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Pinocytotic vacuoles in human dental pulp capillaries

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Summary. Dental pulp capillaries were studied in human. They were of the continuous type, with the exception of a small number which were of the fenestrated type, located in the vicinity of the odontoblasts. A characteristic morphological peculiarity was found in the endothelial cells. In places there was a large quantity of multisized vacuoles. The vacuoles were evidently of pinocytotic origin, and their content was emptied into the extracapillary space. The initiation of their formation was indicated by the creation of cytoplasmic flaps, which could not be characterised as typical pseudopodia, and which in cross sections resembled microvilli. The flaps engulfed a quantity of plasma and then, after bending over, their edge fused with the cell, creating a vacuole. The vacuole, after being moved abluminally, was emptied into the pericapillary area by exocytosis. There was indication that flaps created at the borders of the endothelial cells (flanges) acted likewise, transporting vacuoles through the intercellular spaces. Micropinocytosis, was a distinctly different phenomenon, contributing, to a very small degree, to the intracellular enlargement of the vacuoles. It seems that this vacuolar mechanism of transportation serves an augmented metabolic need of the surrounding tissue.

Key words: Vacuoles, Capillary, Dental pulp

Introduction

The gross vascular architecture of the human pulp was described in detail by Kramer (1960), and the ultrastructure of small vessels and capillaries of the dental pulp in various species by others (Han and Avery, 1963; Riedel et al., 1966; Corpron et al., 1974; Rapp et al., 1977; Kölling and Rask-Andersen, 1983; Seltzer and Bender, 1984; Lyroudia et al., 1989). It is accepted that the endothelium of the dental pulp capillaries is of the continuous type, with the exception of the capillaries located among odontoblasts near the predentin, where in a small proportion they are of the fenestrated type (Riedel et al., 1966: Harris and Griffin, 1971; Dahl and Mjör, 1973; Corpron et al., 1974; Rapp et al., 1977; Kölling and Rask-Andersen, 1983; Ekblom and Hansson, 1984; Oguntebi, 1986).

It has been stated in the literature, and it is accepted, that nutritional material moves from blood vessels to the pulp tissue cells, according to hydrostatic and osmotic pressures, and that transcapillary exchange occurs by diffusion, filtration-absorption and micropinocytosis (Seltzer and Bender, 1984). Micropinocytosis involves a slow mechanism of transport of proteins, and the bidirectional nature of this process has been confirmed (Seltzer and Bender, 1984). Fenestrations existing in the small percentage of capillaries adjacent to odontoblasts, provide an enhanced ability of filtration, which serves augmented nutrient demands (Corpron et al., 1974).

While studying the ultrastructural relations of adrenergic and cholinergic nerve endings with the muscular coat of small vessels in human dental pulp, a morphological particularity of the endothelial cells of the pulp capillaries came to our attention. In places, the endothelial cells presented microvillus-like formations extending to the lumen, and vacuolar cytoplasmic formations. Since these structures were not previously noticed in this form and quantity, neither in literature nor in other capillaries, we proceeded to study them ultrastructurally.

Materials and methods

Five premolars, clinically healthy, extracted for orthodontic purposes from patients aged from 21 to 25 years, were used for the ultrastructural study of capillaries of the human dental pulp.

Immediately after extraction, the teeth were placed in 2.5% cacodylate-buffered glutaraldehyde (pH 7.2). Resection of the apex was performed under continuous flow of the same fixative, aiming at the easier infiltration

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of the fixative into the dental pulp, and then the teeth were reimmersed in identical fresh fixative, for 24 hours.

Using a special microtome (Aceutom, Styers) cross sections (including hard dental tissues and pulp) 150 µm thick were taken. The sections were washed in cacodylate buffer for 1/2 hour and then postfixed in 1% cacodylate buffered OsO_4 (pH 7.2) for 1 hour. «En bloc» staining was effectuated with a saturated aqueous solution of uranyl acetate for 1 hour, and then the sections were dehydrated with a gradual series of alcohol, and embedded in Epon 812.

The tissue blocks were trimmed under a dissecting microscope and all the hard tissues were removed until finally only the dental pulp was left, from which semithin sections were taken.

Semithin sections, 1 µm thick, were cut and stained with Stevenel's blue for light microscopy examination. Sections, 50-75 nm thick, were taken on copper grids for transmission electron microscopy. They were stained with uranyl acetate and were examined in a Jeol 100CX TEM, at 80 kv.

Results

The overall diameter of the pulp capillaries (outer diameter) ranged between 7 and 15 µm. The endothelium of these vessels was of the continuous type in the entire dental pulp, with the exception of a very few places in the odontoblastic vicinity, where it was fenestrated. The endothelial cells were flat and had a more or less oval nucleus, whose membrane often appeared wrinkled and sometimes with more or less deep infoldings (Fig. 1).

Most of the pulp capillaries presented portions of a pericytic cytoplasm in the immediate vicinity of their outer limit. All capillaries were unexceptionally surrounded by a continuous basement membrane, presenting a more or less uniform thickness of approximately 35 nm and which had a constant density. The basement membrane was usually separated from the

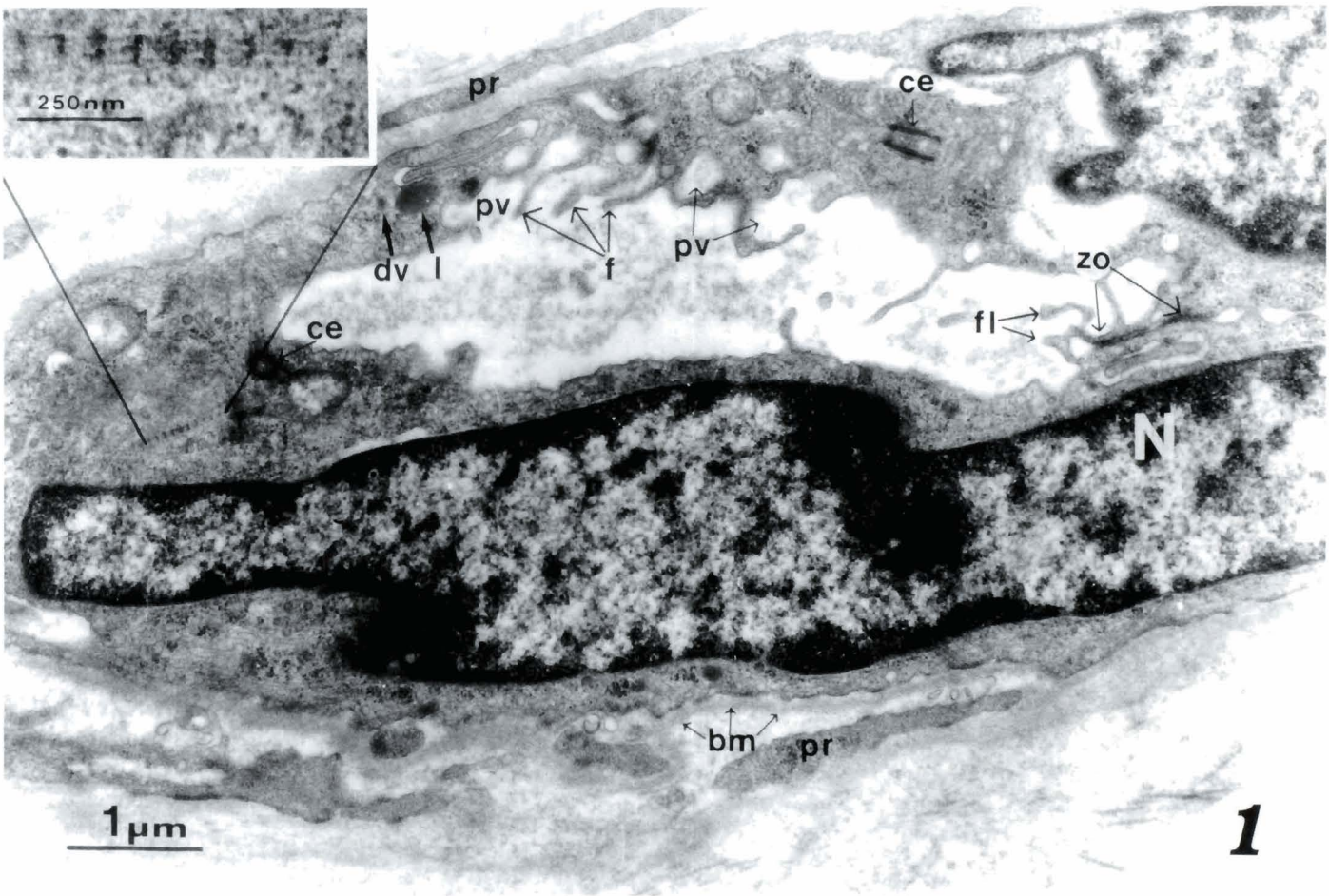


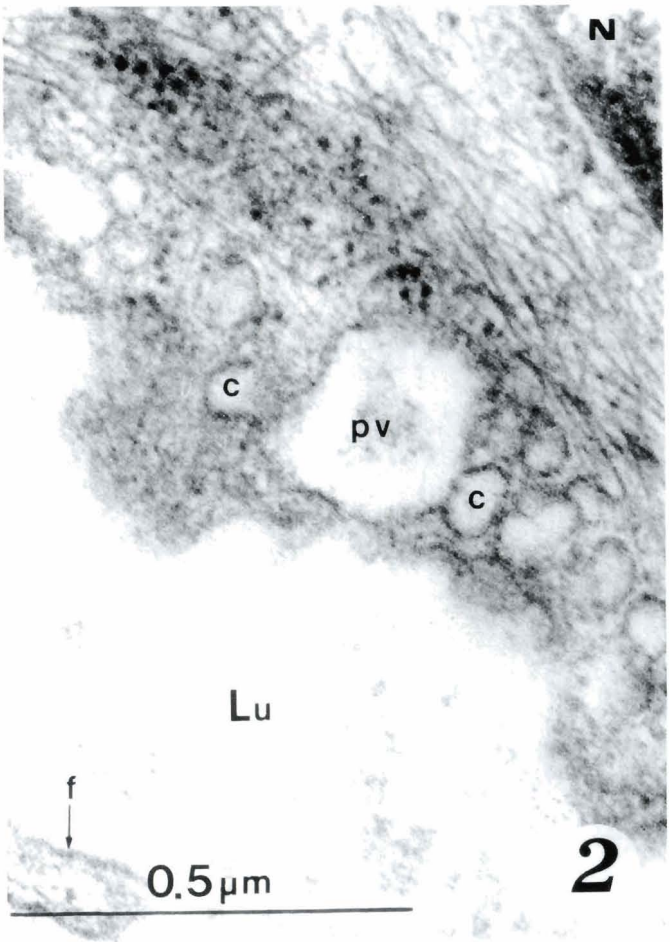
Fig. 1. Cross section of a dental pulp capillary. Many microvillus-like formations: flaps (f); and flanges (fl), are projecting into the lumen. Pinocytotic vacuoles (pv), formed or being formed, are seen. Centrioles (ce) are observed and routlets are presented magnified in the Inset. A dense core vesicle (dv) is shown next to a lysosome (l). Arrows show zonulae occludens (zo). The luminal surface in front of the nucleus presents only one flap in contrast to the population at the other areas. Basement membrane (bm), nucleus of an endothelial cell (N), pericytes (pr).

basal plasma membrane by a clear zone of approximately 15 nm (Fig. 1). The basic morphology of the points of contact between adjacent endothelial cells was similar to other capillaries in other organs. The adjoining cells made a simple contact by creating a uniform lucent zone of 10 to 12 nm which separated their surfaces. This zone seemed to be continuous with the lumen on one side, and with the clear zone between the endothelium and the basement membrane on the other. In places there were attachment zones, forming zonulae occludens, identified by the characteristic densification of intercellular material, adjacent membranes and cytoplasmic matrix of the region (Fig. 1).

Organelles included a number of elements of rough endoplasmic reticulum with short, flat saccular profiles, a few to several small and usually round mitochondria, clusters of ribosomes, small, sparse Golgi apparatus, centrioles and rootlets, occasional dense core vesicles, filaments, numerous micropinocytotic vesicles (caveolae), vacuoles with various sizes, and in places many microvillus-like formations which projected into the lumen (Fig. 1).

The micropinocytotic vesicles measured 30 to 70 nm in diameter and were located along the luminal and abluminal cell membranes. In general they appeared in greater numbers on the luminal cell surface than on the abluminal. Occasionally these vesicles seemed to coalesce not only between each other but with vacuoles as well (Fig. 2).

A varying number of intracellular fibrils were present in many endothelial cells. The diameter of the fibrils was about 6 to 8 nm. In favourably sectioned materials, these long profiles could be seen ramifying into the cytoplasm. Usually they were found in clusters, where the majority were oriented longitudinally, whereas some others were



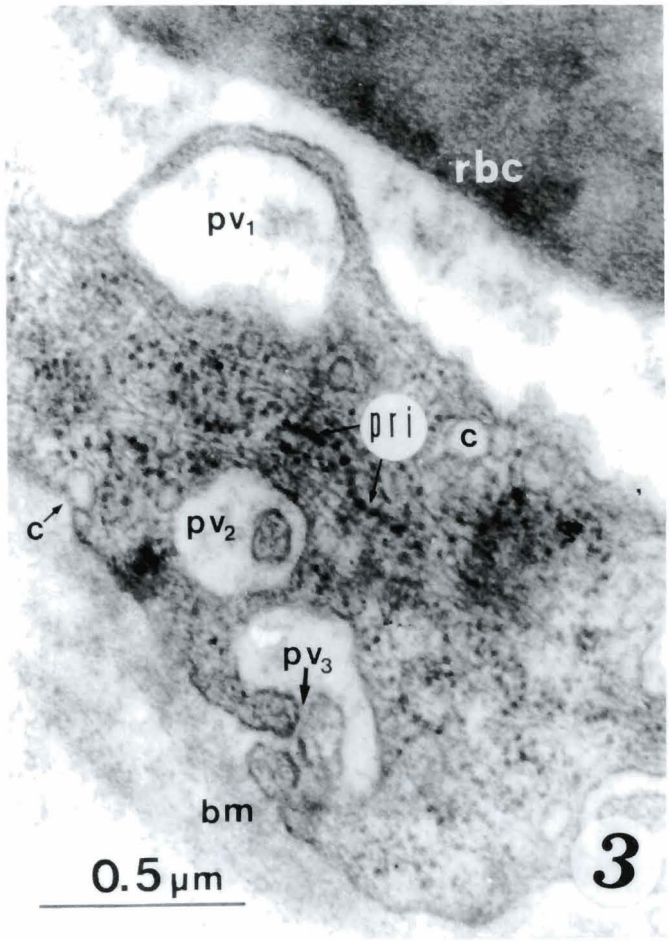
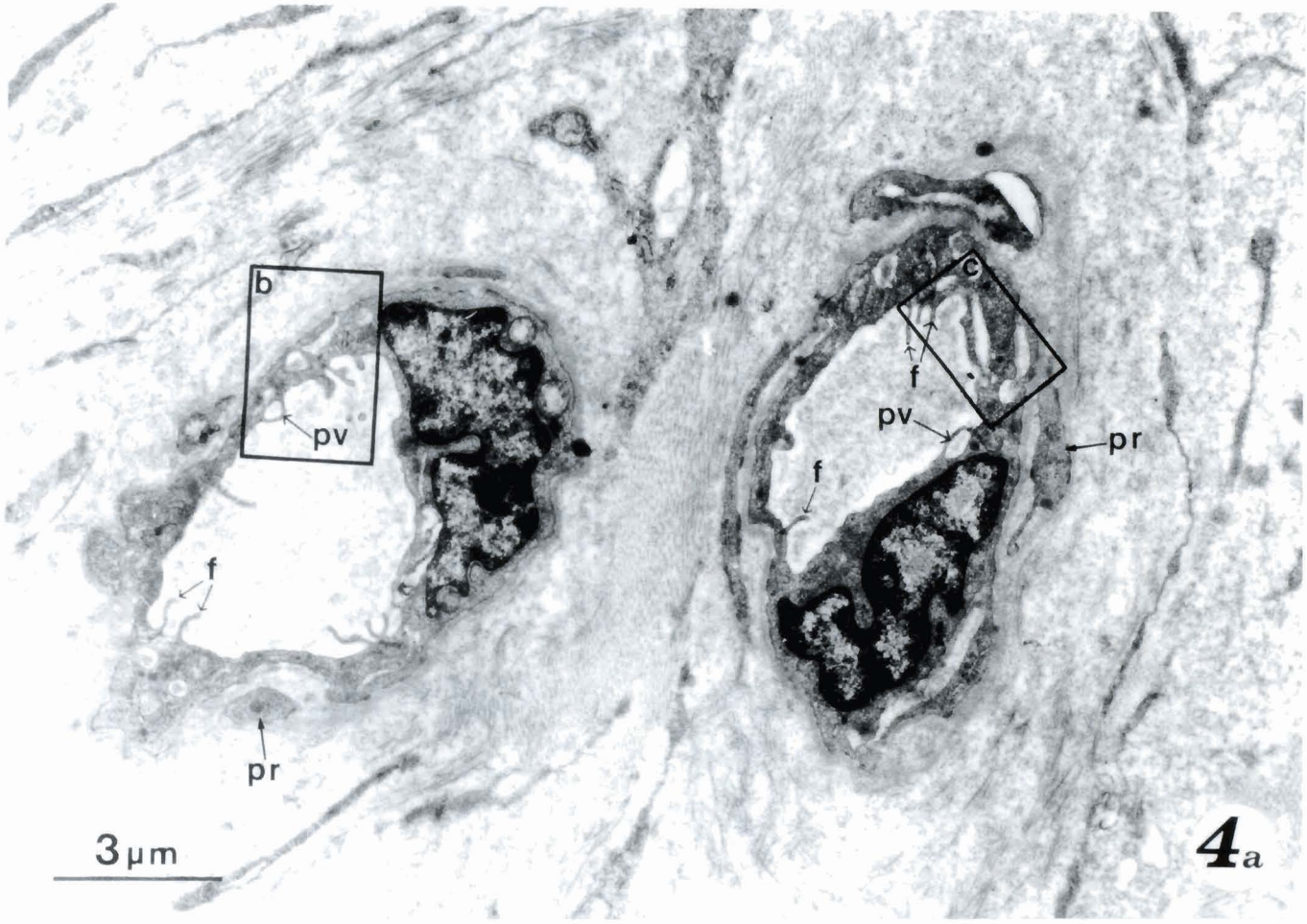


Fig. 2. High manification of part of the cytoplasm of an endothelial capillary cell. Bundles of filaments are extended mostly longitudinally. Two micropinocytotic vesicles (caveolae) (c) are shown to come into contact with and to incorporate into a pinocytotic vacuole (pv). A section of a flap (f) in the lumen (Lu) of the capillary presents its filamentous content. Nucleus of the endothelial cell (N).

Fig. 3. High magnification of the cytoplasm of an endothelial capillary cell, showing ribosomes and polyribosomes (pri), filaments, a newly formed pinocytotic vacuole (pv_1) and an old pinocytotic vacuole (pv_2). The arrow shows the exist of an abluminal vacuole (pv_3). Caveolae (c), red blood cell (rbc), basement membrane (bm).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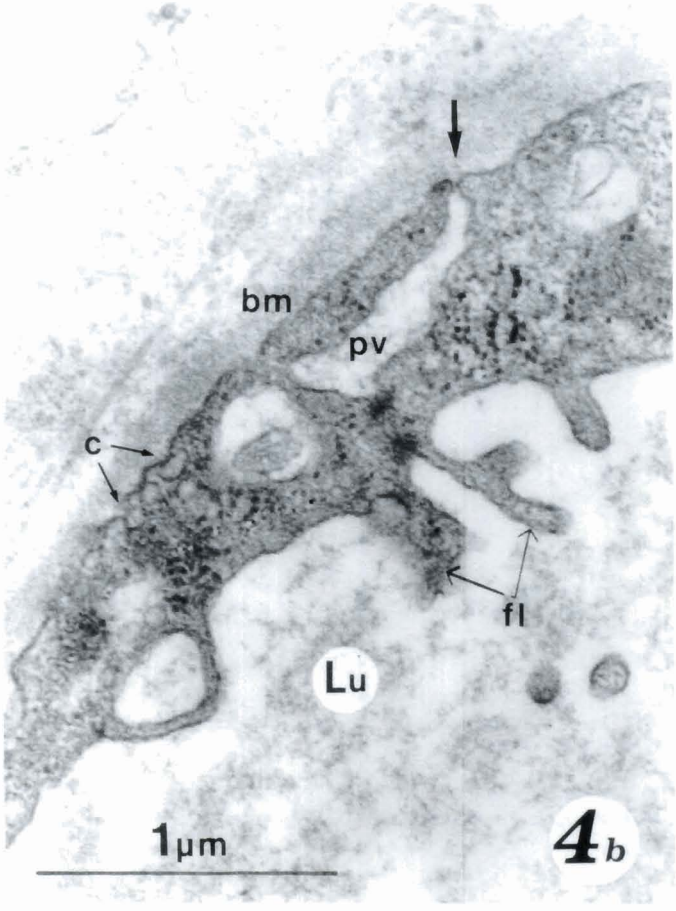
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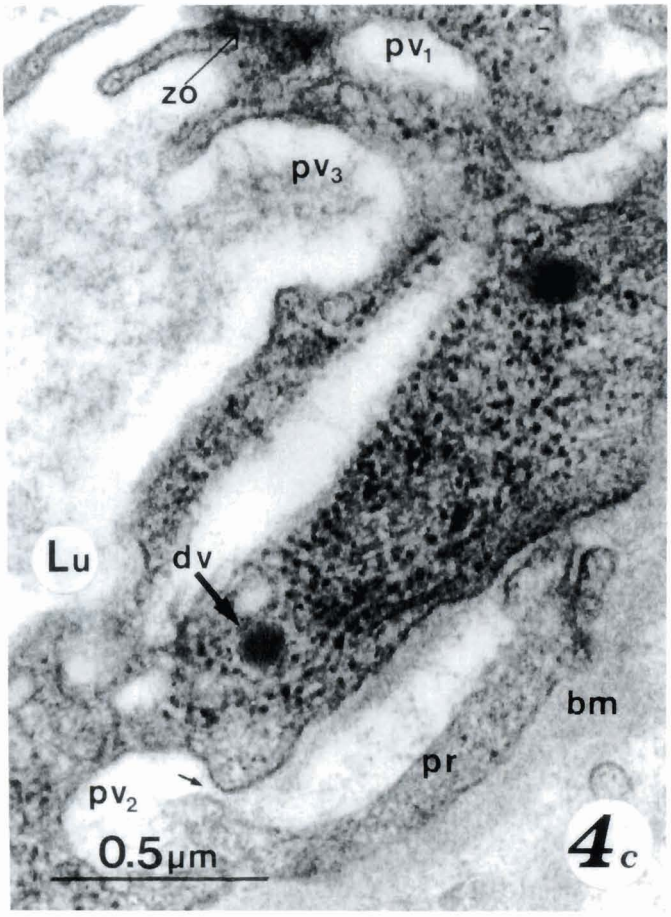
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Fig. 4. a. Cross section of two adjacent capillaries. Flaps (arrows f) projecting within the lumen are visible in both capillaries. Pinocytotic vacuoles are shown by arrows (pv). Parts of pericytes (pr) are seen. Rectangles (b) and (c) are presented magnified in 4b and 4c. **b.** Magnified area of figure 4a (rectangle b). Two flanges (fl), one of which is composite, are formed at the border of the cells, luminally (Lu). A pinocytotic vacuole (pv) occupies the intercellular space and empties its content (arrow). Abluminal micropinocytotic activity (caveolae) (c) is observed. Basement membrane (bm). **c.** Magnificed area of figure 4a (rectangle c). A zonula occludens (zo) is seen and a pinocytotic vacuole (pv_1) occupies the intercellular space. A pinocytotic vacuole (pv_2) is emptied (arrow), exactly in front of a pericyte portion (pr), while another one (pv_3) is being formed luminally (Lu). Arrow indicates a dense core vesicle (dv). Basement membrane (bm).

oriented in different directions. Other cytoplasmic structures, usually ribosomes, or more rarely rough endoplasmic reticulum and mitochondria, were dispersed among them, and sometimes were displaced by them.

A distinctive and very frequent finding was the presence of vacuoles in the cytoplasm of endothelial cells. Morphologically their content, had the same appearance as the plasma. The number of the vacuoles varied from section to section. However, more or less, most of the capillaries presented some of them. Their size varied usually from 200 nm to 1 μm, or occasionally more. Sometimes micropinocytotic vesicles seemed to contribute to the vascular size by releasing their content into the vacuole. Vacuoles seemed to empty their content on the abluminal side of the capillary (Figs. 2, 3).

The most striking feature of our findings associated with the vacuoles, was the presence of what at first sight seemed to be microvillus-like formations or atypical pseudopodia (Fig. 4a). However, by carefully examining these structures it was clear that they consisted of cytoplasmic bulgings, which gradually extended into the lumen to form flaps. The flaps appeared to bend over and return to the surface of the cell, where they fused with it. By this procedure they engulfed a quantity of capillary plasma, thus creating a vacuole. Then the cytoplasmic vacuole seemed to approach the abluminal surface of the capillary, and after its surface had become attenuated it opened, releasing its content into the surrounding intercapillary tissue. The size of the flaps varied, resulting in variations in the size of the vacuoles. Flaps in the borders of the cells, assuming the form of flanges, were a usual phenomena, and they contributed to the formation of vacuoles, which seemed to pass through the intercellular spaces (Figs. 4b, c).

In places where the endothelial cells presented high vacuolar activity, the number of cytoplasmic flaps which protruded into the lumen of the capillary was increased, creating a very characteristic appearance (Fig. 5). In areas presenting high vacuolar activity, besides the characteristic appearance of the capillary luminal surface, the abluminal side of the endothelial cells was also characteristic, presenting many inlets and spikes. This appearance was created by the opening and emptying of the contents of the vacuoles (Fig. 5). In these areas the clear zone between the basement membrane and the endothelial plasma membrane, described above, was at places broader (Fig. 5). The cytoplasmic flaps had a thickness from 50 to 120 nm. In favourably cut sections it sometimes showed the presence of filaments. In capillary sections taken longitudinally, such cytoplasmic flaps could be seen almost in the midstream. They derived from the distant part of the capillary wall (beyond the level of the picture) and appeared at the level of the sections by protruding into the lumen, as was confirmed by serial sections. In such sections it was evident that cytoplasmic flaps had a flat sheet-like appearance with more or less the same thickness and similar internal structure (Fig. 5). The cytoplasmic flaps were not always plain flat formations. In areas presenting high vacuolar activity, composite flaps appeared. They presented secondary or, more rarely, flaps of third or fourth class (Fig. 5). The usual orientation of the flaps was along the stream of the blood. So, in cross sections of the capillaries they presented a microvillus-like appearance.

There was some evidence that the initial structures before the formation of the flaps were real microvilli, because in areas with high vacuolar activity, some microvillus-like formations were seen (Fig. 5).

The population of the flaps and vacuoles was usually great in areas where the endothelial cells were thin. The exocytosing activity of vacuoles was minimal or absent at the abluminal surface of the endothelial cells opposite the nucleus. This surface was usually flat, which implies that usually no vacuoles arrived at this area. Sometimes, while a small vacuolar activity could be identified on the luminal prenuclear zone, there was no indication for similar activity on the correspondent abluminal area (Fig. 5).

High vacuolar activity was found in two of the five teeth examined. In the other teeth, flaps or flanges were a common finding, but vacuoles were very sparse.

Discussion

Capillaries are the exchange vessels responsible for transporting nutrients and oxygen from blood to tissues and for removing waste products in the reverse way. The structural features of their walls, according to which they are characterized as continuous, fenestrated or discontinuous, serve this function. Transcapillary exchange occurs by diffusion, filtration-absorption and micropinocytosis. The latter is a morphologically demonstrable mechanism and involves a slow but active transport of proteins. The bidirectional nature of this mechanism has been confirmed (Seltzer and Bender, 1984).

In the literature it is cited that dental pulp capillaries are of the continuous type and that the augmented metabolic needs of the odontoblastic area are covered by the presence of a small percentage of fenestrated capillaries (Corpron et al., 1974). In human impacted

canines and third molars, Oguntebi (1986) described less than 10% of the capillaries examined as fenestrated. Fenestrations were also reported by Dahl and Mjör (1973), and by Raap et al. (1977). Köling and Rask-Andersen (1983) found fenestrations in the blood capillaries of the subondontoblastic region of the human dental pulp, and Ekblom and Hansson (1984) in the capillaries of both human and feline dental pulp. In this work it has been shown that rather large quantities of plasma material were transferred from the lumen to the extravascular space through a vacuolar mechanism of the capillary endothelium. Evidently this mode of exchange is completely different from micropinocytosis, since this phenomenon has been described in detail in the literature, and its characteristics are clearly defined (Rapp et al., 1977; Köling and Rask-Andersen, 1983; Oguntebi, 1986). Ekblom and Hansson (1984) also reported micropinocytotic vesicles measuring 60-70 nm. Harris and Griffin (1971) reported micropinocytotic vesicles of the same diameter, sometimes reaching a

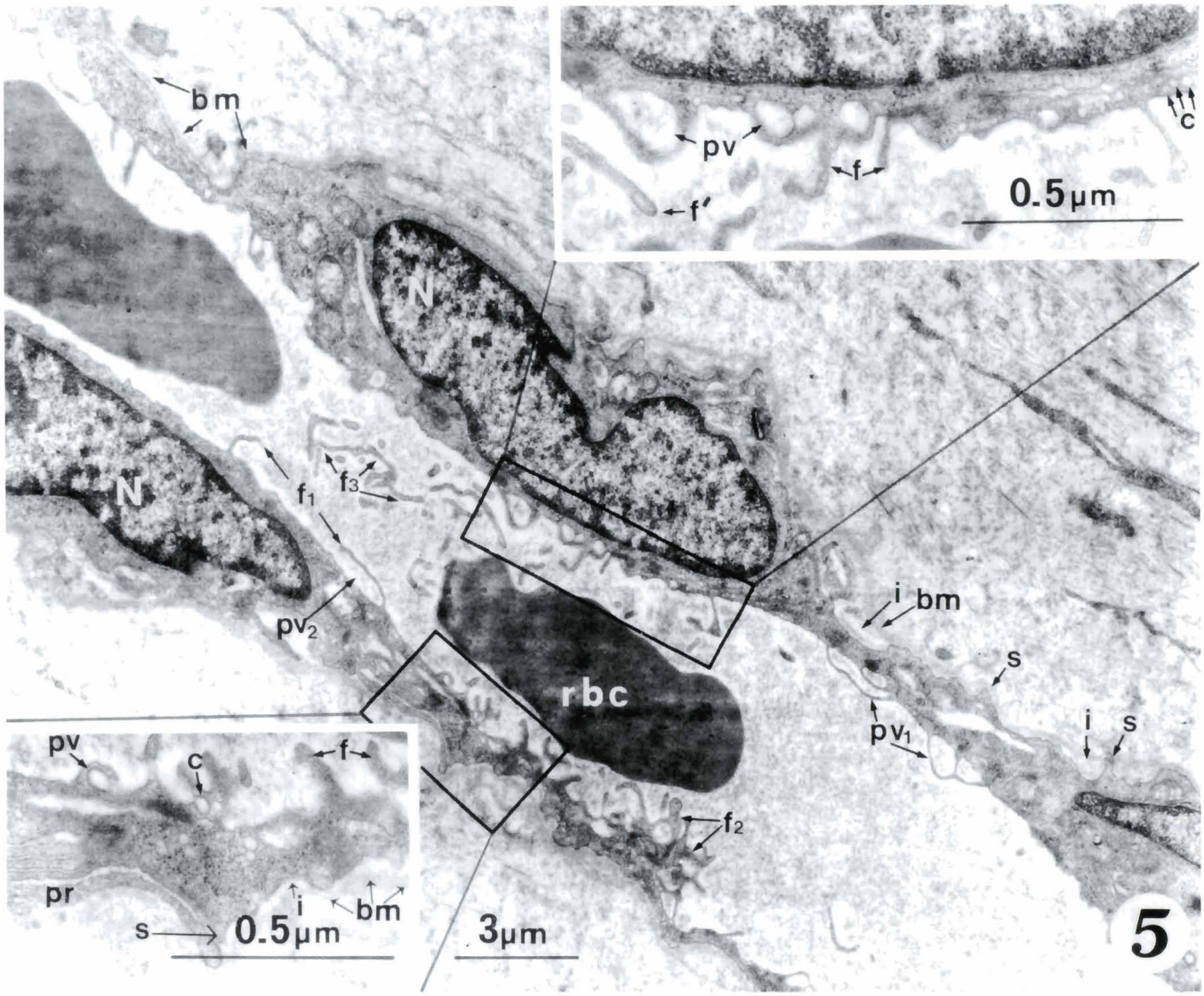

Fig. 5. Longitudinal section of a dental pulp capillary. Characteristic appearance of an area with high vacuolar activity. Many simple flaps (f_1) and composite flaps (f_2) are seen. Some of the flaps (f_3) are located in the midstream, deriving from the distant wall of the capillary. Vacuoles ((pv_1) and (pv_2)) already formed, or in the process of formation, are seen, correspondingly. The areas of the two rectangles are shown in higher magnification in the insets. Red blood cell (rbc), basement membrane (bm), nucleus of endothelial cell (N). The abluminal surface presents spikes (s) and inlets (i). In some of the later basement membrane is slightly removed from the cell surface. **Upper Inset:** Many flaps (f) and pinocytotic vacuoles (pv) are observed. Arrow shows the microvillus-like appearance of a flap (f'). Caveolae (c) are seen. **Lower Inset:** Luminal and abluminal surface of an endothelial cell. The luminal surface has many flaps (f). A pinocytotic vacuole (pv) and a caveola (c) are being formed, indicating the different mode of formation. The pinocytotic vacuole (pv) is created by a flap, while in the caveola (c) the cell surface invaginates. The abluminal surface presents spikes (s) and inlets (i). Basement membrane (bm), pericyte (pr).

diameter of approximately 150 nm. Micropinocytosis also occurred in our specimens, and their morphological characteristics were the classically described ones, but they were completely different from the characteristics of the vacuolar mechanism (Fig. 5). However, there was an indication that micropinocytosis contributed to the vacuolar mechanism, probably implying their synergic nature.

The findings presented in this paper prove that under certain circumstances dental pulp capillaries are capable of forming multisized vacuoles, apparently for enhancing the transendothelial transferring mechanism. There is no doubt as to the mechanism of their formation. Cytoplasmic flaps are developed by the endothelial cells and they end up in a formation of vacuoles. Transverse and longitudinal sections of capillaries, as well as serial sections of the flaps, clearly demonstrated the origin of the flaps and their mode of development and transformation into vacuoles.

On the other hand, it was evident that vacuoles emptied their content into the pericapillary space by way of exocytosis. In the specimens examined there was no indication that any transient passage uniting the lumen with the extravascular space was created. On the contrary, in all sections studied (serial sections included) vacuoles were isolated from the intraluminal space before emptying their content extracapillarly. The direction of transportation was from the capillary lumen to the extravascular space. There was no indication that the reverse procedure could have taken place. The content of vacuoles seemed to be morphologically identical to the plasma content.

Similar flaps have been described in the mouse dental pulp (Han and Avery, 1963). However, their quantity in the capillary lumen was strikingly less than that presented in this work, and furthermore the hypothetical function attributed to them was that they might act as a regulator for the passage of cellular elements through the intercellular space, by obstructing or facilitating the procedure. The findings in this work did not suggest any other function, apart from their essential contribution to the formation of the pinocytotic vacuoles. The endothelium which lines Schlemm's canal of the trabeculum of the eye is a kind of endothelium which presents morphologically similar formations (Tripathi, 1972; Manthos et al., 1980). However, from the functional point of view, this procedure seems to be strictly a pathway mechanism for the exit of aqueous humour to the veins of the eye, serving essentially the aqueous humour circulation. On the contrary, this activity in the dental pulp capillaries seems to be strictly related to metabolic needs.

The morphological characteristics of the vesicles presenting a dense core, found in this work in the endothelial cells, seemed to be the same as those described elsewhere in the literature (Foroglou et al., 1981), but their nature is unknown. Their role could probably be related to the endothelial function and its interaction with the surrounding tissues.

The suggestion that could be made about the appearance of the pinocytotic vacuoles is that they are related to age, and that they are a common phenomenon of the dental pulp capillaries of younger humans, who still have open tooth apices, and whose needs for microcirculation, survival, differentiation of the cells and deposition of dentin, are higher. The number of the examined dental pulp is not sufficient to produce statistically reliable results, a task which may be considered for future investigation.

Reviewing the literature, we did not encounter any micrograph of dental pulp capillary showing such a high vacuolar activity. In fact, as a rule there was none or at the most one vacuole of small and rarely of middle size. However, in most of the pictures presented in bibliography, there were few flaps and flanges. Such vacuolar activity was not mentioned as a possible bulk pathway of plasma transportation. Our findings show that dental pulp capillaries have the ability to show a high activity of vacuolar formation. This suggests that in periods of high tissue nutrient needs, pinocytotic vacuoles may play an important additional role for compesating this situation. One could hypothesize that this capillary reaction is analogous of the role of the fenestrated capillary, which, however, only serves steadily increased needs of the surrounding tissues.

The distribution of capillaries presenting many vacuoles was not suggestive of the possible cause which initiated their formation. Additionally, they were found in two of the teeth examined, and the clinical data collected by these two patients did not suggest any correlation with a causative factor. The previous questions that is, the causative factors, the uni- or bidirectional nature, and their exact content, are subjects for further investigation.

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