Membrane junctions in the subodontoblastic region

A freeze-fracture study of the human dental pulp

Anders Köling and Helge Rask-Andersen
Department of Otolaryngology, University Hospital, Uppsala, Sweden

Köling, A. & Rask-Andersen, H. Membrane junctions in the subodontoblastic region. *Acta Odontol. Scand.* 1983, 41, 99–109. Oslo. ISSN 0001–6357.

The morphology of the subodontoblastic region in the human dental pulp was analysed using the freeze-fracturing technique. Multiple areas of focal intercellular junctions were found between subodontoblastic fibroblasts (or Höhl's cells). The connections were of the gap junction type. Similar junctions were observed between subodontoblastic cells and small-calibre (0.10–0.20 μm) fibres, presumably nerves. Tight junctions were not observed. □ *Dental pulp; freeze-fracture; intercellular junctions; ultrastructure*

Anders Köling, Department of Otolaryngology, University Hospital, S-750 14 Uppsala, Sweden

The subodontoblastic region is a zone of pulpal tissue bordering the odontoblastic cell layer. Classical descriptions of its anatomy (14, 20, 25) reveal both a cell-poor layer and a cell-rich layer with many fine nerve fibres and blood capillaries dispersed in a loosely textured connective tissue. The subodontoblastic area is thought to be important for the proper functioning of the odontogenic zone, with both metabolic and neural involvements (7, 10).

Electron microscopy has been used to further clarify its morphology. The cell-rich zone displays large bipolar cells, often termed Höhl's cells (1). They extend slender cytoplasmic processes, which appear to form contacts with each other and with odontoblasts (19). The connections are thought to be small specialized areas of adjacent cell membranes (6). There are conflicting data concerning the presence of junctions between various cell elements in the subodontoblastic region. Electrophysiological recordings indicate the presence of low-resistance junctions between nerves (17), although the morphological observations appear somewhat contradictory (4).

A recent study, using the freeze-fracture technique, revealed the presence of gap junctions between odontoblasts (16). In the present investigation we tried to obtain more knowledge about the membrane specializations between cells in the subodontoblastic region of the coronal human dental pulp using the same method. This information may shed additional light on the function of the subodontoblastic region.

Material and methods

Thirty caries-free, fully erupted, human molars and premolars extracted in local anaesthesia (2% lidocaine with epinephrine) for orthodontic reasons from healthy individuals aged between 13 and 16 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 a screw-vice with cutting edges and then immersed in the fixative again. After 1 h the coronal pulpal tissue was dissected away from the dentin and cut into small pieces under a dissection microscope. These pieces remained in fixative for at least 48 h. After rinsing with buffer the specimens were transferred to a solution of 25% buffered glycerol for 30 min and then mounted on gold-cup specimen holders. The specimens were subsequently frozen in supercold nitrogen at approximately −190°C, and fractured in a Balzers 360 M freeze-etching apparatus with double replica technique (22). The pulpal tissue was fractured perpendicular to its long

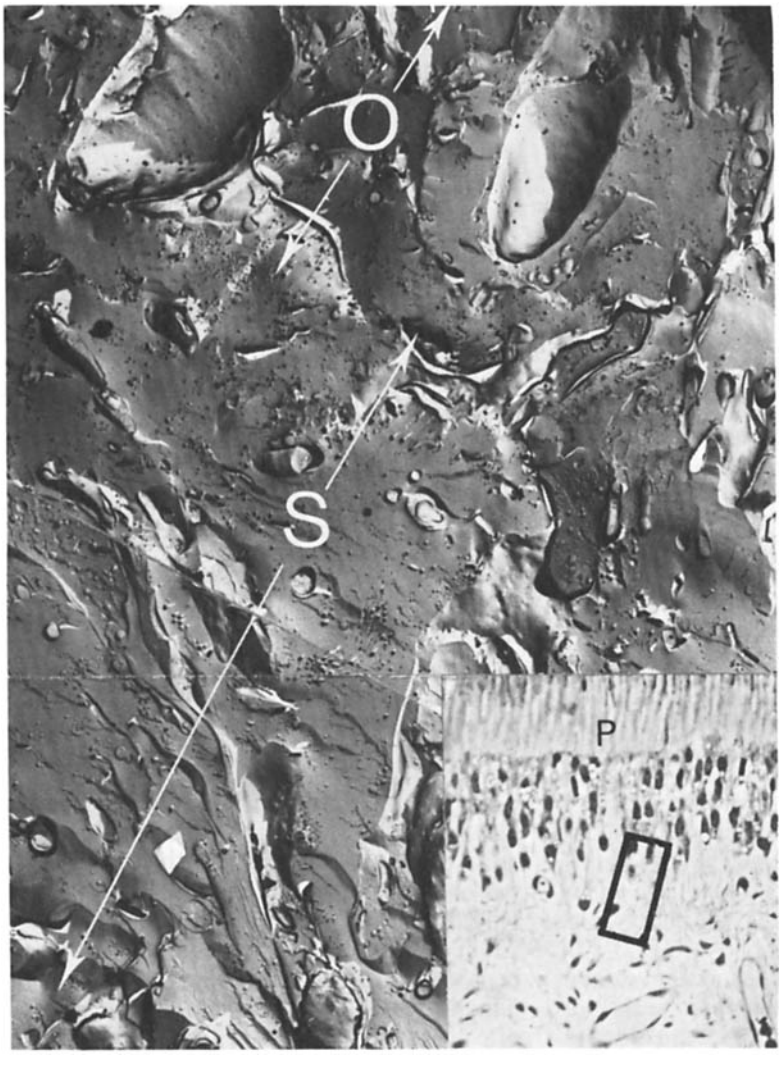

Fig. 1. Low-power view of a freeze-fracture replica from a human dental pulp. O: odontoblastic zone. S: subodontoblastic zone (× 6,200). Inset: semi-thin section of corresponding area. P: predentin.

axis. The resultant replicas were mounted on copper meshgrids and examined in a JEOL-100 B electron microscope at 80 kV.

For proper identification of the subodontoblastic region it was found useful first to identify the odontoblastic layer. These cells line the loose connective tissue which partly builds up the subodontoblastic zone. On freeze-fracture replicas, this zone displays beneath a regular array of odontoblasts a complex relief of broken tubular, lamellar and globular structures demarcated against an even and homogeneous background (Fig. 1).

Observations

In the present paper the terminology used is that suggested by Branton et al. (3). The freeze-fracture technique is believed to expose in principle two different membrane surfaces. One is the interior protoplasmic P-face and the other the outer exoplasmic E-face due to the splitting of the unit plasma membrane in its hydrophobic interior.

Höhl's subodontoblastic cells

General morphology. Large membranous areas of the cells were exposed after frac-

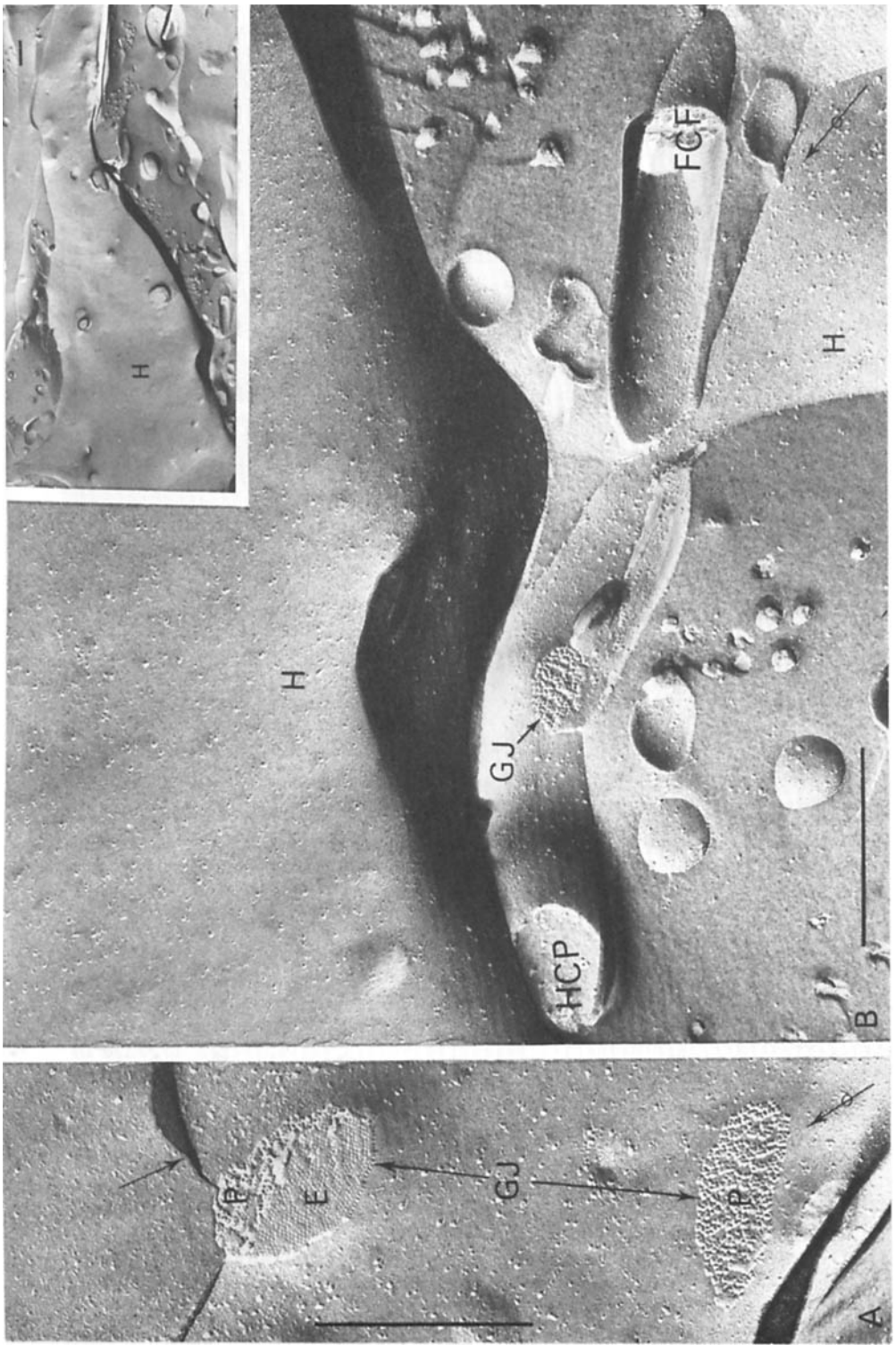

Fig. 2. Electron microscopy of a freeze-fracture replica from the subodontoblastic region of the human dental pulp. Bars = 0.5 µm. □ A. Two macular aggregates of closely-packed intramembranous particles or gap junctions (GJ) are exposed on the surface membrane of a subodontoblastic fibroblast (H). Above: the junction is located on a small elevation of the membrane. Both E and P faces are exposed. A narrowing of the intercellular distance at the region of the junctional area is seen (arrow). □ B. A large fusiform Höhl's cell (H) with several processes can be seen in the subodontoblastic layer (see inset). A subodontoblastic cell (Höhl's cell) process (HCP) is seen below the other subodontoblastic cell. On the process an arrangement of intramembranous particles constituting a gap junction (GJ) is exposed. The junction appears to be formed between the Höhl's cell process and a more regular shaped fine-calibre fibre (FCF). Arrow at bottom right indicates direction of platinum shadowing.

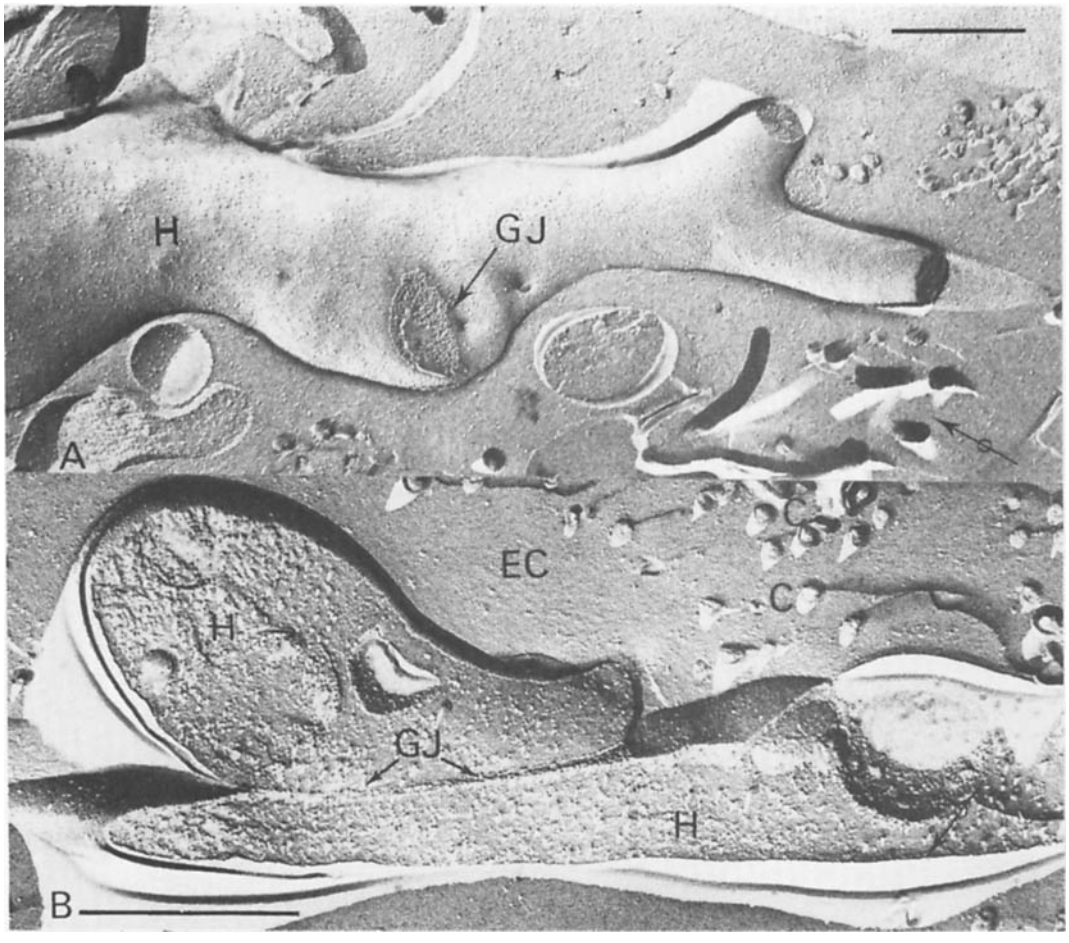

Fig. 3. □ A. Electron microscopy of a freeze-fracture replica from the human dental pulp. In the subodontoblastic region a process of a Höhl's cell (H) is visible on which a macular aggregate of particles or a gap junction (GJ) is manifest. □ B. A connection between two Höhl's (H) cells is formed by a gap junction (GJ) which appears cross-fractured. There is a close apposition between cell structures where the surfaces are separated by a granular arrangement of particles. Some Höhl's cells apparently have a laminar or lamellar structure on which gap junctions are exposed (the lower one). In the loose extra-cellular tissue (EC) a few collagen fibres (C) are seen cross-fractured. Bars = 0.5 μm.

turing. These proceeded from the intramembranous cleaving of large subodontoblastic Höhl's cells. They constituted a conspicuous contribution to the subodontoblastic layer. Their cell bodies were irregular, fusiform and spider-like, with many slender cytoplasmic branches projecting deep into the surrounding intercellular substance (Figs. 2B, 7). This matrix contained collagen fibres (von Korff fibres) (Fig. 3). The cell processes or 'filopodia' were of different calibre, and frequently ramified into smaller fibre-like structures or long palisade-like lamellae of varying width (Fig. 3). The latter often reached the basal portion of the odontoblastic cell layer. The Höhl's cells seemed to extend across the main part of the subodontoblastic zone. Their range was frequently 30–40 μm, including their long dendritic processes. The width at the juxtanuclear portion was around 5–10 μm, while the distal projections were thinner with a diameter around 0.3–1.0 μm. The 'filopodia' sometimes reached the odontoblasts.

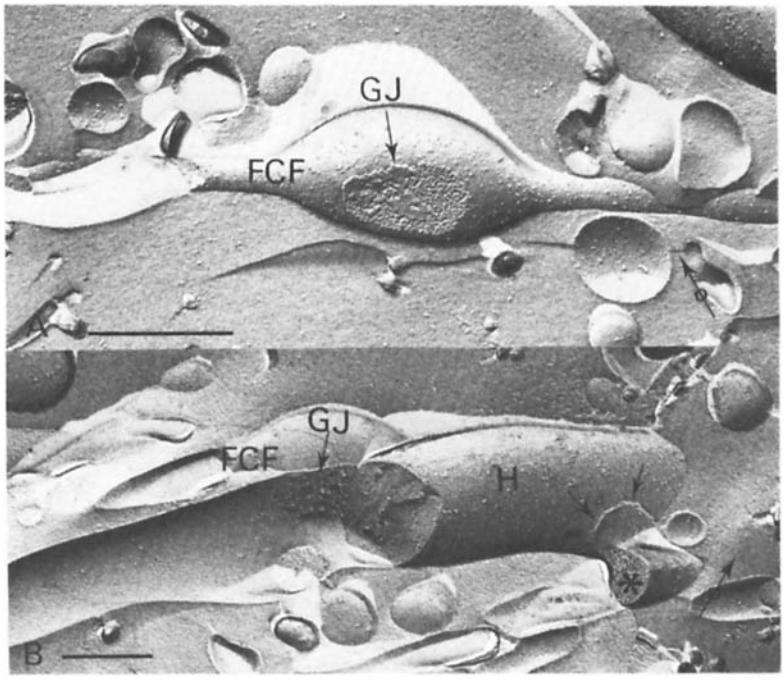

Fig. 4. □ A. Freeze-fracture replica exposing a fine-calibre nerve-like fibre (FCF) of 0.11 μm diameter seen in the subodontoblastic region. A regional swelling or 'bead' is observed, on which a small aggregate of intramembranous particles constitutes a gap junction (GJ). □ B. A subodontoblastic cell process (H) in the human dental pulp is exposed. Closely apposed to this cell process is a fine-calibre fibre (FCF) displaying regional swelling forming apparently a gap junction (GJ) between the nerve-like fibres and the subodontoblastic cell. To the lower right there is a connection (arrow) where the Höhl's cell process (HCP) appears to be fused to a fibre structure (*). The cleaving has not passed through the cellular connection and it cannot be determined whether or not there is a gap junction. Bars = 0.5 μm.

Many appeared to end blindly in the loose connective tissue, but their final fate was frequently difficult or impossible to establish.

Various Höhl's cells were photographed systematically and redrawn in order to obtain a better understanding of the variable morphology of the subodontoblastic cells (Fig. 7).

Membrane junctions. The cell surfaces of the Höhl's cells displayed numerous macular, plaque-like structures of various sizes and shapes (Figs. 2A, 3). On the P-face they had a granular appearance due to the presence of numerous, tightly packed intramembranous particles. These disc-like arrangements of particles measured about 0.2–0.4 μm in diameter. When cross-fractured, a typical narrowing of the intercellular distance to about 2 nm could be observed. Not infrequently, macular aggregates, apparently gap junctions, appeared on small shallow plateaus or elevations formed by the membrane of subodontoblastic cells (Fig. 2A).

The plasma membrane of the Höhl's cells displayed a fairly large number of intramembranous particles. They were usually scattered evenly along the surface membrane but occasionally arranged as regional accumulations of particles. However, they lacked the typical tightly packed arrangement of intramembranous particles as described above. Strands or grooves characteristic of tight junctions were not observed.

Fibre-like structures and membrane junctions

The ground substance between the many branching cells in the subodontoblastic region contained numerous fine-calibre fibres (Figs. 2B, 5, 6). These thread-like stuctures were of uniform size and had a regular diameter of about 0.10–0.20 μm. Their regular shape was often interrupted by

Fig. 5. Freeze-fracture replica exposing the interior of the cell coat as well as a cross-fractioned area of two subodontoblastic cells in the human dental pulp. The border between cells is indicated by arrows. Nu = nucleus of the lower cell. The cross-sectioned area of the upper one exposes a rich content of intracytoplasmatic organelles. Numerous small particles are evenly scattered over the cell surface. On top is a flat, glove-like cell structure adhering the Höhl's cell. The cytoplasmic expansion narrows into a fibre (FCF) in the loose connective tissue in the subodontoblastic zone. It has a diameter of 0.20 μm. Bar = 0.5 μm.

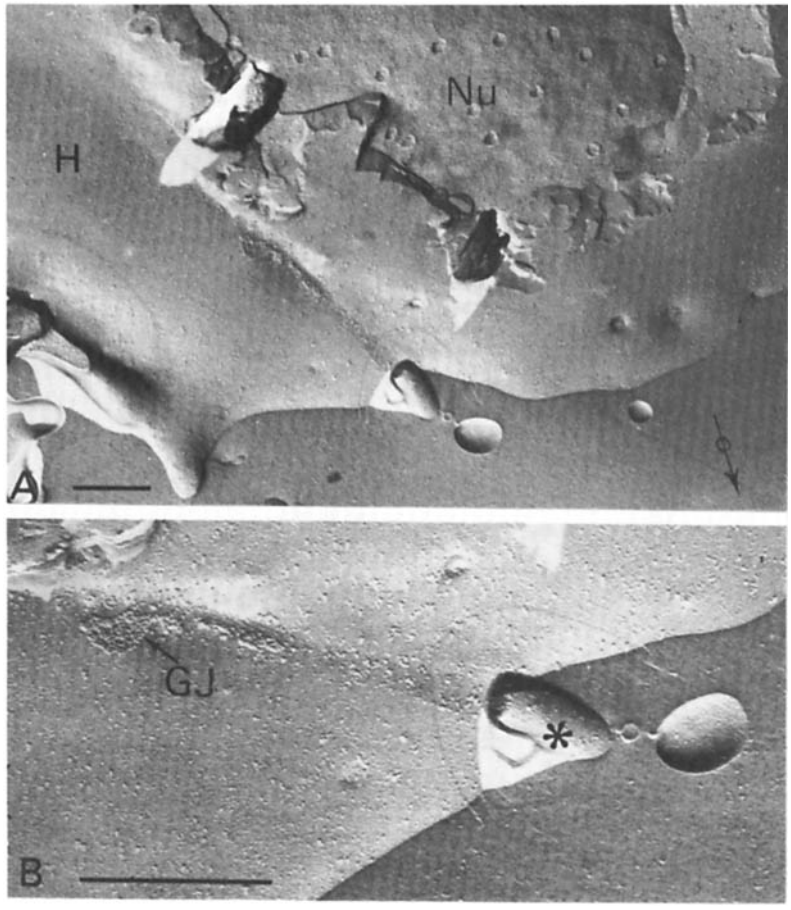

Fig. 6. Electron microscopy of a freeze-fracture replica from the subodontoblastic layer. A Höhl's cell (H) is exposed with a large nucleus (Nu), which demonstrates numerous nuclear pores (Fig. A). A small fibre-like structure 0.17 μm in diameter is shown close to the cell surface of the subodontoblastic fibroblast (* in Fig. B). Its cross-sectioned area seems to emit a continuation line of small intracytoplasmic particles ending as a small expansion of a gap junction (GJ). Bar = 0.5 μm.

regional swellings or 'beads' (Fig. 4A), the diameters of which varied from 0.5 to 0.7 μm. These fibres were often located in close proximity to the Höhl's cells. Membranous connections of gap junction type were seen on the regional expansions and also between the fibres and the Höhl's cells (Fig. 4B). The fibres did not display a cell-covering or Schwann cell. Cross-fracturing revealed many small tubuli and fibrils similar in arrangement to those observed in nerve axons located more centrally in the dental pulp. Gap junctions observed on these fibres were similar in shape and size to those seen on the Höhl's cells.

The various intercellular junctions formed in the subodontoblastic region in the human dental pulp are illustrated schematically in Fig. 8.

Discussion

The development of new staining techniques and freeze-fracturing allows clear differentiation between the various forms of membrane specialization. Gap junctions can be distinguished from tight junctions (or zonulae occludentes), to which group they were earlier believed to belong. Within the gap junctional areas, typically, the intercellular space is narrowed to 2 nm. The plasma membrane in these areas is composed of particles 7–8 nm in diameter, which often present a

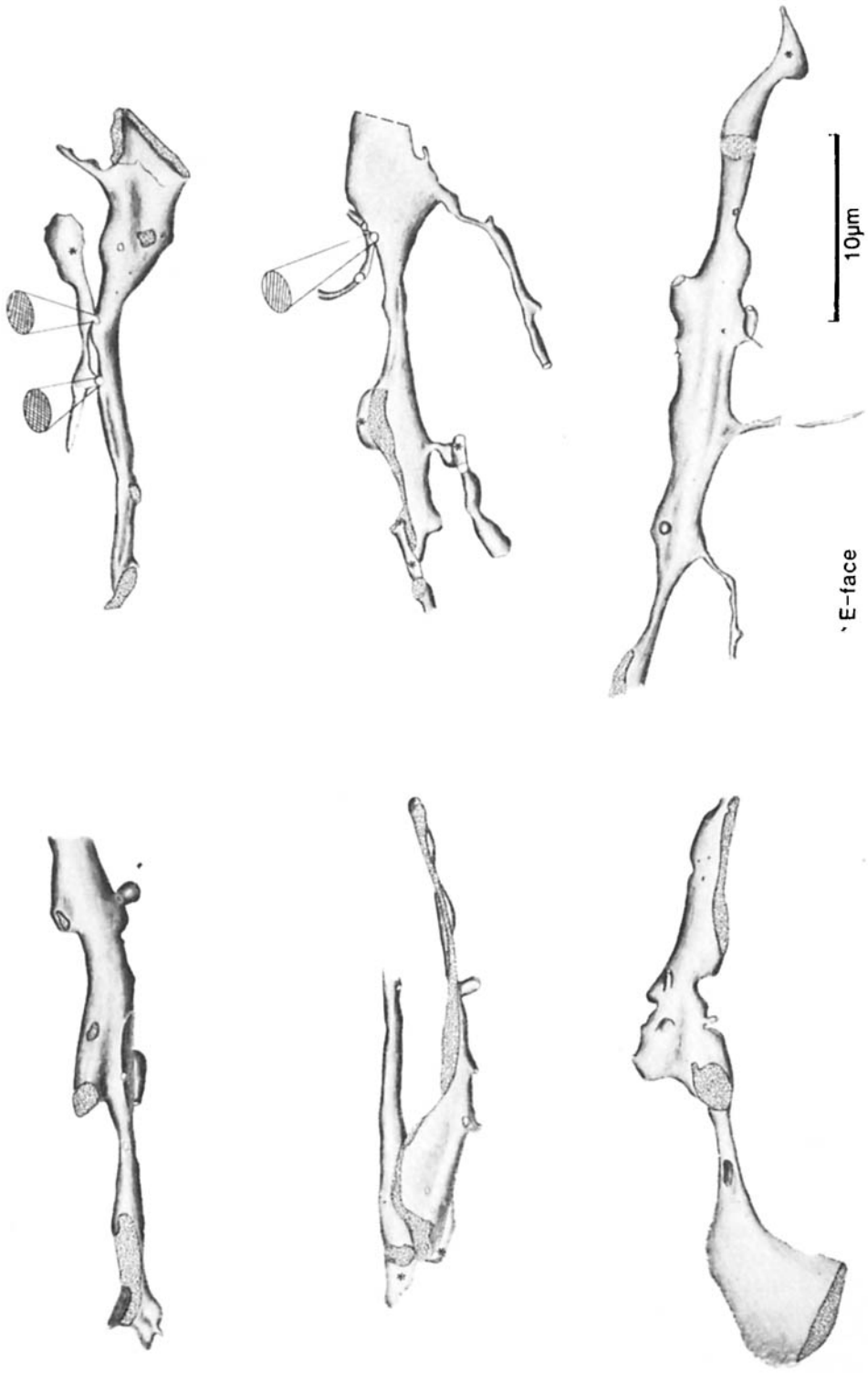

Fig. 7. Graphical full-tone reproductions from freeze-fracture replicas demonstrating the variable anatomy of the subodontoblastic fibroblasts (Höhl's cells). Checkered areas represent gap junctions between cells. Stippled areas indicate fracture surfaces.

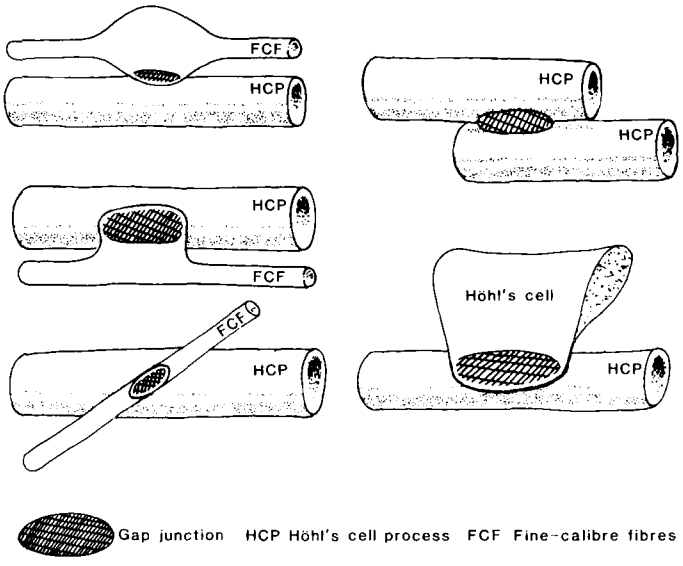

Fig. 8. Variable morphology of junctional connections between cell structures in the subodontoblastic region of the human dental pulp.

hexagonal array. Moreover, high-resolution and X-ray diffraction studies suggest that they are in fact composed of six subunits forming a cylinder about a central hydrophilic channel. It is believed that, on the opposing faces of the participating plasma membranes, these components are in register, such that their hydrophilic channels are confluent (15). The general opinion at present is that freeze-fracturing is the most reliable technique for the characterization of membrane junctions.

The freeze-fracturing technique permits three-dimensional study of cell membranes and their specializations, but involves certain disadvantages in the identification and orientation of structures. It is essential to have a thorough knowledge of the ultrastructural anatomy via the thin-section technique before venturing on freeze-fracturing. Furthermore, areas of great structural complexity are exposed in the human dental pulp.

Ultrastructural embryonic features of the Höhl's cells suggest that they are fibroblasts (5, 6). These subodontoblastic cells differ from other pulpal fibroblasts in their fusiform, bipolar appearance. Gotjamanos's (13) postulation that they could give metabolic assistance to the odontoblasts is supported by histochemical investigations by Goggins & Fullmer (9). Possibly, odontoblasts may be replaced from these cells (6, 21). It has also been suggested that Höhl's cells take part in the synthesis of von Korff's fibres (26, 27).

The Höhl's cells' cytoplasmic processes have been described as forming multiple sites of attachment systems between adjacent cells, and to the basal parts of the odontoblasts (5, 6, 13, 19, 23). Intercellular membrane connections in the subodontoblastic layer may constitute a mechanical anchorage of cells together with the creation of a functional cooperation between subodontoblastic cells and between these cells and odontoblasts.

In the present study we found numerous small macular aggregates of intramembranous particles of gap junction type at sites where cells converge. Gap junctions have been shown to be areas with low electrical resistance between cells, allowing movements of ions, metabolites and even small molecular substances (<1200 D) from one cell to another (8). Thus, the presence of gap junctions between Höhl's cells and odontoblasts may indicate an intercellular communication between the cells and confirm Gotjamanos's (10) theory that subodontoblastic cells are functionally related to the odontoblastic cells and give them metabolic assistance. Furthermore, individual sub-

odontoblastic cells appear to be structurally related or connected to each other through gap junctions. This may reflect a metabolic or electric coupling between Höhl's cells which could unite these many cells into a functional syncytium possibly related to the function of the odontogenic zone. It has also been speculated whether the Höhl's cells might be involved in electrical transmission between nerves and odontoblasts. No synapses between these two cell types have been found, however.

Harris & Griffin (12) described the structure of three different types of nerve endings in the human dental pulp. Terminal expansions in the subodontoblastic layer were preceded structurally by varicosities and constrictions within a single Schwann cell. The terminal fibres were seen to leave the Schwann cell as a constricted axon and to end as a terminal swelling in the ground substance. Pischinger & Stockinger (19) described the ultrastructure of terminal parts of nerves (0.1–1.5 μm diameter) ending in the cell-rich layer of the subodontoblastic region within small compartments formed by thin fibroblastic processes. The nerves were believed to possess sensory functions related to perception of pain and registration of the surrounding environment. No structural connections suggesting intercellular junctions between nerves and fibroblasts were reported by these authors. In our investigation we could detect tissue elements with a nerve-like structure. Their diameters were estimated to be about 0.10–0.20 μm. The fibres displayed local expansions or 'beads' on which gap junctions were observed.

From pure morphological criteria it is difficult to conclude with certainty that these structures are true nerves, although their morphological appearance does indicate this. The sizes of the nerve-like fibres observed in our study are the same as those earlier described as nerves. Evidently, nerves pass through this region in order to reach the odontoblastic zone and the predentinal tubuli where nerves have been located (4).

Ten Cate & Shelton (24) have investigated the cholinesterase activity in human teeth. No such activity was discovered in the odontoblast cell body, nor were cholinergic nerves found immediately below the odontoblasts. It was concluded that if the neuro-anatomical pathway associated with dentin sensitivity traverses the odontoblasts with connections to free nerve-endings in the pulp, the transmission of impulses is not mediated by cholinergic activity. Moreover, in a pharmacological study, Haegerstam et al. (11) conclude that intradental nerve impulses evoked by physical stimuli are not mediated by a cholinergic mechanism. Membrane specializations suggesting the presence of synapses or synaptic membranes have not been described previously, nor were they observed in our present investigation. Gap junctions have been demonstrated to provide electrical coupling between cells in various tissues, including nerves (2, 8, 18). Low resistance junctions, or gap junctions, between terminals of sensory pulpal nerves, which could propagate action potentials from one nerve to another, have been hypothesized (17). Our observations may further strengthen their view, but more information is needed before any definite conclusions can be drawn.

Acknowledgement.—The authors gratefully acknowledge the techinal assistance of Maud Hoffstedt for preparing freeze-fracturing replicas.

References

1. Baume, L. J. The biology of pulp and dentine. Monographs in Oral Science, Vol. 8. S. Karger, Basel 1980, pp. 122–123
2. Bennett, M. V. L. Electrical transmission: A functional analysis and comparison to chemical transmission. In: Kendel, E. R. ed. Handbook of Physiology I, Williams & Wilkins, Baltimore 1977, pp. 357–416
3. Branton, D., Bullivant, S., Gilula, N. B., Karnovsky, M. J., Moor, H., Mühlethaler, K., Northcote, D. H., Packer, L., Satir, B., Satir, P., Speth, V., Staehelin, L. A., Steere, R. L. & Weinstein, R. S. Freeze-etching nomenclature. Science 1975, 190, 54–56
4. Byers, M. R. Fine structure of trigeminal receptors in rat teeth. In: Anderson, D. J. & Matthews, B. eds. Symposium on Pain in the Trigeminal Region. Elsevier/North-Holland, Amsterdam 1977, pp. 13–25
5. Cahen, P. & Frank, R. M. Microscopie electronique

de la pulp dentaire humaine normale. Bull. Grp. Int. Rech. Sci. Stomatol. 1970, 12, 412–443
6. Eifinger, F. F. Die Mikromorphologie der menschlichen Zahnpulpa. Hanser, München 1970, pp. 57–74, 82–88
7. Frank, R. M. Etude au microscope electronique de l'odontoblast er du canlicule dentinaire humain. Archs Oral Biol. 1966, 13, 833–834
8. Gilula, N. B., Reeves, O. R. & Steinbach, A. Metabolic coupling, ionic coupling, and cell contacts. Nature 1972, 235, 262–265
9. Goggins, J. F. & Fullmer, H. M. Dehydrogenase histochemistry of the rat molar pulp. Archs Oral Biol. 1966, 11, 1365–1370
10. Gotjamanos, T. Cellular organization in the subodontoblastic zone of the dental pulp. I. A. study of cell-free and cell-rich layers in pulps of adult rat and deciduous monkey teeth. Archs Oral Biol. 1969, 14, 1007–1010
11. Haegerstam, G., Olgart, L. & Edwall, L. The excitatory action of acetylcholine on intradental sensory units. Acta Physiol. Scand. 1975, 93, 113–118
12. Harris, R. & Griffin, C. J. Fine structure of nerve endings in the human dental pulp. Archs Oral Biol. 1968, 773–778
13. Harris, R. & Griffin, C. J. The fine structure of the mature odontoblasts and cell rich zone of the human dental pulp. Aust. Dent. J. 1969, 14, 168–177
14. Höhl, E. Beitrag zur Histologie der Pulpa und des Dentins. Arch. Anat. Physiol. 1896, 32, 31–54
15. Hopkins, C. R. Structure and Function of Cells. Saunders, London, Philadelphia, Toronto 1978, pp. 94–104
16. Köling, A., Rask-Andersen, H. & Bagger-Sjöbäck, D. Membrane junctions on odontoblasts. A freeze-fracture study. Acta Odontol. Scand. 1981, 39, 355–360
17. Matthews, B. & Holland, G. R. Coupling between nerves in teeth. Brain Res. 1975, 98, 354–358
18. McNutt, N. S. & Weinstein, R. Membrane ultrastructure at mammalian intercellular junctions. Progr. Biophys. Mol. Biol. 1973, 26, 47–101
19. Pischinger, A. & Stockinger, L. Die Nerven der menschlichen Zahnpulpa. Zeitschrift für Zellforschung, 1968, 89, 44–61
20. Raschkow, I. Meletemata circa mammalium dentium evolutionem: dissertation inauguralis anatomico-physiologica. Friedlander, Vratislaviae (Breslau) 1835. Cited by Fearnhead, R. W. Innervation of dental tissues. In: Miles, A. E. W. ed. Structural and Chemical Organization of Teeth. Academic Press, New York and London 1967, p. 249
21. Schroeder, H. E. Orale Strukturbiologie. Entwicklungsgeschichte, Struktur und Funktion normaler Hart- und Weichgewebe der Mundhöhle. Thieme, Stuttgart 1976
22. Sleytr, U. B. & Umrath, W. A simple fracturing device for obtaining complementary replicas of freeze-fracture and freeze-etched suspension and tissue fragments. J. Microsc. 1974, 101, 177–186
23. Takuma, S. & Nagai, N. Ultrastructure of rat odontoblasts in various stages of their development and maturation. Archs Oral Biol. 1971, 16, 993–1011
24. Ten Cate, A. R. & Shelton, L. Cholinesterase activity in human teeth. Archs Oral Biol. 1966, 11, 423–428
25. Weil, A. Zur Histologie der Zahnpulpa. Dt. Mschr. Zahnheilk (Leipzig and Berlin), 1887, 5, pp. 335–356; 1888, 6, pp. 10–21, 403–413
26. Zerlotti, E. Histochemical study of the connective tissue of the dental pulp. Archs Oral Biol. 1964, 9, 149–162
27. Zerosi, C. Observations histochemiques sur les fibres de von Korff de la pulpe. Bull. Group. Int. Rech. Sci. Stomatol. 1967, 10, 197–210

Received for publication 4 November 1981