Acta odont scand 1972 *30:* 291—311

Keywords:
Dentin
Odontoblasts
Nerve endings

From:
The Department of Oral Histopathology, School of Dentistry, Stockholm, Sweden, and Dental Clinic of Milan, Milan, Italy

THE DENTINAL TUBULES AND THE ODONTOBLAST PROCESSES

A SCANNING ELECTRON MICROSCOPIC STUDY

Martin Brännström
Riccardo Garberoglio

Scanning electron microscopy of the dentinal tubules and the odontoblast processes was performed on young premolars that had been fractured to open the tubules in the crown dentine parallel to theiraxis. The tubules were viewed in an outward direction, starting from the attached pulp tissue at the buccal pulp horn. The odontoblast processes in the tubules were found within a distance from the pulp equelling about one quarter the total length of the tubule; in only a few tubules the process was more than 0.4 mm from the pulp and none were seen beyond 0.7 mm from the pulp. The process almost completely filled the tubule in the predentine-dentine area and up to a distance of 0.2 mm from the pulp. The processes were tube-like.

In the predentine-dentine area the processes were surrounded by a thin membrane-like structure. Further into the dentine the tubule wall seemed to have a coating of an amorphous substance containing a few circumferentially, and in places also longitudinally, arranged fibres. The tubules apparently contained no nerve fibres.

The picture of the dentinal tubules and their contents is still unclear and the information in the literature is controversial. As pointed out by *Scott* (1955), opinions differ on whether the odontoblast process, the Tomes' fibre, is solid or tube-like, and whether or not it fills the entire lumen out to the enamel border.

Some electron microscopic studies have indicated that there is no membrane-like lining of the tubule wall (*Helmcke & Jahn,* 1952; *Scott,* 1955). On the other hand, studies with the replica technique have brought to light a kind of sheath surrounding the odontoblast process, which, in turn, is directly attached to the tubule wall, thus completely filling the tubule throughout its length, leaving no room for tissue fluid (*Syrrist & Gustavson,* 1951;

Received for publication, March 5, 1972.

Schroff et al., 1956; *Awazawa*, 1969). A similar conclusion has been reached by *Arwill* and *Bloom* (1954) on the basis of an electron microscopic study of decalcified dentine.

Using the electron microscope, but in undecalcified dentine, *Frank* (1959) has noticed that in the middle and outer portion of the dentine the odontoblast process assumed a tubular form; he has found the part near the predentine to be a rod of cytoplasm, but sometimes apparently absent. In a second study in 1966 *Frank* has concluded that the dentinal tubule contained the cellular extension of the odontoblast. In the predentine area he has also found structures in the tubules resembling unmyelinated nerve fibrils.

From a similar study *Johansen* & *Parks* (1962) have concluded that, except in the predentine area, the lumen of the dentinal tubule was lined by a membranous structure containing collagenous elements and closely associated with the highly calcified peritubular zone. In the predentine area the only membranous structure present was the plasma membrane of the odontoblast process. In no section did these workers find evidence of two separate membranes in the tubule.

Collagenous fibrils arranged circumferentially or longitudinally on the tubule wall, or what has been termed »the periprocessal area», have been noticed in many studies (*Scott*, 1955; *Frank*, 1959; *Takuma*, 1960). Such fibrils have also been observed in scanning electron microscopic examinations of fractured dentine (*Boyde* & *Lester*, 1967).

In a histochemical study of undecalcified sections *Symons* (1962) has found that the odontoblast process had a solid appearance and terminated about 120 and 200 μm from the predentine junction. Peripherally to this zone the contents were tubular and stained strongly at the boundary between the peritubular zone and the tubule contents. Symons suggested that this lining might as well be a thin limit boundary of the peritubular matrix as the outer limit of the odontoblast process.

In a study of longitudinally sectioned dentinal tubules in the scanning electron microscope, *Kubota*, *Ajisaka* and *Inoue* (1969) have found that the inner walls of the dentinal tubules often had fibrillar plica structures and many lateral branches, but no Tomes' fibres were seen.

With the same technique *Isokawa et al.* (1970) studied etched dentine surfaces with cross-fractured dentinal tubules. After etching almost all the peritubular dentine diappeared, but an acid-resistant layer remained in the innermost aspect of the peritubular dentine. At some apertures of the non-etched and cross-fractured dentinal tubules they also noticed structures which they interpreted as Tomes' fibres.

The object of the present study was, by means of the scanning electron microscope, to examine the dentinal tubules and, in particular, the extent of the odontoblast process, and to record evidence of the presence or absence of nerve-like fibres in the tubules.

MATERIAL AND METHOD

The study was performed on young permanent premolars. Immediately after the tooth had been extracted the root was cut off about 2 mm from the enamel border by means of a diamond wheel with constant water cooling. In the same way a narrow groove was cut in the buccolingual or mesiodistal direction in two surfaces and leaving either the buccal or one of the proximal surfaces intact. The tooth was then placed in liquid propane or liquid nitrogen to fracture spontaneously. If this did not occur, the tooth was removed from the liquid and the fracture was effected with a forceps. Some teeth were fractured before being placed in propane; others were first fixed in 10 per cent formalin or 5 per cent glutaraldehyde for a few hours. The teeth were then treated with a freeze-drying procedure before mounting for examination in the scanning microscope.

The surface of fracture was covered *in vacuo* with a gold film about 200 Å thick. A Cambridge scanning microscope was used with an accelerating voltage of 20 kV. After examination under a binocular microscope the surfaces were chosen for further examination by scanning; they should have the pulp well attached to the wall, and at the same level as the fractured surface. Areas from the middle of the crown pulp or the buccal pulp horn were examined starting along the pulpodentinal wall, and then following the tubules in an outward direction. Photographs were taken with a slight overlap, following the direction of the tubules. Twenty to 40 photographs were taken in such series, usually with magnifications of about 1000 and 2000, in some areas 5000 and 10,000.

RESULTS

The predentine — dentine area

The odontoblast processes in the predentine were well defined, and continued into the tubules (Figs. 1—7). At the upper level of the surface of fracture the processes were interrupted, the ends being visible both on the pulp side and in the tubules at the dentinal border (Figs. 2—5, 7—9). Processes could also be seen fractured in the predentine. The process appeared to be tubular

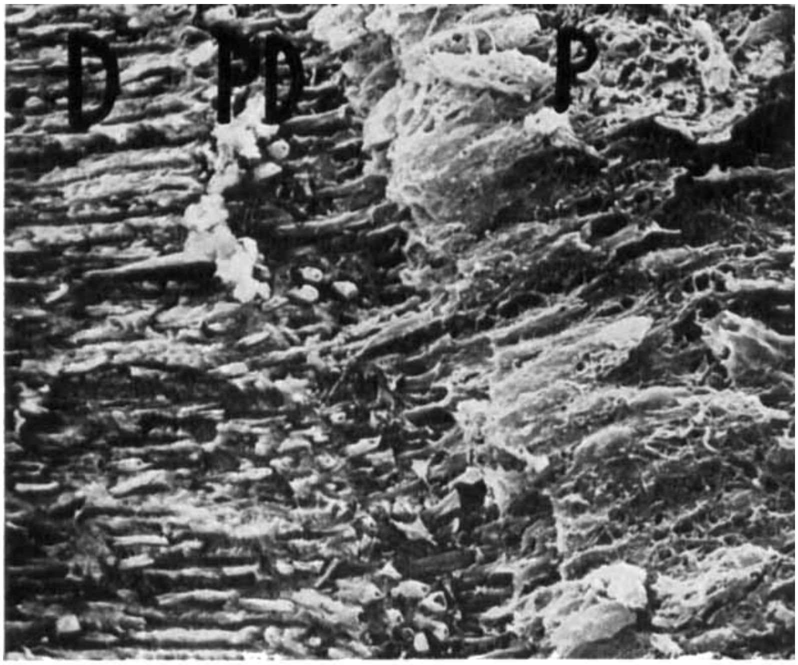

Fig. 1

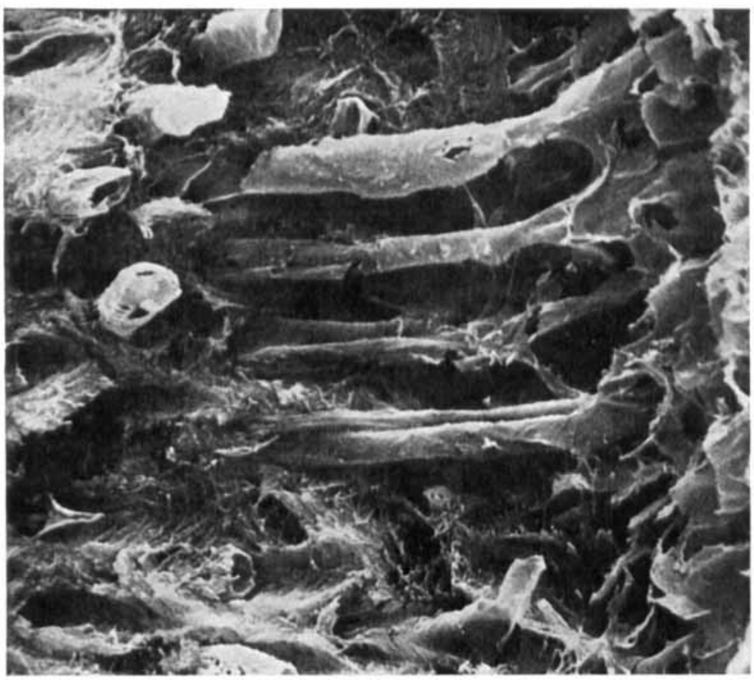

Fig. 2

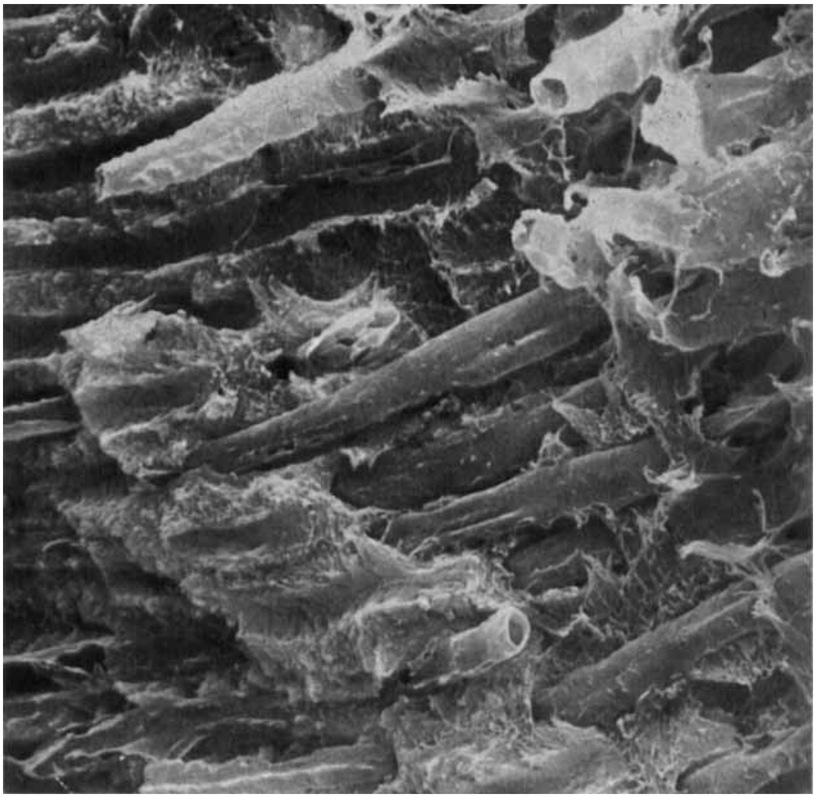

Fig. 3. Exposed predentine. Pulp tissue to the right. Cytoplasmic processes without any membranous coating are entering the tubules. × 2200.

Fig. 1. Exposed dentine (D), predentine (PD) and pulp (P) in an area at the buccal side in the middle of the crown. Odontoblast processes are seen exposed in the predentine and in the dentine. × 550.

Fig. 2. Pulp-predentine area. Pulp tissue to the right. Folded processes are entering the tubules. × 2200.

Fig. 4

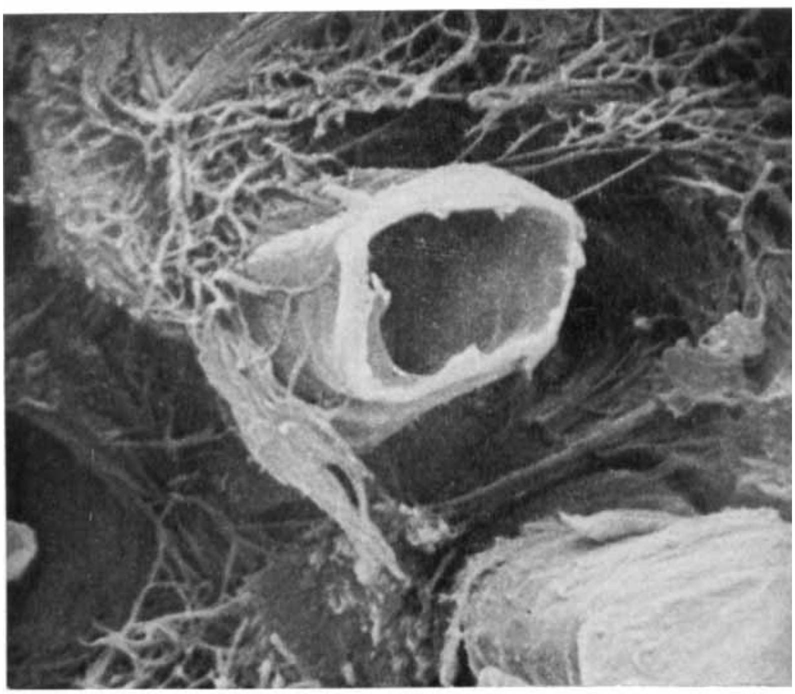

Fig. 5

Fig. 6. Predentine area at point A in Fig. 10. Folded and »naked» cytoplasmic processes are entering the tubules. To the left they continue into the dentine. At the upper level of the fracture, opened and empty tubules, their walls being fairly smooth. Notice that the membranous structure on the tubule wall or on the process seems to be built up of mainly circumferentially oriented fibrils coated with an amorphous material. × 2200.

Fig. 4. Exposed predentine. Pulp tissue to the right with interrupted processes. The tubule apertures contain the other end of the fractured, tube-like processes. Underneath the processes are continuous. A network of fibrils is seen between the tubules. The fibrils close to the process are coated with an amorphous material contributing to a membranous structure surrounding the cytoplasmic process (arrow) × 2200.

Fig. 5. Detail from Fig. 4. The process surrounded by a network of fibrils closely attached to the process. × 11,000.

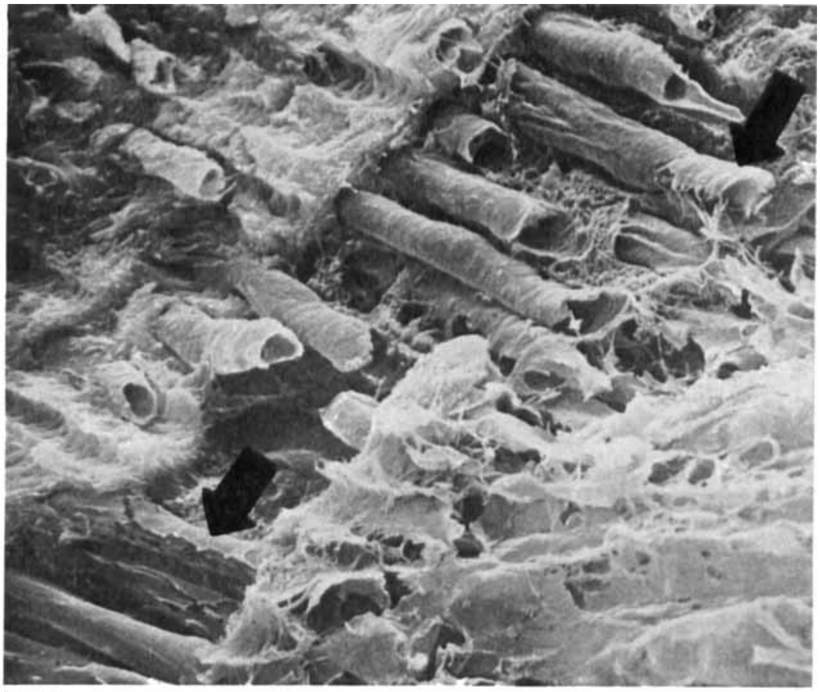

Fig. 7

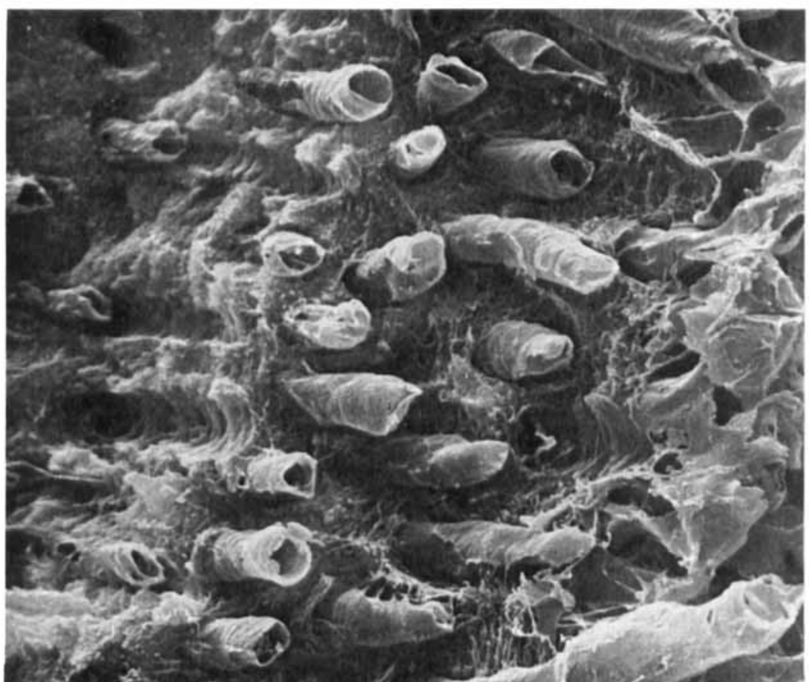

Fig. 8

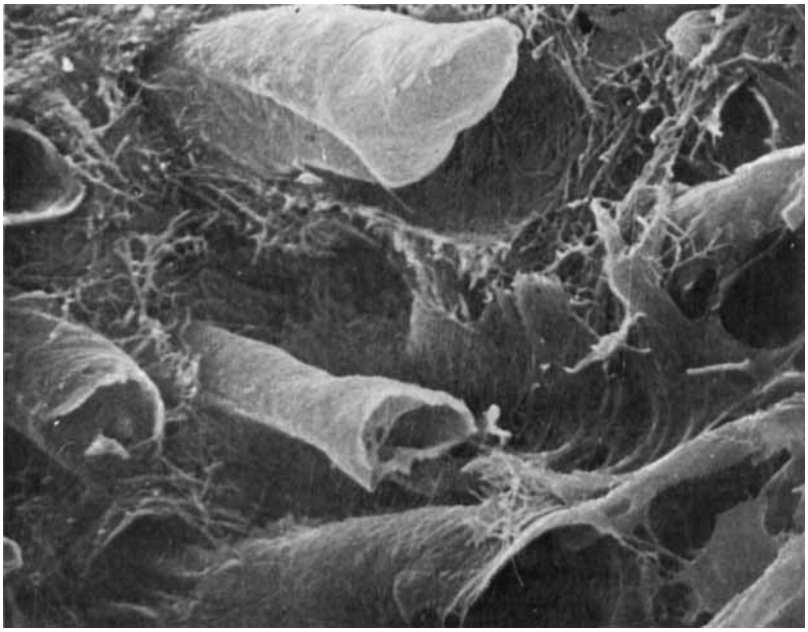

Fig. 9. Exposed predentine with odontoblast processes embedded in a network of fibrils. Amorphous material on fibrils close to the processes. × 5400.

Fig. 7. The predentine area exposed at point B in Fig. 10. Pulp tissue to the right. Cylindrical processes are seen to fill the tubules almost entirely. A membranous structure seems to line the process (arrow). × 2180.

Fig. 8. Exposed predentine with some cross-fractured tubules and processes. Pulp tissue to the right. The processes seem to have a »collar» at the pulp-predentine junction, possibly corresponding to the pulp-dentinal membrane. × 2150.

Fig. 10. Surface of fracture with longitudinally opened dentinal tubules on the buccal side of the crown. Pulp tissue to the right. Figs. 1 to 9 were taken in the predentine area at A and B. Figs. 11 to 17 were taken along the lines from A and B. The tubules at the end of the lines, 0.6—0.7 mm from the pulp, and further outward were empty. × 54.

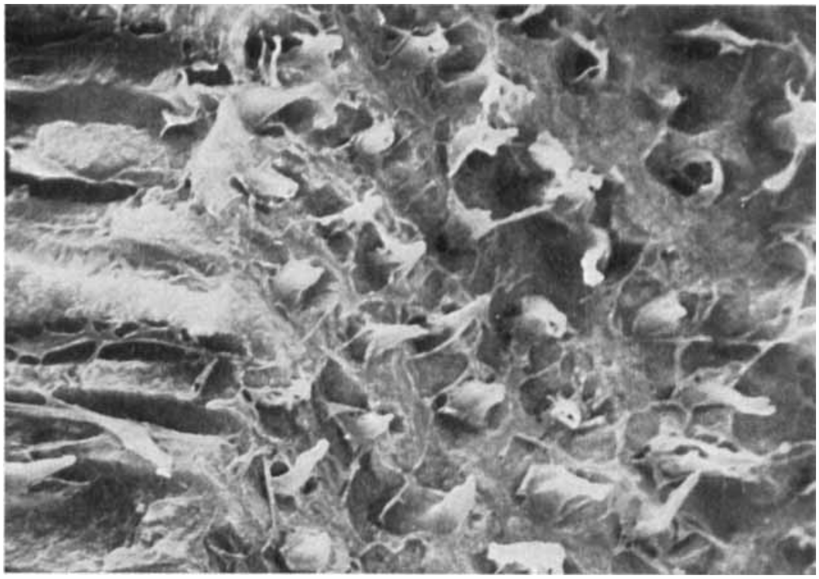

Fig. 11. Exposed dentine, 0.3 mm from the pulp, at A in Fig. 10 (arrow). Almost all tubules contain an odontoblast process. The tubules in the main part of the figure are cross-fractured due to a step in the fractured surface as seen in Fig. 10. × 2200.

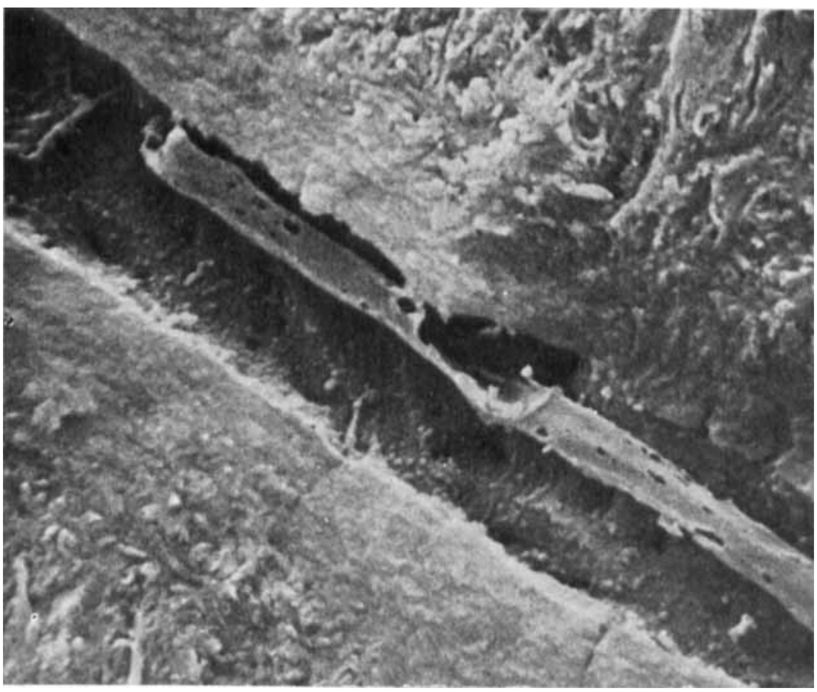

Fig. 12. Exposed dentine, 0.5 mm from the pulp, along the line from A in Fig. 10. A tube-like odontoblast process is seen. In this area only a few tubules contained a process. × 10,800.

Fig. 13. Exposed dentine, 0.6 mm from the pulp along the line from A in Fig. 10. In this area and further outward the tubules were empty. × 2200.

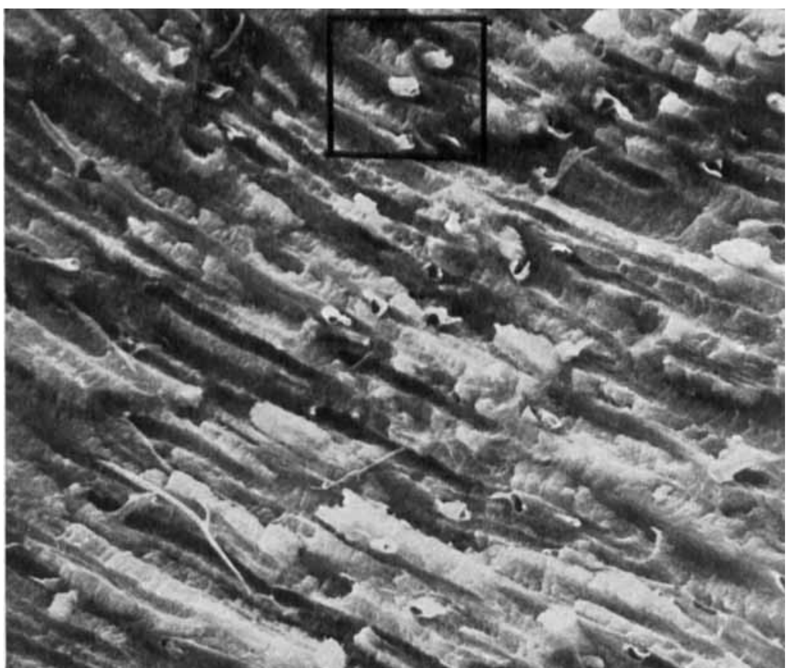

Fig. 14. Exposed dentine, 0.15—0.20 mm from the pulp, at point B in Fig. 10 (arrow). Tubules with odontoblast process that almost entirely fills the tubule. × 1080.

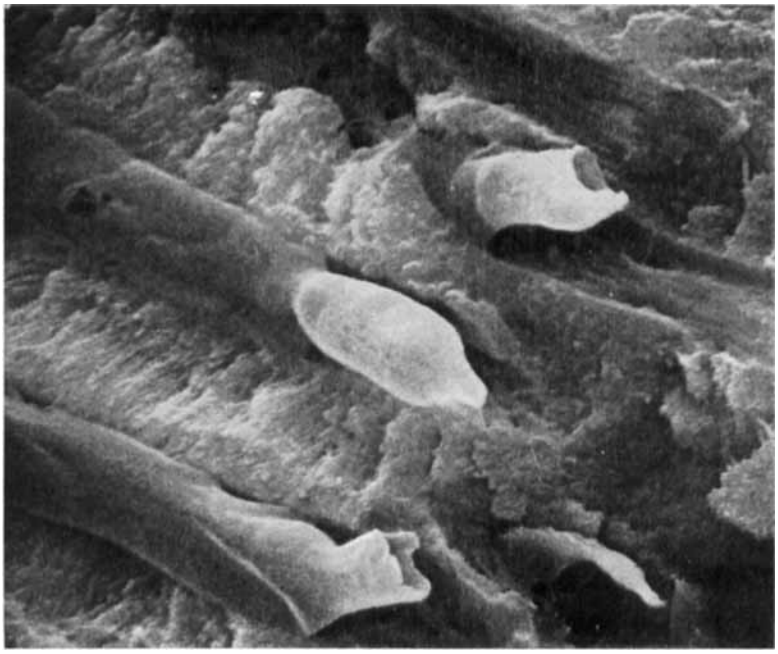

Fig. 15. Detail from Fig. 14, framed area. × 5040.

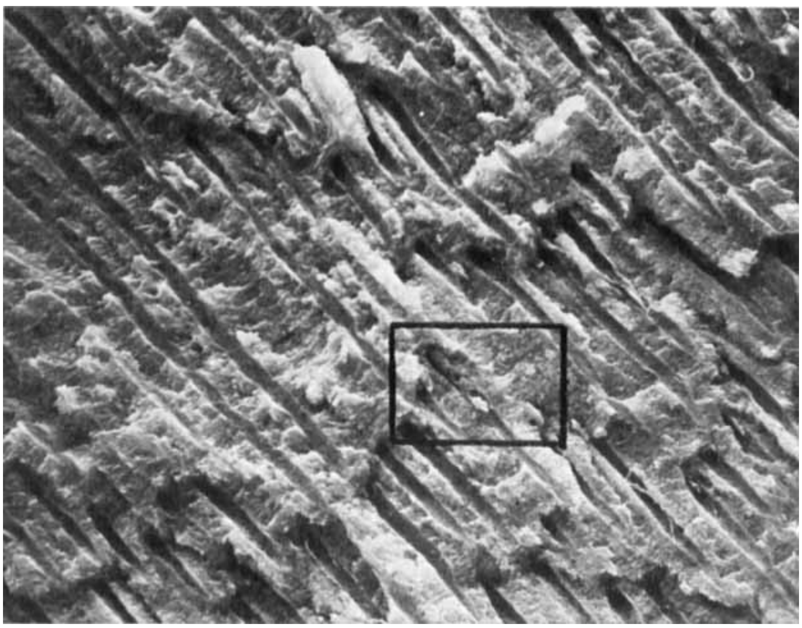

Fig. 16. Exposed dentine further outward along the line from B in Fig. 10, 0.5 to 0.6 mm from the pulp. *Only one tubule was found to contain a process in this area.* Further outward all tubules were empty. × 1080.

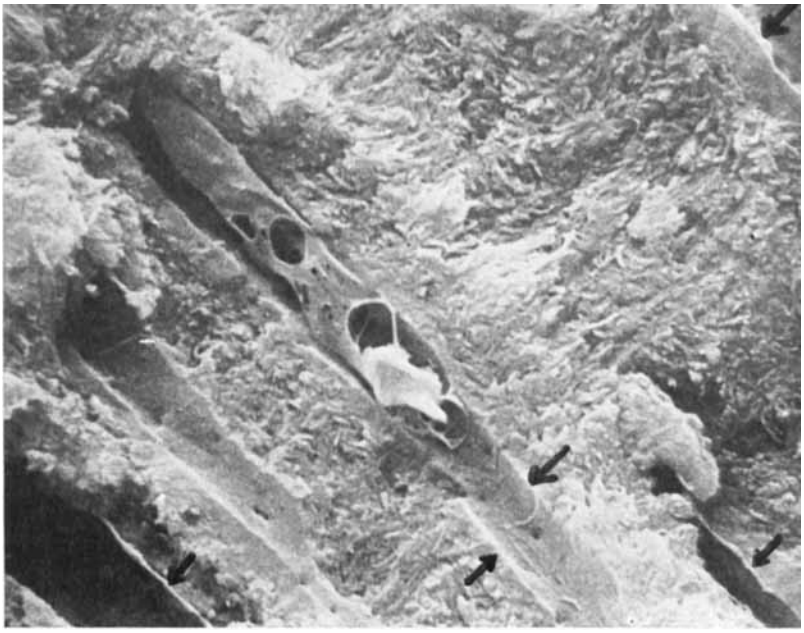

Fig. 17. Detail from Fig. 16, framed area. Notice the profile of the lining of the tubules, 0.1—0.2 um thick (arrow). × 5400.

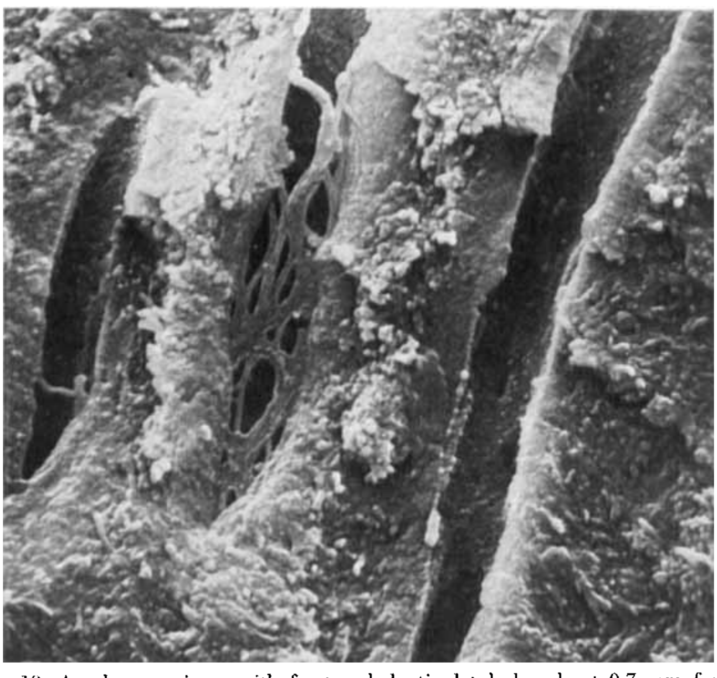

Fig. 18. Another specimen with fractured dentinal tubules about 0.7 mm from the pulp. A few fibrils are exposed in the material lining the tubule wall. × 5800.

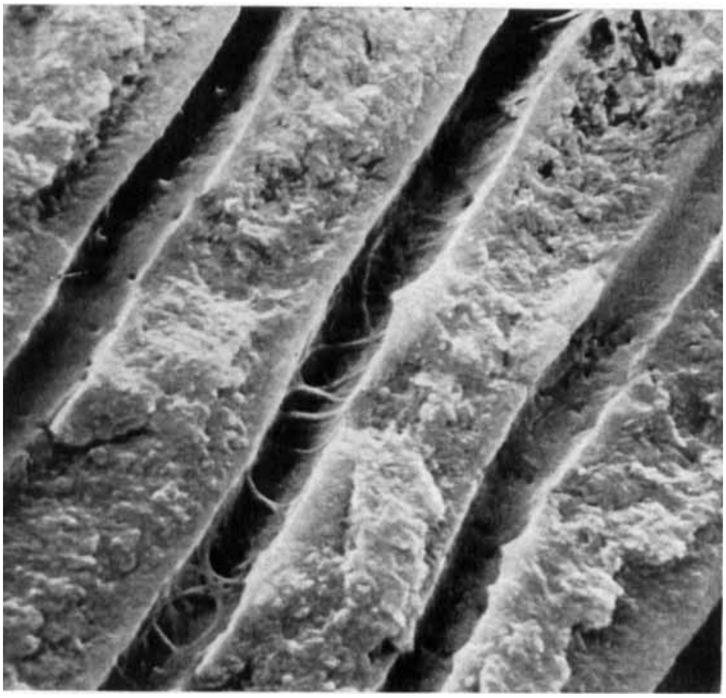

Fig. 19. Another specimen with tubules exposed 1.2 mm from the pulp. Fibrils are seen in the tubule lining in one of the tubules. The other tubules have a fairly smooth wall. × 6000.

Fig. 20. A tubule about 2.3 mm from the pulp; the same specimen as seen in Fig. 18. Fibrils in the exposed wall. × 11,500.

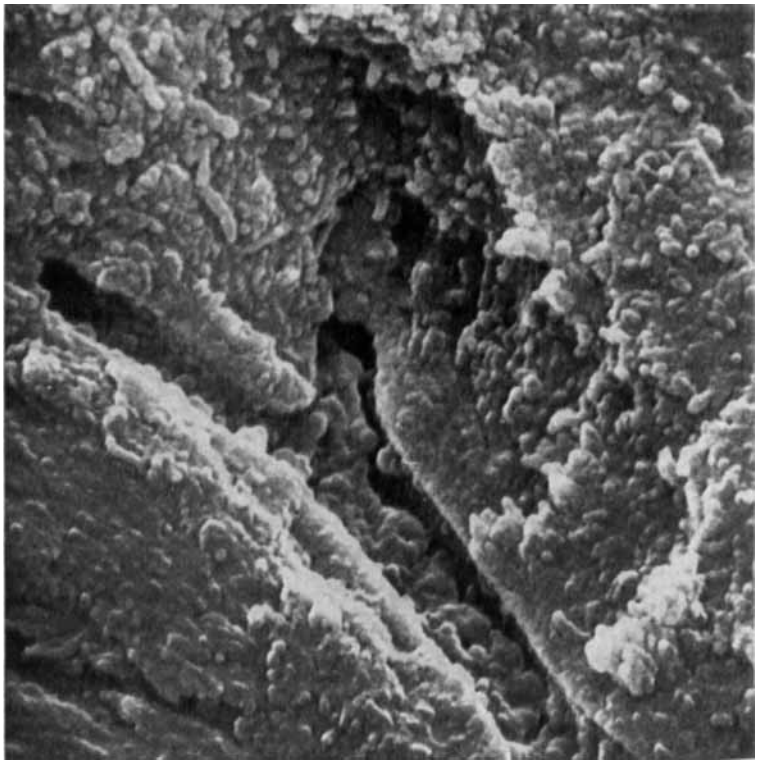

Fig. 21. A dentinal tubule devided in an area near the enamel border. The tubule is partly filled with rounded bodies, calcospherites, of the same type as is seen in peritubular and intertubular dentine. × 11,000.

in form and was in this area covered by a membrane-like structure (Fig.4, arrow). This membrane surrounding the odontoblast process seemed to be a thin wall of the tubule built up mainly by circumferential fibrils embedded in an amorphous material (Figs. 6, 7; arrow). Outside this membrane there was a network of fibrils (Figs. 4—9). The uninterrupted and »naked» processes were folded (Figs. 2—4, 6). In places where they seemed to be covered with the membrane-like structure they looked more cylindrical and with a slight indication of circumferential fibrils on the surface (Figs. 7, 9). The processes seemed to fill the tubules almost completely and the diameter at the predentine-dentine border was about 2.7 μm. No double processes or fibres reminiscent of nerve structure were seen to enter the tubules.

The dentine

Following the dentinal tubules from the predentine areas (Fig. 10; A and B) described above and illustrated in Figs. 1 to 9, the odontoblast processes could be seen extending further outward. At a distance from the pulp of about 0.2 mm most tubules contained processes (Figs. 11, 14, 15). They seemed to fill the tubules almost completely but did not reveal any membrane-like lining of the type seen in the predentine area (Figs. 14, 15). Visible processes diminished greatly in number at about 0.4 mm from the pulp until, at 0.6 to 0.7 mm, there were none at all (Figs. 13, 16). The distal part of the process did not seem to fill the tubule completely (Figs. 12, 17). More than 0.7 mm from the pulp all tubules were empty and had a fairly smooth coating or lining with a thickness of 0.1 to 0.2 μm (Figs. 13, 17; arrow). Some fractured surfaces revealed that this lining may contain a few circumferential and longitudinal fibrils (Figs. 17—20). These fibrils with a diameter of 0.1 to 0.4 μm were occasionally observed in the tubules near the predentine as well as in the peripheral dentine.

The high mineralized homogenous peritubular wall was not always distinct around longitudinally fractured tubules but seemed to appear about 0.2 mm from the pulp and became more clear with a thickness of about 0.5 μm after a further 0.1 mm.

At a distance from the pulp of 0.4 to 0.6 mm the diameter of the tubules varied from 1.8 to 2.0 μm. One millimetre from the pulp the diameter was about 1.5 μm; further outward it diminished and near the enamel border it was around 1.0 μm (Fig. 21).

Near the enamel some tubules were divided into two, and some tubules occasionally contained rounded bodies with a diameter of 0.1 to 0.3 μm. Such rounded structures could also be seen on the fractured surface of the peritubular and intertubular dentine (Fig. 21).

Although small grooves or »apertures» were seen on the walls in some tubules (Fig. 19), very few canaliculi were noticed.

No nerve-like fibre was seen in the dentinal tubules, which either contained a single tubular odontoblast process or were empty.

DISCUSSION

The folding and the tubular shape of the odontoblast process in the predentine area might well be an artefact, possibly due to the fixation or the freezing procedure. As has been noticed by *Scott* (1955), a transversely sectioned tubule may sometimes give the impression of containing a number of processes; this effect is possibly due to the folding of the process, representing cross-sections in the predentine and the adjacent dentine — an area of particular interest judging from a large number of electron microscopic studies.

The observations by *Scott* (1955), *Boyde* and *Lester* (1967) and others, that the process was surrounded by several fibrils enclosed within the membrane-like coating of the tubule walls, would seem to borne out by the present findings. Throughout its length the wall of the tubule might be coated with an amorphous lining, about 0.1 to 0.2 μm thick; this also contained a few mainly circumferentially oriented fibrils. Such fibrils have been observed in previous studies and identified as collagenous fibres (*Johansen & Parks*, 1962; *Lester & Boyde*, 1968). In a histochemical study *Symons* (1962) found in peripheral dentine on the tubule wall a substance that stained more strongly than the rest of the peritubular dentine. It cannot be excluded that this membrane-like structure is what some researchers have called the odontoblast process membrane.

Johansen & Parks (1962) found only one membrane in the predentine area where the present authors have seen two, including the cytoplasmic membrane (Fig. 4). To judge from Figs. 6—9, the outer membrane-like structure closely attached to the process membrane seems to be the lining of the tubule wall in this area. This membranous structure may thus correspond to the junction between intertubular and peritubular dentine further peripherally in the dentine where peritubular dentine is developed.

The fact that the lining of the tubules seemed to be closely attached to the cytoplasmic process in the predentine area and about 0.2 mm further outward suggests that over this length the process almost entirely fills the tubule. Further outward that is, from a point where the peritubular dentine has begun to develop the odontoblast process did not entirely fill the tubule. At this distance from the pulp, the cytoplasmic process was not coated with any extra lining.

The rounded bodies, observed in a few tubules near the enamel seem to be of the same type as the »irregular outgrowths» noticed by *Lester* and *Boyde* (1968). They believed that such outgrowths »may represent the first stage in the occlusion of tubules with material of the same nature as the peritubular dentine». The present authors found this structure not only in the tubule and the peritubular dentine but also in intertubular dentine (Figs. 20, 21). If occlusion occurs in the tubules of young intact permanent teeth, it is likely to be first observed in tubules near the enamel.

Of particular interest is the observation that the odontoblast process was found in the crown dentine only within a distance of less than one quarter the length of the tubule from the pulp. *Symons* (1962) noticed solid staining of what he interpreted as being an odontoblast process extending about 120 to 200 μm into the dentine. *Johansen* and *Parks* (1962) found a cytoplasmic membrane only in an area where there was no peritubular, highly mineralized and homogenous dentine, i.e., according to *Atkinson* and *Harcourt* (1961) 100 to 250 μm into the dentine, according to the present observations 200 to 300 μm from the pulp. Further outward some tubules still had their process, but they were fewer and none was seen more than 0.7 mm from the pulp. Although the process might have been displaced from some tubules during fracturing or the following procedures, this would hardly be confined only to the tubules more distant from the pulp. Apart from the globule-like structures described above and present only in a few tubules near the enamel in one specimen, all tubules at a distance from the pulp of more than 0.7 mm were quite empty. It must be stressed that not only areas described and illustrated in the figures were examined. Many other areas were studied in several specimens, and special interest was focused on tubules 0.5 mm from the pulp and further outward. These findings seem to motivate a new concept of the Tomes' fibre, which in many textbooks is said completely to fill the dentinal tubules out to the enamel.

Frank (1968) pointed out that he »usually located intradentinal nerve fibres in tubules devoid of peritubular dentine (that is about 0.2 mm from the pulp), and assumed that they did not extend further». The present authors could not find any nerve-like fibre together with the »naked» cytoplasmic process or in cross-fractured processes in the predentine. It cannot be ruled out, however, that in areas, where the processes were covered with an »extra» membrane-like structure, a nerve-fibre was hidden inside this membrane. As the odontoblast process was not covered with any visible membrane beyond 0.2 mm into the dentine and at a greater distance did not fill the tubule entirely, any nerve-like fibre should have been detected. This would be the case at least further outward where no odontoblast processes at all were present. Small fibre-like structures were occasionally seen in the dentine containing odontoblast processes but never together with such a process (Fig. 14). Such »fibers» seemed to be displacements of processes, remnants of them or the tubule lining material.

Nerve-like structures have occasionally been observed in light microscopic examinations of decalcified sections stained in various ways. *Langeland et al.* (1971) recently described thread-like structures extending into the peripheral 1/3 of the dentine. These structures were observed to run transversely crossing a great number of dentinal tubules, in some cases about 20 in the published photographs. In view of the present knowledge of the ultrastructure of the dentine, as revealed by recent electron microscopic studies (including scanning), it must be questioned if the structures observed actually represent true nerve fibers. Nerve fibers in mineralized tissue, free from any concomitant visible tubule structures does indeed seem strange.

It has been suggested that the tubule is mainly filled with interstitial fluid (*Brännström*, 1968). Unlike cytoplasm, this has a low potassium content but is rich in sodium. The dentinal fluid has been found to be similar in this respect (*Schneider*, 1955; *Paunio & Näntö*, 1965; *Coffey, Ingram & Bjorndahl*, 1970). *Haljamäe* and *Röckert* (1969), who collected dentinal fluid from exposed dentine *in vivo*, found two types of liquid in the dentine, one of which had an electrolyte composition similar to that of extracellular fluid. As the tubules have been reckoned to occupy about 21 per cent of the dentine (*Hoppe & Stüben*, 1965) and the dentinal fluid accounts for about 25 per cent of the volume of the dentine, most of this fluid is evidently located in the tubules. This physiological approach would seem to throw light on the hydrodynamics of the dentine and the mechanism underlying the sensitivity of this tissue (*Brännström*, 1968, *Brännström & Åström*, 1972).

REFERENCES

Arwill, T. & G. Bloom, 1954: Some remarks on the structure of dentine as revealed by the electron microscope. Acta odont scand *12:* 185.

Atkinson, H. F. & J. K. Harcourt, 1961: Some observations on the peritubular zone in human dentine. Austral. D. J. *6:* 194.

Awazawa, Y., 1962: Electron microscope investigation of the dentin with particular regard to the nature of area surrounding the odontoblast process. J. Nipon Univ. School Dentistry *5:* 31.

Boyde, A. & K. S. Lester, 1967: An electron microscope study of fractured dentinal surfaces. Calc. Tiss. Res. *1:* 122.

Brännström, M., 1968: Physio-pathological aspects of dentinal and pulpal response to irritants. In B. B. Symons: Dentine and Pulp. London, 1968. Livingstone Ltd. pp. 231—246.

Brännström, M. & A. Åström, 1972: The hydrodynamics of the dentine; its possible relationship to dentital pain. Int. J. dent. *22:* 219.

Coffey, C. T., M. J. Ingram & A. M. Bjorndahl, 1970: Analysis of human dentinal fluid. O. Surg. *30:* 835.

Frank, R. M., 1959: Electron microscopy of undecalcified sections of human adult dentine. Arch. oral Biol. *1:* 29.

Frank, R. M., 1966: Etude au microscope électronique de l'odontoblaste et du canalicule dentinaire humain. Arch. oral. Biol. *11:* 179.

Frank, R. M., 1968: Ultrastructural relationship between the odontoblast, its process and the nerve fibre. In B. B. Symons: Dentine and Pulp. London, 1968. Livingstone Ltd. pp. 115—167; discussion p. 229.

Haljamäe, H. & H. Röckert, 1970: Potassium and sodium content in dentinal fluid. Odont. revy *21:* 369.

Helmcke, J. G. & B. Jahn, 1952: Elektronenmikroskopische Untersuchungen über das Dentin im menschlichen Zahn. Naturwissensch. *39:* 492.

Hoppe, W. F. & J. Stüben, 1965: Über die Messung des Volumens der Dentinkanälchen und über das Verhältnis des Kanalvolumens zum Gesamtdentinvolumen. Stomatol. *18:* 38.

Isokawa, S., Y. Toda, & K. Kubota, 1970: A scanning electron microscopic observation of etched human peritubular dentine. Arch. oral. Biol. *15:* 1503.

Johansen, E. & H. F. Parks, 1962: Electron-microscopic observations on sound human dentine. Arch. oral Biol. *7:* 185.

Kubota, K., Ajisaka, M. & Y. Inoue, 1969: Scanning electron microscopic observation of longitudinally sectioned dentinal tubules. J. Nipon Univ. School Dentistry *11:* 140.

Langeland, K., W. E. Dowden, L. Tronstad & L. K. Langeland, 1971: Human pulp changes of iatrogenic origin. Oral Surg. *32:* 943.

Lester, K. S. & A. Boyde, 1968: The surface morphology of some crystalline components of dentine. In B. B. Symons: Dentine and Pulp. London, 1968, Livingstone Ltd. pp. 197—219.

Paunio, K. & V. Näntö, 1965: Studies on the isolation and composition of interstitial fluid in swine dentine. Acta odout scand *23:* 411.

Schneider, D., 1955: Experimentelle Untersuchungen zur Bestimmung der Dentinliquormenge, sowie zur quantitativen Bestimmung des Gehalten an Natrium und Stickstoff im Dentinliquor. Diss. Kiel, 1955.

Shroff, F. R., K. I. Williamson, W. S. Bertaud & D. M. Hall, 1956: Further electron microscope studies of dentine. O. Surg. *9:* 432.

Scott, D. B., 1955: The electron microscopy of enamel and dentin. Ann. N.Y. Acad. Sci. *60:* 575.

Symons, N. B. B., 1962: A histochemical study of the odontoblast process. Arch. oral Biol. *7:* 455.

Syrrist, A. and *G. Gustafson,* 1951: A contribution to the technique of the electron microcopy of dentine. Odont. Tidskr. *59:* 500.

Takuma, S., 1960: Electron microscopy of the structure around the dentinal tubule. J. dent. Res. *39:* 973.

Address:
School of Dentistry,
Box 3207,
103 64 Stockholm 3, Sweden