Membrane structures in the pulp–dentin border zone
A freeze-fracture study of demineralized human teeth

Anders Köling and Helge Rask-Andersen
Department of Otolaryngology, University Hospital, Uppsala, and
Department of Physiology II, Karolinska Institutet, Stockholm, Sweden

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The membrane morphology of cells in the pulp–dentin border zone in human teeth was scrutinized by means of the freeze-fracture technique. The tissue was fractured and replicated after mild demineralization in Na-EDTA. This procedure did not seem to influence the preservation of the tissue significantly. The odontoblastic cell bodies and their long processes lying within the predentinal and dentinal tubules were exposed. The technique made it possible to analyze the structural features of the apical part of the odontoblastic layer, including the 'terminal bar' region. This area exhibited large, irregularly shaped gap junctions. In some regions clusters of many small membranous pits or caveolae apparently representing pinocytotic vesicles were seen. The odontoblastic process displayed membrane protuberances projecting outward and abutting the inner tubular wall. In the predentinal and the adjacent dentinal regions, fine-caliber fibers (approximately 0.1–0.4 μm in diameter), presumably nerves, appeared in intimate relationship with the odontoblastic cell processes. At these sites the cell membrane displayed aggregations of membrane-associated particles, presumably gap junctions. □ *Dental pulp; dentin; intercellular junctions; ultrastructure*

Anders Köling, Department of Otolaryngology, University Hospital, S-751 85 Uppsala, Sweden

In some recent investigations the freeze-fracture technique was applied to the study of the morphology of the human dental pulp (1–4). This method offers distinct advantages in some respects over the conventional transmission electron microscopy (TEM) technique—for instance, in the study of cell membranes and their specializations. The freeze-fracturing technique is normally limited to investigations of soft tissues. This has hitherto precluded an investigation of the border zone between pulp and predentin–dentin.

In the present investigation a technique was used to demineralize the hard tissue without adversely affecting the preservation of the dental soft tissue. This made it possible to visualize the membrane structures and their specializations of cells in the most peripheral part of the human dental pulp, together with dentin and predentin. Special attention was devoted to the nerve-like fibers existing in the dentinal tubules and to their possible morphological relationship to the odontoblastic process. Enhanced knowledge of the membrane morphology in the pulp–dentin border zone may shed additional light on the innervation pattern of the dentin.

Materials and methods

Ten caries-free, fully erupted molars and premolars extracted under local anesthesia (2% lidocaine with epinephrine) for orthodontic reasons from healthy individuals aged between 13 and 20 years were studied. The teeth were immediately placed in a solution containing 2.5% v/v glutaraldehyde and 1% v/v formaldehyde in 0.1 M of Sörensen's phosphate buffer at pH 7.2–7.4. Within 3 min the teeth were split in the following manner. Grooves perpendicular to the long axis of the tooth were prepared in the enamel with a dental diamond wheel under continuous cooling with physiological saline solution. The hard tissue was fractured in a vice, and slices 1–2 mm thick were obtained. These slices of enamel, dentin, and pulp tissue were immediately reimmersed in the fixative. In some instances the soft tissue did not separate from adjacent slices. In these

cases the entire tooth was immersed in the fixative for a couple of hours, after which the soft tissue was sectioned with a razor blade to obtain complete separation of the slices. The specimens remained in the fixative for 24 h. Demineralization was performed in a solution of 0.1 M Na-EDTA (Na–ethylenediaminetetraacetic acid), 2.5% v/v glutaraldehyde, and 0.1 M Sörensen's phosphate buffer at pH 7.2–7.4 for 3 weeks. After demineralization small rectangular pieces of dentin and pulp tissue were prepared. Their long axes were either parallel to or at right angles to the dentinal tubules (Fig. 1). This technique was designed to obtain replices exposing both longitudinally and cross-fractured dentinal tubules and odontoblastic processes.

After being rinsed with buffer the specimens were transferred to a solution of 25% buffered glycerol for 30 min and then mounted on goldcup specimen holders. The specimens were subsequently frozen in supercold nitrogen at approximately −190°C and fractured in a Balzer's 360M or 301 freeze-etching apparatus with a double replica technique (5). The resultant replicas were mounted on copper grids and examined in a Jeol-100 B or Philips 301 electron microscope at 80 kV. Ten successful replicas were examined. Morphometric data were collected from measurements on micrographs of the freeze-fracture replicas.

The terminology according to Branton et al. (6) is used.

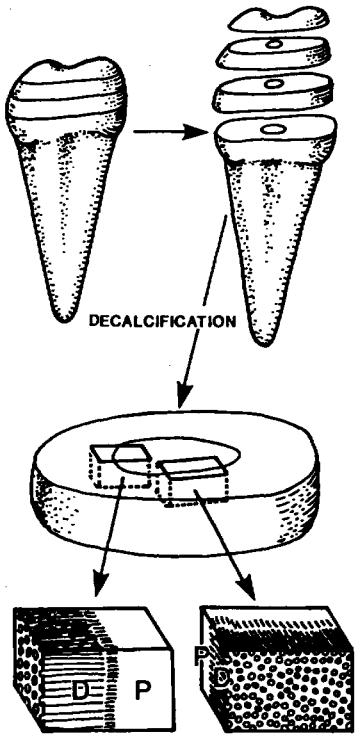

Fig. 1. Schematic drawing illustrating the processing of the teeth investigated in this study.

Observations

Odontoblastic cell body

Freeze-fracturing of the apical portion of the odontoblastic layer, where cells converge near the predentin, displayed numerous gap junctions between adjoining cells (Fig. 2). These gap junctions were irregularly shaped and appeared to be generally larger than those seen nearer the central pulp (Fig. 3). Sometimes as many as seven or ten gap junctions could be observed simultaneously at the apical region of a partially exposed odontoblast. Some junctions appeared as many small (20–30) 'mini-junctions' consisting of aggregations of particles located some distance apart on the cell membrane (Fig. 4). Morphometric data of the gap junctions are summarized in Fig. 5. No tight junctions were observed.

A few cells displayed invaginations or pits on their apical cell membrane, presumably representing micropinocytotic vesicles (Fig. 6).

Between odontoblasts we observed smaller associated cells, some of which were tubular or fiber-like, while others were flatter and more lamellar in shape. These structures were often positioned close to the odontoblastic cell body surface, where adjacent cell membranes were differentiated into typical gap junctions. Such junctions between nerve-like structures and the odontoblastic cell body were reported on in detail in a recent paper (4).

Odontoblastic cell process

The membrane surface of the odonto-

Fig. 2. Low-power electron micrograph of a freeze-fracture replica from the odontoblastic and predentinal layer of a human molar, treated in Na-EDTA. OD = odontoblastic cell body; NU = odontoblast nucleus; OP = odontoblastic process; PD = predentin. Frame: see legend to Fig. 2. Scale bar, 2 μm.

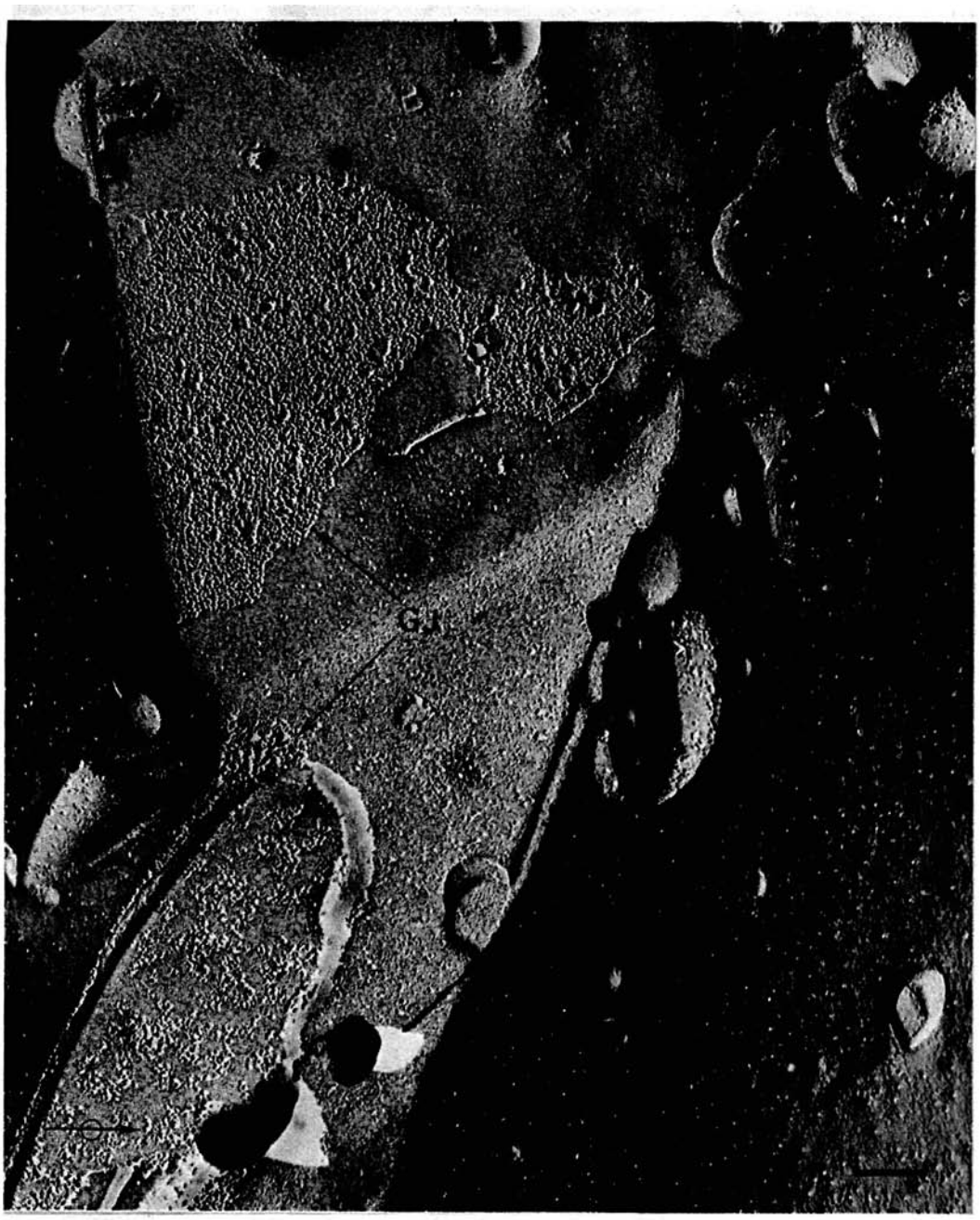

Fig. 3. Close-up view of framed area in Fig. 2, showing a gap junctional area in the apical part of odontoblastic layer. Note large, irregularly shaped gap junction (GJ) at the region near the predentin, together with a partially exposed gap junction located more inferiorly. Scale bar, 0.1 μm.

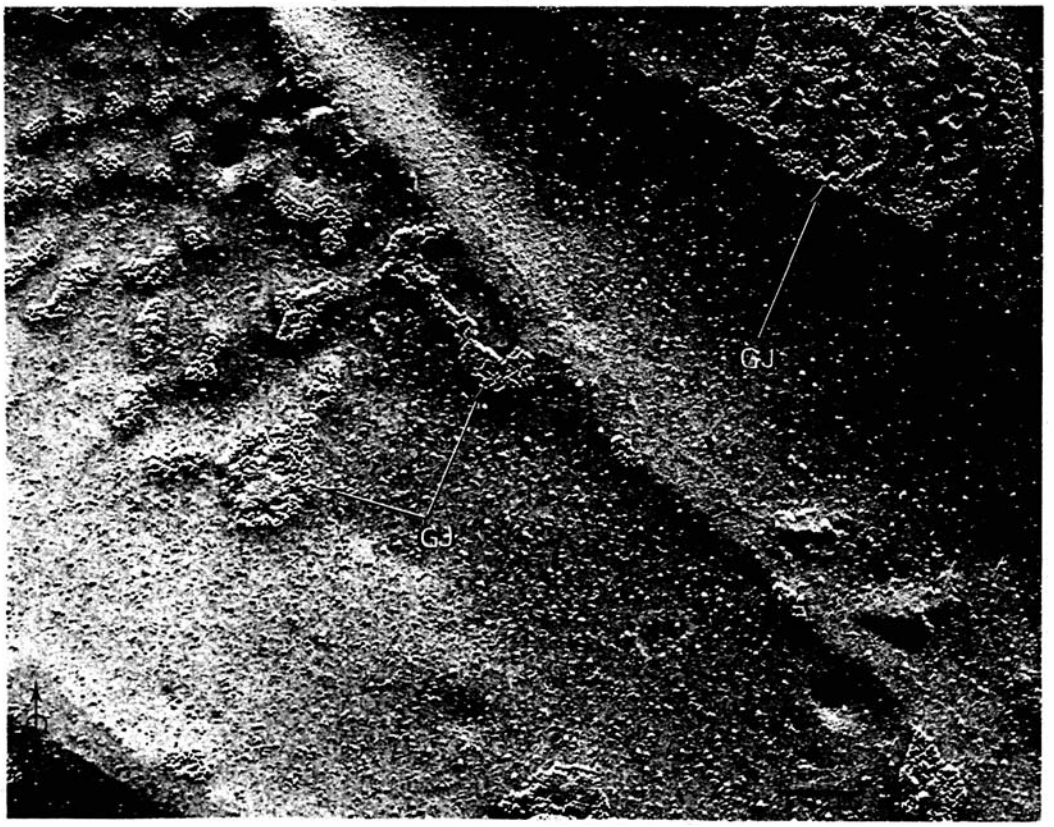

Fig. 4. Freeze-fracture replica demonstrating the varied morphology of the gap junctions in the apical region of the odontoblastic layer. At top right a gap junction (GJ) where both the E- and the P-face are exposed. To the left many small aggregations of intramembranous particles are seen. On some aggregations both E- and P-face can be seen simultaneously, demonstrating the characteristic features of the junctional specializations. Scale bar, 0.2 μm.

Anatomic region	Mean diameter μm (range)	Shape
Subodontoblastic fibroblasts	0.25 x 0.44 (0.19-0.27 x 0.42-0.46)	
Odontoblastic cell body		
Supranuclear portion	0.63 x 1.31 (0.40-0.97 x 0.81-2.10)	
Infranuclear portion	0.36 (0.18-0.57)	
Odontoblastic cell process		
Intratubular portion	0.06 x 0.10 (0.04-0.07 x 0.05-0.16)	

Fig. 5. Morphometric data of gap junctions in peripheral dental pulp and dentin.

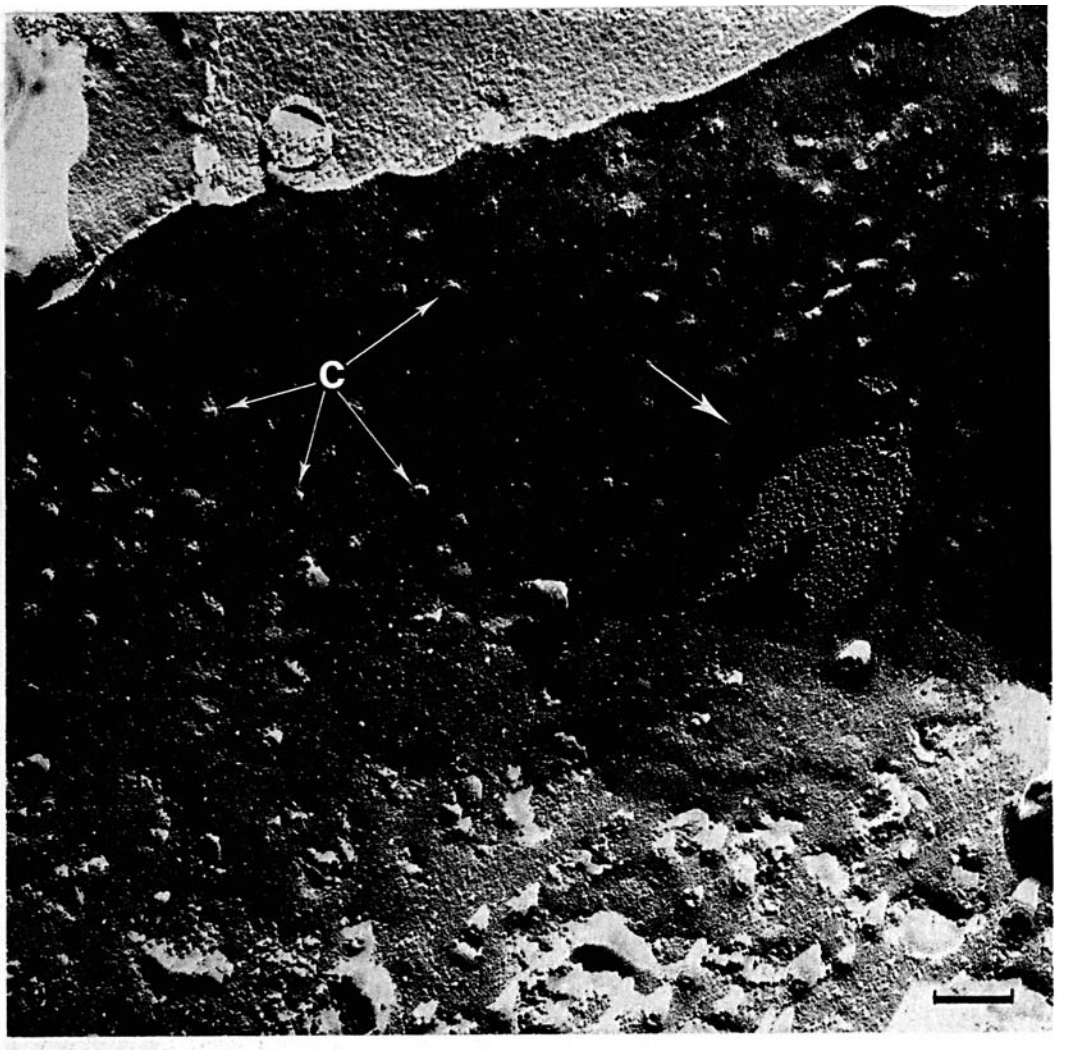

Fig. 6. Electron micrograph of a freeze-fracture replica displaying a cell membrane at the apical part of an odontoblastic cell body. Note many small caveolae (c) or pits on the membrane, probably representing micropinocytotic vesicles. The large arrow marks a typical gap junction. Scale bar, 0.2 μm.

blastic cell process was generally smooth, with an abundance of exposed intramembranous particles. Short bud-like protuberances projected outwards and abutted the predentinal and dentinal tubular inner wall (Fig. 7). These projections could be most clearly demonstrated in the predentinal and inner dentinal regions. Their number seemed to decrease in the apical direction. In the predentin, the odontoblastic cell process appeared to be closely attached to the inner tubular wall, whereas more peripherally the process did not seem to fill the tubule completely, and a small space seemed to widen out in direction toward the dentin–enamel border (Fig. 8).

The number of intramembranous particles seemed to be relatively high, approximately 400/μm^2.

Fine-caliber fibers in the predentinal and dentinal tubules

In the predentin, small thread-like fibers

Fig. 7. Freeze-fracturing of demineralized human inner dentinal tubule containing an odontoblastic process. On the cell surface numerous small bud-like protuberances can be observed (arrows). Scale bar, 0.2 μm.

appeared among the odontoblastic processes. In general, their diameter was 0.1–0.4 μm. They were regular and tubular in shape. The fibers joined the odontoblastic cell process at the entrance to the predentinal tubules. They were thereby closely associated with the odontoblastic cell membrane (Fig. 9), on which they formed shallow impression or furrows (Fig. 10). In these sulci, minor aggregations of particles were spread or scattered along the P-face of the odontoblastic cell membrane. Since both the P- and the E-face could be observed simultaneously, we felt justified in classifying them as gap junctions.

A total of 30 fine-caliber fibers could be visualized on freeze-fracture replicas. This corresponds to a ratio of approximately 1 fiber to 20 tubules.

Discussion

It has previously been alleged that tight junctions are present between odontoblasts both in the human dental pulp and in other mammals (7–10). Holland (11) and Cox et al. (12) suggested that some of these represent small gap junctions. All these observations are based on morphological studies with the use of TEM and the thin-section technique. However, freeze-fracturing appears to be a more suitable method for optimal visualization of intercellular junctional complexes.

It has been observed in various epithelia that the apical part of adjacent cell bodies converge and fuse, thus sealing the luminal area from the intercellular space. In this region neighboring cell membranes are specialized into so-called tight junctions or

Fig. 8. Freeze-fracture replica demonstrating cross-fractured odontoblastic process (Op) relatively far out in the dentinal tubule. At this level the odontoblastic process does not completely fill out the tubule but leaves circumferentially a homogeneous substance, presumably dentinal liquor (L). D = dentin. Scale bar, 1 μm.

zonulae occludentes (13). These junctions, when freeze-fracturing is used, can be seen as grooves or strands forming belt-like structures around the cells.

In the present study we did not find any membrane specializations suggesting the presence of tight junctions or zonulae occludentes on the odontoblasts in the human dental pulp. Considering that the odontoblastic layer is not a true epithelial cell layer but possibly a form of echtomesenchymal tissue derived from the neural crest (14), the absence of such junctions may be possible. Tight junctions, in various epithelia, may enable gradients of different substances to be built up across the cell layer (15). There does not seem to be any need for such a barrier function in the odontoblastic layer. On the contrary, a free flux of metabolites and other substances along the odontoblasts may be essential for the proper function of the odontogenic zone. In addition, many small nerve fibers and von Korff's fibers are allowed to pass unrestricted through the odontoblastic layer from the subodontoblastic region to the predentin. Instead, numerous membrane specializations representing gap junctions were found on the distal periphery of the odontoblasts. Gap junctions, observed near the pulp–dentin border zone, were generally larger than those found in the subodontoblastic region and on the infranuclear portion of the odontoblasts (Fig. 5). The many gap junctions demonstrated in this study seem to indicate an important physiological function.

Desmosomes have been demonstrated between odontoblasts (8). No attempt was made to investigate them further in our study since freeze-fracturing is not an ideal method for examination of such junctions (15).

The odontoblastic cell body displayed on its cell membrane small caveolae or pits, presumably representing micropinocytotic vesicles. Baume (7) concluded from several reports that the extrusion of secretory products from the odontoblast must be assumed to take place by a process of exocytosis. Jessen (16) and Takuma & Nagai (17) visualized exocytotic vesicles in the odontoblastic cell wall with the help of TEM. Thus, the membranous craters found in our study may be the morphological expression of a secretory activity related to dentin matrix formation and mineralization.

Despite laborious efforts to clarify the mechanism behind the sensitivity of the peripheral dentin, where no nerve fibers are present, many problems remain unsolved. It is generally agreed that any stimulus, mechanical or osmotic, causing streams in the dentinal liquor can give rise to pain impulses (18). The mode of action of this transformation of motive energy into somatosensory nerve impulses is not known.

The first convincing evidence of the innervation of the inner dentin was given by Fearnhead (19). Using light microscopy and serial sectioning, he traced continuity between nerve-like structures within the dentinal tubules and bundles of fibers, which were indisputably nerves, in the central pulp. In animal experiments these intradentinal fibers were absent after sectioning of the main bundles to the tooth. Ultrastructural

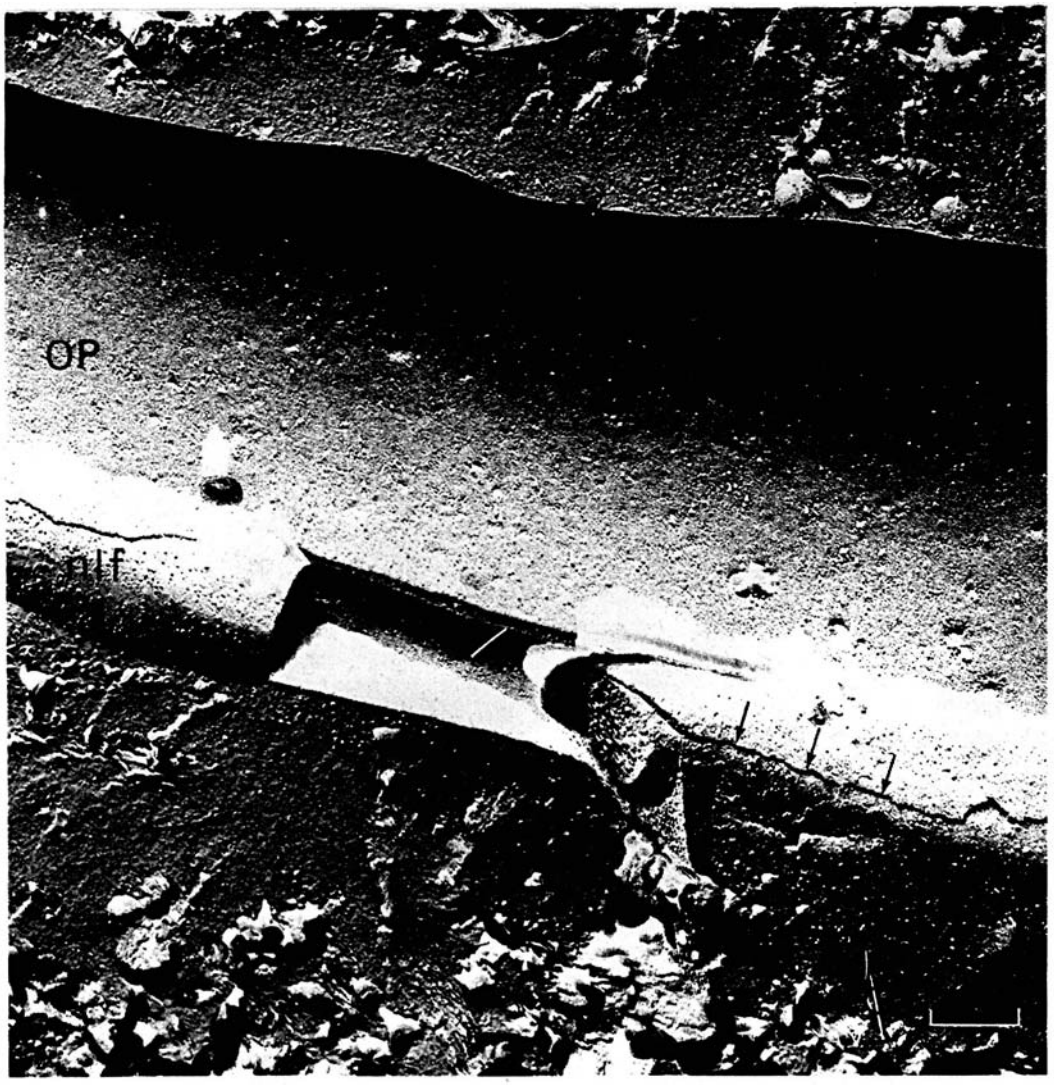

Fig. 9. Electron micrograph of an odontoblastic process (OP) and an associated nerve-like fiber (nlf). There is a close morphological relationship between the two cell structures (arrows). Scale bar, 0.2 μm.

denervation investigations confirmed the presence of nerves in the predentinal and proximal dentinal tubules (20, 21).

Several investigators have described the structural relationship between odontoblastic processes and assumed nerves. Frank (9, 22) suggested the existence of an intercellular connection between unmyelinated nerves and the odontoblastic process. Later, both Roane et al. (23) and Dahl & Mjör (24) reported on possible junctional sites between odontoblastic cell processes and neural elements, whereas Gunji (25) denied their existence. We did not find chemical synapses, which is consistent with earlier studies. Nor did we locate any sign of nerve ending specializations between cells in the odontoblastic cell layer or in the predentinal–dentinal tubules. On the other hand, we observed on the odontoblastic process small aggregations of intramembranous particles, located in shallow furrows, where

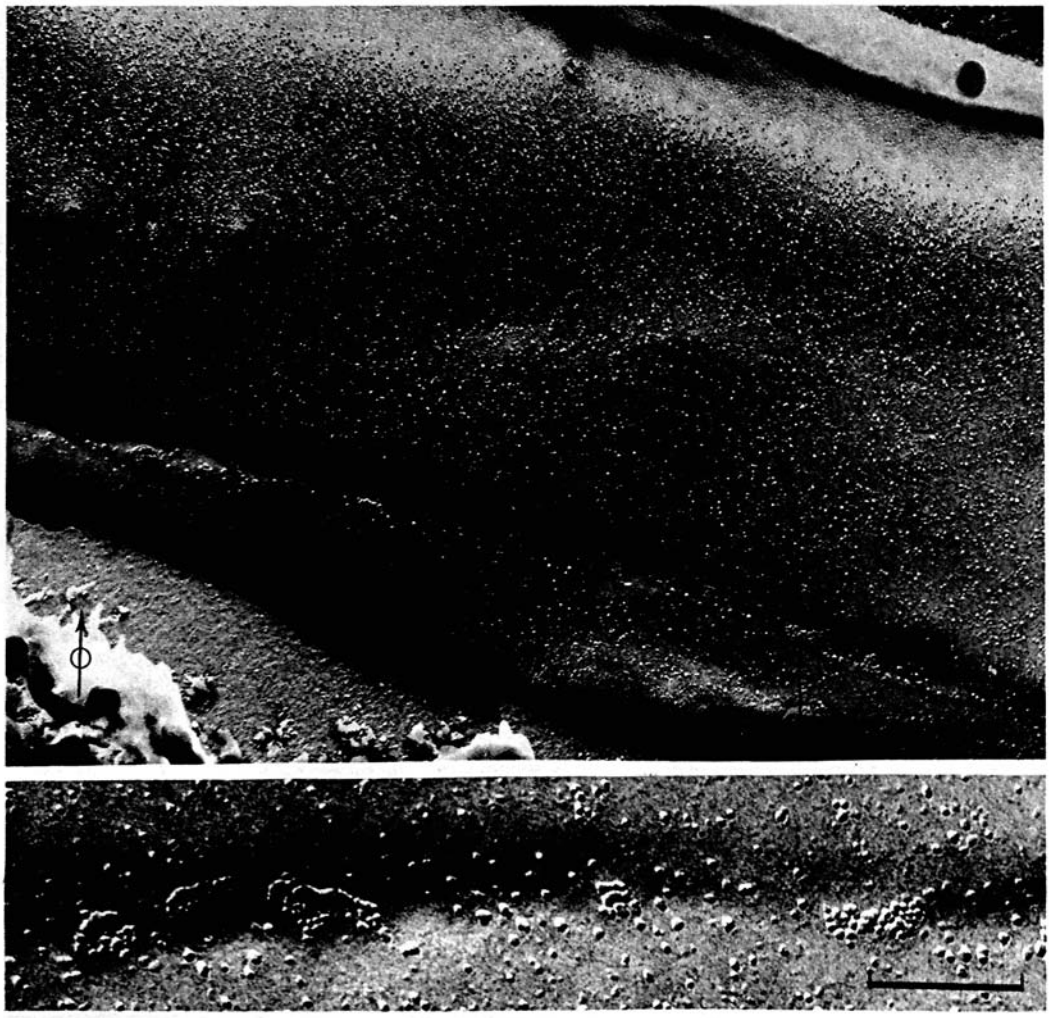

Fig. 10. Freeze-fracturing of an odontoblastic cell process (OP) in inner dentin. A shallow furrow is seen on the surface, where intramembranous particles are gathered into aggregates (arrows). Close-up view reveals both P- and E-face on these aggregates (bottom). Scale bars, 0.5 μm (top) and 0.2 μm (bottom).

also nerve-like fibers could be observed. It was possible to visualize both the E- and the P-face at these sites. It therefore seems justifiable to classify them as gap junctions. Recently, we reported on similar junctions between nerve-like structures and odontoblasts within the odontoblastic layer (4) (see Fig. 11).

Cell-to-cell communication has been ascertained to be a common property of both excitable and non-excitable tissue (26, 27). In excitable tissue gap junctions are considered to function as low-resistance, electrical pathways, permitting action potentials to spread from one cell to another with no synaptic delay (13). The function of gap-junction communication in non-excitable tissue is not quite clear. The classical phenomenon of 'metabolic cooperation' has been described in tissue cultures (28). The biological significance of such metabolic cooperation is largely unknown.

The question of whether the gap junctions in the peripheral human dental pulp and

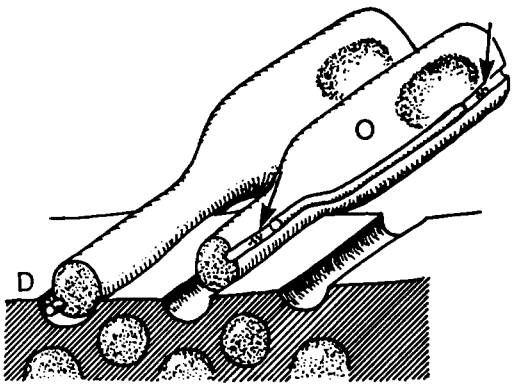

Fig. 11. Illustration of the interrelationship between the odontoblasts (O) and adjacent nerve-like fibers. Arrows indicate gap junctions. D = dentin.

dentin are involved in the neurosensory supply of pulp and dentin or are manifestations of a metabolic exchange between cells without excitatory involvement needs further investigation by appropriate physiological methods. It is interesting that the nerve-like fibers described in this paper, as far as form and distribution are concerned, are in accordance with the sensory nerve fibres described by Arwill et al. (20). In the light of our findings we can hardly refrain from speculating about the possibility that gap junctions between nerves and odontoblasts may constitute a significant link in the propagation of afferent pain impulses elicited from the dentin.

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