The blood capillaries in the subodontoblastic region of the human dental pulp, as demonstrated by freeze-fracturing

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The microvasculature of the subodontoblastic region in the human dental pulp was studied, using freeze-fracturing. This technique allows an analysis of the fine structure of the vascular endothelium with special reference to the membrane structure. The blood capillaries were noted to be of the non-fenestrated or continuous type, although a few fenestrated vessels were observed. The endothelial plasmalemma often exhibited bundles of fibrillar structures, presumably myofilaments. There was a relatively large number of micropinocytotic vesicles and the interendothelial spaces were closed juxtaluminally by tight junctions (*zonulae occludentes*). The junctions appeared mostly as two to four strands, seen as ridges or grooves on the cell membrane. Thin-walled, irregular, tissue channels lacking the typical, blood-vessel configuration were disclosed. These vessels were believed to represent lymphatics.
☐ *Freeze-fracture; dental pulp; blood vessels; endothelial cells; ultrastructure*

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A capillary network is formed subjacent to the odontoblastic cell-layer from many small, ramifying vessels emanating from the main vascular trunks. These blood vessels are located in the loose, subodontoblastic tissue and are of obvious importance for the proper functioning of the cells responsible for the development and growth of dentin.

The capillary endothelial wall greatly influences the exchange of nutrients between the blood and the surrounding cells. The high tissue pressure (3) and the fact that the soft pulp tissue is encapsulated by a rigid, dentin-enamel mantle would seem to imply that there are special physiological and mechanical demands on these vessels.

Thin-section, transmission electron microscopy (TEM) has already given us new and valuable information about the ultrastructure of the vascular, endothelial cell in the human dental pulp (5, 8, 15, 16). In the present study, we used the freeze-fracturing technique to obtain more information about the endothelial structure in this tissue.

Materials and methods

Twenty caries-free, fully erupted, human molars and premolars extracted under local anaesthesia (2% lidocaine with epinephrine) for orthodontic reasons from healthy individuals aged between 13 and 18 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 mol 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. Immersion fixation was performed immediately in the above-mentioned solution. 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

Fig. 1. Freeze-fracture replica demonstrating a capillary, endothelial cell membrane, E (E-face, luminal view) located close to laminated, subodontoblastic cells (SO). The cell membrane displays many crater-like structures representing micropinocytotic openings. No fenestrae are seen. Scale bar 1 μm. Arrow at bottom right indicates direction of platinum shadowing.

in a Balzers 360 M freeze-etching apparatus with double-replica technique (18). The pulpal tissue was fractured perpendicular to its long axis. The resultant replicas were mounted on copper-mesh grids and examined in a JEOL-100B electron microscope at 80 kV. The relative frequency of vesicle stomata was calculated on micrographs and compared with previously known data from other tissues.

The widely accepted terminology proposed by Branton et al. (1) was used in this report. The freeze-fracture technique exposes, in principle, four different surfaces

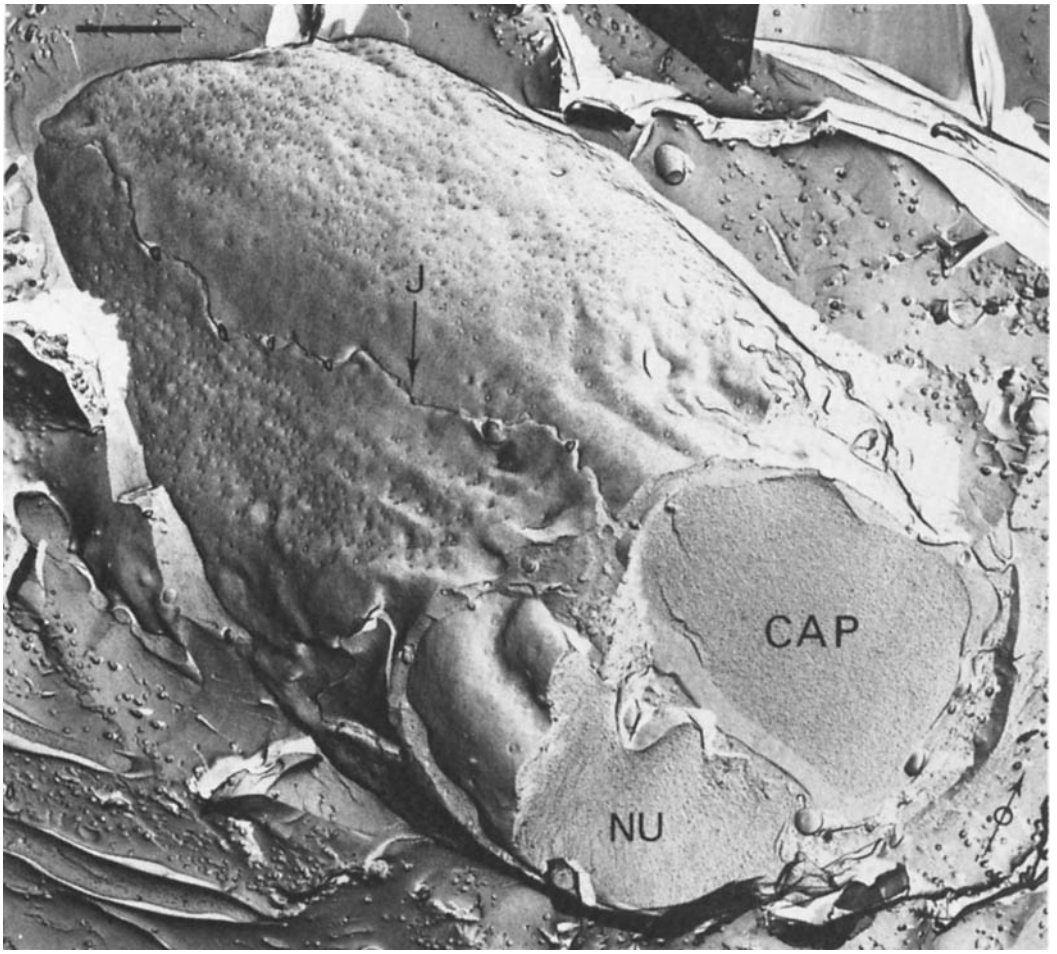


Fig. 2. Freeze-fracture. A small capillary in the vicinity of the odontoblastic cell layer. The cell membrane (P-face) exposes the endothelial-junctional fissure (J), as well as numerous, bleb-like vesicles at a certain distance from the junctional cleft. CAP = capillary; NU = nucleus of endothelial cell. Scale bar 1 μm.

of the endothelial cell, namely the exo- and protoplasmic faces of the luminal and abluminal walls. The protoplasmic-fracture face (P-face) is defined as the internal, protoplasmic half of the membrane, while the exoplasmic-fracture face (E-face) represents the membrane surface facing the extracellular tissue.

In performing electron microscopy of freeze-fracture replicas from dental pulp, difficulties may arise in the identification of the subodontoblastic zone. The odontoblastic cell layer may serve as a helpful guide, but is not always replicated. In these cases, we classified the loose tissue displaying many multibranched, fibroblastic cells, fine vessels, mainly non-myelinated nerves and other small-calibre fibres as the subodontoblastic region.

Observations

General morphology

The blood vessels in the subodontoblastic layer ranged in luminal diameter between 3 and 15 μm. These vessels displayed a thin endothelial cell surrounded by a basement membrane. Occasional pericytes were seen.

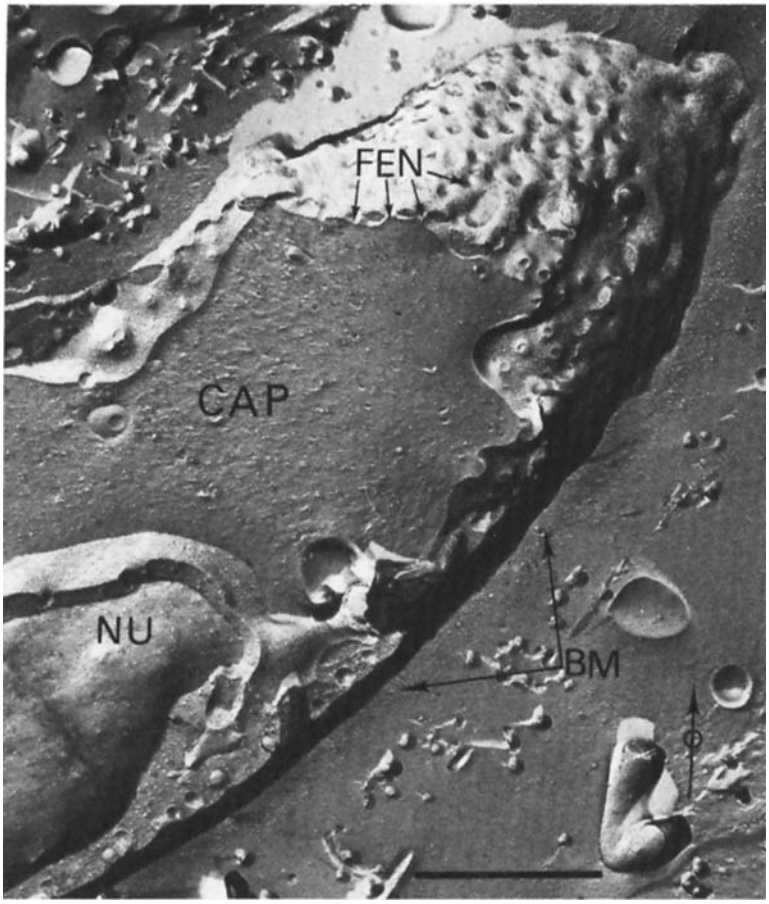


Fig. 3. Electron micrograph showing a fenestrated capillary (CAP) in the subodontoblastic zone. Both E-face and P-face are exposed (FEN = fenestrae). The abluminal zone close to the outer endothelial membrane appears slightly granular and presumably represents the basal membrane (BM). NU = nucleus of endothelial cell. Scale bar 1 μm.

Although it cannot be ruled out that some of the larger vessels in this area actually belong to the prevenular or venular part of the vascular tree, they were all classified as capillaries and included in this study.

Most vessels were cross-fractured, but sometimes considerable areas of their endothelial membranes (Fig. 1) were exposed. This made it possible to study part of their three-dimensional outline. The capillaries appeared mostly as regularly shaped, cylinder-like tubes, varying little in shape and diameter (Fig. 2). The endothelial nucleus often bulged deeply into the narrowed, luminal space. The width of the endothelial wall varied and was estimated at about 0.2–0.6 μm. A basement membrane was revealed abluminally as a narrow, homogeneous, somewhat granular zone, having a width of about 0.1–0.2 μm (Fig. 3). Pericytes ensheathed those vessels that had a slightly larger diameter (>5 μm).

Fenestrations

The great majority of capillaries in the subodontoblastic region were continuous or non-fenestrated. Sometimes (in about one out of twenty vessels) the endothelial cell appeared very attenuated and, in these regions, small pores or fenestrations were sometimes noted (Fig. 3). The pores appeared as circular depressions on the P-face and as elevated or crater-like structures

Table 1. Frequency of vesicle stomata and fenestrae on endothelial surface. Data of dental pump from present investigation, other data from Simionescu et al. (17)

Type of capillary	Species	Aggregated area examined, μm^2	Vesicular stomata/μm^2		Fenestrae/μm^2
			Blood front (range)	Tissue front (range)	
Muscular					
Diaphragm	Rat	87.8	59 (34–94)	97 (66–132)	—
Myocardium	Rat	122.5	67	110	—
Visceral					
Pancreas	Rat	80.5	32 (0–66)	21 (5–67)	15 (2–31)
Jejunal mucosa	Rat	91.8	11 (0–18)	9 (0–22)	26 (6–56)
Dental pulp	Man	60	76 (66–87)	33 (17–52)	* (see below)

* About 5% of capillaries seem to be fenestrated, but the examined, fenestrated, endothelial surface areas are not large enough to permit an adequate estimation of the number per square area.

on the E-face, such as have been described before.

The fenestrations were closed by a thin diaphragm, as observed by thin-section TEM, but this was difficult to discern on replicas with certainty. The diameter of the pores was about 60 nm.

Micropinocytotic vesicles

The capillaries in the subodontoblastic zone displayed a rather high number of vesicles or caveolae distributed on both the luminal and abluminal cell surfaces (Figs. 1, 2). The vesicles were of uniform size and appeared as cup-like formations about 70 nm in diameter. They were often clustered in areas. Here the mean density was estimated as 66–70/μm^2 (luminal surface). In general the micropinocytotic vesicles appeared in greater numbers on the luminal cell surface than on the abluminal cell surface (Table 1).

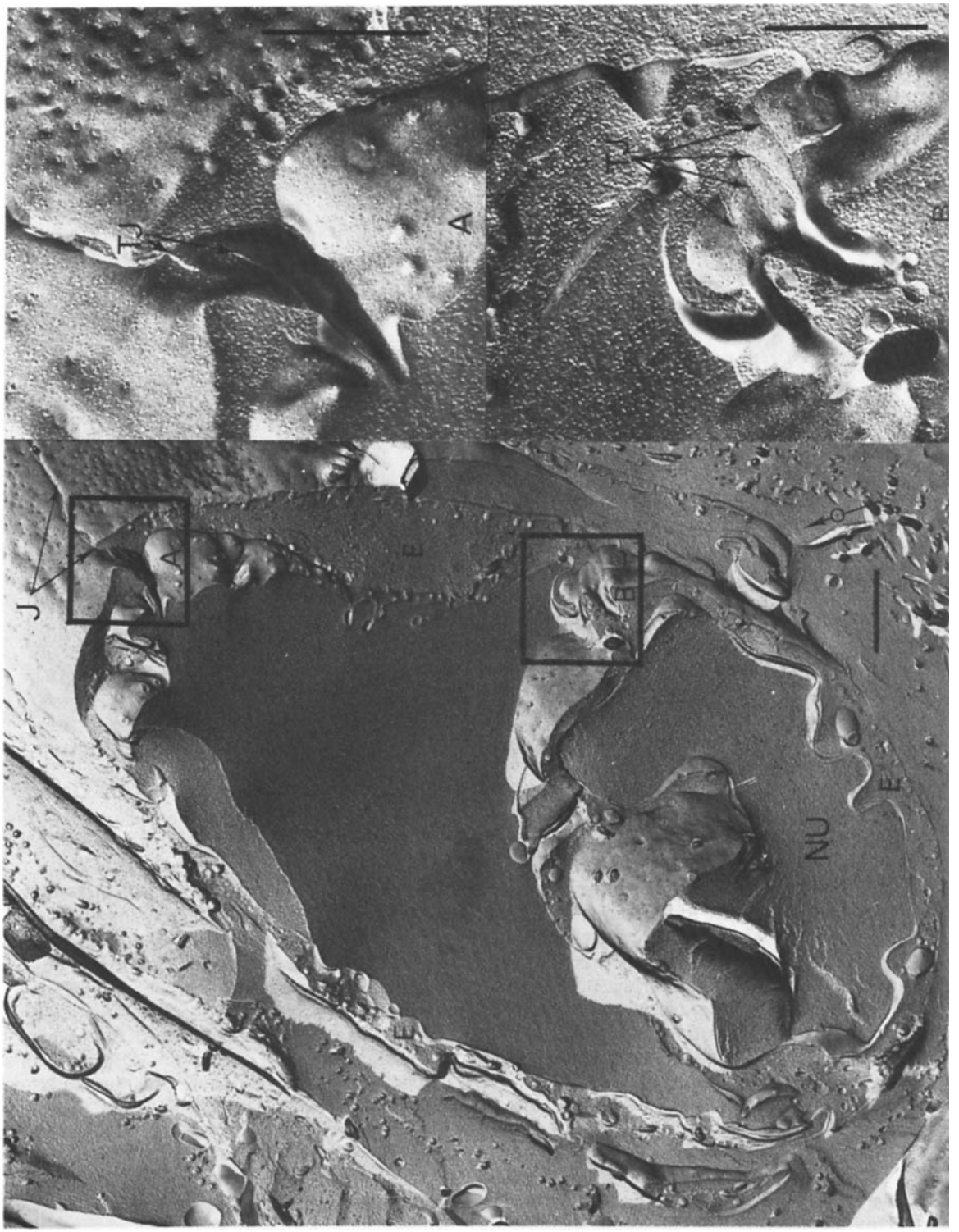
Endothelial cell junctions (zonula occludentes)

Sometimes fracturing of tissue occurs in the region between two endothelial cells. This exposes the endothelial membrane, where the cells come together and fuse, forming 'tight junctions' or *zonulae occludents* (Fig. 4). These areas between adjoining cells usually displayed 2–4 continuous strands of grooves or ridges juxtaluminally in the cell membrane. Junctional depths were about 0.25 μm depending on this number, but the mean distance between individual strands was estimated as 0.06–0.10 μm.

Irregular tissue channels

Irregular tissue channels or continuous, slender vessels having a luminal diameter of about 2–6 μm were disclosed on some replicas in the subodontoblastic region (Fig. 5). The luminal spaces showed a homogeneous mass lacking red blood corpuscles. The abluminal cell membrane showed few pits and there was no clear-cut, delineated, basement membrane.

The width of the endothelium was about 0.2–0.3 μm. The endothelial cells often showed cytoplasmic processes projecting into the surrounding tissue and extracellular fibres often adhered to their tissue front. There were few intracytoplasmic organelles. Interendothelial gaps were not observed.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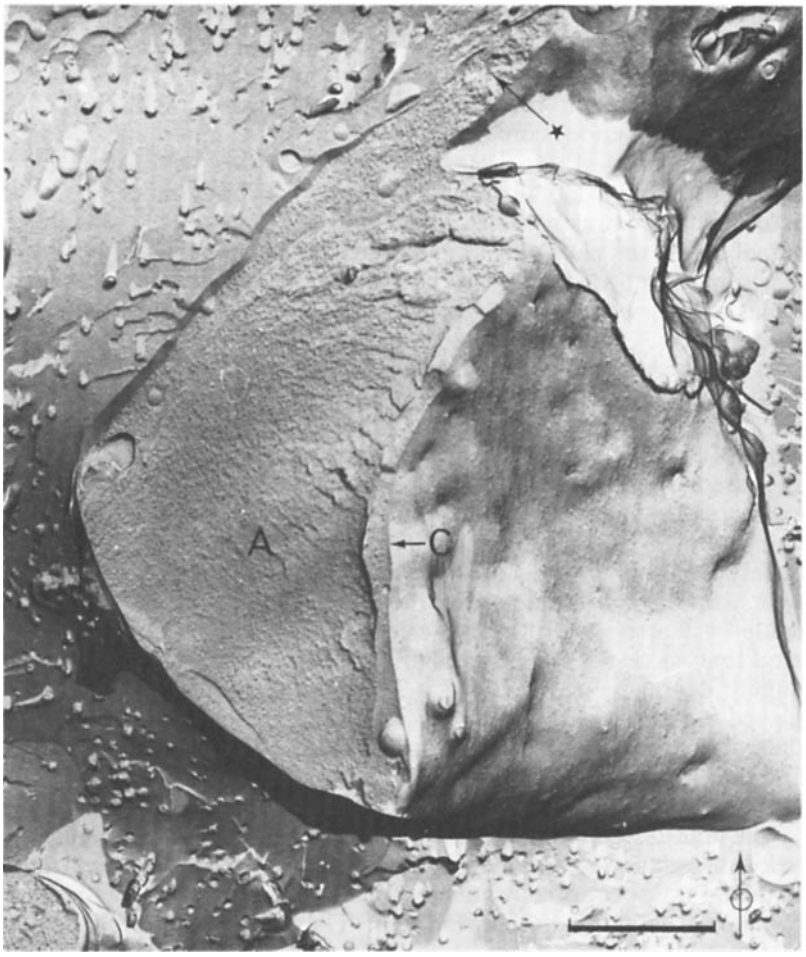

Fig. 5. Electron micrograph of a tubular, irregular vessel-like structure partly cross-fractured. The broken surface exhibits a central homogeneous area (A) surrounded by a thin cell layer (C) whose outer surface is also exposed. Its cytoplasm contains a few organelles. The cells seem to project into surrounding extracellular tissue (*). No clearly demarcated basement membrane is seen. These structures are believed to represent irregular tissue channels, possibly lymphatics. Scale bar 1 μm.

The vessels were supposed to represent lymphatics.

Endothelial filaments

Filaments (≈7 nm in diameter) were observed in the capillary endothelial cells. The diameters of these bundles were in the range 0.1–0.2 μm and were most often orientated longitudinally. Each bundle contained 10–40 filaments. No associations were found between endothelial filaments and the cell membrane. Identical filaments in abundant masses were found in the muscular cell layer as well as in the endothelial cell cytoplasm of arterioles and arteries in the central pulp.

Fig. 4. Freeze-fracturing displays a cross-fractured capillary in the subodontoblastic region. It is composed of three endothelial cells (E) separated by junctional areas (J). Scale bar 1 μm. □ A. A junctional region built up by two continuous, separate strands representing tight junctions (TJ) is seen. Scale bar 0.5 μm. □ B. Junctional site demonstrating at least four separate slightly granular strands (TJ). E-face. Scale bar 0.5 μm.

Discussion

The gross vascular architecture of the human pulp was described in detail by Kramer (10). He noted a plexus of fine vessels spread along the periphery of the coronal part of

the pulp. This network was found to be located in the subodontoblastic region, but a small number of capillary loops could be seen to penetrate into the odontoblastic cell layer. Arterio-venous connections were disclosed. Riedel et al. (16), Harris & Griffin (8), Dahl & Mjör (5) and Rapp et al. (15) described the ultrastructure of the human, pulpal blood vessels. A striking feature was that some small capillaries in the subodontoblastic layer showed endothelial pores or fenestrae.

In the present study, using the freeze-fracturing technique, we were able to confirm the findings of, in principle, two different types of capillaries. There were continuous and discontinuous vessels, the latter displaying pores or fenestrae in their endothelial lining. However, only one capillary out of twenty was found to be of the fenestrated type, which is in agreement with the findings made by Harris & Griffin (8), who discovered that about 4% of the capillaries in the human dental pulp observed by TEM displayed fenestrations. This seems to indicate that discontinuous vessels are indeed few and that capillaries in general in the peripheral dental pulp lack endothelial pores.

The fenestrated blood vessels are believed to offer reduced resistance to the transendothelial transport of various substances, as compared with the non-fenestrated. They are found in various organs of the body that are engaged in the production and absorption of fluids, such as the kidneys, the choroid, the ciliary body of the eye, the gall bladder and the intestine, i.e. in organs known to be functionally specialized in water transport or flux. However, they can also be found in endocrine glands and in sensory ganglia (13, 14). The fact that endothelial pores appear arranged in local arrays with some areas devoid of fenestrations in freeze-fracture replicas may indicate that continuous and discontinuous vessels do not represent two distinctly different types of vessels, but instead represent morphological adaptation to local variations in the functional needs.

Transport may also occur across cell boundaries by means of cytosis or micropinocytotic vesicles. These are formed on one side of the cell wall, from where they migrate and release their contents on the other (9). The vessels in the subodontoblastic region displayed a fairly even distribution of micropinocytotic openings. Sometimes they were arranged linearly or in groups. The number and relative frequency of vesicles in the vessels of the peripheral pulp, compared with those in other organs of the rat, are shown in Table 1.

Several authors have described filaments in the endothelial cytoplasm in the small vessels of the pulp (5, 7, 11). The function of these fibres has not been conclusively explained and it has not been determined whether they represent tono-filaments or myo-filaments. Replicated arteries and arterioles in the central pulp displayed filaments in their endothelial cell cytoplasm and muscular wall. The filaments appeared more infrequently in the peripheral vascular tree, and in the capillary region relatively few bundles of filaments could be seen. The filaments observed in the cytoplasm of the capillaries seemed to be morphologically identical to those observed in the cytoplasm of the muscular layer of the arteries and arterioles, which makes it reasonable to suggest that they may be myo-filaments. It has been suggested that these filaments give greater elasticity to these cells, which are generally subject to continuous pressure and morphological variations (4).

Encapsulation by hard tissue seems to make the dental pulp especially vulnerable to increasing pressures. Inflammatory swelling induced by any stimulus may lead to local or general vascular strangulation, with concomitant ischaemia and tissue necrosis due to the rigid enclavement, if compensatory mechanisms are not present. Thus, bundles of filaments in the endothelial cytoplasm in subodontoblastic vessels may increase vascular stability, in order to maintain morphological integrity versus high intraluminal pressure. In this respect, tight junctions anchoring adjacent, endothelial cells to each other may be an important factor, too. These junctions play a role in sealing off the luminal space of the vessels from the abluminal, extracellular compartment. They allow the vascular wall to create gradients between

intra- and extra-vascular spaces. A protein gradient is a necessity for the maintenance of an osmotic pressure-gradient over the endothelial wall. We found 2–4 separate junctional strands included in the junctional strands. These were arranged typically near the luminal border. Their number is in agreement with the number generally seen in the capillary region (17). Thus, there are anatomical modifications in the endothelial structure which may serve the purpose to withstand the high, intraluminal pressure demonstrated by Brown (2).

Another factor that has to be considered for the maintenance of a protein gradient over the cell wall is the presence of lymph vessels, which could eliminate a surplus of protein in the interstitial fluid. Freeze-fracturing of the subodontoblastic region displayed thin-walled, slender vessels, which may represent lymphatics. Such vessels have earlier been described in pulpal tissue (5, 6, 12, 16).

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