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The Innermost Layer of Cementum in Rat Molars: Its Ultrastructure, Development, and Calcification

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Summary. The present study describes the ultrastructure of the innermost layer of cementum (ILC) in the rat molars and discusses its developmental process and calcification mechanisms. The following points are the main results of this study:

1. The ILC is a thin layer, about 2.0 μm thick, intensely stained with hematoxylin but not stained by silver impregnation.
2. Electron microscopically, it is composed of substances stained with ruthenium red and chromic phosphotungstic acid presumed to be proteoglycans and a few thin collagen fibrils.
3. Periodontal fibers penetrate the ILC only a short distance, and do not reach the root dentin surface.
4. The ILC begins to form on the root dentin surface, just after the disintegration of Hertwig's epithelial root sheath. At the same time, matrix vesicles and spherical bodies, which may be derived from the matrix vesicles, appear on the surface of the developing ILC.
5. Dental sac cells show higher cell activities than the epithelial sheath cells. Observations support the view that the dental sac cells secrete the ruthenium red positive material.
6. On the basis of the above findings, the ILC is suggested to be formed by the dental sac cells and calcified by the matrix vesicles derived from these cells.
7. The ILC can be regarded as a specialized cementum between the root dentin and the cementum in the strict sense, serving the connection of the two tissues.

Previous investigators have noticed that the thin, innermost layer of cementum (ILC) could be ultrastructurally differentiated from the cementum in the strict sense in rat teeth (PAYNTER and PUDY, 1958; STERN, 1964) and in mouse teeth (SELVIG, 1964; TEN CATE, 1978).

Recently, OWENS (1980), LINDSKOG (1982), and TEN CATE (1985) reported that the ILC was probably formed by the inner enamel epithelial cells of Hertwig's epithelial root sheath, not by the cementoblasts. OWENS (1980) suggested that the inner enamel epithelial cells in the rat molar teeth possessed a secretory activity as suggested by the structures of their organelles. Furthermore, he found a granular material between the inner enamel epithelial cells and the calcified root dentin. Since the material resembled the enamel-precursor-material secreted by the ameloblasts, he concluded that the inner enamel epithelial cells might secrete the granular material to form the layer in

question. LINDSKOG (1982) proposed some ultrastructural similarities between the inner enamel epithelial cells and the ameloblasts in monkey teeth. He suggested that the inner enamel epithelial cells participated in the formation of the layer. As described above, many investigators have reported on the ultrastructural characteristics of the ILC, some of them insisting that it was formed by the inner enamel epithelial cells. However, the findings on its ultrastructure differ slightly from author to author. It is clear that previous studies lack the precise morphological evidence to conclude that the inner enamel epithelial cells produce the layer. The present study therefore aims to determine the ultrastructural and histochemical nature of the ILC more clearly and discuss its origin, developmental process, and calcification mechanisms.

MATERIALS AND METHODS

The first molar teeth of the upper jaws from 10, 15, 20-day-old and adult Wistar rats were used in this study.

1. Light microscopy

The animals were perfused with 10% formol-saline for 15 min and their upper jaws were excised. The specimens were then immersed in the same fixative for 1 week, decalcified in a formic acid-sodium citrate mixture solution (MORSE, 1945) for 2 weeks, and embedded in paraffin. Serial sections were cut at a 7 μm thickness in the mesio-distal plane of the tooth and stained with hematoxylin and eosin or by a silver impregnation method for reticular fibers (PERDRAUV, 1921).

2. Electron microscopy

The animals were perfused with 2.5% glutaraldehyde in 0.06M cacodylate buffer at pH 7.4 containing 0.5% sucrose for 30 min. The upper jaws were removed and immersed in the same fixative for 4 hrs. After decalcification in 5% EDTA for 2 weeks and postfixation in 1% OsO_4 for 3 hrs, the specimens were embedded in Epon 812. Ultrathin sections cut medio-distally were stained with uranyl acetate-lead citrate or 2% phosphotungstic acid to be observed with a JEM-100SX electron microscope. Semithin sections were stained with 0.5% toluidine blue for light microscopic observation.

Ruthenium red stain: After perfusion with 2.5% glutaraldehyde in 0.06M cacodylate buffer at pH 7.4 containing 0.5% sucrose and 500 ppm ruthenium red, the upper jaws were immersed in the same fixative. Some specimens were decalcified in 5% EDTA and others were not. They were then post-fixed in 1% OsO_4 containing 500 ppm ruthenium red and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate-lead citrate.

Chromic phosphotungstic acid stain: After perfusion with 2.5% glutaraldehyde in 0.06M cacodylate buffer at pH 7.4 containing 0.5% sucrose, the upper jaws were immersed in the same fixative, decalcified, and embedded in glycol methacrylate. Ultrathin sections were stained doubly with 10% chromic acid and 1% phosphotungstic acid.

RESULTS

1. Light microscopy

A thin layer of approximately 2 μm thickness, intensely stained with hematoxylin but not impregnated with silver, was recognized as the innermost layer of acellular and

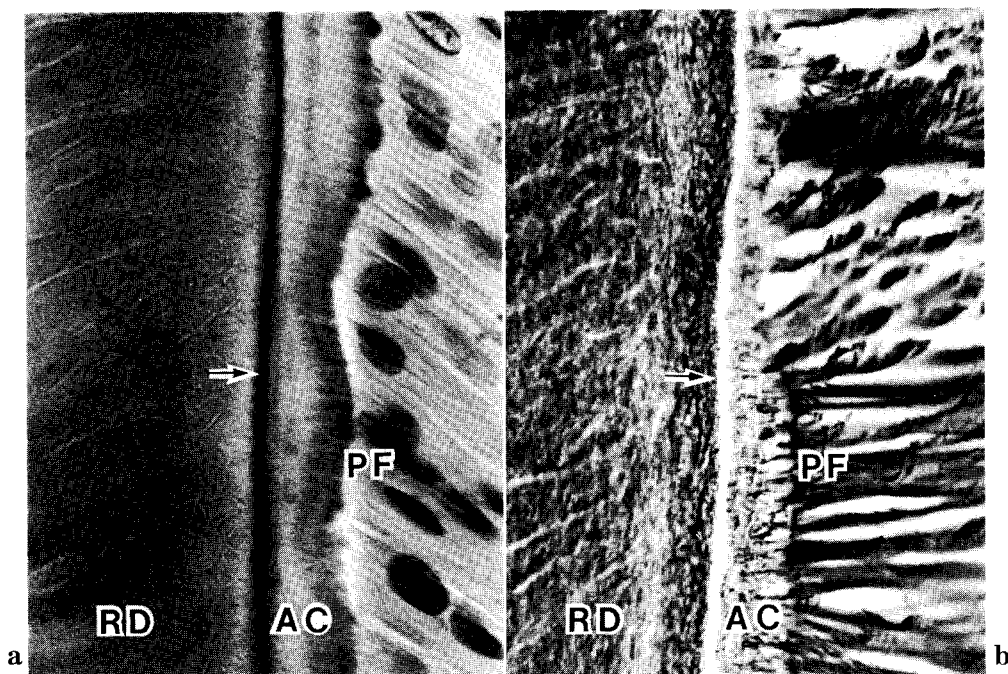


Fig. 1. Light micrographs showing the cervical area in the adult rat teeth. The innermost layer (arrows) of acellular cementum (*AC*) is seen on the root dentin (*RD*) surface. This layer is intensely stained with hematoxylin (**a**), but not impregnated with silver (**b**). Periodontal fibers (*PF*) do not enter this layer. **a**: Hematoxylin and eosin, **b**: silver impregnation. **a**, **b**: $\times 830$

cellular cementum (Fig. 1, 2). This layer, which is called the innermost layer of cementum (ILC), was shown to consist of a basophilic, non-fibrous material. It covers almost all the surface of the root dentin in the rat molar teeth. Periodontal fibers terminated on the surface of this layer.

ILC formation began on the root dentin surface as the root dentin calcification advanced (Fig. 3). The ILC developed to its full thickness in the adult teeth (Fig. 4). These findings indicate that the ILC forms gradually on the root dentin surface from the coronal to the apical side, and then remains unchanged after reaching its full thickness. The cementum in the strict sense covered the ILC subsequently.

2. Electron microscopy

a. The ultrastructural and histochemical nature of the ILC

The cervical area of the 20-day-old rat teeth was examined. In sections with uranyl acetate-lead citrate staining, the ILC was slightly more electron dense than the root dentin, though the junction between the two tissues was not clear (Fig. 5a). At high magnifications, the ILC showed a reticular structure composed of a fine granular or filamentous material. The distribution of collagen fibrils in the layer was obscure in these sections. Many spherical bodies about $0.5 \mu\text{m}$ in diameter were observed in the layer closest to the root dentin surface. These bodies were represented by round, electron lucent areas containing an electron dense core (Fig. 6a).

Phosphotungstic acid preparations revealed that the ILC contained a few fine collagen fibrils, while the root dentin consisted of densely packed thick collagen fibrils.

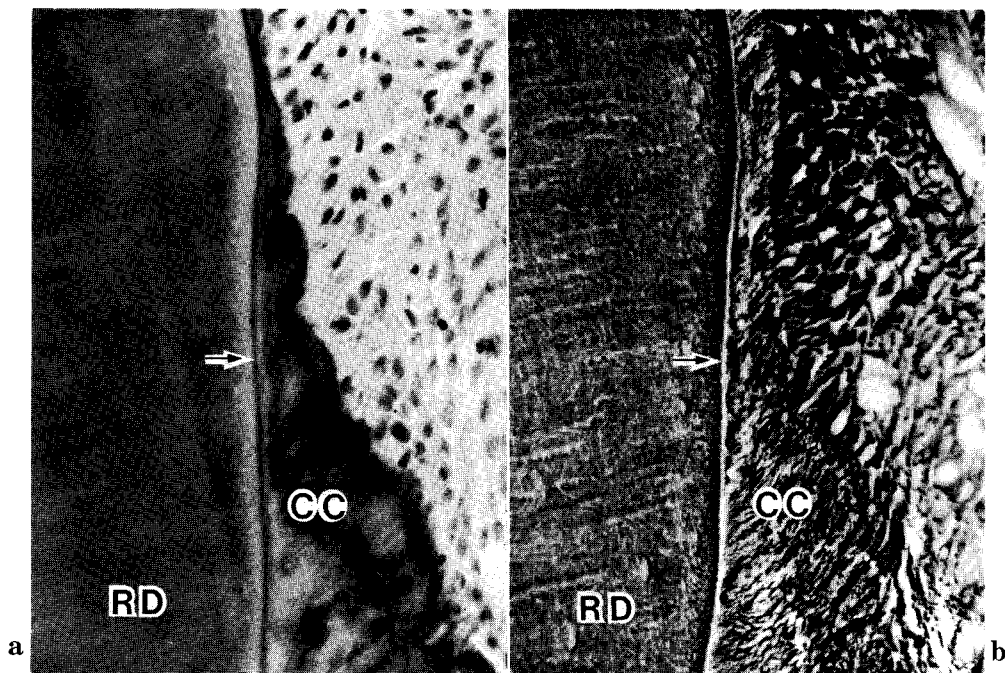


Fig. 2. Light micrographs showing the apical area in the adult rat teeth. The layer shown in Figure 1 is also seen as the innermost layer (arrows) of cellular cementum (*CC*). *RD* root dentin. **a:** Hematoxylin and eosin, **b:** silver impregnation. **a, b:** $\times 330$

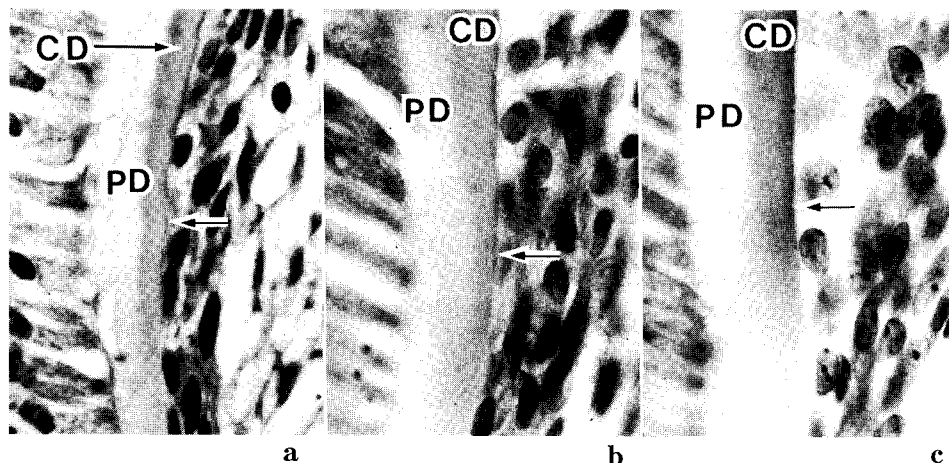


Fig. 3. Light micrographs showing the apical areas in 10 (**a**), 15 (**b**), and 20 (**c**)-day-old rat teeth. The ILC (arrows) begins to form on the root dentin surface, as the root dentin calcification advances. *CD* calcified dentin, *PD* predentin. Hematoxylin and eosin. $\times 830$

Thus the ILC could be distinguished from the root dentin (Fig. 5b). The fibrils in the ILC lacked regular striations and were dispersed randomly. Thick periodontal fibers showing regular striations and which terminated on the ILC surface under the light microscope, entered the ILC only a short distance, failing to reach the root dentin surface. The fibers loosened to finer elements at these ends (Fig. 7).

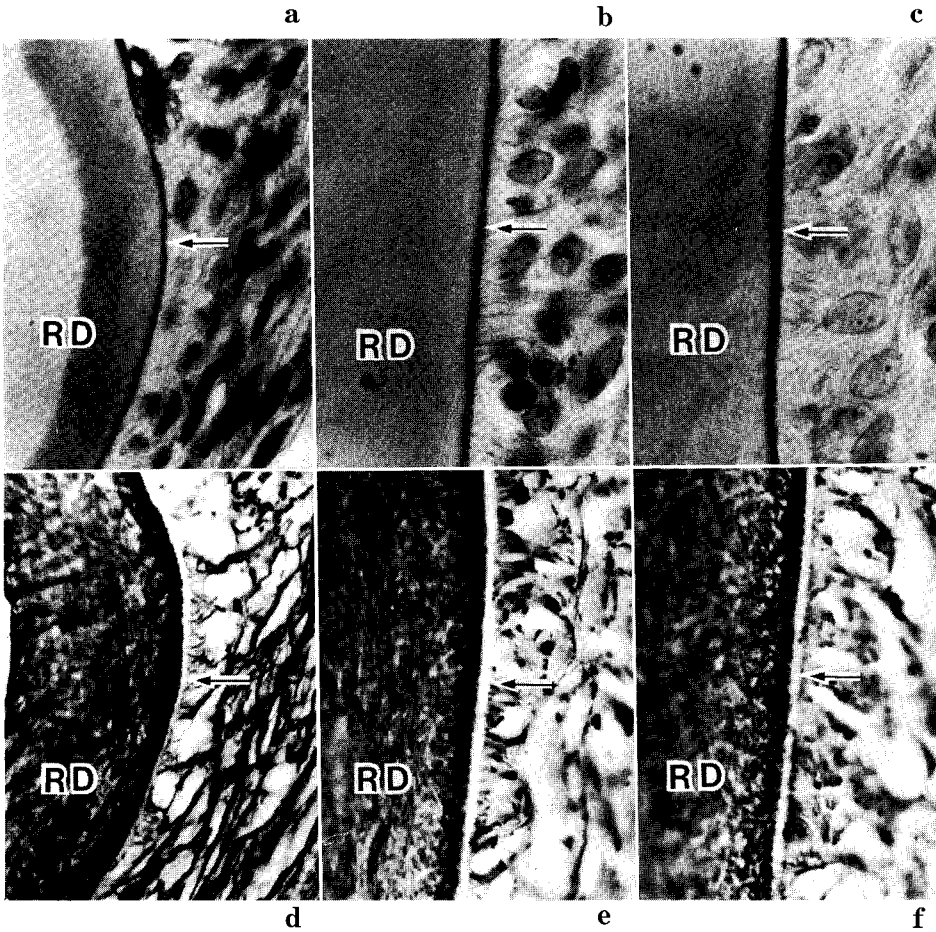


Fig. 4. Light micrographs showing the cervical areas in 10 (**a, d**), 15 (**b, e**), and 20 (**c, f**)-day-old rat teeth. The ILC (arrows) in the 10-day-old rat teeth does not still reach the full thickness shown in Figure 1. The ILC in the 15 and 20-day-old rat teeth approximately reaches it. *RD* root dentin. **a-c:** Hematoxylin and eosin, **d-f:** silver impregnation. a-f: $\times 830$

Ruthenium red and chromic phosphotungstic acid preparations made the ILC much more easily identifiable, clearly showing its border against the root dentin (Fig. 5c, d). Many spherical bodies were seen in the ILC closest to the root dentin surface (Fig. 6b, c). They showed a concentric circle composed of an outer electron dense zone, an intermediate electron lucent zone, and an electron dense core. These bodies seemed to be fundamentally identical with the spherical bodies observed in sections with uranyl acetate-lead citrate staining. The outer zone not stained with uranyl acetate-lead citrate may probably consist of a ruthenium red or chromic phosphotungstic acid positive material. Small electron dense masses were seen in the root dentin (Fig. 6b). They were similar to the core of the spherical bodies, but lacked the intermediate and outer zone.

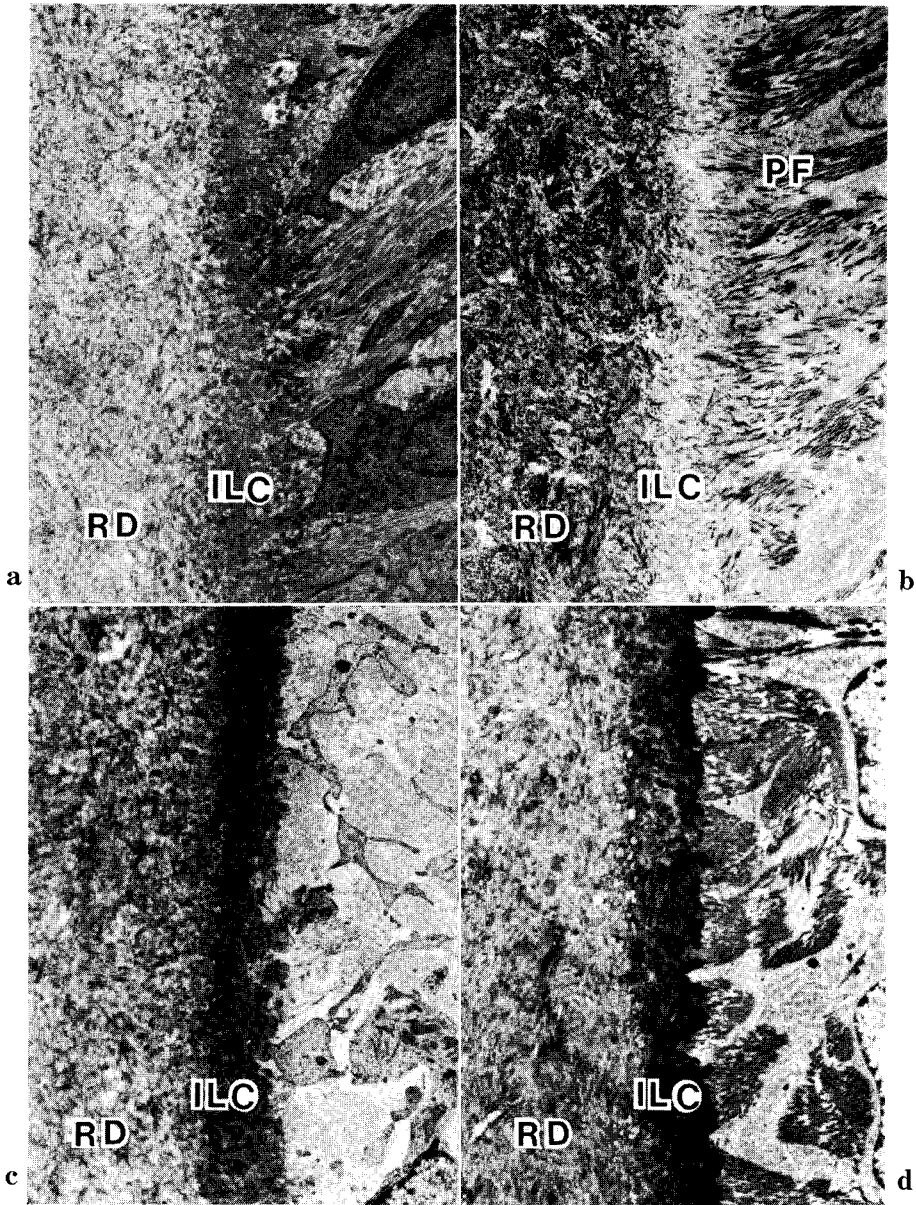


Fig. 5. Electron micrographs showing the cervical area in 20-day-old rat teeth. *RD* root dentin. Decalcified. $\times 4,000$. **a.** Uranyl acetate-lead citrate staining does not show the border between the ILC and the root dentin. **b.** The ILC contains a few collagen fibrils, while the root dentin is composed of densely packed collagen fibrils. Periodontal fibers (*PF*) tend to be arranged perpendicularly to the ILC. Phosphotungstic acid. **c** and **d.** The ILC is intensely stained with ruthenium red (**c**) and chromic phosphotungstic acid (**d**).

b. The development and calcification of the ILC

To avoid confusion in the following description, the root of the 20-day-old rat teeth was divided into three areas (Fig. 8).

Area 1 where no ILC formation is seen (Fig. 9a).

Area 2 where the ILC appears and rapidly increases in thickness (Fig. 9b).

Area 3 where the ILC reaches almost its full thickness (Fig. 9c).

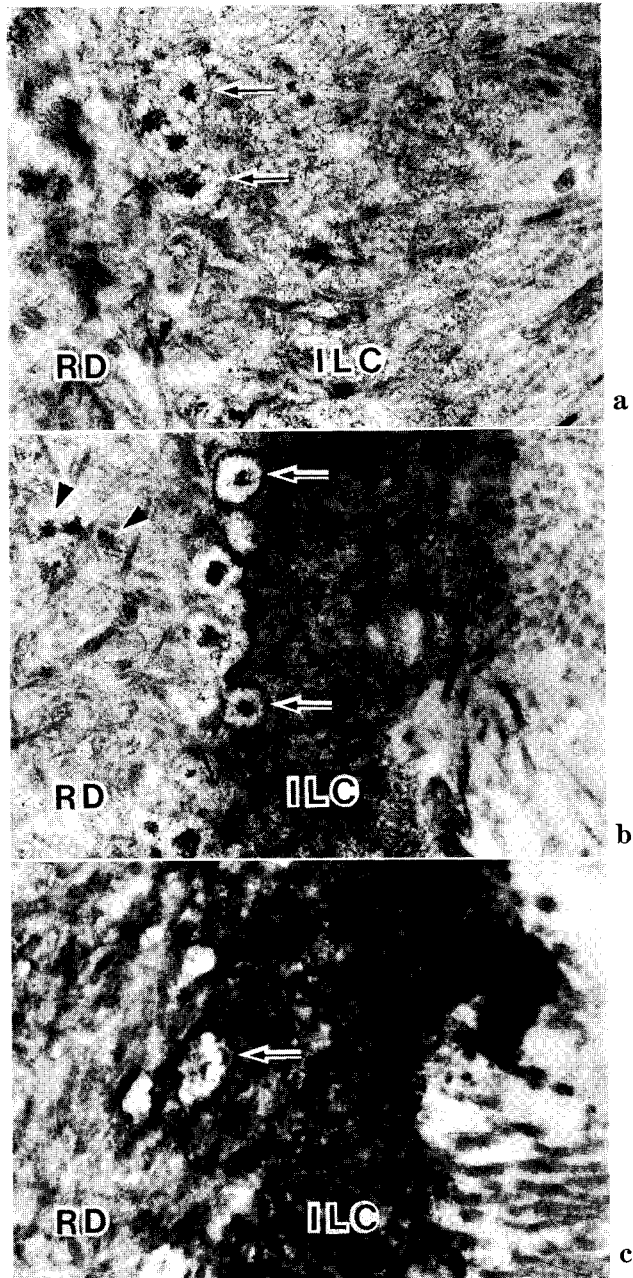


Fig. 6. High power electron micrographs showing the ILC and the root dentin (RD). Decalcified. $\times 19,000$. **a.** The ILC consists of a fine granular or filamentous material. Spherical bodies (arrows) are seen in the ILC closest to the root dentin surface. They are composed of electron lucent areas containing an electron dense core. Uranyl acetate-lead citrate. **b** and **c.** The outer electron dense zone (arrows) intensely reacts to ruthenium red (**b**) and chromic phosphotungstic acid (**c**). Small electron dense masses (**b** arrowheads) similar to the core of the spherical bodies are seen.

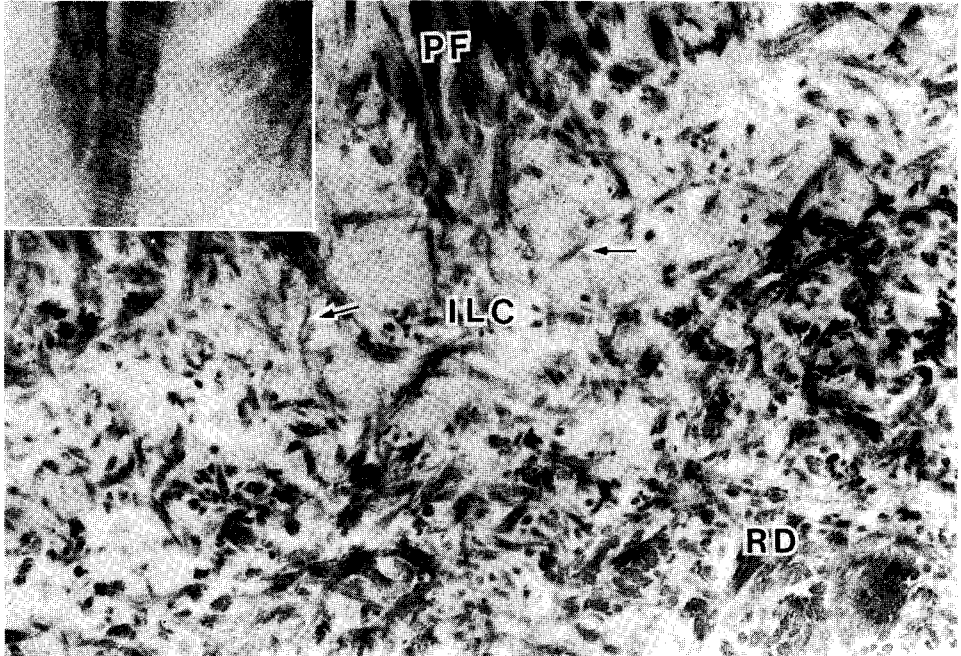


Fig. 7. Periodontal fibers (*PF*) enter the ILC only a short distance, not reaching the root dentin (*RD*) surface. There are a few thin collagen fibrils (arrows) in the ILC, distributed randomly in it. Phosphotungstic acid. $\times 19,000$. **Inset.** The ends of periodontal fibers loosen to finer elements. $\times 95,000$

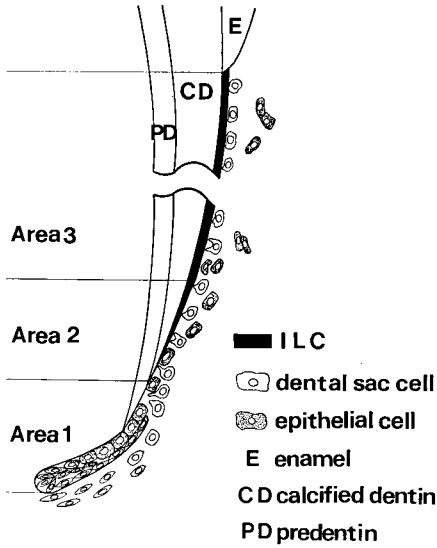


Fig. 8. A schematic diagram showing three divisions of the root.

Area 1: Near the end of Hertwig's epithelial root sheath, the sheath was composed of a few layers of squamous epithelial cells. In the midst of Area 1, the sheath became reduced in thickness and was composed of inner and outer enamel epithelial cells (Fig. 10, 11). These cells connected with one another forming desmosomes and gap junctions. A basal lamina surrounded the sheath on both sides, facing the dental sac and the dental papilla (Fig. 11). The outer enamel epithelial cells were flattened and contained a few intracellular organelles, i.e., mitochondria, Golgi apparatuses, rough endoplasmic reticulum. On the other hand, the inner enamel epithelial cells were cuboidal, somewhat larger, and contained more organelles (Fig. 10). These inner cells also contained more organelles than those at the end of the sheath. A few tonofilaments were seen in these cells (Fig. 11).

The cell surface facing the dental papilla was initially smooth, whereas near Area 2 it

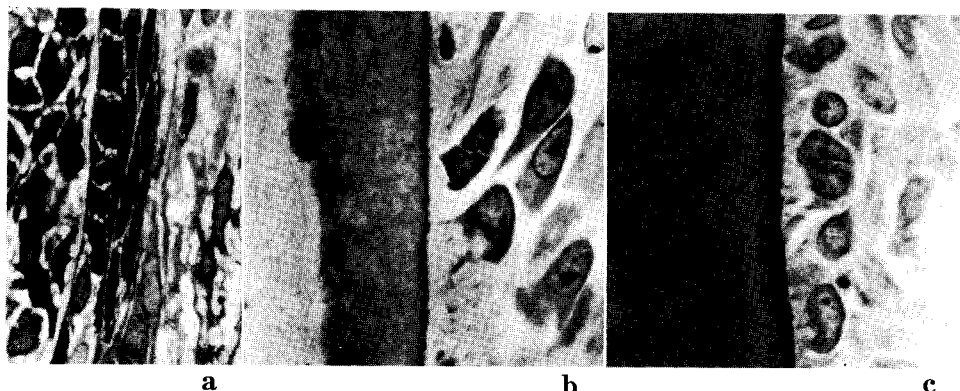


Fig. 9. Light micrographs showing each area (a: Area 1, b: Area 2, c: Area 3) in staining with toluidine blue. $\times 830$

showed an irregular appearance with coated pits and coated vesicles. At the same time, matrix vesicles appeared in the root dentin matrix (Fig. 12). Just before Area 2, small processes of the inner enamel epithelial cells perforated the basal lamina facing the dental papilla (Fig. 13).

Initially, the dental sac cells exhibited a flattened shape and were arranged parallel to the sheath. Collagen fibrils in the dental sac were also arranged parallel to the sheath (Fig. 14). Near Area 2, these cells gradually rounded and became larger. They contained more organelles than the epithelial cells. Just before Area 2, the epithelial sheath disintegrated. In the spaces caused by the disintegration of the sheath, the basal lamina disappeared on both sides. The dental sac cells extended projections to the root dentin surface through the spaces (Fig. 15).

Area 2: The ILC formation began just after the disintegration of the epithelial sheath. A layer of root dentin matrix was entirely calcified (Fig. 17). The inner enamel epithelial cells decreased in their amount of cytoplasm and organelles, while tonofilaments increased markedly, thus indicating a diminution in cell activity (Fig. 16). These cells were separated from one another due to the destruction of the junctional devices, drifting away from the root surface.

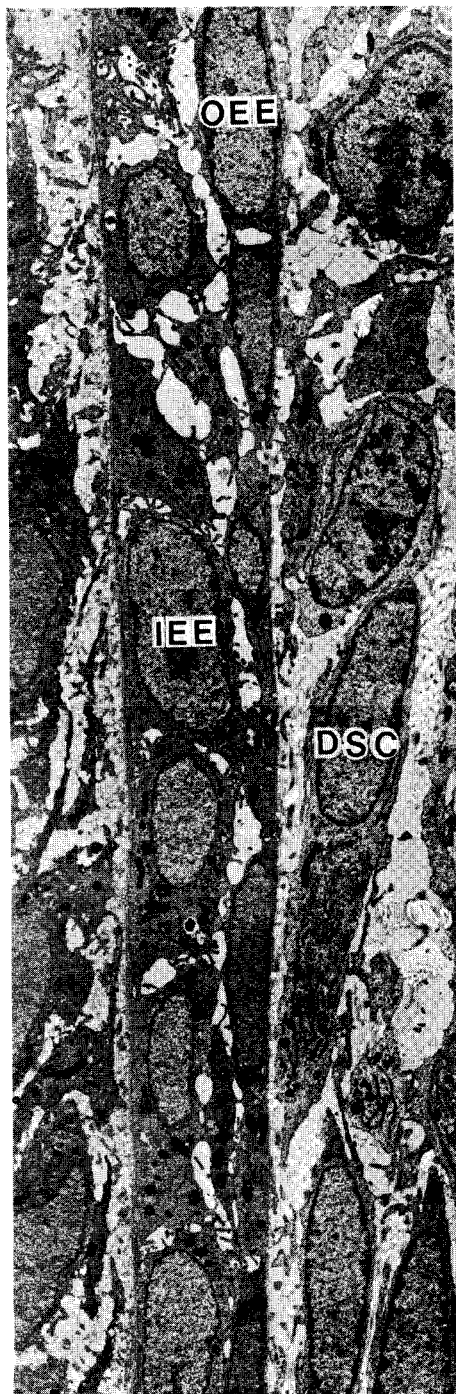
The dental sac cells were larger and their organelles more highly developed than those in Area 1. The rough endoplasmic reticulum was distended and the Golgi apparatus exhibited both its lamellar and vacuolar components (Fig. 18). Figure 19 suggests that the dental sac cells secrete a ruthenium red positive material from the tip of the projections. Calciferous spherules, matrix vesicles, and the spherical bodies were observed on the ILC surface. Some matrix vesicles contained crystal-like structures (Fig. 20, 21). On the other hand, matrix vesicles could be no longer seen in the root dentin. Approaching Area 3, the spherical bodies became embedded within the ILC.

Collagen fibrils were arranged perpendicularly to the ILC surface, attaching to it rapidly (Fig. 22).

Area 3: In this area, the ILC reached its full thickness and the epithelial cells drifted away from the root surface completely. These cells were present independently or in groups of a few cells and contained thick bundles of aggregated tonofilaments and some lysosomal granules. In contrast, the dental sac cells were arranged on the ILC surface (Fig. 23, 24). Well developed bundles of collagen fibrils passed between the dental sac cells.

As calcium crystals were deposited in relation to collagen fibrils, the ILC surface showed a serrated appearance (Fig. 25). Calciferous spherules and matrix vesicles could no longer be seen. All spherical bodies were embedded within the ILC and located closest to the root dentin surface (Fig.

6b, c).



DISCUSSION

1. The ultrastructure of the ILC

PAYNTER and PUDY (1958) first described the existence in the rat molar teeth of a thin specialized layer, about 1.5-3.0 μm thick, between the root dentin and cementum. According to their study, this layer was intensely stained with hematoxylin, not impregnated with silver, reacted positively to both PAS and alcian blue, and was markedly metachromatic. Designating this layer the "innermost layer of cementum" (ILC), they reported that this layer, being formed first, was a fiber-free amorphous tissue composed of mucopolysaccharides. They considered it to be the basement membrane of the epithelial root sheath. SHIBATA and STERN (1967) examined the ILC in rat incisors and presented findings similar to PAYNTER and PUDY's. By electron microscopy, on the other hand, SELVIG (1964) using mouse molars and STERN (1964) using rat incisors, observed the ILC as a layer composed of irregularly arranged fibrils showing a meshwork appearance. TEN CATE (1978) reported that the ILC was filled with a granular material and contained a few fine collagen fibrils in the mouse molars. Although these findings agreed in their revealing a specialized layer between the root dentin and cementum in the rat or mouse teeth, they were not necessarily in agreement with regard to the fine structure of the layer.

In the present study, the ILC was recog-

Fig. 10. Hertwig's epithelial root sheath is composed of inner and outer enamel epithelial cells (*IEE*, *OEE*). These cells contain less organelles than dental sac cells (*DSC*). The dental sac cells tend to become round as traced coronally (top). Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 3,800$

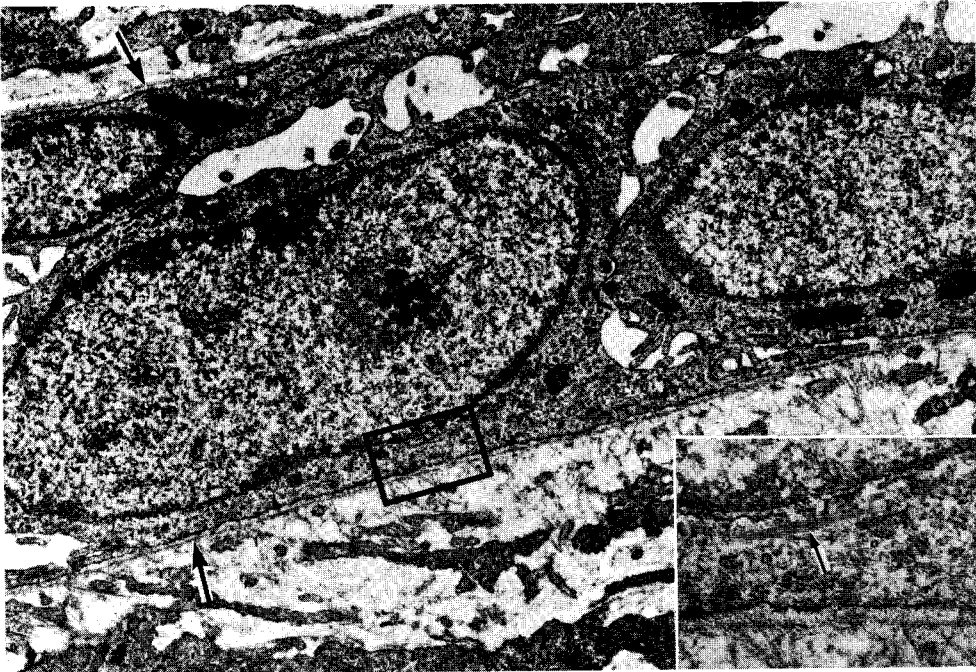


Fig. 11. A basal lamina (arrows) surrounds the sheath. Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 10,000$. **Inset.** High magnification of the box. An arrow shows tonofilaments. $\times 33,000$

nized as a thin layer, about $2 \mu\text{m}$ thick, showing an intense affinity to hematoxylin but no affinity to silver. Electron microscopically, the ILC consisted of a ruthenium red or chromic phosphotungstic acid positive material, i.e., proteoglycans (LUFT, 1971; TAKANO, 1979) and of a few fine collagen fibrils distributed at random. These fibrils did not assume the meshwork structure reported by SELVIG (1964) and STERN (1964). The present study thus confirms the findings described by TEN CATE (1978). The fibrils within the ILC did not connect with either periodontal fibers or fibrils in the root dentin; thus the author considers that they are the intrinsic fibrils of the ILC. Consequently, the ILC can not possibly be a tissue originating from the basement membrane as reported by PAYNTER and PUDY (1958), and SHIBATA and STERN (1967).

Periodontal fibers entered the ILC only a short distance, terminated in frayed ends, and did not reach the root dentin surface. In human teeth, periodontal fibers have been found to reach the root dentin surface (DEWEY, 1926; SELVIG, 1965; FURSETH, 1974). The author, therefore, concludes that the rat teeth possess the ILC which is not seen in human teeth and that it is a specialized tissue which is ultrastructurally different from both the root dentin and the cementum as it is generally called.

2. The development of the ILC

The ILC formation, or the deposition of a ruthenium red positive material on the root dentin surface, begins just after the disintegration of Hertwig's epithelial root sheath. Since the inner enamel epithelial cells decreased in their cytoplasm and number of organelles and increased in bundles of tonofilaments, these cells showed little cell activity. OWENS (1980) observed a granular material between the inner enamel epithelial

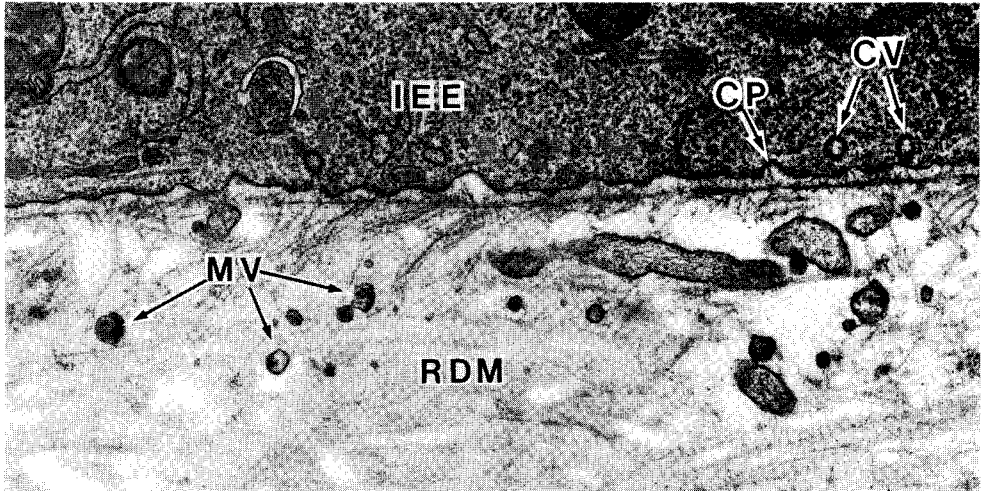


Fig. 12. Coated pits (*CP*) and coated vesicles (*CV*) are seen on the cell surface. Matrix vesicles (*MV*) appear in the root dentin matrix (*RDM*). *IEE* inner enamel epithelial cell. Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 24,000$

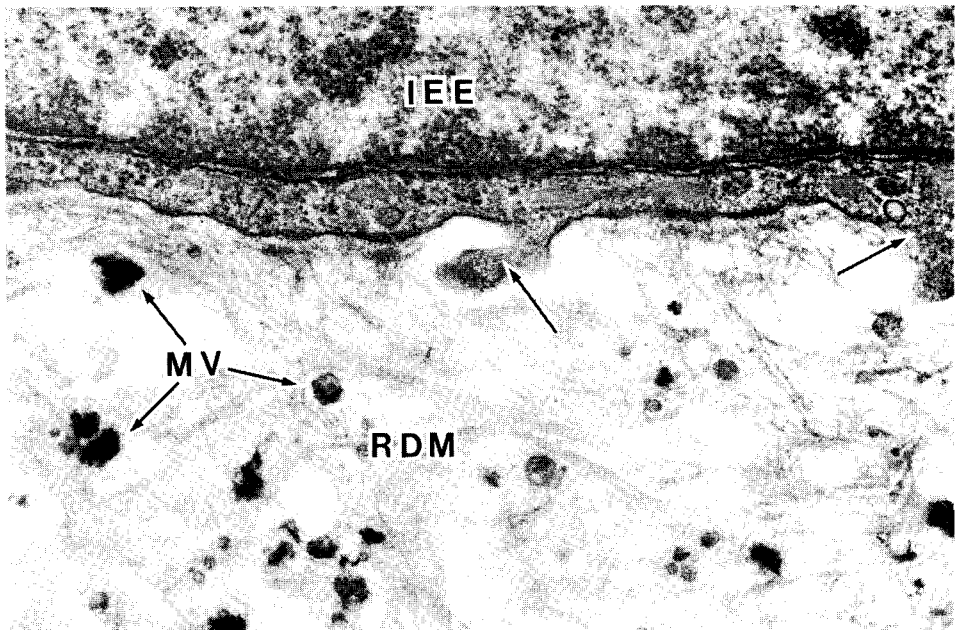


Fig. 13. Small processes (arrows) of an inner enamel epithelial cell (*IEE*) perforate a basal lamina on the dentinal side. Many matrix vesicles (*MV*) are seen in the root dentin matrix (*RDM*). Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 30,000$

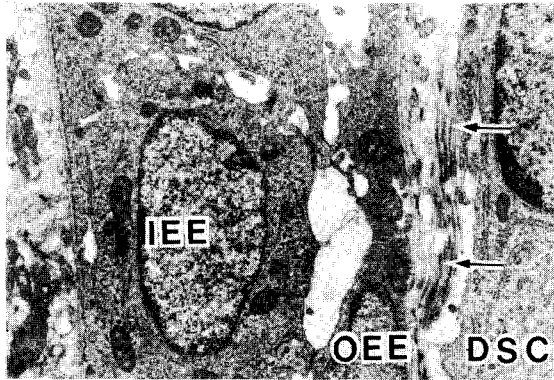


Fig. 14. Collagen fibrils (arrows) are arranged parallel to the epithelial sheath. *IEE* inner enamel epithelial cell, *OEE* outer enamel epithelial cell, *DSC* dental sac cell. Phosphotungstic acid. $\times 8,000$

cells and the calcified root dentin which resembled the enamel-precursor-material secreted by the ameloblasts (REITH, 1967). As the inner enamel epithelial cells contained more organelles than those cells at the end of the epithelial sheath, he suggested that these cells secreted the granular material to form the ILC. However, he failed to prove that the material actually formed the ILC. In the present study, although the inner enamel epithelial cells showed higher activity than the cells at the end of the sheath, these cells became inactive again in the area where the ILC formation began. Therefore, the author disagrees with the suggestion by OWENS (1980) that the inner enamel epithelial cells form the ILC. LINDSKOG (1982) noted that the inner enamel epithelial cells, like the ameloblasts, extended small processes and thus postulated that these cells had a secretory activity. He, however, could not show an actual image of the inner enamel epithelial cell secretion. The author too could not reveal the function of the small processes. However, because the inner enamel epithelial cells are unlikely in many respects to form the ILC, the author assumes that the processes have functions other than secretory. The significance of the coated pits and coated vesicles observed on the cell surface also remains to be elucidated.

The dental sac cells in Area 2 contained many organelles, so they are assumed to be a secretory cell type. These cells extended projections to the root surface and appeared to secrete the ruthenium red positive material from their tips. Matrix vesicles were also seen on the root surface. Considering the rapid ILC formation and the degeneration of the epithelial cells in Area 2, the author would like to propose that the dental sac cells form the ILC. The dental sac cells secreting the ruthenium red positive material can be regarded as cementoblasts. This is to say that, although the ILC is a specialized tissue which has different structures and functions from the cementum, it is nothing but a part of the cementum. This supports the concept that the initial layer of cementum forms immediately after the disintegration of Hertwig's epithelial sheath (HOFFMAN and SCHOUR, 1940; SELVIG, 1964; FORMICOLLA et al., 1971; KAWASAKI et al., 1976). In relation to the manner of attachment of periodontal fibers, the present study confirms previous findings (TROT, 1962; SELVIG, 1963; LESTER, 1969). Collagen fibrils were at first arranged parallel to Hertwig's epithelial root sheath, and immediately after the disintegration of the sheath, the fibrils became arranged at right angles to the ILC surface where they rapidly became attached to it.

3. The calcification of the ILC

When staining the femora of osteolytic rats with ruthenium red, MARTINO et al. (1979) demonstrated doughnut-like structures, 0.5–1.5 μm in diameter and composed of

three zones. They called these structures "calcification nodules" and suggested that the nodules derived from matrix vesicles. They further proposed that the peripheral dark zone of the three zones was the accumulation of protein polysaccharides and phospholipids, the intermediate light zone was one of non-collagenous material, and the central dark zone was the organic remnants of ruptured matrix vesicles. They proposed that the nodules were essential for the rapid formation of woven bone. Since the

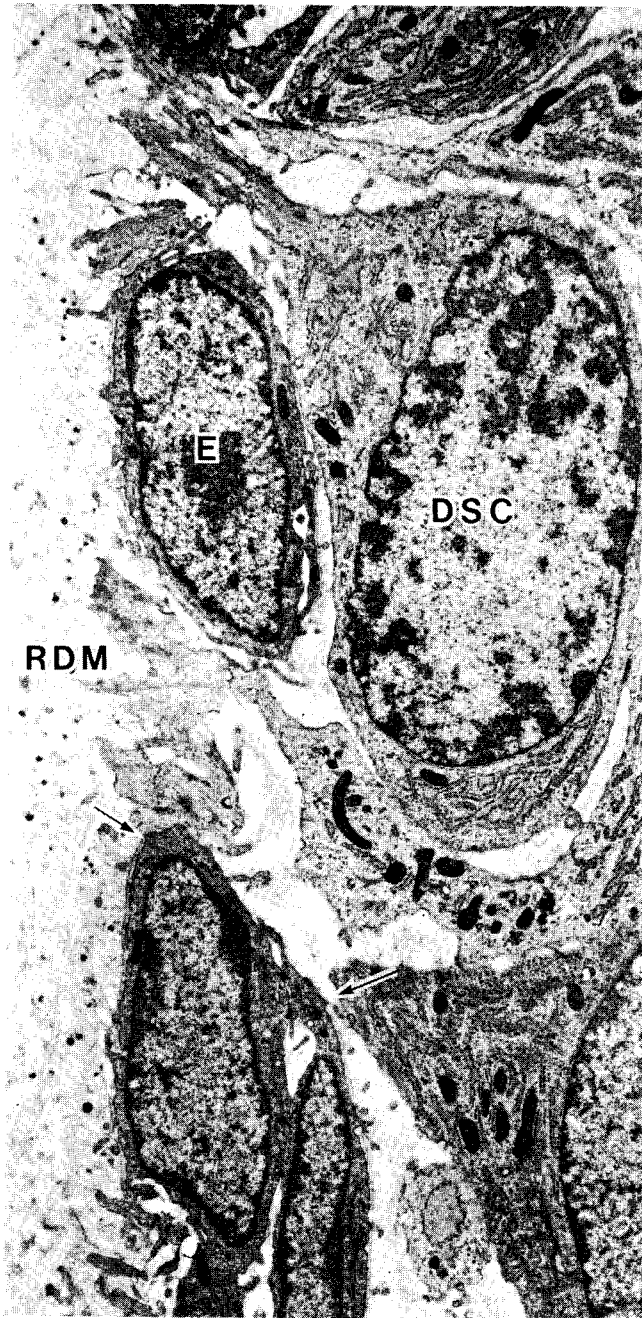


Fig. 15. Dental sac cells (*DSC*) extend projections to the root dentin matrix (*RDM*). A basal lamina (arrows) disappears in the discontinuous region of the epithelial sheath. The dental sac cells are larger and contain more highly developed organelles than the epithelial cells (*E*). Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 6,500$

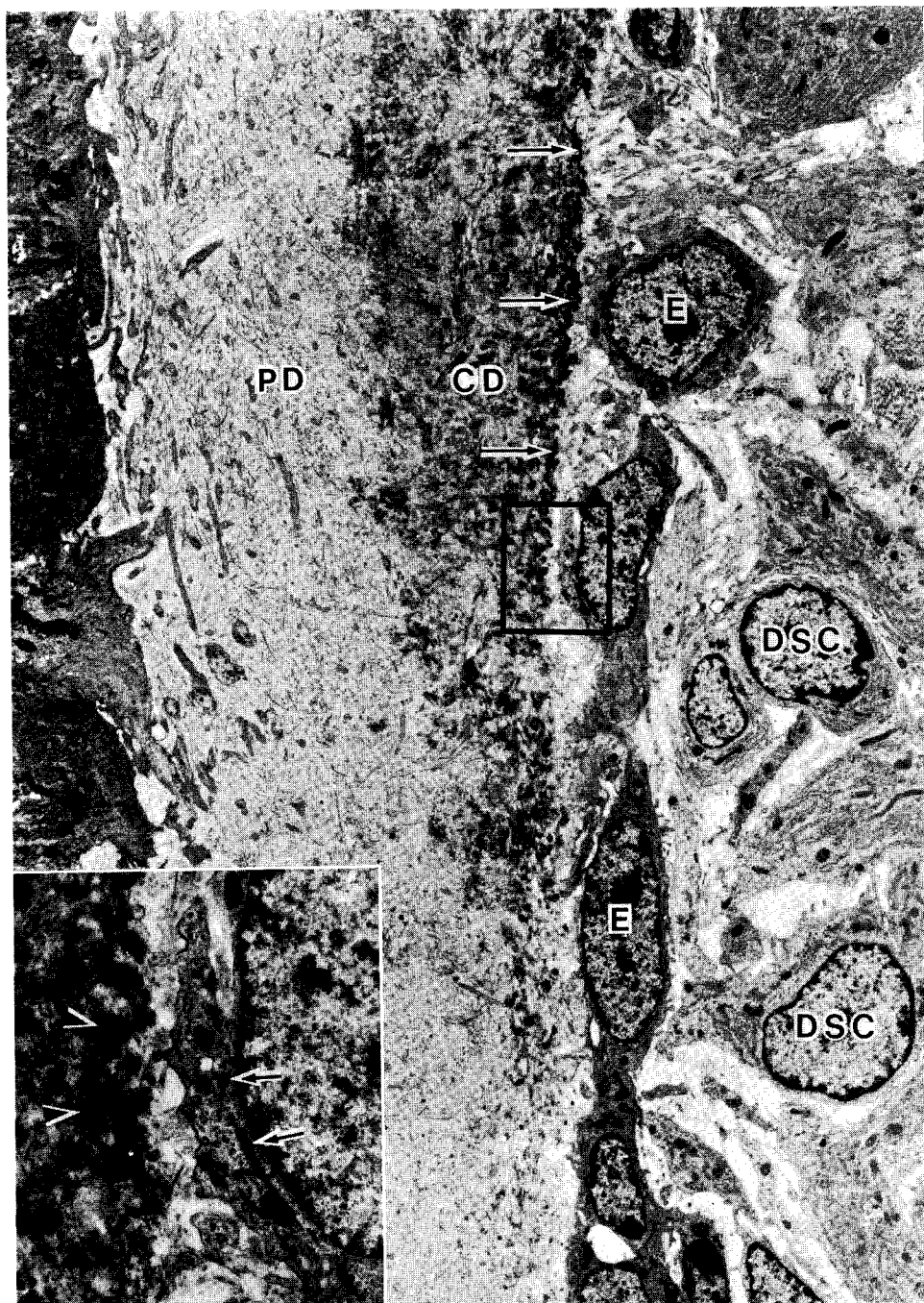


Fig. 16. An electron micrograph showing the starting portion of Area 2. A ruthenium red positive material (arrows) appears on the root dentin surface. Dental sac cells (*DSC*) contain more highly developed organelles than epithelial cells (*E*). The dark region shows calcified dentin (*CD*) and the light region shows predentin (*PD*). Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 3,500$. **Inset.** High magnification of the box. The ruthenium red positive material (arrow heads) is seen. The epithelial cell contains some bundles of tonofilaments (arrows). $\times 11,000$

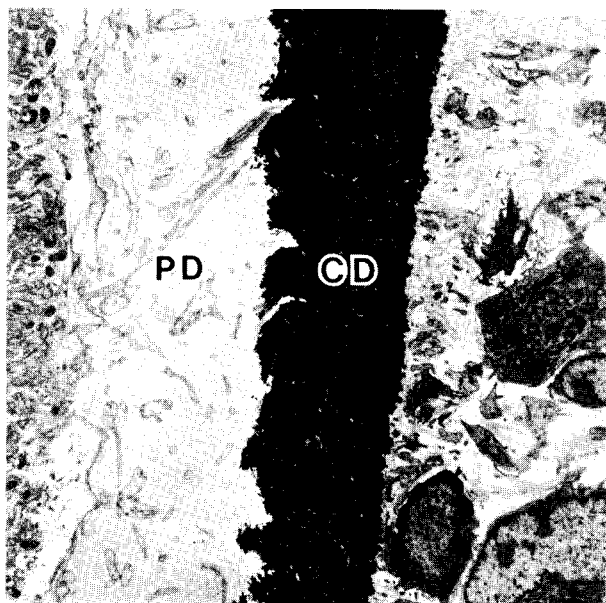


Fig. 17. An electron micrograph showing Area 2 in an undecalcified section. A layer of the root dentin matrix is entirely calcified. *CD* calcified dentin, *PD* predentin. Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 2,300$

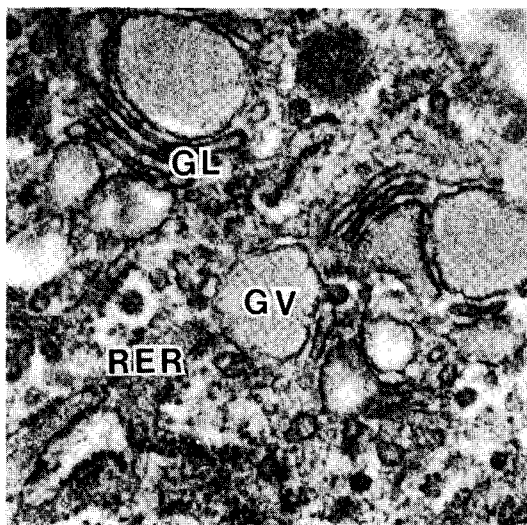


Fig. 18. A high power electron micrograph showing the organelles of a dental sac cell. Rough endoplasmic reticulum (*RER*) and Golgi apparatus are well developed. *GV* Golgi vacuole, *GL* Golgi lamella. Ruthenium red and uranyl acetate-lead citrate. $\times 15,000$

spherical bodies in the present study closely resemble the calcification nodules in size, shape, and stain affinity, the author believes that the bodies correspond to the nodules and derive from the matrix vesicles. This is supported by the existence of matrix vesicles on the surface of the developing ILC. The ground substance of the ILC is the proteoglycans, which were reported to be a calcification-inhibiting factor (DI SALVO and SCHUBERT, 1967; HOWELL et al., 1969). The collagen fibrils, which are known to be closely associated with the successive calcification, are rare in the ILC. Therefore, successive calcification from the root dentin to the ILC can not possibly occur. It is well known that the matrix vesicles are the initial calcification sites (OZAWA, 1985). Thus, to remove the inhibitory action of the proteoglycans (FUJIWARA, 1982) and to induce the calcification of the ILC anew, matrix vesicles would be necessary in ILC calcification. More-

over, the rapid ILC calcification associated with the spherical bodies may contribute to the rapid and firm attachment of periodontal fibers to the ILC surface. Whether the cementum calcification is associated with matrix vesicles (LISTGARTEN, 1974; HAYASHI, 1985) or not (BERNARD and MARVASO, 1981; TEN CATE, 1985) remains controversial. The

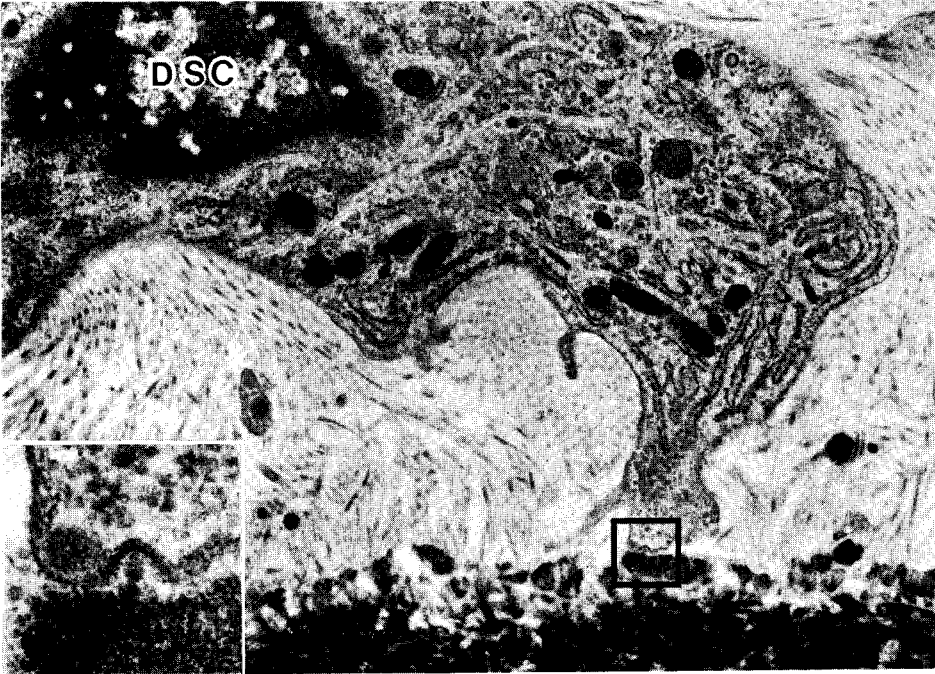


Fig. 19. A dental sac cell (*DSC*) extends a projection to the surface of the developing ILC. Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 11,000$. **Inset.** High magnification of the box. A ruthenium red positive material appears to be secreted from the tip of the projection. $\times 68,000$

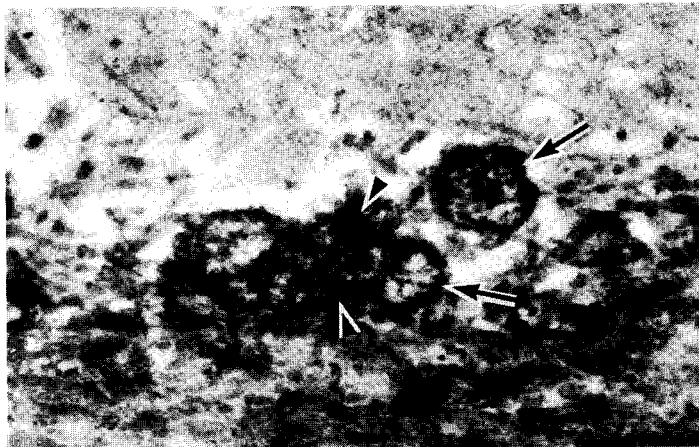


Fig. 20. Spherical bodies (arrows) and a chromic phosphotungstic acid positive material (arrow heads) are seen on the root dentin surface. Chromic phosphotungstic acid. $\times 28,000$

author is of the opinion that the cementum calcification in the rat molar teeth is initiated by matrix vesicles.

Figure 21 shows the occurrence of matrix vesicles on the surface of the developing ILC. In accordance with the findings by BERNARD (1972) and SUZUKI (1985), the author observed that when a layer of the root dentin matrix was entirely calcified, matrix vesicles could no longer be seen in it. Therefore, if the matrix vesicles on the surface of the developing ILC do derive from the dental papilla side, it is hard to explain why only these matrix vesicles remain while other ones disappear. Thus, it is reasonable to consider that the matrix vesicles on the ILC surface are derived slightly later from

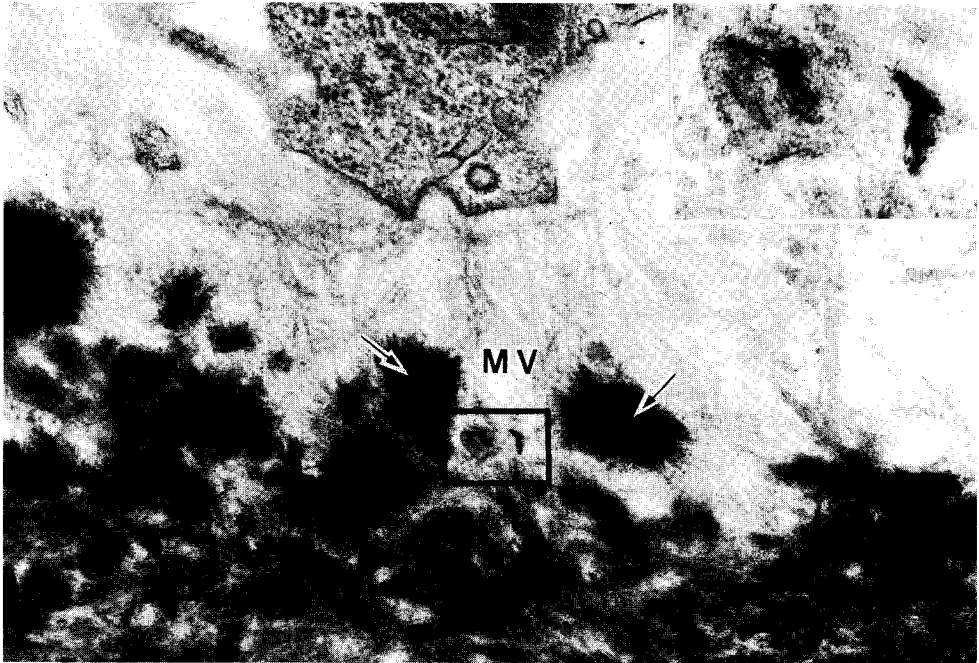


Fig. 21. Calciferous spherules (arrows) and matrix vesicles (*MV*) are seen on the ILC surface. Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 36,000$. Inset. High magnification of the box. The matrix vesicles contain crystal-like structures. $\times 143,000$

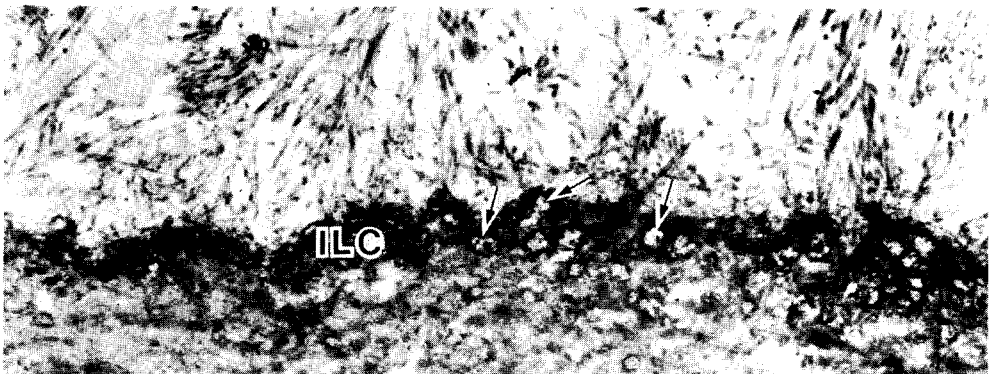


Fig. 22. Spherical bodies (arrows) become embedded in the ILC. Collagen fibrils begin to attach to the ILC surface. Chromic phosphotungstic acid. $\times 11,000$

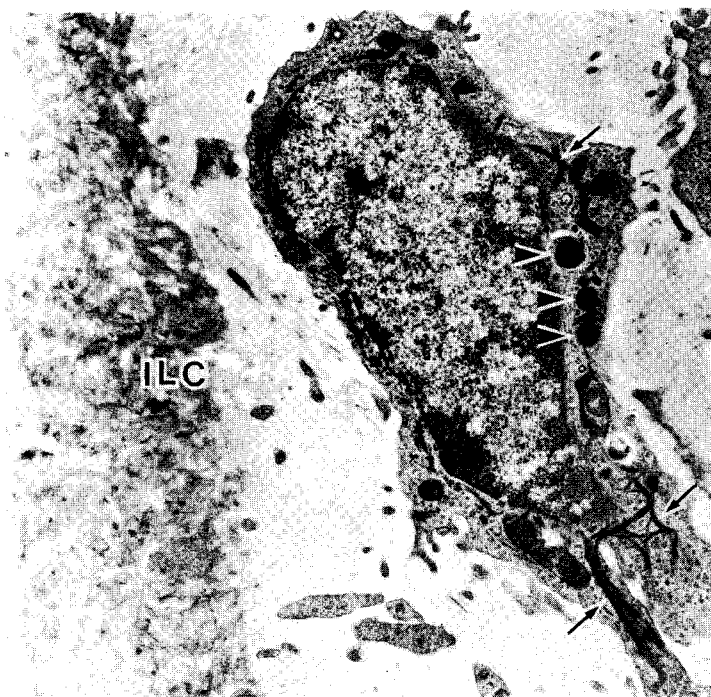


Fig. 23. An epithelial cell contains thick bundles of tonofilaments (arrows) and lysosomal granules (arrow heads). Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 9,000$

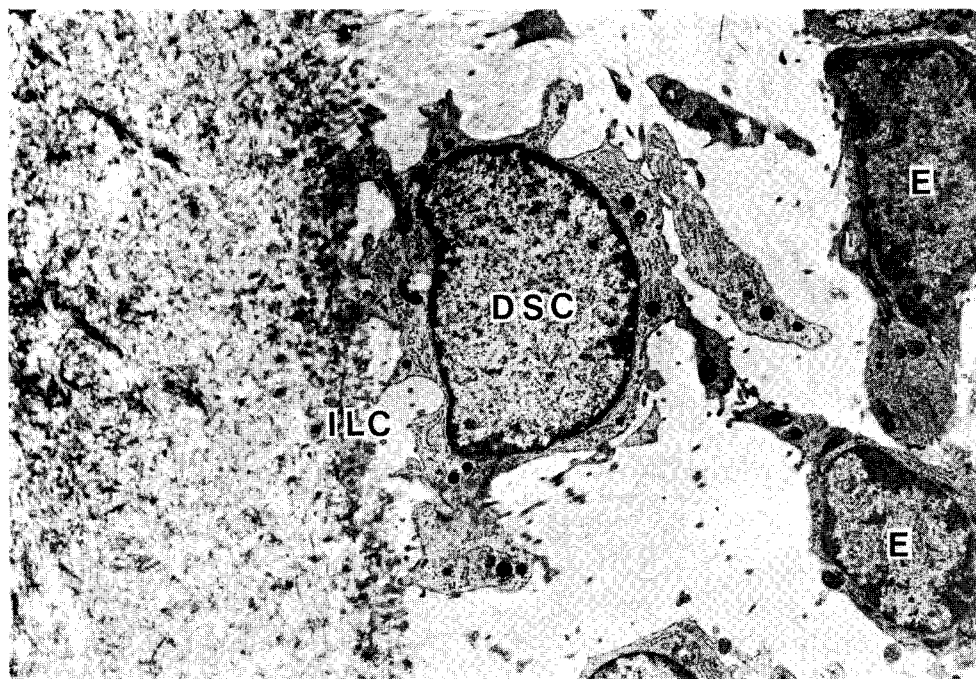


Fig. 24. A dental sac cell (*DSC*) is arranged on the ILC surface. *E* epithelial cell. Decalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 4,900$

the dental sac side than the matrix vesicles observed in the root dentin matrix. That is, the spherical bodies are associated with the matrix vesicles from the dental sac side. On the other hand, those matrix vesicles which derive from the dental papilla side are associated with the root dentin calcification and result in the electron dense masses.

The chondrocytes, osteoblasts, and odontoblasts, which can produce matrix vesicles, are the mesenchymal cells. SUZUKI (1985) suggested that the epithelial sheath cells could not produce matrix vesicles. Therefore, the matrix vesicles involved in the ILC calcification may arise from the cementoblasts. After the ILC calcification, the subsequent calcification in the cementum in the strict sense proceeds, as reported by SELVIG (1964) and FURSETH (1970), in close association with collagen fibrils.

4. The function of the ILC

KURIHARA and ENLOW (1980a, b), and KURIHARA et al. (1983) studied the reattachment between periodontal fibers and physiologically or artificially resorbed alveolar bone in the rat molar region. According to their studies, in the reattachment process of periodontal fibers onto the resorptive bone surfaces, a thin ruthenium red reactive layer containing few collagen fibrils formed first on the naked bone and then periodontal fibers with frayed ends were attached to this layer. They considered that the thin layer was a specialized structure for joining the periodontal fibers on the resorptive bone surfaces. The thin layer closely resembles the ILC.

In the present study, a thin layer which was very similar to the ILC was observed on the resorbed surfaces of the root dentin and cellular cementum. Periodontal fibers reattached to the layer and acellular cementum reformed successively on its surface (Fig. 26). These findings suggest that the ILC triggers the attachment and fixation of periodontal fibers on the root surface. As the ILC calcification and the cementum formation proceed, the attachment and fixation are reinforced (Fig. 27). At the same time, the ILC serves to join the root dentin and the cementum as cement mortars two bricks. This must be the most important function of the ILC.

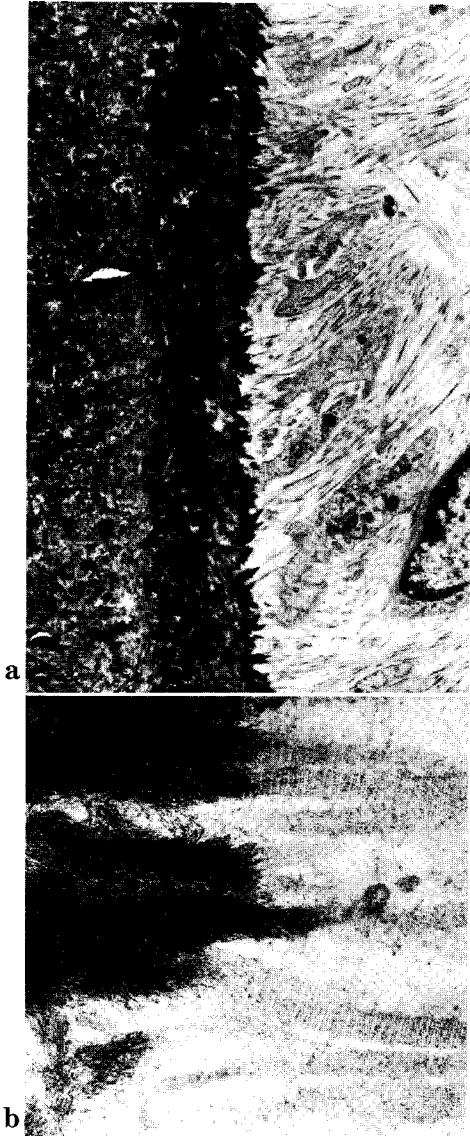


Fig. 25. **a.** The ILC surface shows a serrated appearance. $\times 4,600$. **b.** Calcium crystals are deposited in relation to collagen fibrils. **a** and **b.** Undecalcified. Ruthenium red and uranyl acetate-lead citrate. $\times 59,000$

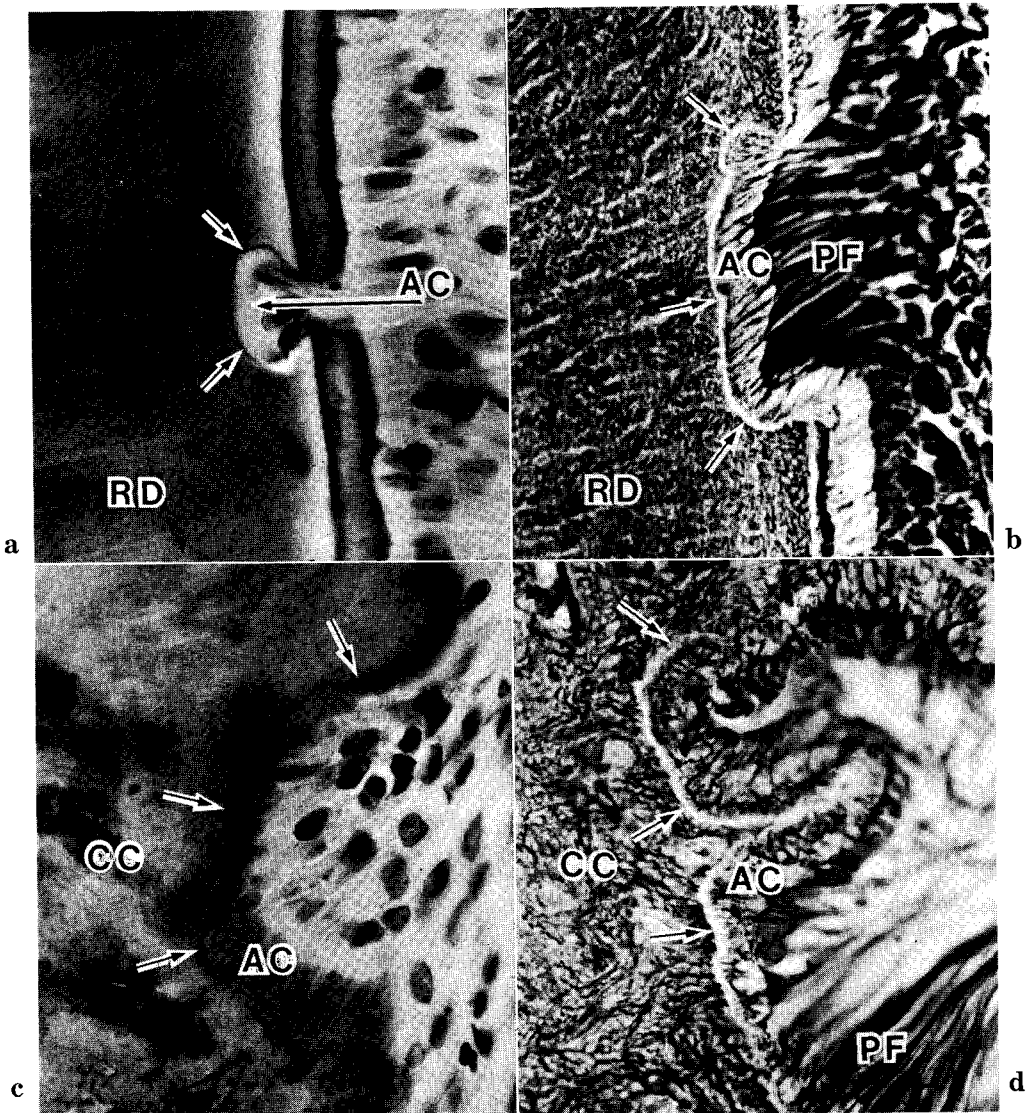


Fig. 26. Light micrographs showing resorptive regions of the root dentin (a, b) and cellular cementum (c, d). A thin layer (arrows), which shows the same stain affinity as the ILC, is seen on the resorptive surfaces. Periodontal fibers (*PF*) reattach to this layer and acellular cementum forms anew on it. *CC* cellular cementum, *AC* acellular cementum, *RD* root dentin. **a** and **c**: Hematoxylin and eosin, **b** and **d**: silver impregnation. a-d: $\times 670$

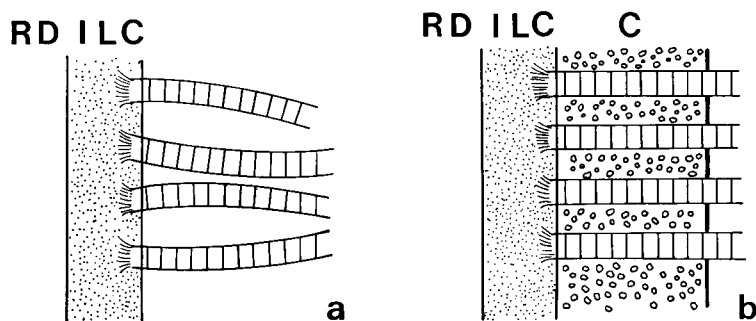


Fig. 27. Schematic diagrams showing the manner of attachment of periodontal fibers to the ILC. **a.** Only the ends are embedded in the ILC, immediately after the completion of the ILC formation. **b.** As the popularly called "cementum" (*C*) forms, the attachment and fixation of the fibers are reinforced. *RD* root dentin.

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