Membrane junctions between odontoblasts and associated cells

A freeze-fracture study of the human odontoblastic cell layer with special reference to its nerve supply

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The relationship between odontoblasts and adjacent cell structures within the odontoblastic cell layer was analyzed by means of the freeze-fracturing technique. Two principal forms of interodontoblastic cell structures were found. The first was tubular or thread-like in appearance, having a general diameter around 0.1–1.0 μm. From morphological criteria these were believed to represent small, unmyelinated nerve fibers. The second type of cell structure found between odontoblasts was more irregular and heterogeneous in outline, and often lamellar or branched. These slender formations sometimes proved to constitute cellular projections from adjacent odontoblasts or neighboring, subodontoblastic fibroblasts. Both the nerve-like fibers and the irregular branched cells between the odontoblasts showed morphological contact areas with odontoblastic cell bodies. At these sites the intracellular distances were reduced, and characteristic gap junctional complexes occurred. Nerve ending specialization or membrane structures indicating the presence of chemical synapses on the odontoblastic cell surface were not observed. □ *Dental pulp; intercellular junctions; ultrastructure*

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The site of generation of and the mechanisms behind the transmission of dentinal pain are still an enigma. Evidence has been submitted that various physical stimuli give rise to a flow of liquor in the dentinal tubules, which in turn may elicit somatosensory pain impulses in the pulpal nerves, whence they are mediated to the central nervous system (10).

Both light microscopy and ultrastructural studies have demonstrated the existence of myelinated (A-delta fibers) and unmyelinated nerves (C-fibers) in the mammalian dental pulp (11). Presumed nerve endings have also been observed in the subodontoblastic region, among odontoblasts, and in the predentinal tubules, but not in the outer dentine (3, 5, 15, 16, 24, 33).

Since the fully mineralized outer dentin has no direct innervation, some mediation of stimuli must take place from dentin to nerve fibers. The odontoblast is nowadays generally considered to act as intermediary (9, 10, 21). However, the modes of transformation of stimuli into nerve impulses and transmission to the pulpal nerve fibers are still not clear. Several different hypotheses about this mechanism have been submitted.

A chemical synapsis between odontoblast and nerve was postulated by Avery & Rapp (4). Others suggested that nerves and odontoblasts form no synapse and that the nerves terminate only as sensory, free endings in the pulp–dentinal border zone (9, 18, 21). Olgart (30) proposed that when the odontoblast is deformed by dentinal-liquor currents, it leaks potassium ions, which in turn may stimulate nerve endings in the extracellular space. Morphological connections or junctional complexes between nerve fibers and odontoblasts have been reported (15–17, 25, 29).

Membrane junctions between subodontoblastic fibroblasts (Höhl's cells) and fine-caliber fibers, possibly nerves, were identified in a recent investigation using freeze-fracturing technique (28). The intercellular connections were established by membrane specializations of gap junction type. In the

present study we used the same method to obtain more information about the morphological interrelationship between odontoblasts and other cell structures within the odontoblastic cell layer.

A prerequisite for this investigation was that large pieces of the odontoblast layer could be successfully replicated to permit the analysis of a greater number of odontoblasts than in earlier studies (27). This was accomplished by means of a modification of the preparation technique.

Materials and methods

Ten caries-free, fully erupted, human molars extracted under local anesthesia (2% lidocaine with epinephrine) for orthodontic reasons from healthy individuals aged between 13 and 35 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 Sörensen's phosphate buffer at pH 7.2–7.4. Within 3 min the crowns of the teeth were split in the following manner.

Grooves perpendicular to the long axis of the tooth were cut in the enamel with the help of a dental diamond wheel. The teeth were continuously cooled with physiological saline. The hard tissue was fractured in a vice and 1- to 2-mm-thick slices obtained. These slices of enamel, dentin, and pulp tissue were immediatedly reimmersed in the fixative. In general, the pulpal soft tissue remained *in situ* along the inner surface of the dentin. In some instances the soft tissue did not separate from adjacent slices. The entire fractured tooth remained immersed in the fixative for a couple of hours, followed by sectioning of the soft tissue with a razor blade to achieve complete division of the slices. The pulp was thoroughly separated from the dentinal inner surface by means of a razor blade. Most of the central pulp was dissected from the disc of soft tissue, thereby leaving the peripheral zone (about 1 mm) for replication.

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. They were subsequently frozen in supercold nitrogen at approximately −190°C and fractured in a Balzer 360 M or 300 freeze-etching apparatus by the double replica technique (35).

The resultant replicas were mounted on copper mesh grids and examined in a JEOL-100 B or Philips 301 transmission electron microscope at 80 kV.

Morphometric data were collected from micrographs of freeze-fracture replicas. Measurements were performed on stratified samples on cross-fractured areas of the odontoblastic cell-layer.

The terminology is in accordance with that of Branton et al. (8).

Results

The freeze-fracture replicas of the pulpal tissue often exposed considerable areas of the odontoblast layer (Fig. 1). As a rule, the cells were fractured along their axis, but in a few instances the random fracture face laid bare numerous cross-fractured ondontoblastic cell bodies, which prompted the quantitative study of interodontoblastic structures (Fig. 2).

Two principal types of interodontoblastic cell structures were found. The first was tubular, thread-like in shape, having a general diameter around 0.1–1.0 μm. Their cross-fractured surface exhibited intracytoplasmic filaments or tubules resembling those of the small myelinated nerve axons in the central pulp (Köling & Rask-Andersen, unpublished observations). The diameters of the fibers were even. No regional swellings or 'beads' within the odontoblastic cell layer were observed. When longitudinally split, it was possible to observe the fibers running in intimate relationship with the odontoblastic cell bodies, sometimes for almost their entire length (~20 μm) (Fig. 3). This type of intercellular fiber was believed to represent small unmyelinated nerve fibers.

The other form of interodontoblastic cell structure was more irregular and heterogeneous in character, being often lamellar or branched (Fig. 4). These were thought to be

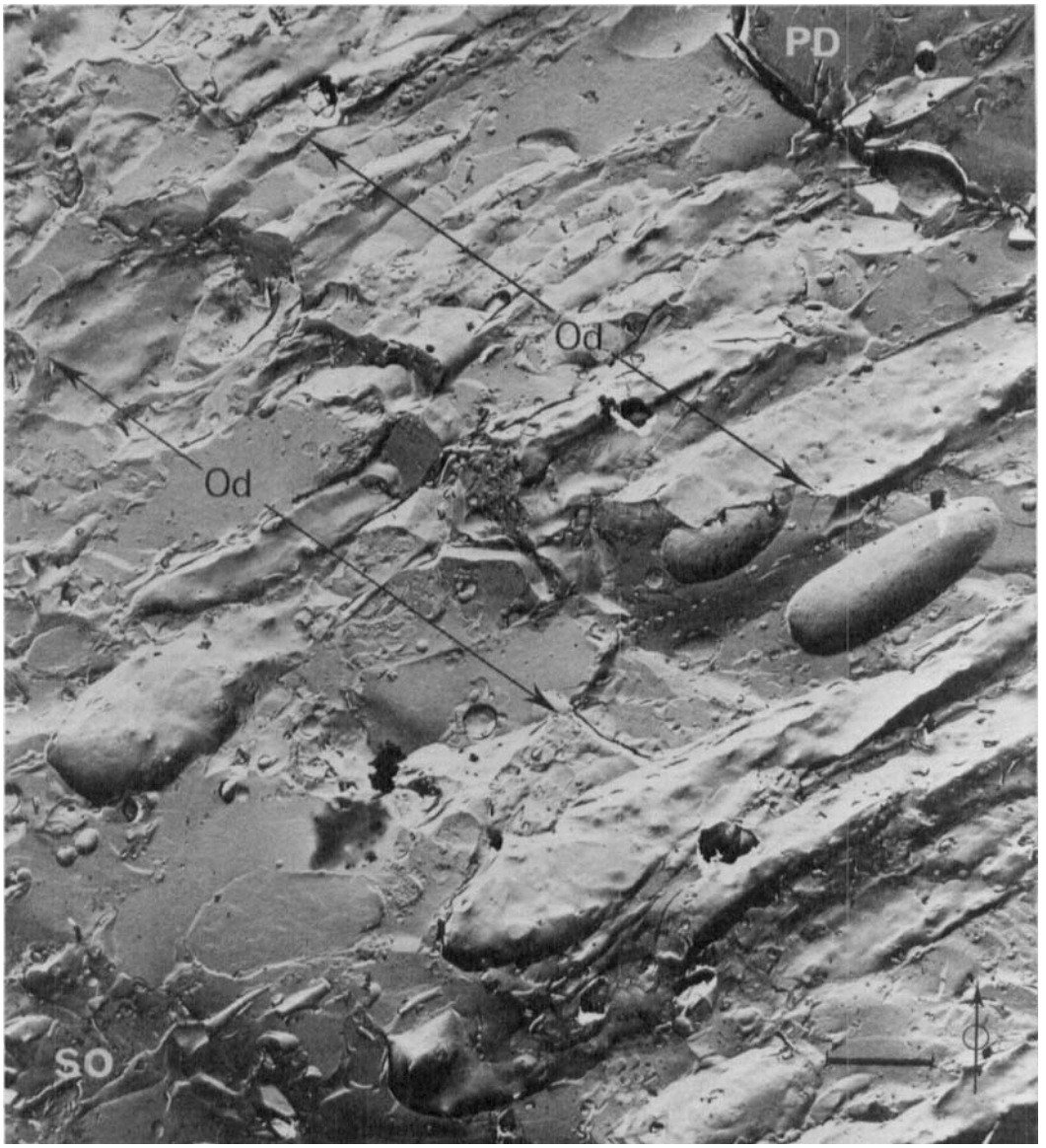

Fig. 1. Low-power electron micrograph of a freeze-fracture replica from the peripheral human dental pulp. The replica exposes about 50 × 50 μm of the odontoblast layer. Numerous tightly packed odontoblasts are split longitudinally, most along their outer cell membrane (top Od). Below (bottom Od) are seen mostly cross-fractured odontoblastic cells exposing their cytoplasmic interior. At top right a small area of the pulp-dentin (PD) border zone is visualized. SO=subodontoblastic region. Scale bar, 5 μm. Arrow indicates direction of shadowing.

derived from cellular projections of adjacent odontoblasts and/or proximal subjacent subodontoblastic fibroblasts. Their origin was sometimes ascertained, since they could be continuously followed to odontoblasts or fibroblasts (Fig. 3).

The nerve-like fibers were generally arranged parallel to the long axis of the odontoblasts. Even though the fibers were exposed for a considerable distance, they could not be followed inside the subodontoblastic layer. They were not observed to

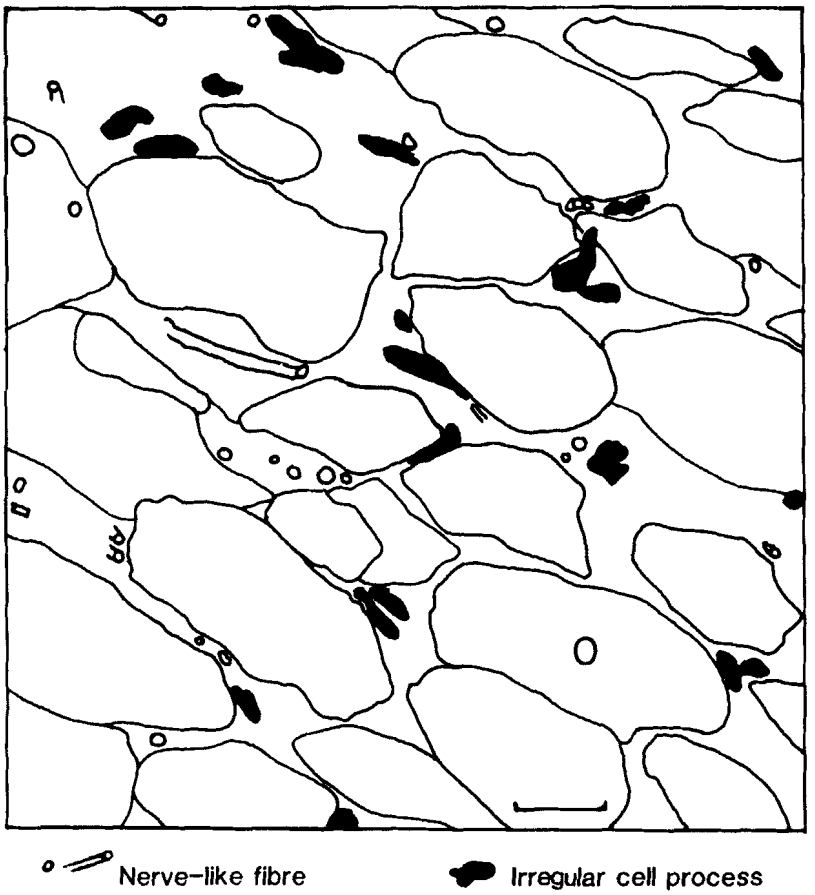

Fig. 2. Drawing of a freeze-fracture replica in which the odontoblasts have been cross-fractured. Between individual odontoblasts (O) are groups of irregular cell processes and fiber structures. The latter range from about 0.1 to 1.0 μm in diameter. Scale bar, 1 μm.

display any kind of specialized endings in contact with the odontoblast surface. Nor were any membrane structures seen that indicated the presence of chemical synapses between the fibers and the odontoblastic cell body. In general, the fibers appeared to run uninterrupted, and in close topographic relationship to the odontoblasts towards the predentinal layer. Many cross-fractured fibers were observed on the periphery of the odontoblast layer. Their straight course was in marked contrast to the irregular cell structures that emerged in many directions.

The fibroblastic and odontoblastic processes together with the nerve-like structures formed morphological contact areas with adjacent odontoblastic cell bodies. The intercellular distance was reduced, and characteristic gap junctional complexes were observed (Figs. 4 and 5).

Junctions between the nerve-like fibers and the odontoblasts were best studied when both the P-face and adjacent E-face of nearby odontoblasts were visualized with an intervening nerve-like fiber. Such a circumstance can be seen in Figs. 4 and 5, which show a nerve-like fiber crossing over one odontoblast at a certain angle.

Some morphological data obtained from freeze-fracture replicas are given in Table 1.

Discussion

The overriding problem in morphological investigations of the odontoblastic layer is

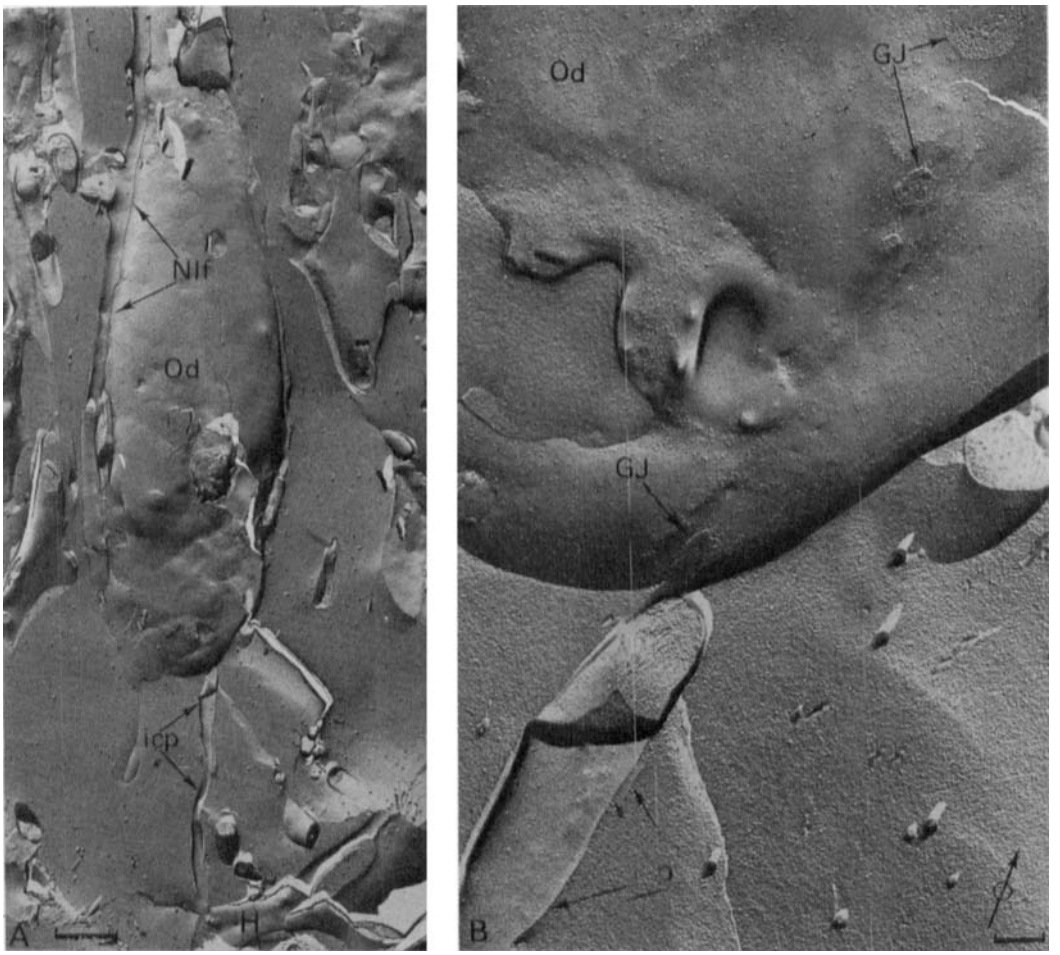

Fig. 3A. Freeze-fracture electron micrograph demonstrating a flask-shaped odontoblast (Od). Note the intimate relationship between the thread-like structure (Nlf), possibly a nerve, and the cell body surface. Below, a somewhat more irregularly shaped cell process (icp) is in close contact with the basal portion of the odontoblastic cell body (Od). This cell process appears to be in continuity basally with a subdontoblastic fibroblast or Höhl's cell (H). Scale bar, 2 μm. 3B. At higher magnification of the contact area between the irregular cell process (icp) and the odontoblast (Od) a gap junction (GJ) can be visualized. At top right several gap junctions (GJ) on the odontoblast can be observed. Scale bar, 0.2 μm.

the identification of nerve fibers. Other cell processes, fibroblastic and odontoblastic, display a structure resembling nerve fibers. It must be emphasized that these difficulties are encountered also when performing freeze-fracturing. A definite advantage with this method is the three-dimensional visualization of the tissue, which enables one to study both the structure and the course of various cell elements in different planes.

The morphology of the odontoblastic region and the relationship between odontoblasts and assumed nerves have been described for several species. Arwill (2) perceived, among human odontoblasts, 'associated cells', which he suggested might be of nervous origin. Corpron et al. (14), after transection of the inferior alveolar nerve in mice, found degenerative changes of unmyelinated nerves between odontoblasts. The ultrastructure of the dentinal–pulpal border zone after sensory and autonomic nerve tran-

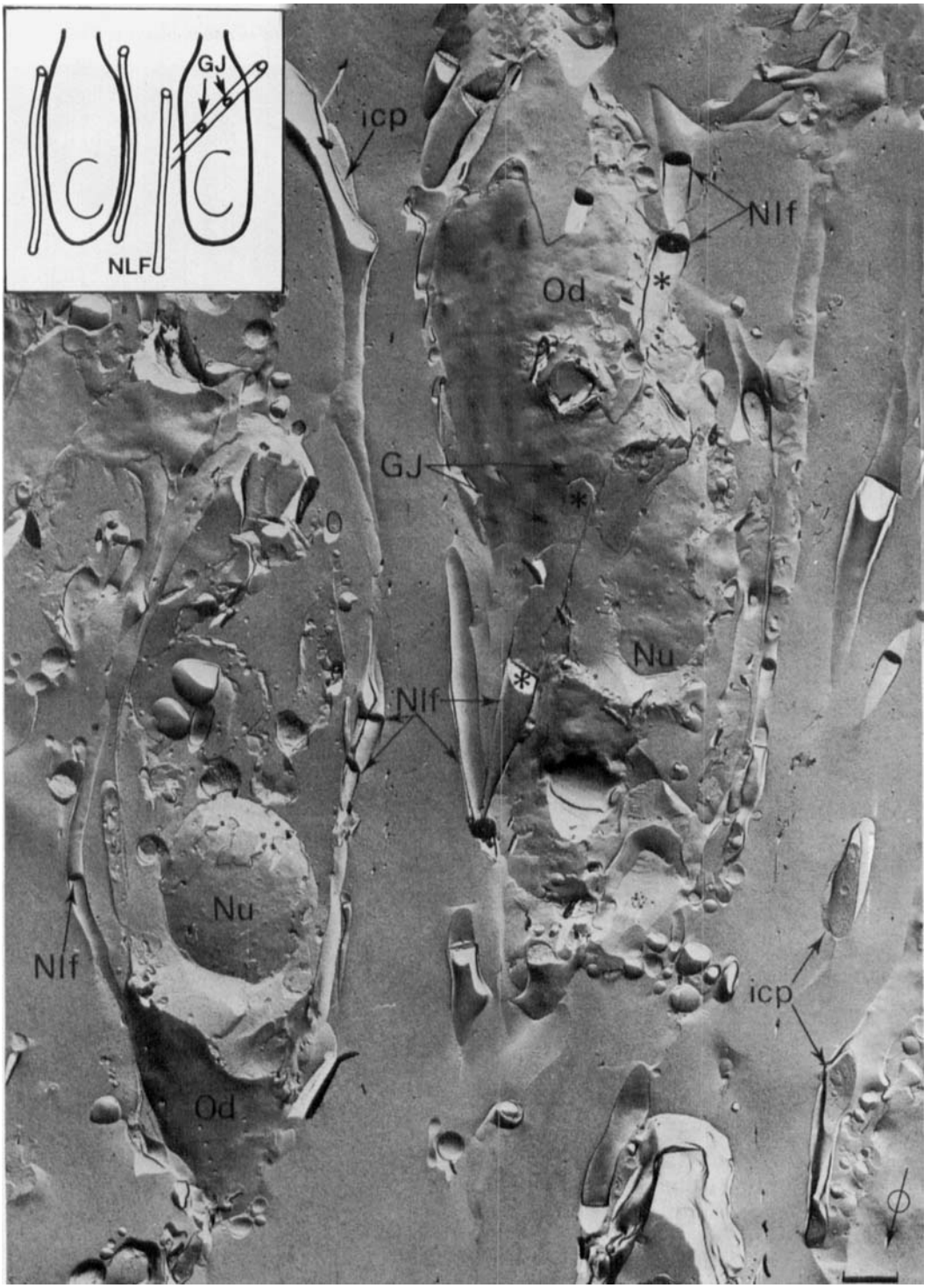

Fig. 4. Freeze-fracture replica of a human dental pulp showing two odontoblasts (Od). One cell (left) is partly fractured through its cytoplasmic interior, exposing the nucleus (Nu) and intracytoplasmic organelles at the supranuclear level. Tubular structures pass in the vicinity of the odontoblasts. Some of them are cross-fractured (top right). One tubular structure passes close to and along the outer cytoplasmic membrane of the odontoblast to the right (asterisks). Gap junctions (GJ) are formed between the tubular nerve-like fiber (Nlf) and adjacent odontoblasts. Irregular cell processes (icp) can be seen at bottom right. Scale bar, 1 μm.

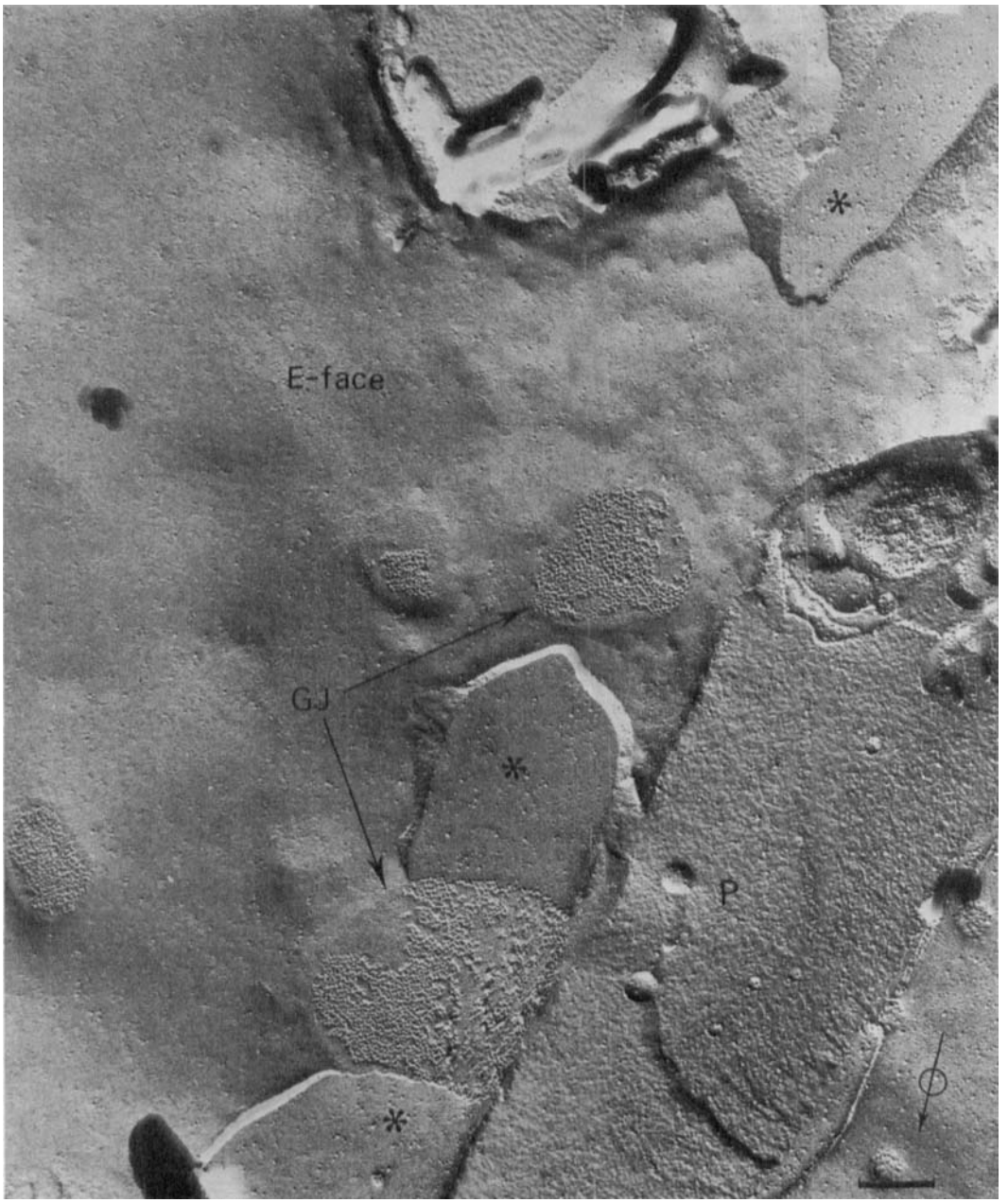

Fig. 5. Higher magnification of the junctional region visualized in Fig. 4. A nerve-like structure passes from top right to bottom left (asterisk). Fortunate fracturing occasionally shows the fiber passing between two odontoblasts (of which the E-face of one odontoblast and the protoplasmic interior (P) of an adjacent odontoblast are exposed). Two gap junctions (GJ) are formed between the odontoblastic cell and the nerve-like fiber. To the left are two additional junctions. Note characteristic narrowing of intracellular space at the junctional level (lower asterisk). Both E- and P-faces of the junctional structure are observed. Scale bar, 0.2 μm.

Table 1. Morphometric data obtained from freeze-fracture replicas from the human odontoblastic cell-layer (average from different parts of the coronal pulp)

	Mean	Range
No. of nerve-like fibers/μm^2*	0.4	
No. of irregular cell processes/μm^2*	0.3	
Diameter of nerve-like fibers, μm*	0.4	0.1–1.1
Diameter of gap junction on odontoblasts, μm	0.36	0.18–0.57

* Measurements were based on calculations from 300-μm^2 cross-fractured areas of the odontoblastic cell-layer.

section in the cat was investigated by Arwill et al. (3). The intratubular 'associate cells' then showed degenerative changes or were absent. It was suggested that these cells, earlier described in human teeth, were sensory neurons. Recently, electron microscopic autoradiography was used to identify nerves among rat odontoblasts (12).

Dahl & Mjör (15) noted membrane contacts between supposed intradentinal nerves and odontoblastic processes. Matthews & Holland (29) presented evidence of electrical coupling between terminals of sensory nerves in the teeth of the cat and morphological couplings (gap junction-like) between odontoblasts and nerve-like structures in an electrophysiological and ultrastructural study. Byers (13) reported 'wide appositions' between nerves and odontoblasts in rat molars. 'Close appositions', or gap junctions, occurred both between odontoblasts and between adjacent nerves in the same area. No labeled nerves were found to form gap junctions with other cell structures in rat molars.

Gap junctions in the odontoblast layer of the cat involve cell processes that may be nerve fibers (25). In a recent study Holland (26) tried to establish the nature of unidentified cell processes participating in gap junctions in the odontoblast layer. He concluded that the findings did not prove that the 'gap members' were nerve fibers but supported the hypothesis that the processes taking part in the gap junctions in the peripheral pulp were nerve fibers.

In our present study gap junctional structures were regularly found on odontoblasts. These junctions were established between both individual odontoblasts and odontoblasts and nerve-like structures. They also occurred between odontoblasts and presumed (and sometimes proved) fibroblastic and odontoblastic processes. The great number of junctions in the studied area may indicate an important physiological function of these membrane specializations. Our findings could support an electric synapse theory to the effect that electrical stimuli are mediated via gap junctions between nerve fibers and odontoblasts. However, the physiological role of gap junctions in this area is still unclear. Whether they have excitatory or other functions remains to be elucidated. Hitherto, most discussions in the literature about nerve fibers in the odontoblastic cell layer have pertained to dentinal sensitivity and transmission of pain impulses. It must, however, be borne in mind that other possibilities exist.

The electron microscope has revealed that the gap junction consists of a pair of differentiated plasma membranes involved in intercellular communication. This structure is a lattice of subunits called connexons, which bridge a 2-nm gap between apposed junctional membranes and penetrate both of the junctional membranes. The gap junction membrane contains a polygonal array of particles matching a similar arrangement in the adjoining membrane. These particles house cylindrical channels 1.5 nm in diameter, composed of six main subunits. They may allow free exchange of small molecular substances between neighboring cells (32). Microinjection studies have demonstrated a 1200-D maximum size for molecules that move from cell to cell via a gap junction. A 14-Å size of the pores has been deduced from these data, which suggest a sharing of

small metabolites (ions, monosaccharides, amino acids, nucleotides, and their metabolic products) while still maintaining the macromolecular integrity of each cell (20).

Recent studies suggest a dynamic quality of the gap junction structure, and electrophysiologists have submitted evidence that the gap junctions are able to switch rapidly from a low- to a high-resistance pathway (31). According to Rose & Loewenstein (34), the Ca^{++} ion may play a significant role during the switch from low- to high-resistant pathways.

In functional terms, gap junctions provide cells with the capacity for direct two-way communication via ions and low-molecular weight substances. In excitable tissues the gap junction functions as a low-resistance electrical pathway that permits action potentials to spread with negligible synaptic delay. This is exploited in situations in which near-synchrony of adjoining cells is important. Thus, they are found in the muscle fibers of the heart and in serially arranged neurons in the central nervous system subserving escape reflexes (7, 36). Measurements and calculations suggest that in the latter case junctions may be used in place of chemical synapses, because electrical activation is the most efficient way to excite large nerve cells with high-voltage thresholds, as found in the escape reflex circuits (38). In non-excitable tissues the role of the gap junctions is less obvious. According to Gilula et al. (19), metabolic cooperation and low-resistance ionic pathways are interrelated with morphologically demonstrated gap junctions.

The possibility that nerve structures and odontoblasts could form gap junctions may support the hypothesis that the odontoblasts serve as a mechanoreceptor to register pressure changes in the dentinal tubuli and to transduce them into signals to adjacent nerve fibers via those junctions. On the other hand, the fact that gap junctions make a two-way communication possible provokes further interesting speculations. An efferent pathway could exist, and neural efferent influence on dentinogenesis has actually been hypothesized (6). Further physiological studies seem necessary before any definite conclusions can be drawn about the function of gap junctions in the odontoblastic area.

Ten Cate & Shelton (37) investigated the cholinesterase activity in human teeth. They concluded that if the neuroanatomical pathway associated with dentinal sensitivity transverses the odontoblasts with connections to free nerve endings in the pulp, the transmission is not mediated by a cholinergic activity. Moreover, Haegerstam et al. (22) found in a pharmacological study that intradental nerve impulses evoked by physical stimuli are not mediated by cholinergic activity. According to Haegerstam (23), the sensory receptors in the tooth do not seem similar to the nociceptors of the skin, thus making the tooth a unique pain-sensitive organ.

The characteristic membrane specializations of chemical synapses observed in freeze-fracturing replicas (1) could not be identified in the cell structures in our material.

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