously of extreme importance to this aspect of the study and unfortunately the zone of dentine nearest the enamel is most inaccessible to fixatives. Consequently, until the difficulties involved in obtaining rapid fixation in the peripheral region of the dentine are overcome, it is impossible to draw very precise conclusions about its histology.

SUMMARY

Using a modified Holmes silver technique very small beaded intra-tubular nerve fibrils have been demonstrated in human dentine. These fibrils have an approximate diameter of 0·2 μ and are situated in the tubules between the odontoblast process and tubule wall. They were traced in continuity from the predentine into the dentinal tubules for varying distances up to 0·4 mm. Fibres with similar morphology were identified even farther into the dentine. For the present it is not possible to determine whether fine terminal filaments from the intra-tubular nerve fibrils extend as far as the enamel-dentine or cementum-dentine junction. Using various conventional

histological methods care was taken to exclude sources of artefact which could lead to misinterpretation of the silver impregnated fibrils.

I wish to express my thanks to Prof. A. E. W. Miles and Prof. R. J. Harrison for their encouragement, helpful advice and criticism. I am indebted to Dr P. Grodzinski and Dr J. F. H. Custers of Industrial Distributors Ltd. for the preparation of the diamond knife with which the sections of undecalcified dentine were cut. My thanks are also due to Mr W. K. Mansfield of Queen Mary College for his generous assistance in the use of the electron microscope. Text-fig. 2 was drawn by Miss P. Archer, and the expenses were partly defrayed by a grant from the Yarrow Research Fund, The London Hospital Medical College.

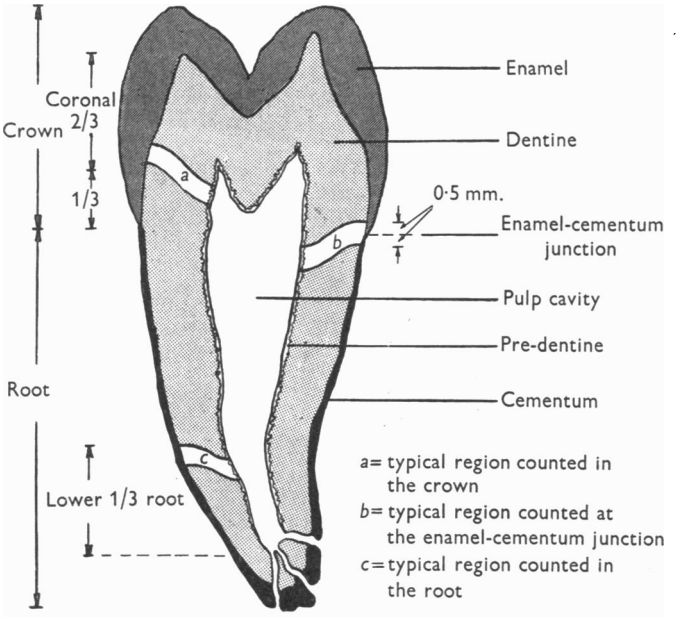
APPENDIX

Dentinal tubule and intra-tubular nerve fibril counts

Ten longitudinal sections, 10 μ thick, were selected from the mid-portion of each of the four teeth on which the counts were made. Ten different fields, measuring 0·1 mm. in diameter, were chosen in each of the three regions, five fields being selected from opposite sides of the tooth for each region. The method of selecting fields was determined to a large extent by the plane of the section because the dentinal tubules do not pursue a straight course. Fields were chosen therefore from areas where the dentinal tubules were cut longitudinally at the junction between the predentine and dentine. The counts of intra-tubular nerve fibrils and dentinal tubules are listed in Table 1 *a–c*. These totals represent the result of 100 fields within each region for each tooth counted.

In order to avoid overlapping of regions the third of the crown nearest the root was never included in the crown counts (Text-fig. 3). The region designated enamel-cementum junction included dentinal tubules which were situated within 0·5 mm. from the true junction in both directions, that is, coronally or rootwards. In practice these tubules were located by first moving the junction on the outer surface of the tooth into the centre of the field, and then following the curvature of the dentinal tubules by manipulating the stage micrometer until the predentine-dentine junction was reached. In the root the counts were taken from the third of the root farthest away from the crown. Differences in the totals between the crown region and the enamel-cementum junction (Table 1 *a*) and the enamel-cementum junction and the root region (Table 1 *b*) were tested separately by means of the χ^2 test. It will be seen that only in one case (Table 1 *b*), specimen 2, where the χ^2 value is 0·751, is the P value greater than 0·3. In all other cases the statistical value can be regarded as highly significant. For completeness the χ^2 value for the difference between crown and root of specimen 2 is included (Table 1 *c*). Calculated with 1 D.F. the value for $P = 0{\cdot}05$ is 3·84.

I am greatly indebted to Miss V. Cane, Department of Mathematics, Queen Mary College, London, and to Dr F. Steel, Anatomy Department, London Hospital Medical College, for their considerable help and advice with the statistical treatment.



Text-fig. 3.

Table 1*a*

	Crown		Enamel-cementum junction		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
1	212	2563	28	2722	152·49
2	105	2197	5	2460	102·89
3	90	2220	15	2348	57·91
4	188	2301	24	2299	71·539

Table 1*b*

	Enamel-cementum junction		Root		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
1	28	2722	5	2379	13·14
2	5	2460	2	1998	0·751
3	15	2348	2	2201	8·887
4	24	2299	2	8679	16·72

Table 1*c*

	Crown		Root		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
2	105	2197	2	1998	89·81

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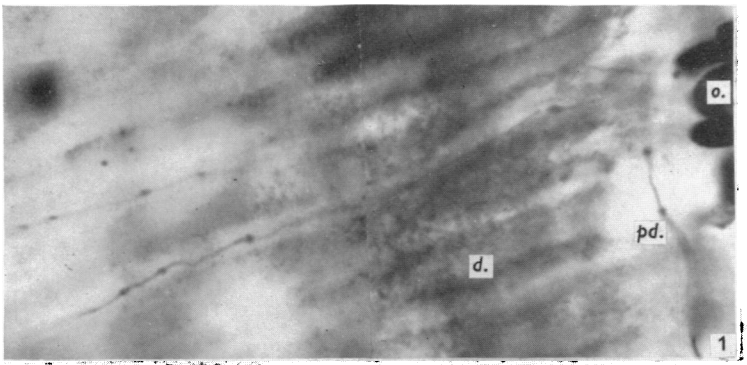

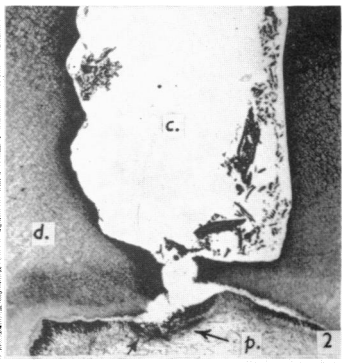

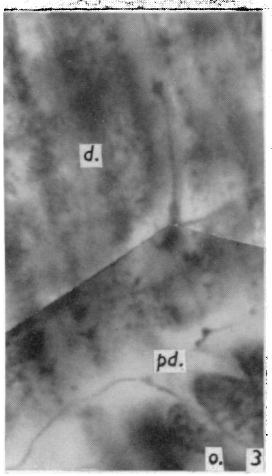

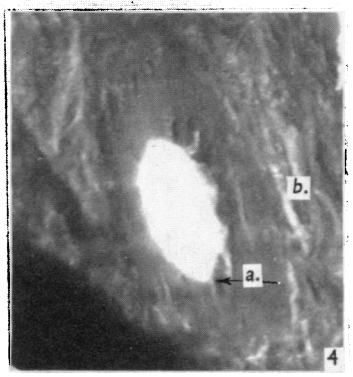

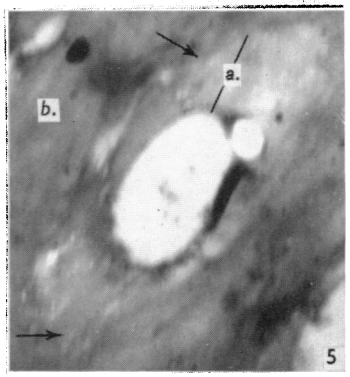

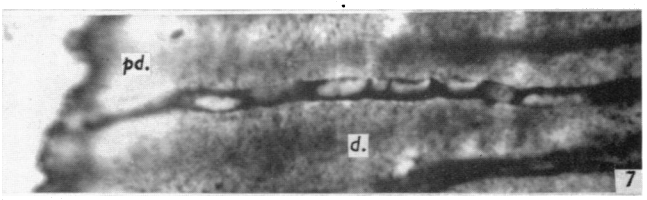

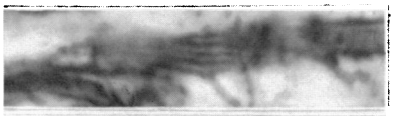

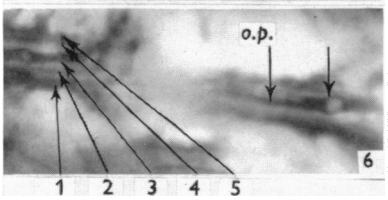

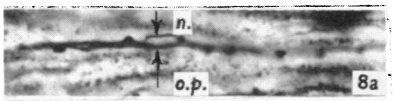

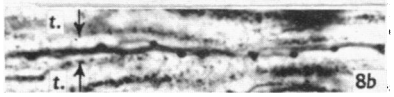

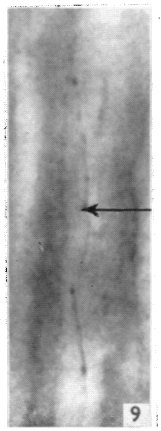

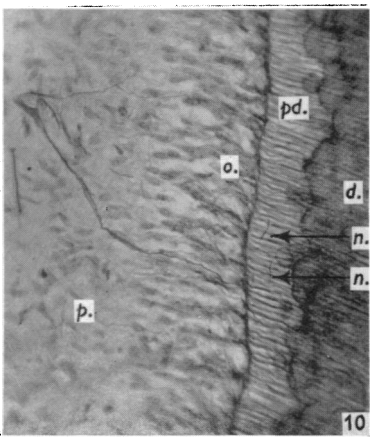

FEARNHEAD—Histological evidence for the innervation of human dentine

(*Facing p.* 277)

LIST OF ABBREVIATIONS

a. 'translucent area' or peritubular dentine
b. inter-tubular dentine matrix
c. cavity
d. dentine
o. odontoblasts
pd. predentine
p. pulp
op. odontoblast process
n. nerve fibril
t. dentinal tubule

EXPLANATION OF PLATE

Fig. 1. See also Text-fig. 1. Two photomicrographs taken at different focus levels and joined to show intra-tubular beaded fibrils, and similar fibrils in predentine. Silver impregnated section from decalcified human premolar. (×1500.)

Fig. 2. Section of dentine and pulp from a tooth in which a small hole was drilled prior to fixation. Note the difference in the character of the staining of the dentine adjacent to the hole, and the zone of damage in the pulp indicated by the small arrows. Silver impregnation. (×20.)

Fig. 3. Two photomicrographs of the same field taken at different focus levels and joined to show a beaded intra-tubular nerve fibril arising as a branch from a similar fibril in the predentine. Silver impregnation human premolar. (×1500.)

Fig. 4. Electron micrograph of undecalcified dentine from the crown of a lower third molar tooth from a male aged 21 years. The area (*a*) around the tubule which appears electron dense represents the peritubular dentine equivalent to the 'translucent area' seen in ground sections. The arrow from (*a*) ends on the tubule wall. (×10,000.)

Fig. 5. Electron micrograph of a section adjacent to that in fig. 4. The methacrylate was removed and the section decalcified for 30 sec. in 10% sodium formate/formic acid solution. Note the electron dense character of area (*a*) in fig. 4 has disappeared and the junction between the dentine matrix and the region of the 'peritubular dentine' is more clearly defined. Arrows indicate the position of 'Neumann's sheath'. (×10,000.)

Fig. 6. Decalcified section of dentine from human premolar stained with Held's molybdic acid haematoxylin. The five lines represent the junctions between the outer margin of inter-tubular dentine and the peritubular dentine, the matrix of the peritubular dentine, and the tubule wall, and the odontoblast process. The arrow indicates a small, spherical, unstained droplet closely applied to the surface of the odontoblast process. (×1800.)

Fig. 7. Reduced silver trapped in the dentinal tubule between globular remnants of the odontoblast process. Decalcified section of human premolar, silver impregnation. (×1500.) See also Text-fig. 2.

Fig. 8*a*, *b*. Two photomicrographs of the same field at different focus levels. A silver impregnated beaded fibril which has the characteristic morphology of a small nerve, and a more delicately impregnated odontoblast process in the same tubule. This appearance is not due to trapped stain, or the matrix of the 'peritubular dentine', nor is it due to staining of the odontoblast process since this can still be identified as a discrete object within the same tubule. Decalcified section of dentine from a human premolar approximately 0·4 mm. from the predentine. (×1000.)

Fig. 9. A small impregnated beaded fibril with a morphology similar to a very small nerve fibre in a dentinal tubule. This fibre was found situated 1·5 mm. from the pulp-dentinal junction. (×1800.)

Fig. 10. A nerve fibre in the pulp; branches from this nerve are situated between the odontoblasts and on the surface of the predentine, and small terminal branches with short lengths of their course in focus can be seen lying across the dentinal tubules in the predentine. Silver impregnation, human molar. (×400.)