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# Radioautographic Study of [$^3$H]Mannose Utilization During Cementoblast Differentiation, Formation of Acellular Cementum, and Development of Periodontal Ligament Principal Fibers

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*ABSTRACT* The formation of acellular cementum and the deposition of [$^3$H]mannose-labeled extracellular matrix were studied in 14-day-old Sprague-Dawley rats. The sequential events of cementogenesis and periodontal ligament formation observed by light and electron microscopy were described from the stage of an intact root sheath to postcementogenesis. Ultrastructural examination of cementoblasts and periodontal ligament fibroblasts revealed [$^3$H]mannose labeling of the Golgi apparatus at 10 minutes, collagen secretion granules at 30 minutes, and the extracellular matrix beginning at 30 minutes. The extracellular matrix between cementoblasts and dentin was heavily labeled at 1 and 4 hours. Newly formed principal fibers of the periodontal ligament were also heavily labeled at 4 hours. Fully differentiated cementoblasts exhibited the largest sectional profiles and the highest number of silver grains per unit area of cytoplasm. The morphologic and radioautographic data suggest that during the formation of acellular cementum, the cementoblast phenotype is expressed for a short period of time, after which cementoblasts appear to mix with the fibroblasts of the periodontal ligament.

In a recent monograph on the periodontium, it was pointed out that additional studies of cementogenesis are needed (Schroeder, 1986). In discussing acellular cementum, Schroeder noted that, ". . . its initial formation and further developmental stages on the external surface of a developing and fully formed root have never been followed directly and in a dynamic fashion (i.e., based on markers for cellular identification, proliferation, and metabolism, as well as on labeling of the accumulation of products)." It is our hope that the present report, as well as a related paper (Cho and Garant, 1988) will help to explain the genesis of acellular cementum and stimulate new interest in this area.

We recently observed that cementoblast differentiation involves directed cell migration of precementoblasts toward the dentin matrix, causing disruption of the epithelial root sheath and the eventual contact of newly differentiated cementoblasts to the dentin surface of the root (Cho and Garant, 1988). Migration of precementoblasts from the dental follicle is probably initiated by a chemoattractant and/or promoted by an adhesion gradient within the local extracellular matrix. We believe that the concept of directed cell migration during cementogenesis provides a fresh approach to understand the events that take place during the development of cementum and the associated periodontal ligament. Armed with this new information, we conducted a light and electron microscopic radioautographic study of the uptake and secretion of [$^3$H]mannose-labeled substances during the early root formation. Our objective was to examine the synthetic activity of newly developed cementoblasts and to observe the deposition of an acellular layer of cementum on the dentin surface. Our results document the secretory activity of newly differentiated cementoblasts, and, much to our surprise, reveal that soon after the onset of cementum deposition newly differentiated cementoblasts detach from the root surface and appear to join the fibroblastic population of the periodontal ligament. Pulse labeling of the extracellular matrix (ECM) with [$^3$H]mannose coupled with the lapse of various periods of time after intravenous injection prior to killing the animals provided a means of approximating the duration of various differentiation steps. The results of our study indicate that the formation of acellular cementum involves an initial phase of directed cell migration, followed by a short period of attachment and cementum matrix deposition during which the cementoblast morphotype is clearly expressed, culminating in detachment concurrent with assumption of a fibroblast-like morphotype.

## MATERIALS AND METHODS

### *Tissue Preparation*

Eight 14-day-old male Sprague-Dawley rats weighing 25.5 ± 0.5 gm were anesthetized with ether and injected via the jugular vein with 2.5 Mci of D-[2,6-$^3$H]mannose (S.A. = 45.9 Ci/mmole, Amersham Corporation, Arlington Heights, IL) in 0.1 ml of sterile saline solution. At 5,

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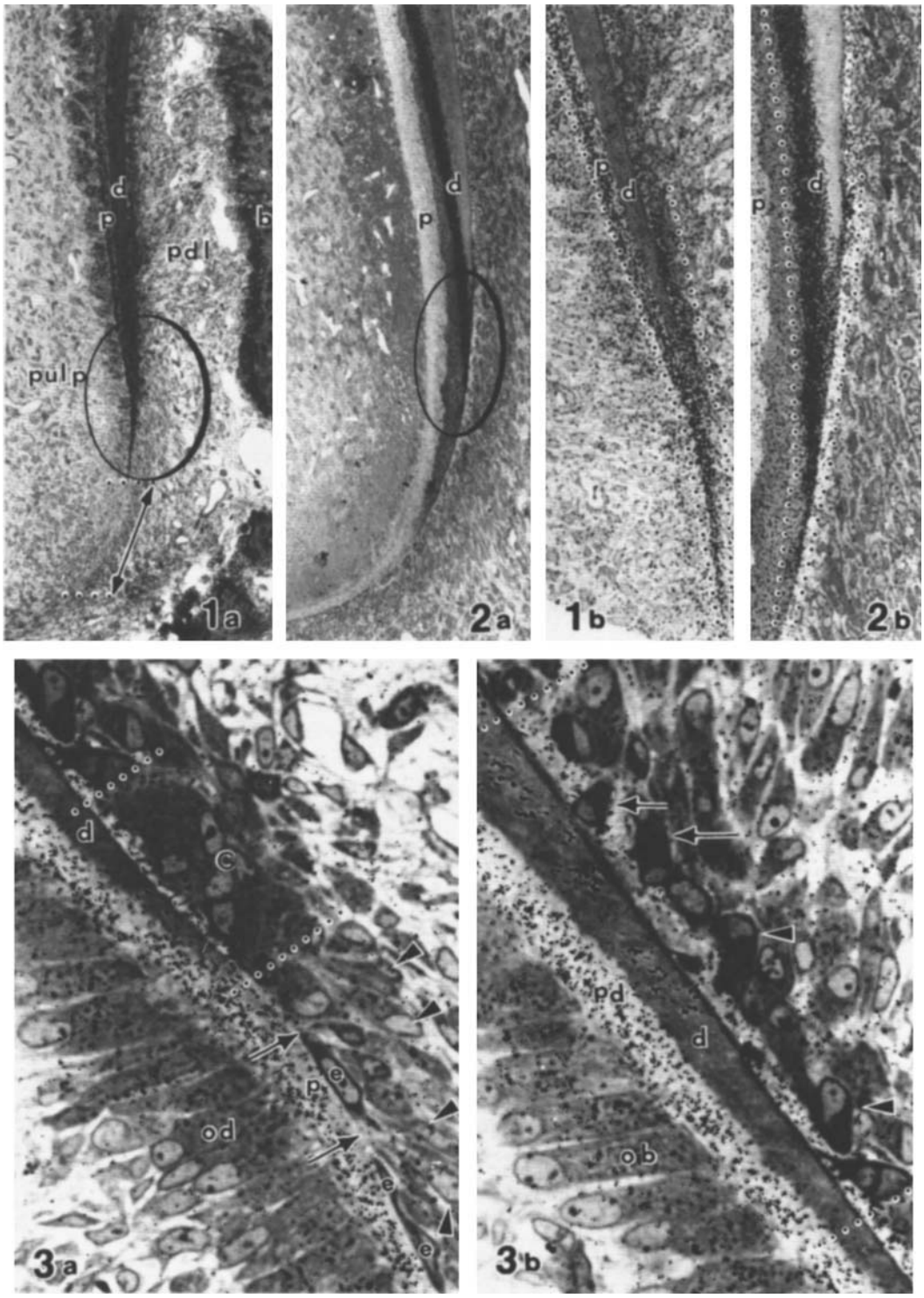



Fig. 1. Light microscopic radioautographs demonstrating the general labeling pattern with [$^3$H]mannose over a developing root at 30 minutes after intravenous injection of [$^3$H]mannose. **a:** Note "Y"-shaped pattern of heavy labeling along the predentin (p) and dentin (d) surface facing the periodontal ligament (pdl) at the cervical portion of the developing root. Hertwig's root sheath, whose position is shown by double arrow, is only labeled slightly. b, Bone. ×240. **b:** Higher magnification of the circular area in a depicting the "Y"-shaped labeling pattern (dotted line) over both the predentin (p) and dentin (d) surface facing the periodontal ligament. ×360.

Fig. 2. Light microscopic radioautographs demonstrating the general labeling pattern with [$^3$H]mannose over a developing root at 2 days after intravenous injection of [$^3$H]mannose. **a:** Note the "Y"-shaped heavy labeling over dentin (d) and on the dentin surface facing the periodontal ligament in the middle portion of the developing root. p, predintin. ×240. **b:** Higher magnification of the circular area in a. Note the "Y"-shaped labeling (dotted line) over the deep portion of dentin (d) and the dentin surface facing the periodontal ligament, which is occupied by well-polarized periodontal ligament fibroblasts. ×360.

**TABLE 1. Quantitative analysis of light microscopic radioautographic silver grains over cells at 10 minutes after intravenous injection of [$^3$H]-mannose and cell sizes during development of periodontal ligament of the rat[1]**

| Zone | Cell area ($\mu m^2$) | No. of grains per cell | No. of grains per 10 $\mu m^2$ |
|---|---|---|---|
| I | | | |
| Cells of DFP | 280 ± 44 | 4.8 ± 2.8 | 1.7 |
| Cells of PFA | 301 ± 41 | 2.8 ± 1.6 | 0.9 |
| II | | | |
| Precementoblasts | 489 ± 75 | 13.2 ± 4.6 | 2.7 |
| Cells of PFA | 476 ± 51 | 7.7 ± 2.5 | 1.6 |
| III | | | |
| Cementoblasts | 1198 ± 53 | 41.8 ± 7.1 | 3.5 |
| Cells of PFM | 903 ± 52 | 20.9 ± 5.1 | 2.3 |
| IV and V | | | |
| PDL fibroblasts | 987 ± 112 | 25.4 ± 4.3 | 2.6 |

[1]Quantitative analysis of silver grains was based on 3-day exposed radioautographs.

10, 20, and 30 minutes, 1 and 4 hours, and 2 days after the administration of [$^3$H]mannose, one animal for each time period (except two animals for the 10-minute period) was sacrificed by intracardiac perfusion with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The maxillae were dissected free of surrounding tissues and further fixed in Karnovsky's fixative (Karnovsky, 1965) for an additional 3 to 4 hours. After rinsing in 0.1 M sodium cacodylate buffer for 10 minutes, the maxillae were decalcified for 2 weeks at 4°C in 0.1 M ethylene diaminetetraacetic acid (EDTA) containing 3% glutaraldehyde. The first molars were then mediodistally sectioned into slices of 1-mm thickness and postfixed for 1½ hours in 1% $OsO_4$ in s-collidine buffer, pH 7.4. Tissues were then dehydrated in a graded series of cold ethanols and propylene oxide prior to infiltration with Poly Bed mixture at room temperature for 4 hours and placed in flat embedding molds containing fresh embedding mixture. Polymerization was accomplished at 60°C for 48 hours.

### *Light Microscopic Radioautography*

Three to four 1-$\mu$m-thick sections from each block were mounted on glass slides, coated with Kodak NTB-2 liquid emulsion, exposed at 4°C, and developed as previously described (Cho and Garant, 1981b). The radioautographs were subsequently stained with 1% toluidine blue in 0.1 M sodium acetate buffer.

Fig. 3. Light microscopic radioautographs showing cementoblasts at different stages of differentiation on the dentin surface of the same root and the pattern of silver grain deposition at 30 minutes after intravenous injection of [$^3$H]mannose. **a:** Cervical portion of developing root. Note precementoblasts (arrowheads) oriented toward the dentin surface between the inner epithelial cells (e), which have become discontinuous (arrows). Numerous silver grains are located over predentin (pd), and within the odontoblasts (ob). Also note zone of fully differentiated cementoblasts (C, bordered by dotted lines) coronal to the precementoblasts. d, dentin. ×1,000. **b:** Portion of the same developing root just coronal to a. Cementoblasts (bordered by dotted lines) near the dentin (d) surface have an elongate shape and densely stained cytoplasm, and are now oriented toward either the dentin (arrowheads) or the periodontal ligament (arrows). × 1,000.

### *Electron Microscopic Radioautography*

To prepare electron microscopic radioautographs, the loop method of Caro and Van Tubergen (1962) was employed, as described previously (Cho and Garant, 1981b). Thin sections approximately 100-nm-thick were cut from blocks of each time period and were coated with a crystalline monolayer of Ilford L4 emulsion. After exposure at 4°C, the sections were developed with physical developer for compact grains (Kopriwa, 1975).

### *Quantitative Analysis of Silver Grains*

The synthesis of mannose-containing glycoproteins during cell differentiation in the dental follicle proper and in the perifollicular mesenchyme was expressed by counting silver grains over cells and expressing the results as the number of grains per unit area of 100 $\mu m^2$. This quantitative analysis was performed in the two animals killed 10 minutes after [$^3$H]mannose injection, because at this time period essentially all the radiolabeled precursor had been incorporated and was located intracellularly. A total of 16 blocks (four from each maxilla) was used for this purpose. Fibroblasts in the dental follicle proper and perifollicular mesenchyme were sectioned in a midlongitudinal plane (i.e., containing in profile a nucleus, Golgi, and/or cell body cytoplasmic mass, and a major cell process), photographed at 1,000 × in a Zeiss photomicroscope (Carl Zeiss, Inc., New York, NY), and printed at a final magnification of 5,000 ×. Twenty fibroblasts were thus recorded in each of four zones of root development (see Results).

The number of silver grains over each cell was counted, and the cell area was measured with a Knotron MOP analyzer (Carl Zeiss). The mean of three area measurements was used to calculate the number of silver grains per 100 $\mu m^2$ of cytoplasm. The results are shown in Table 1.

## RESULTS

Root development in the maxillary first molar of 14-day-old rats was well advanced (Figs. 1a and 2a). Fibroblasts and collagen fibers of the periodontal ligament were arranged obliquely from the root surface coronally toward the alveolar bone in areas where the root was

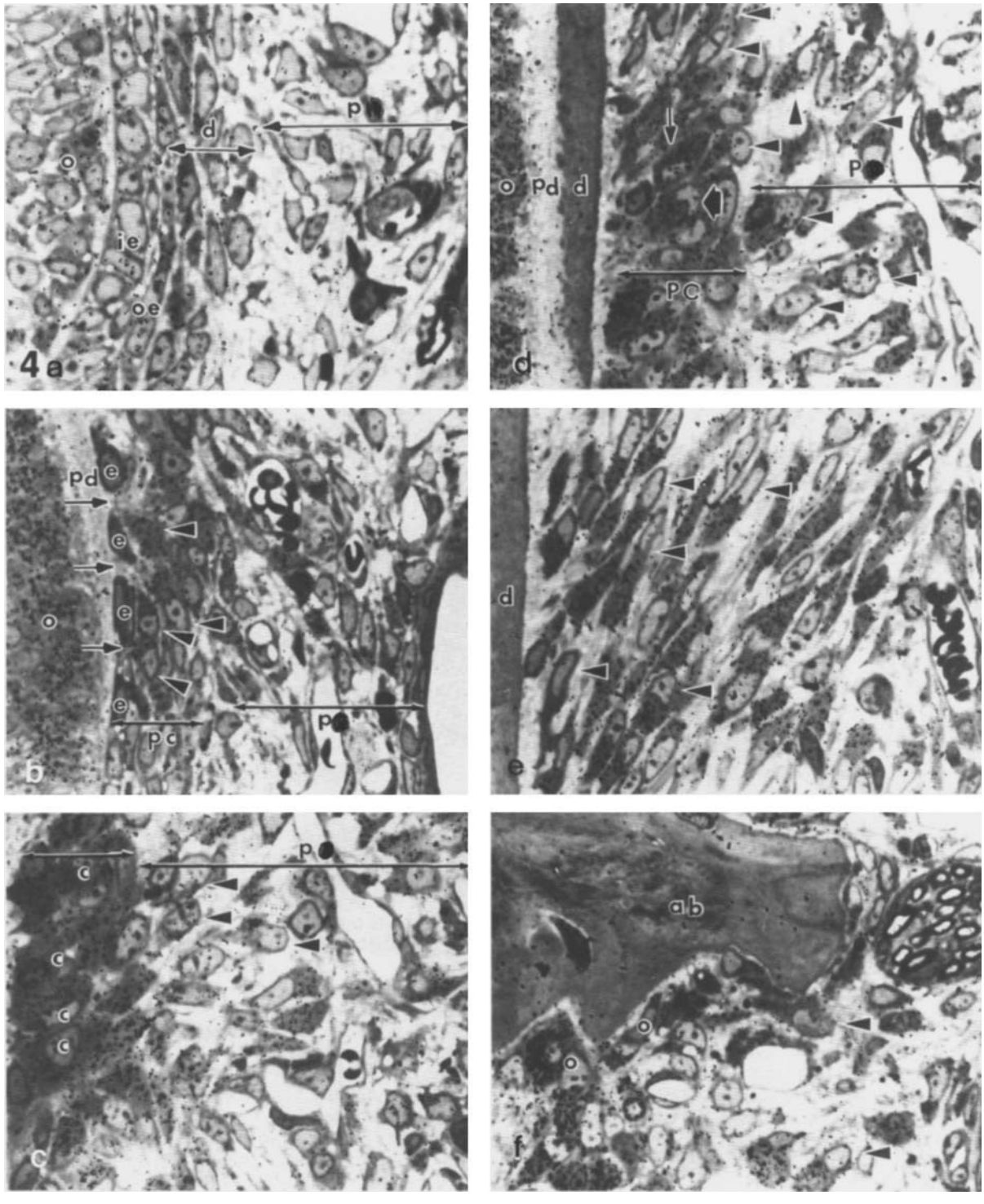
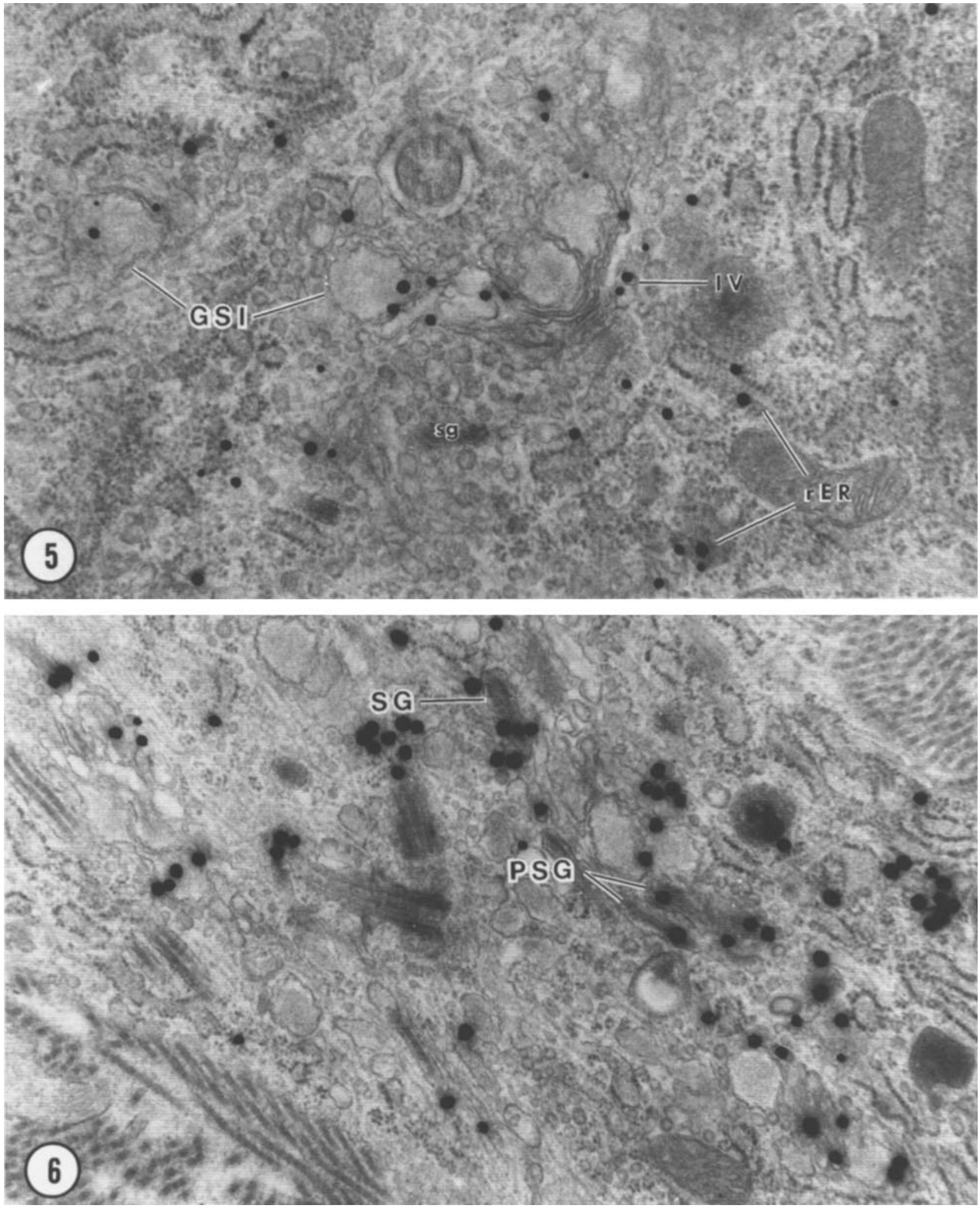


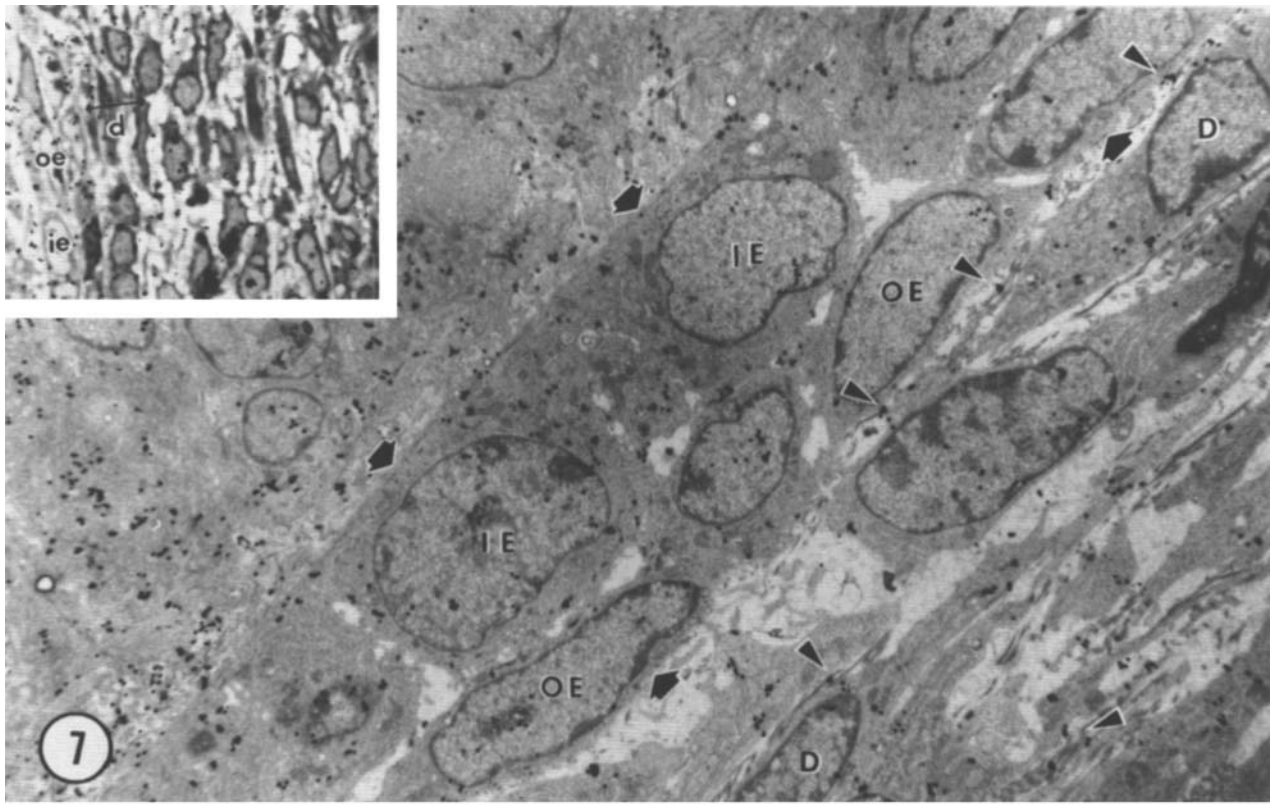

Fig. 4. Light microscopic radioautographs demonstrating uptake of [$^3$H]mannose during development of the periodontal ligament at 10 minutes after intravenous injection. **a:** Note small number of silver grains over the predontoblast (o), the inner (ie) and outer (oe) epithelial cells of Hertwig's root sheath, the cells in the dental follicle proper (d), and mesenchymal cells in the perifollicular area (p). ×1,000. **b:** Note the presence of an increased number of silver grains over the precementoblasts (pc) (arrowheads), which demonstrate polarity toward the dentin. Penetration of precementoblasts between the inner epithelial cells (e) occurs as the root sheath becomes discontinuous (arrows). Cells in the perifollicular area (p) show a low level of silver grains, while odontoblasts (o) are heavily labeled. Predentin (pd) remains unlabeled. ×1,000. **c:** Note heavy labeling of the cementoblasts (c) and the fibroblasts (arrowheads) adjacent to the cementoblasts. Cells in the perifollicular area also show increased labeling (p). The fibroblasts (arrowheads) exhibit an increase in cell size, and many are oriented toward the dentin surface. ×1,000. **d:** The region adjacent to the root surface (labeled PC) contains heavily labeled cells that we presume have previously functioned as cementoblasts. These cells, which are still heavily labeled with [$^3$H]mannose, are no longer uniformly polarized toward the dentin (d) surface. Some cells (thin arrow) are oriented away from the dentin, while another (thick arrow) remains polarized toward the root surface. Fibroblasts (arrowheads) near the root surface as well as in the bone half of the periodontal ligament are heavily labeled. pd, predentin; o, odontoblast. ×1,000. **e:** A homogenous population of elongate fibroblasts (arrowheads) with a high degree of cytoplasmic polarity occupies the periodontal ligament in the region coronal to that depicted in d. Note that their cytoplasm is heavily labeled. d, dentin. ×1,000. **f:** Osteoblasts (o) lining the surface of alveolar bone (ab) and fibroblastic cells (arrowheads) adjacent to the bone show heavy labeling of their cytoplasm. ×1,000.




Figs. 5 and 6. Radioautographs depicting location of [$^3$H]mannose at 10 minutes (Fig. 5) and 30 minutes (Fig. 6) after intravenous injection.

Fig. 5. At 10 minutes, the majority of silver grains is localized over rER, immature Golgi saccules type 1 (GS1), and intermediate vesicles (IV). Secretory granules (sg) remain unlabeled. ×32,000.

Fig. 6. At 30 minutes, numerous silver grains are present on either presecretory granules (PSG) or secretory granules (SG). ×32,000.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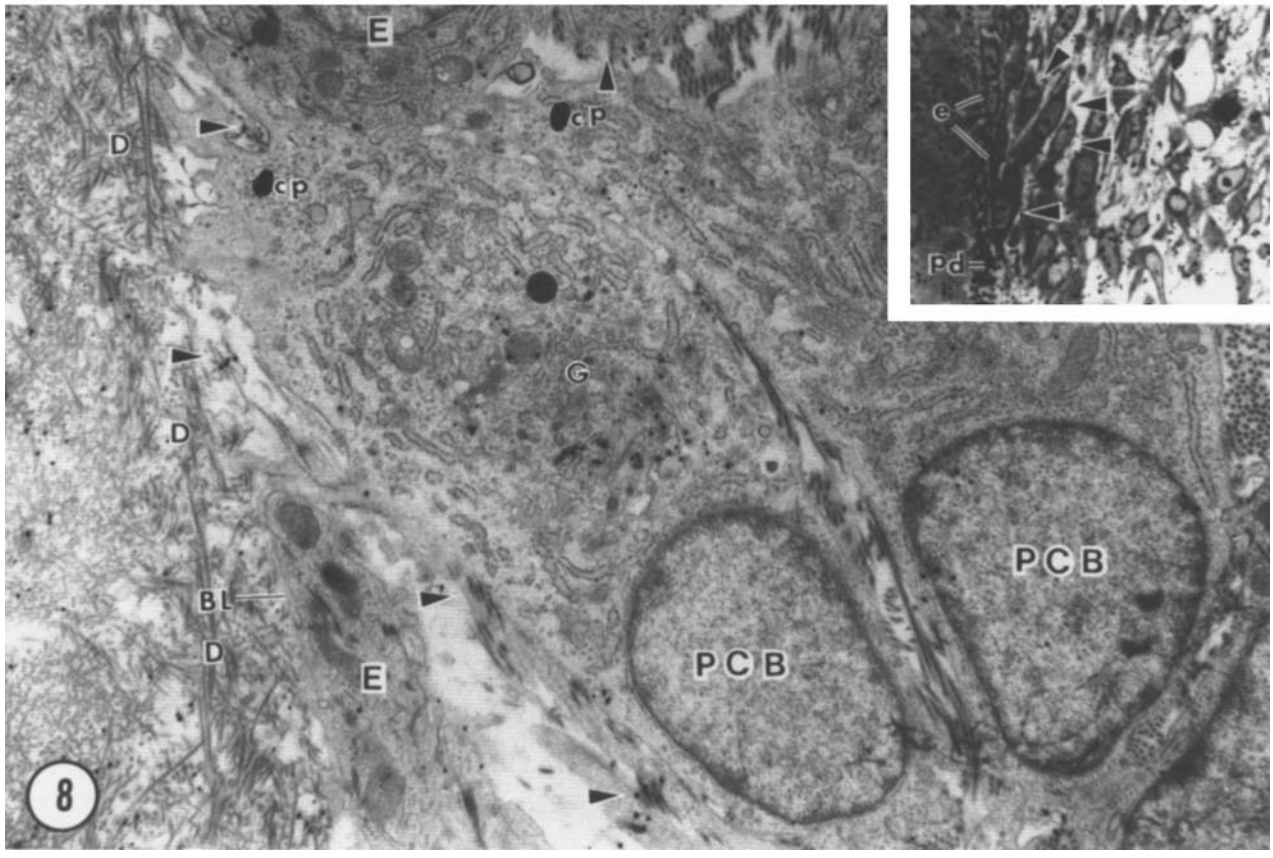
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fully formed (Figs. 1a and b). Apically, in the area of continued root formation, an intact epithelial root sheath and adjacent layers of elongated fibroblasts (the dental follicle proper) were present (Figs. 1a and 2a). In order to describe the sequential events involved in cementogenesis and periodontal ligament formation, we have chosen to subdivide the area of active root formation into the following five zones: I, dental follicle proper; II, cementoblast differentiation; III, cementogenesis; IV, post-cementogenesis transition; and V, newly formed periodontal ligament. The general morphological criteria used in demarcating these zones, and the level of cell differentiation in each zone, are described below. These subdivisions and the changes occurring within them are summarized in diagrammatic form in Figure 14.

### *I: Dental Follicle Proper*

This zone was characterized by an intact epithelial root sheath located between the preodontoblasts of the dental papilla and the dental follicle proper. An internal basement membrane separated the inner layer of the epithelial root sheath from the preodontoblasts, and an external basement membrane was present along the outer layer of epithelial cells (Figs. 4a and 7). Preodontoblasts were beginning to concentrate along the internal basement membrane, but at this stage no deposition of predentin matrix was evident. The mesenchymal elements external to the epithelial root sheath were segregated into two populations of cells, the dental follicle proper (DFP) and mesenchymal cells of the perifollicular area (PFA). The cells of the DFP were elongated and closely parallel to the epithelial root sheath. The cells of the PFA bounded by the DFP and the developing alveolar bone were stellate-shaped, small, and randomly oriented (Fig. 4a). In contrast to the DFP, the cells of the PFA were rather widely separated.

### *II: Cementoblast Differentiation*

In this zone, the continuity of the epithelial root sheath and its basement membranes was disrupted, first in the outer epithelial layer and second in the inner layer, by hypertrophic cells of the DFP (Fig. 4b). Fully differentiated odontoblasts were depositing the first layers of dentin matrix (Fig. 3a). The cells of the DFP were polarized toward the dentin and several cytoplasmic processes were oriented toward that surface (Fig. 3a and 4b). These cell processes, rich in cytoplasmic filaments, penetrated between epithelial cells and made contact with the dentin surface (Fig. 8) (Cho and Garant, 1988). During this process, the epithelial cells of the epithelial root sheath were separated from each other and were displaced from their original location.

### *III: Cementogenesis*

In this zone, the dentin surface was covered by fully differentiated cementoblasts (Figs. 3a and 4c). These cells were mostly low cuboidal in shape and were well polarized toward the dentin surface. A large, well-developed Golgi complex was located between the dentin surface and the nucleus (Fig. 9). Cementoblast cytoplasm, rich in granular endoplasmic reticulum and free ribosomes (Fig. 9 inset) stained more intensely with toluidine blue than the surrounding cells. Cells in the perifollicular area increased in number and size and began to demonstrate cytoplasmic polarization as they gave rise to the periodontal ligament fibroblast population (Fig. 4C).

### *IV: Postcementogenesis Transition*

In this zone, the cementoblasts were no longer in close contact with the newly deposited cementum and became mixed into the periodontal ligament fibroblast population. During this process, cementoblasts loose their plump cuboidal shape and assume a more elongated outline, resembling the fibroblasts of the developing periodontal ligament (Figs. 3b and 4d). Thus, in this zone the periodontal ligament space adjacent to the root surface was populated by more elongated polarized cells (Fig. 4d). Many of these cells continued to stain more intensely with toluidine blue, suggesting a cementoblast lineage. For ease in description and discussion, we will refer to these cells as postcementogenic fibroblasts. Some periodontal ligament fibroblasts were observed to be oriented toward the cementum with long cytoplasmic processes extending between postcementogenic fibroblasts toward the cementum surface (Fig. 9).

### *V: Newly Formed Periodontal Ligament*

Fibroblasts in newly formed periodontal ligament appeared fully differentiated with an elongated profile parallel to recently deposited collagen fibers. These cells were highly polarized and oriented either toward the root surface or the alveolar bone. Cementoblasts were no longer identifiable along the root surface (Fig. 4e). Periodontal ligament cells adjacent to the bone surface were frequently oriented toward the bone surface and were also labeled with mannose (Fig. 4f).

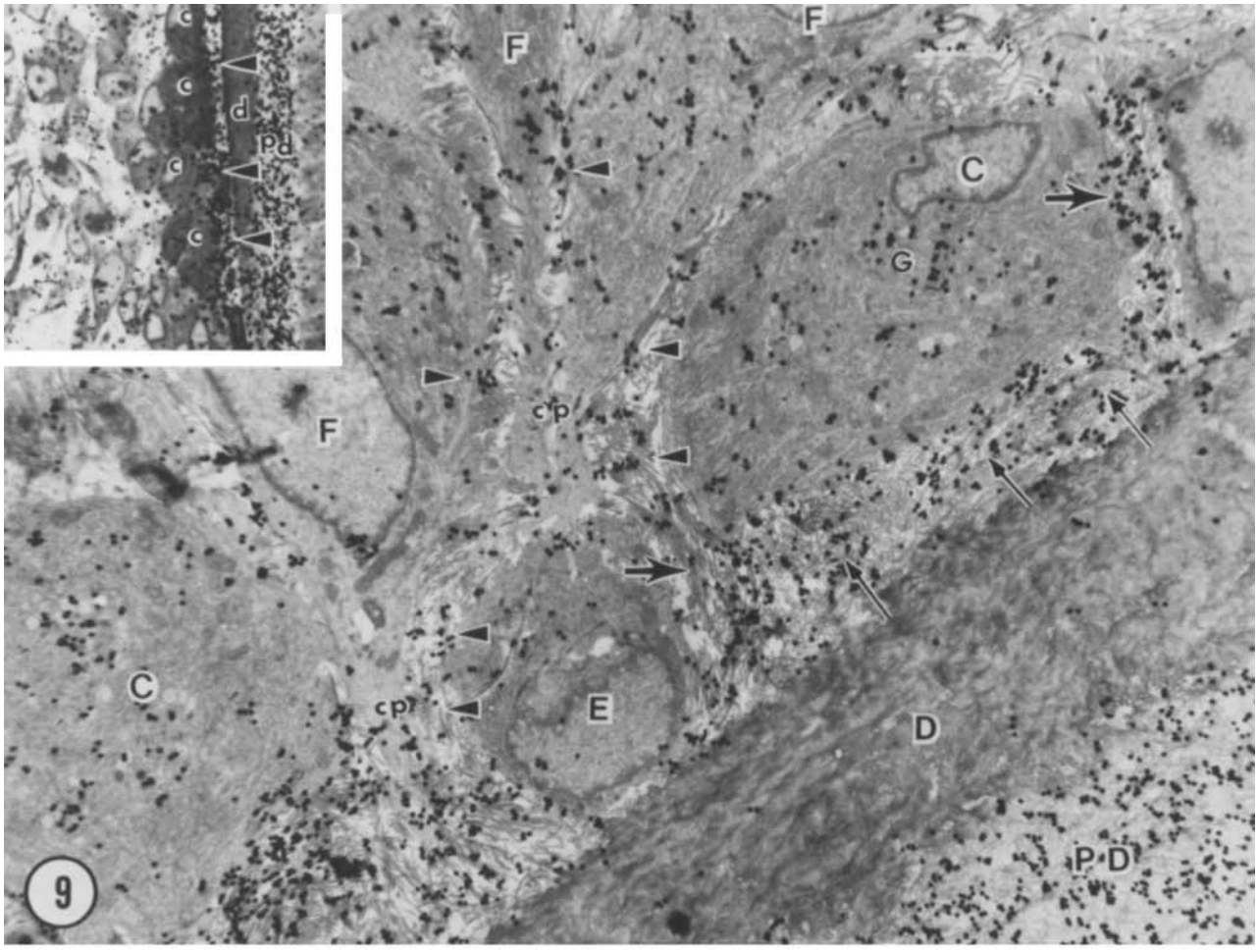
### *Radioautographic Studies*

#### Pattern of [$^3$H]mannose deposition at the developing dentino-cementum interface

At 30 minutes after injection, [$^3$H]mannose was incorporated as a dense band in newly secreted predentin matrix beneath fully differentiated odontoblasts (Fig. 3a

Fig. 7. Radioautographs showing the deposition of mannose-containing glycoprotein(s) by the cells in the dental follicle proper at 30 minutes afer intravenous injection of [$^3$H]mannose. Note the deposition of mannose-containing extracellular matrix (ECM) components (arrowheads) by the cells in the dental follicle proper (D). Both the inner (IE) and outer (OE) epithelial cells and their basal laminae (arrows) remain intact. ×5,600. **Inset:** Light microscopic radioautograph at 30 minutes, indicating the location of the inner (ie) and outer (oe) epithelial cells, and the cells in the dental follicle proper (d). ×500.

Fig. 8. Radioautographs showing deposition of mannose-containing glycoproteins by the precementoblasts at 30 minutes after intravenous injection of [$^3$H]mannose. Note the deposition of mannose-containing ECM products (arrowhead) over small collagen fibrils on the dentin surface (D) and alongside the precementoblasts (PCB). Cell processes (cp) of precementoblasts contact the dentin surface between epithelial cells (E). Note labeling of the Golgi complex (G) and polarity of the precementoblasts. BL, basal lamina. ×9,600. **Inset:** Light microscopic radioautogaph at 30 minutes, demonstrating the location of precementoblasts (arrowheads). e, inner epithelial cells; pd, predentin. ×500.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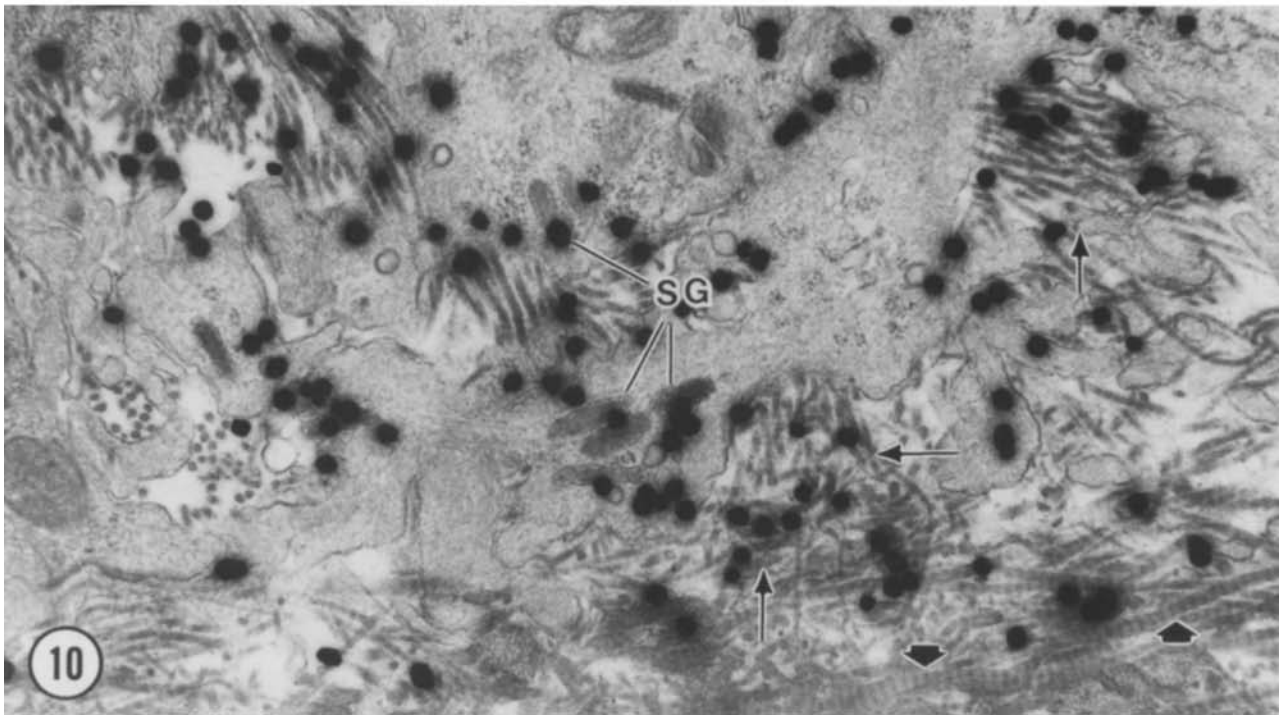
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and b). Another band of radiolabeled matrix was deposited beneath the newly differentiated cementoblasts (Figs. 3a, and b and 9). The shorter band of radiolabeled cementum joined the longer band of labeled predentin at a point on the root surface where the epithelial root sheath was no longer intact, producing a "Y"-shaped pattern of silver grains in the radioautographs at 30 minutes (Fig. 1b). The band of labeled cementum was observed only along the root segment covered by cementoblasts (i.e., zone III: cementogenesis) Figs. 1a and b and 3a). The fact that the older root surface (zone V) was not labeled (Fig. 4e) correlated with the disappearance of cementoblasts at that site. The "Y" pattern of newly deposited matrix was still visible 2 days after [$^3$H]mannose injection (Fig. 2a and b). At the pulpal side, the band was located in older mineralized dentin, and the cementum band was present on the root surface, associated with the newly formed periodontal ligament (Fig. 2a and b).

### Electron microscopic localization of [$^3$H]mannose

Ultrastructural examination of radioautographs revealed labeling of immature Golgi saccules (Golgi saccules I) at 10 minutes, (Fig. 5) and the labeling of presecretory and secretory granules at 30 minutes (Fig. 6). In zone II, ECM alongside precementoblasts and between the dentin and the cell processes of the leading edge was also labeled at 30 minutes (Fig. 8). Precementoblast Golgi structures also contained [$^3$H]mannose (Fig. 8). Heavy labeling of the ECM, especially collagen fibrils, was observed at 1 hour (Fig. 9). The ECM between cementoblasts and dentin, and within the intercellular spaces between cementoblasts, was heavily labeled (Fig. 9). Cells with a more elongated fibroblastic appearance also appeared to contribute to deposition of ECM at these two sites via cellular processes extending between cementoblasts (Fig. 9). High magnification of cementoblast cytoplasm abutting the dentinal surface revealed the accumulation of labeled secretory granules at that surface and a heavy labeling of collagen fibrils between the cell membrane and the dentin surface beginning at 30 minutes (Fig. 10). In zones IV and V of the newly differentiating periodontal ligament, [$^3$H]mannose-labeled material was deposited in association with collagen fibrillogenesis (Figs. 11 and 12). At 4 hours after injection, newly forming principal fibers were covered by silver grains (Figs. 11 and 13).

### Analysis of cell size and [$^3$H]mannose labeling during cell differentiation

Prior to overt differentiation, the cells of the DFP and those of the adjacent PFA were relatively small. The area of cell profiles in the DFP averaged 280 $\mu m^2$, and those in the PFA averaged 301 $\mu m^2$ (Fig. 4a and Table 1). As the cells of the DFP differentiated into precementoblasts, the area of their cellular profiles increased to an average size of 489 $\mu m^2$ (Fig. 4b and Table 1). A similar increase in size to an average of 476 $\mu m^2$ was noted for the PFA cells in zone II (Table 1). Fully differentiated cementoblasts (zone III) exhibited the largest sectional profiles, averaging 1,198 $\mu m^2$ (Fig. 4c and Table 1). Fibroblasts occupying the adjacent developing periodontal ligament, presumed to be derived from the PFA, exhibited an average sectional area of 903 $\mu m^2$. Periodontal ligament fibroblasts in the newly formed periodontal ligament (zone V) had an average sectional outline area of 987 $\mu m^2$ (Fig. 4e and Table 1).

At 10 minutes after its intravenous injection, most of the [$^3$H]mannose was taken up inside cells (Fig. 4). By comparing the number of silver grains over cells at 10 minutes after [$^3$H]mannose injection, we obtained a semiquantitative picture of the relative synthetic activities in each cell type (Table 1). Cells in zone I had the lowest number of grains per unit area (100 $\mu m^2$) of cytoplasm. A steady increase in [$^3$H]mannose uptake per unit area was observed from zone I to zone III in the dental follicle proper, precementoblast, and cementoblast cell lines (Table 1). Fully differentiated cementoblasts had the highest level of activity, demonstrating a nearly tenfold increase in grains per cell and a doubling of grains per 100 $\mu m^2$ of cytoplasm when compared with cells of the DFP (Table 1).

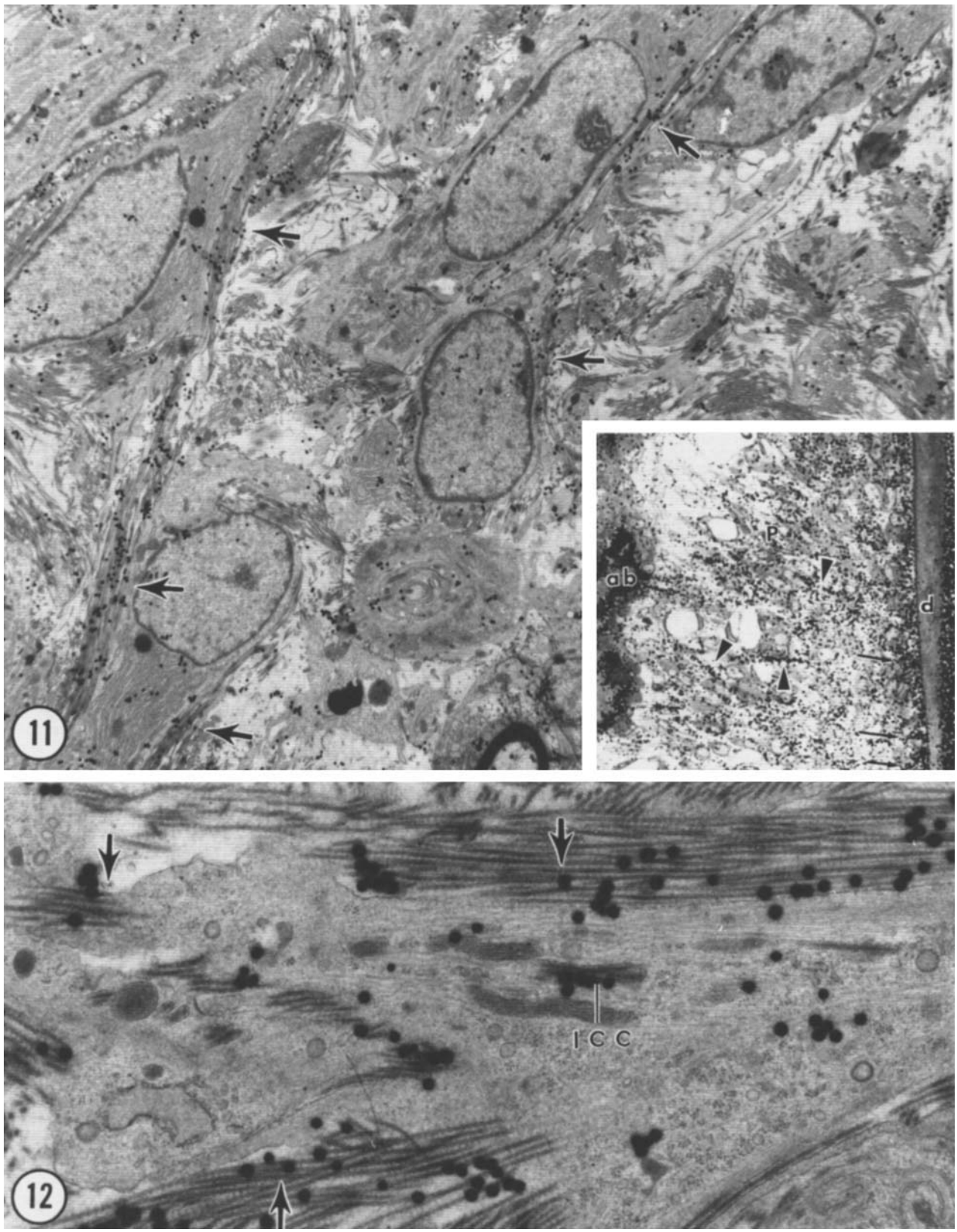
A similar increase in cell size and [$^3$H]mannose uptake per unit area of cytoplasm was also noted in the cells of the PFM as they differentiated into fibroblasts of the periodontal ligament (Fig. 4 and Table 1). In zones IV and V, fully differentiated periodontal ligament fibroblasts had an area three to five times larger than the cells of the DFP and PFA. In addition, the periodontal ligament fibroblasts showed a two to three timies higher level of grains per 100 $\mu m^2$ than their less differentiated precursors (Table 1).

## DISCUSSION

Numerous descriptions of root formation and cementogenesis have been published (Selvig, 1964; Shibata and Stern, 1967; Lester, 1969; Freeman and Ten Cate, 1971; Owens, 1974; Ten Cate, 1980; Furseth et al., 1986). A critical and thorough review of this literature as well as the report of new observations on the development of the periodontal ligament and cementogenesis is contained in a recently published monograph (Schroeder, 1986). In this report, we have followed Schroeder's terminology in using the term "dental follicle proper" in referring to layers of spindle-shaped ectomesenchymal cells that flare out from the base of the dental papilla to envelop the enamel organ as well as the root sheath, and the term "perifollicular mesenchyme" to refer to

Fig. 9. Electron microscopic radioautograph demonstrating the deposition of mannose-containing glycoproteins on the dentin surface by cementoblasts and in the intercellular spaces by the fibroblasts migrating toward the dentin at 1 hour after intravenous injection of [$^3$H]mannose. Note heavy labeling of numerous collagen fibrils (thin arrows) deposited on the dentin (D) surface, and in the intercellular spaces (thick arrows) between cementoblasts (C) and epithelial cells (E), or between the fibroblasts (F) and cementoblasts (arrowheads). PD, predentin; G, Golgi complex; CP, ×4,500. **Inset:** Light microscopic radioautograph at 1 hour, indicating the location of cementoblasts (c) and deposition of mannose-containing ECM products (arrowheads) on the dentin (d). Pd, predentin. ×850.

Fig. 10. Electron microscopic radioautograph demonstrating the deposition of mannose-containing ECM products on the dentin surface at 30 minutes after intravenous injection of [$^3$H]mannose. Note the presence of numerous silver grains over the secretory granules (SG) in the cell process, and collagen fibrils (thin arrows) deposited directly on the dentin surface (thick arrows) and around cell surface (thin arrows). ×24,000.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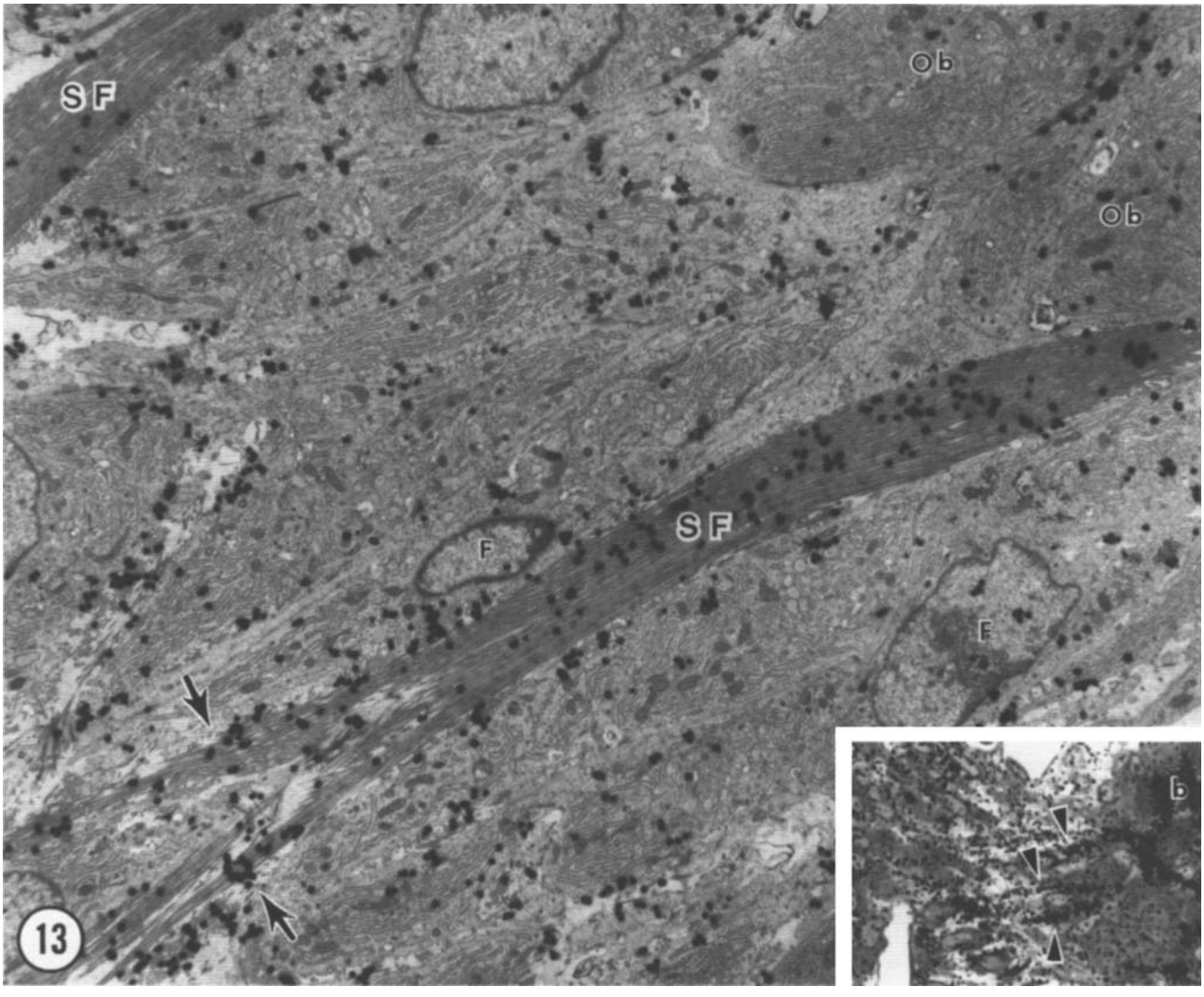


Fig. 13. Electron microscopic radioautograph demonstrating heavy labeling over Sharpey's fibers near alveolar bone at 4 hours after intravenous injection of [$^3$H]mannose. Note labeling over Sharpey's fibers (SF) coursing between fibroblastic cells (F) as well as osteoblasts (Ob). Sharpey's fiber appears to split into two thinner fibers toward the middle region of the periodontal ligament (arrows). ×46,000. **Inset:** Light microscopic radioautograph at 4 hours, indicating formation of thick Sharpey's fibers (arrowheads) in the bone surface (b). ×500.

Figs. 11 and 12. Electron microscopic radioautographs demonstrating deposition of [$^3$H]mannose labeled material in association with fibrillogenesis in the middle third of the developing periodontal ligament at 4 hours after intravenous injection of [$^3$H]mannose.

Fig. 11. The majority of silver grains are located over extracellular collagen fibrils. Heavier labeling over collagen fibers (arrows) in close and paralleel arrangement to the fibroblasts. ×7,200. **Inset:** Light microscopic radioautograph indicating the location of fibrillogenesis in the middle third of the periodontal ligament (arrowheads) as well as on the dentin (d) surface (arrows). ab, alveolar bone; p, periodontal ligament. ×415.

Fig. 12. Note the location of silver grains over collagen fibrils close to the cell surface (arrows). Intracellular collagen fibrils (ICC) are also labeled. ×19,600.

more sparsely distributed and less elongated cells occupying the area between developing alveolar bone and the dental follicle proper. Although numerous investigators have noted the morphological distinction of these two tissues, their specific contribution to the fully formed periodontium continues to be debated.

There is now good evidence that the dental follicle proper, like the dental papilla, is of ectomesenchymal origin. In fact, the dental papilla appears to generate the dental follicle proper (Osborn and Price, 1988). Osborn (1984), in his studies of comparative odontogenesis, has concluded that primitive tooth mesenchyme eventually gives rise to two differentiation compartments: an alveolar clade producing fibroblasts and osteoblasts, and a cement clade producing fibroblasts and cementoblasts. Our results suggest that the dental follicle proper

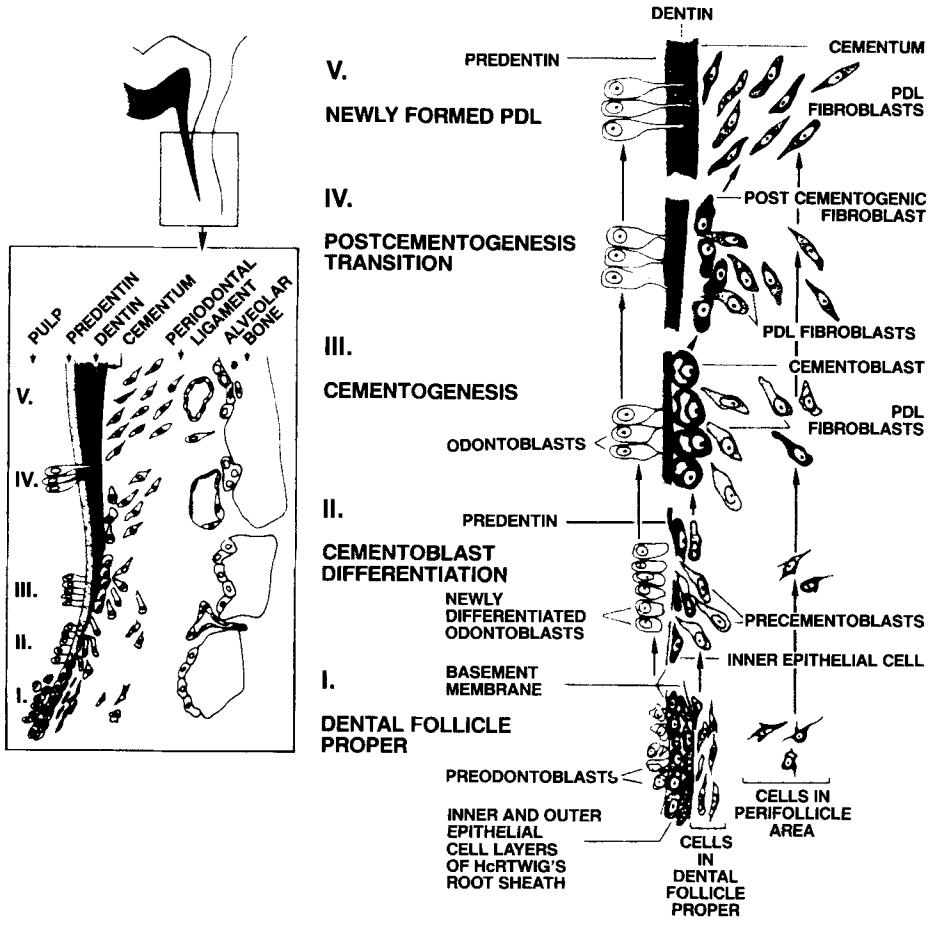


Fig. 14. Diagrammatic representation of root formation, depicting the stages in the genesis of acellular cementum and the dual source of periodontal ligament fibroblasts.

may be the morphological equivalent of the cement clade, and the perifollicular mesenchyme may represent the alveolar clade. Thus, both the dental follicle proper and perifollicular mesenchyme contribute to the pool of periodontal ligament fibroblasts. Ten Cate (1972) and Perrera and Tonge (1981a,b) have shown that the apical region of the developing periodontal ligament contains a proliferation compartment from which periodontal ligament fibroblasts originate. Our observations indicate that both the dental follicle proper and perifollicular mesenchyme contribute to this pool.

In an electron microscopic study of the development of the periodontium, Freeman and Ten Cate (1971) reported that cells in the dental follicle differentiated into periodontal ligament fibroblasts and cementoblasts. They described the amplification of the rough endoplasmic reticulum and the Golgi apparatus during cementoblast formation. Although they also reported that dental follicle cells appeared to insinuate themselves between epithelial cells of the root sheath, they did not describe the morphology of cells processes in the leading edge and the development of cytoplasmic polarity. Directed cell migration from the dental follicle proper and its implications in disruption of the root sheath and in cementogenesis were not discussed.

In the present study, we have confined our observations to the development of the cervical half of the root, and therefore our results pertain solely to the question of the genesis of acellular cementum. We have divided the region of periodontal ligament development into five zones for ease and clarity of description. During stages I and II, cementoblast differentiation involved the development of cell polarity, amplification of organelles responsible for extracellular matrix synthesis and secretion, and an increase in cell processes rich in cytoskeletal elements directed toward the dentin matrix. In a previous paper we described the ultrastructural changes in precementoblasts, which support the idea that they are undergoing directed cell migration (Cho and Garant, 1988). The differentiation of cementoblasts and the deposition of an extracellular matrix is evident during stage III. Although the layer of new matrix is roughly no more than 1 $\mu$m thick, the ultrastructural evidence of [$^3$H]mannose-labeled secretion granules in cell processes contacting the dentin and the abundance of label associated with collagen fibrils in the extracel-

lular space abutting the dentin are clear proofs of the formation of this layer by the newly differentiated cementoblasts. Owens, in studies of developing roots in dogs (1974, 1975) and rats (1979) and Schroeder, in his studies of human teeth (1986) concluded that during initial root formation the periodontal ligament fibers attach directly to a layer of unmineralized dentin matrix and that acellular cementum is not formed until a later stage, when cellular cementum has been formed at the apex of the root. Furthermore, Owens (1979) reported deposition of granular material on the dentin surface by the inner epithelial cells of the root sheath. We were unable to confirm secretion of an ECM product by the root sheath. Several explanations may be given for the difference in our observations and conclusions. It appears from published micrographs that Owens, in his study of 12-day-old rat molar teeth (1979), may have limited his observations to the area of root development just apical to the enamel. Perhaps a lingering ameloblastic function is displayed in the inner epithelial cells of the root sheath at this site. Re-examination of dog and human periodontal ligament formation in optimally fixed specimens with high resolution and the knowledge that cementoblast function during acellular cementum formation is only a brief event may lead to reinterpretation of the data.

We have previously studied the utilization of [$^3$H]mannose by periodontal ligament fibroblasts by electron microscopic radioautography (Cho and Garant, 1987). The routes and time periods for the intracellular translocation of mannose-containing glycoproteins in the periodontal ligament fibroblasts were found to be comparable to those for procollagen (Cho and Garant, 1981b, c). Among the known mannose-containing glycoproteins of connective tissues are fibronectin (Moczar et al., 1979; Labat-Robert, 1981), structural glycoprotein (Anderson and Jackson, 1971), and the carboxyl terminal propeptides of procollagen (Olsen et al., 1977; Moczar et al., 1979). Thus the silver grains over the extracellular matrix around fibroblasts and cementoblasts observed after 30 minutes represent [$^3$H]mannose incorporation into one or more of the above components. Other mannose-containing glycoproteins specific to cementum might also exist. Attachment of the periodontal ligament connective tissue to the dentin surface obviously requires the localized secretion of new collagen molecules at that surface, and perhaps there is also a need for simultaneous deposition of an adherence factor such as fibronectin or some other glycoprotein specific to cementum. Formicola et al. (1971) reported a gradual increase in thickness of rat molar acellular cementum with time. There appears to be no doubt that the extrinsic fibers are of periodontal ligament fibroblast origin. However, additional research is needed to identify the source and composition of the organic matrix (cementum) that surrounds the extrinsic fibers and undergoes mineralization.

In 1979, in noting that active periodontal ligament fibroblasts were highly polarized cells, we speculated on their possible migratory behavior (Garant and Cho, 1979a). We also reported that the development of Sharpey's fibers in the periodontal ligament was coordinated to bone formation and apparently to migration of fibroblasts (Garant and Cho, 1979b). In subsequent publications, we determined that collagen secretion occurred in a highly polarized fashion under control of the microtubular network, and furthermore, that the secretory pole of the periodontal ligament fibroblast was also the leading edge of the cell during migration (Cho and Garant, 1981a, b, 1985). Migration of collagen-secreting cells toward alveolar bone and cementum could lead to the formation of Sharpey's fibers. In the context of the present results, it appears that directed migration of precementoblasts toward the dentin surface is associated with the deposition of fibers oriented approximately perpendicular to the long axis of the root. Continued deposition of collagen molecules as the cementoblasts reach the dentinal surface increases the width of these fibers near the point of insertion.

We readily admit that this description contains numerous assumptions based on stratic micrographs. Nevertheless, we emphasize that when reviewed in the context of our previous studies and the large body of knowledge that has accumulated in the last 10 years on fibroblast migration and cell-matrix interaction, the assumptions are reasonable, and the concept suggests numerous additional avenues of study.

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